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BIOCHEMICAL AND PHYSIOLOGICAL ASPECTS OF ENDURANCE EXERCISE IN THE HORSE

by

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Presented for the degree of Doctor of Philosophy

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August, 1985

"A great, golden-bearded Centaur, with man's sweat on his forehead and horse's sweat on his chestnut flanks ....."

C.S. Lewis, "The Last Battle", p.19.

Dedication

To Gordondene (Highland pony register no. B134), who probably has this thesis to thank for the fact that he has never had to take long distance riding seriously;

Also to Rolfe, for getting off the page long enough for me to write something;

And to my parents, for tea and sympathy.

Acknowledgements

I would like to thank my supervisors and those other members of the academic staff who gave me considerable help and encouragement - Dr C.D. Munro, Dr D.H. Snow, Professor T.A. Douglas, Dr D.McE. Jenkinson and Professor J.A. Bogan.

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A financial contribution towards the cost of the work presented in Section 2 was generously made by the Endurance Horse and Pony Society of Great Britain. Grateful thanks are also due to the owners and riders of horses entered for EHPS events who allowed blood samples to be collected from their horses and who assisted with details of diet and training regimes and the collection of urine and sweat samples.

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Throughout this thesis the usage of the word "parameter" is not strictly correct. The words "constituent" or "variable" would have been more appropriate and this sense should be applied to the word wherever it appears.

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LIST OF ABBREVIATIONS

AAI	Technicon autoanalyser I system
AAII	Technicon autoanalyser II system
ACTH	Adrenocorticotrophic hormone
ADP	Adenosine diphosphate
ALP	Alkaline phosphatase
AMP	Adenosine monophosphate
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BHS	British Horse Society
cAMP	Cyclic adenosine monophosphate
CK	Creatine kinase
CNS	Central nervous system
CTR	Competitive trail ride
c.v.	Coefficient of variation
DEA	Diethanolamine
ECF	Extracellular fluid
EHPS	Endurance Horse and Pony Society of Great Britain
ELDRIC	European Long Distance Rides Committee
ER	Endurance ride
F+30	30 minutes after the finish of an endurance ride
FEI	International Equestrian Federation
FFA	Free (non-esterified) fatty acids
g.l.c.	Gas liquid chromatography
ICF	Intracellular fluid
LDH	Lactate dehydrogenase
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
NAD	Nicotinamide adenine dinucleotide
NADH	Reduced NAD
PCV	Packed cell volume (haematocrit)
PEP	Phospho-enol pyruvate
PK	Pyruvate kinase
r	Correlation coefficient
RIA	Radioimmunoassay
RNA	Ribonucleic acid
SD	Standard deviation
SDF	Synchronous diaphragmatic flutter
SEM	Standard error of the mean
SG	Specific gravity
TB	Thoroughbred
t.l.c.	Thin layer chromatography
$\bar{x}$	Arithmetic mean

In addition, SI (Systeme International d'Unites) units and their commonly accepted abbreviations are used in this thesis.

SUMMARY

A number of biochemical and haematological changes are known to occur in horses involved in long distance riding competitions of 40-160 km, particularly changes associated with disturbances in fluid/electrolyte balance, catabolism of body fuel stores and alterations in the integrity of the muscle cell membrane. This study investigated these changes in more detail in 50 horses involved in competitive rides and in four horses undergoing two 80 km rides under controlled conditions. In addition, experiments were carried out on horses and ponies exposed to a hot, humid environment (41°C, 33°C wet bulb) and during intravenous adrenaline infusion, in order to study further the fluid/electrolyte alterations associated with sweating and in particular the composition of equine sweat. Changes in plasma and urine biochemistry were also studied over 24 hours in horses at rest for comparative purposes.

Significant changes were shown in 13 of the 14 plasma parameters measured in the resting horses. Most of these could be related to feeding, in particular to hay feeding which caused alterations in fluid/electrolyte balance associated with salivary secretion. Urine composition changed very markedly during the 24 hours. Urine flow rate and creatinine and urea excretion were higher during the day and increased following drinking, as in man. Urine potassium and chloride excretion were much higher than sodium excretion and all three electrolytes (and pH, which was alkaline) showed diurnal variations markedly different from those in man.

The competing endurance horses demonstrated moderate haemoconcentration, but plasma electrolyte alterations, particularly an increase in sodium concentration, were not always consistent with the production of

apparently hypertonic sweat. The pattern of fuel utilisation was one of exhaustion of liver glycogen after about 40 km with extensive fat mobilisation and the use of glycerol for gluconeogenesis. Breakdown of phosphocreatine was extensive and evidence of protein catabolism was observed. Large variable increases in plasma CK and AST activities unassociated with fatigue suggested a non-pathological alteration in muscle cell membrane integrity in a number of horses.

During heat exposure changes in PCV and plasma proteins were poorly related to fluid losses but changes in electrolyte concentrations were consistent with the sweat tonicity. Hypertonic chloride and potassium, and isotonic sodium concentrations (relative to plasma) were maintained in the sweat for 4.5 hours. In contrast sweat magnesium and protein concentrations were initially high but decreased exponentially with time. There was a very close correlation between these two parameters.

Most of the changes in plasma parameters seen during adrenaline infusion were attributable to the adrenaline per se, but the profuse sweating induced in the horses caused some haemoconcentration. Small ponies sweated much less in response to adrenaline than Thoroughbred horses. Hypertonic sweat concentrations of chloride, sodium and potassium were maintained for 3 hours and significant differences, particularly in Na/K ratio, were found between heat and adrenaline-induced sweat. Sweat urea concentration was related to plasma urea concentration and glucose appeared in the sweat when the plasma glucose concentration exceeded 10 - 12 mmol/l. The sweat magnesium was not protein-bound and the two main electrophoretic fractions of the sweat protein were not present in serum. The possible function of this protein as a wetting agent was discussed.

During the controlled 80 km rides total fluid loss was 33.5% of the total ECF volume: 78% of this was sweat and 22% respiratory evaporation. Although sweat electrolyte concentrations were again hypertonic to

plasma, theoretical concentrations in total body (sweat plus respiratory) water loss were much closer to plasma concentrations - slightly hypotonic for sodium, slightly hypertonic for chloride. As a result changes in plasma electrolyte concentrations were small, an increase of 9 mmol/l (5.7%) in sodium and a decrease of 4 mmol/l (3.5%) in chloride. Potassium appeared to move out of the intracellular fluid at the start of exercise and back in immediately afterwards and this obscured any effect of sweat losses on plasma concentration. The only urinary constituent which was conserved in the exercising horses was chloride, and the absence of any decrease in urea excretion suggested that the increase in plasma urea concentration was due to increased protein catabolism, probably from the liver. Body fuel utilisation was similar to that seen in the competing horses and plasma CK and AST activities again suggested that a non-pathological disruption of muscle cell membrane integrity was occurring in some horses which continued intermittently for several months.

It was concluded that in the horse, unlike man, the thermoregulatory fluid is approximately isotonic to plasma, which minimises electrolyte imbalances and allows prolonged exercise with less need for drinking. After exercise the large caecum and colon and the sodium contained in them appear to be important in the controlled replacement of the extensive water and electrolyte losses which result.

GENERAL INTRODUCTION

THE EVOLUTION OF THE MODERN HORSE AND THE DEVELOPMENT OF THE SPORT OF LONG DISTANCE RIDING

THE EVOLUTION OF THE MODERN HORSE AND THE DEVELOPMENT OF THE SPORT OF LONG DISTANCE RIDING

Until the spread of the railways in the middle of last century the only motivating force for land transport of both people and goods was muscle power, in one form or another. This muscle power was provided by animals from a variety of species such as cattle, camels, elephants, dogs, reindeer and of course man himself, but probably the most useful of all animals was the horse. Until very recently horses have been virtually indispensable to human civilisation, and it could be argued that, after the discovery of fire, the domestication of the horse was the single most important influence in the advance from the Stone Age to the time of the Industrial Revolution.

Evolution of the Horse

The fossil records of the ancestry of the modern domestic horse (*Equus caballus*) are unusually complete, and the development of the species can be traced in an unbroken line from the Eocene period over 50 million years ago. This development is summarised by Hardman (1981) (Figure 1). The major alterations which have taken place have been an increase in size (*Eohippus* was only about 30 cm high), specialisation of the cheek teeth to favour a grazing rather than a browsing type of mastication, and the reduction of the number of weight-bearing digits on each limb from three or four to one. It is generally believed that these changes are indicative of a move from forest dwelling to a prairie habitat, with the single hoof being particularly well suited for fast locomotion on the hard surface of plains grassland (Romer, 1933; Grove *et al*, 1966).

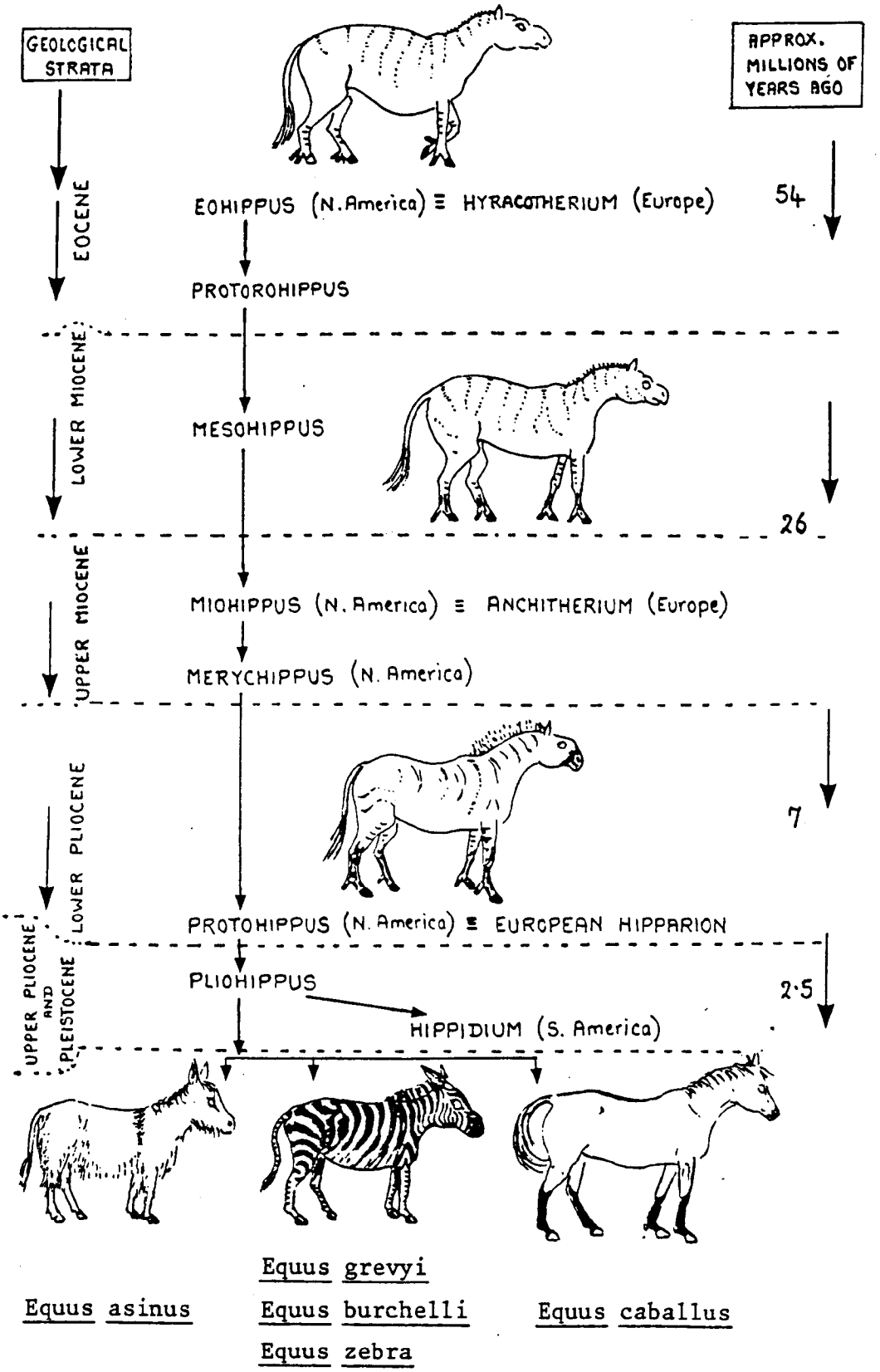


Figure 1. Stages in the evolution of the modern horse.
(from Hardman, 1981).

Eohippus is known to have been present in Europe, in north east Asia and in North America, these last two of which were joined by a land bridge over what is now the Bering Strait. However, it appears that the species did not survive in the Old World and from the Eocene to the Pliocene periods the evolution of the horse took place in North America. During the Pliocene the Pliohippus seems to have migrated widely both to South America and back across Asia into Europe and Africa, and by the time of the Ice Age it was present in every continent except Australia. During this Ice Age the Bering Sea was formed which cut Alaska off from Siberia and resulted in the complete isolation of the New World from the Old, and after this separation it appears that Pliohippus and its descendants became extinct in the Americas. The reason for the disappearance of the species from an environment which was apparently eminently suitable is unknown, although Hardman (1981) suggests a panzootic infection, but as a result the subsequent development of the horse into the forerunners of the many breeds and types evident today occurred in the Old World. Five distinct pre-domestication types of horse emerged from the migration of Pliohippus from Siberia to Europe and Africa: the Celtic Pony, the Heavy Horse, the Asiatic Wild Horse, the Barb and the Arabian (Hardman, 1981), and their geographical distribution is shown in Figure 2. Hayes (1897) postulates that the location of a breed has a consistent effect on the resulting appearance, e.g. that high altitudes produce small horses and hot climates result in taller, leggy animals. He also suggests that humid climates are unsuitable for horse breeding as the horse's major means of thermoregulation is by sweating and a humid atmosphere prevents evaporation, rendering sweat ineffective in cooling the animal.

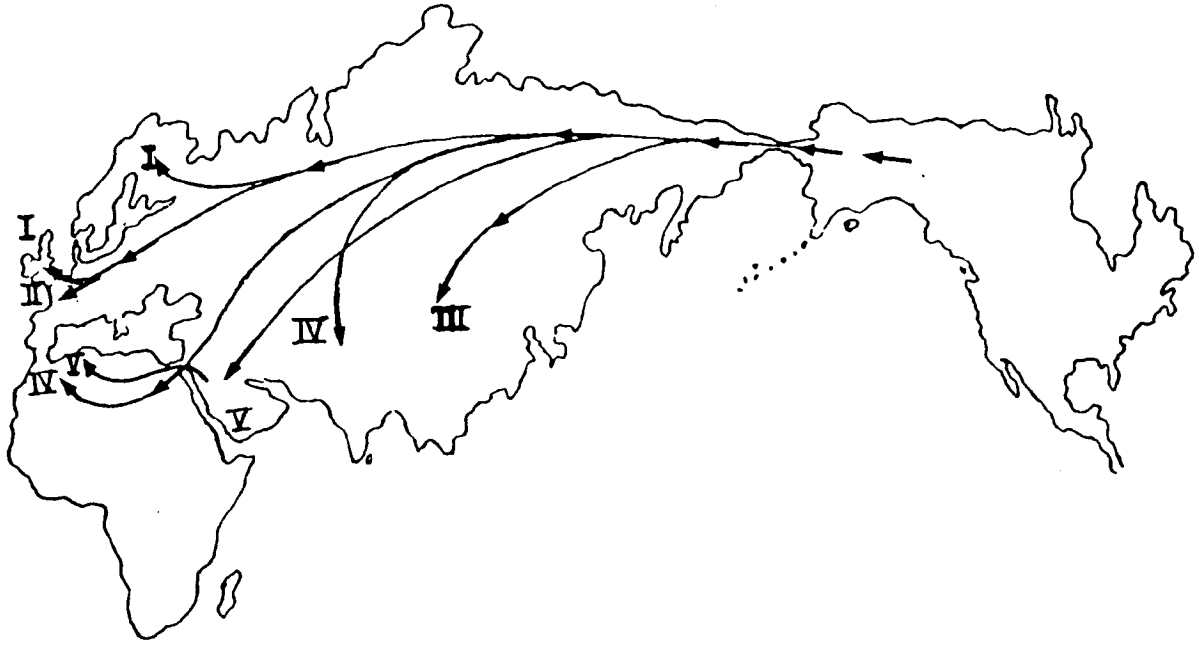


Figure 2. Migration paths of Pliohippus from America into Europe, Asia and Africa, where five distinct pre-domestication types emerged: I, Celtic Pony; II, Heavy Horse; III, Asiatic Wild Horse; IV, Barb; V, Arabian (from Hardman, 1981).

Originally the most important factor influencing the development of the horse was the changing environment, but for the last 5,000 years man and his requirements have been the dominant force. Horses were hunted as food by Stone Age man, but the earliest records of the horse as a domesticated animal appear around 3,000 B.C. (Romer, 1933). One of the best known surviving examples of the Hittite language, an early form of Indo-European, is the "Hippologia hethitica", a manual on the care and training of chariot horses by a man named Kikkuli, a Hurrian from Mitanni who was in charge of the royal stables (Lockwood, 1972). This text, which dates back to 1360 BC, prescribes a diet of concentrates (wheat, barley and chopped grass) and hay, and describes an exercise routine which bears a strong resemblance to the "interval training" system which has recently come into favour with a number of endurance riders. For five millenia horses have been used by man for draught, for agricultural work, for hunting, for personal transport, for war and for recreation, and selective breeding has been practised to improve the suitability of the original pre-domestication types for these purposes. It is therefore impossible to separate the effects of natural selection from the results of deliberate controlled breeding when considering the appearance of the modern horse.

Breeds of Horse

Summerhays (1968) describes the distinguishing features and breeding history of 126 recognisably different breeds and types of horse now in existence. Some of these, such as the Exmoor pony, are believed to differ little from the early wild inhabitants of the areas where they are still found. Others are of more recent origin and several have been very closely line-bred from one (the Morgan horse) or a very few (the English Thoroughbred and the Lipizzaner) particularly

prepotent stallions. Another recent means of establishing a breed has been to select for individuals of a particularly prized colour such as Palomino or Appaloosa.

In the early stages of the development of a breed outcrossing on the female side is common and progeny will be accepted so long as they conform reasonably closely to the standards for that breed. However, the modern trend is for the adoption of closed stud books, which means that only foals whose parents are both registered members of the breed can be registered in their turn. This means that the gene pool is restricted, which tends to lead to a greater uniformity of appearance within the breed. Most breeds also have written "breed standards" which describe the desirable characteristics to be sought by breeders and show judges, and it is noticeable from photographic records that some breeds have altered quite dramatically over the past century or so as a result of exaggeration of points mentioned in the breed standard. As almost all horses are still required to work to some extent, the worst excesses of certain dog breeds have been avoided, but many horsemen maintain that successful show animals are poor workers. Show Clydesdales, for example, are held in contempt by many of those who still use horses for farm work, and serious endurance competitors often have little time for show Arabs.

The British Isles are extraordinarily rich in the number of indigenous breeds of horse and pony to be found here. Classically, nine native mountain and moorland pony breeds are described (Shetland, Highland, Fell, Dale, Welsh, New Forest, Exmoor, Dartmoor and Connemara), each of which originates from a different area and is quite distinctive in appearance. These breeds, particularly the Welsh, can be sub-divided further, and there are also a few extra breeds favoured by enthusiasts (Lundy pony, Eriskay pony and the now extinct Galloway pony). In addition mainland Britain is the home of several breeds of

heavy draught horse (Clydesdale, Shire and Suffolk Punch) and light riding or coaching horse (English Thoroughbred, Cleveland Bay, Yorkshire Coach Horse and Hackney).

Among the wide variety of breeds now in existence it is still possible to make the general distinction between "hot-blooded" horses (those primarily of Arabian or Barb descent, i.e. types IV and V in Figure 2) and "cold-blooded" horses (mainly descended from the Celtic Pony, the Heavy Horse and the Asiatic Wild Horse i.e. types I, II and III in Figure 2). This distinction does not of course refer to any difference in body temperature between the animals, but is descriptive of differences in build and temperament. The division is not in any way absolute, in particular many originally cold-blooded pony breeds have had infusions of Arab blood to "improve the stock", as hot-blooded types are generally preferred for riding purposes. However, there have been some suggestions of basic anatomical and metabolic differences between the two types, particularly with regard to blood volume (Julian et al, 1956) and carbohydrate and fat metabolism (Robie et al, 1975). The breeds used as experimental animals in this present study may be considered good representatives of these two types as the English Thoroughbred is almost exclusively of Arabian/Barb descent while the Shetland pony has remained virtually undisturbed in its present habitat since the Bronze Age and is believed to be very similar to the original Celtic Pony.

Changing role of the horse in the 20th Century

Until early in this present century the absolutely central role of the horse in human communications was essentially the same as it had been since pre-Roman times. Long distances were covered not for recreational purposes but through necessity, and often the horse's welfare was of secondary consideration compared to the demands of the job in hand, whether of the over-romanticised "the mail must go

through" variety or the much maligned cabbie trying to make a living on the streets of a large city. Under these circumstances horse management was an important skill, the principles of which were passed from generation to generation, and horse maintenance (feed supply, farriery, harness making, stabling, etc.) was an important industry employing a large number of people. Organised equine sport was mainly confined to sprint racing and to hunting, and although endurance races were not unknown they were uncommon and usually involved extremely long distances covered with little concern for the horses' welfare.

The successive advent of the railways, telecommunications and most significantly the internal combustion engine have changed this situation out of all recognition in the space, almost, of a single generation. The horse is virtually redundant as a means of transport and its use is now almost entirely leisure-oriented. From the horse's point of view this is not an entirely bad thing. The equine population has certainly decreased in numbers, but as the pressures of the "mail must go through" type have been shifted to non-sentient machinery the horses of today are far less likely to be overworked or underfed out of sheer economic necessity than those of last century. Most horses are still required to work, but the nature of the work has fundamentally altered. Privately owned horses are now likely to be ridden only two or three times per week, or less in bad weather. Horses employed for commercial purposes are also mostly connected to the leisure industry, from Flat racing to pony trekking, and the consumer can easily be alienated by any suggestion of exploitation of the animal. Indeed, there seems to be a general increase in public pressure for the humane treatment of all species of animal.

On the other hand there are now fewer skilled grooms and farriers available, and many owners who look after their own horses have lost touch with the old traditions of "horse sense". In recent years the

popularity of equestrian activities has greatly increased among people whose parents had little contact with horses and unsatisfactory husbandry conditions are now often the result of ignorance of the requirements of the horse in a mechanical age. Even racing and show jumping stables, where the use of the horse is most commercialised, sometimes have difficulty in recruiting experienced staff, and perhaps only in the armed forces, where the horse has been retained in a purely ceremonial capacity, have the traditions of horsemastership been carried on unbroken. It is against this background of a complete alteration in the horse's role in society that the sport of long distance riding has emerged in its modern form.

It is therefore almost certainly the case that very few of today's endurance horses are as fit as the average working horse of last century. The sport is entirely amateur, and all the necessary training (and often all the stable work as well) has to be fitted in to the rider's spare time. Under these circumstances it is very difficult to attain the same level of fitness as a horse which was routinely required to carry a cowboy or a country vet 20 or 30 miles daily in the course of his work. It therefore seems highly likely that the major key to success in endurance riding lies in the devotion of a very considerable amount of time to training. Many riders, however, are not prepared to carry their interest in the sport to these lengths and for them the shorter distance rides provide a pleasant means of seeing new country in company over a well-planned route.

Endurance riding as a sport

The first organised endurance races were held around the turn of the century, and the history of the development of the sport since that time is well documented by Kydd (1979). The early races carried large amounts of prize money, and as a result many horses were ridden hard with little concern for their welfare. In 1892 25 horses died during

the Berlin to Vienna race, and this was followed in 1901 by the deaths of 30 horses in the Brussels to Ostend event. At this time public concern over animal welfare issues was beginning to emerge and there was a general outcry in the British press in condemnation of these races. As a result the races were discontinued, and when the concept of competitive long distance riding was revived some 50 years later great care was taken in the formulation of ride rules to eliminate any possibility of a repetition of these tragedies. Even today the term "race" is seldom used to describe a long distance riding event.

Until the inter-war years feats of endurance travel on horseback were still quite commonly performed through necessity, particularly for military purposes. Hyland (1975) describes a cavalry training march of 90 miles across the North African desert which had to be accomplished in 12 hours with each horse carrying over 300 lb of rider and equipment, and which was still carried out in the late 1930's. This level of performance was probably not attractive to amateur riders, and it is noteworthy that endurance riding as a popular sport did not really emerge until some time after the Second World War when all military transport was completely mechanised.

One legendary feat which was accomplished in 1925-26 was the ride of Professor A.F. Tschiffley from Buenos Aires to New York, using two Criollo ponies. The distance of 13,350 miles was covered at an average speed of 26.5 miles per day, and at one stage they travelled 93 miles across a desert in Ecuador in only 20 hours, without water in an environmental temperature of almost 50°C. Occasional very long distance events are still organised such as the "Bite the Bullet" ride from Sydney to Melbourne in 1975 (600 miles in 12 days riding time) to promote a film with an endurance ride as its main theme, and the "Great American Horse Race" of 1976 (3,600 miles) which was won by a team of

two mules in a time of 98 days. However, modern organised endurance riding generally involves distances of 25 - 100 miles (40 - 160 km) and the longest events are of no more than three days' duration.

Endurance riding as it is known today originated in the 1950's and 60's in the USA and Australia, and the level of competition is still much higher in these countries than in Britain. The terrain in these countries is much more demanding, particularly in terms of gradient, and the climate is considerably hotter. In spite of this the leading competitors generally finish in much shorter times, and the number of horses competing in each event is large by British standards.

Kydd (1979) gives 1973, the year of the foundation of the Endurance Horse and Pony Society of Great Britain (EHPS) as the beginning of modern endurance riding in this country. As Hyland (1975) points out, the Golden Horseshoe Ride run jointly by the Arab Horse Society and the British Horse Society (BHS) dates back to 1965, but strictly speaking this is not actually an endurance ride but a form of competitive trail ride. At present these organising bodies exist side by side and operate different sets of ride rules.

Rules of modern long distance rides

Detailed explanation of the rules of endurance events is probably inappropriate as these rules are still in the process of evolution. The EHPS in particular has been making major rule changes every two or three years in response to feedback from competitors and judges, and this has occasionally generated some confusion. In 1979 another governing body was added to the sport with the formation of the European Long Distance Rides Committee (ELDRIC), of which the EHPS and the BHS are both members and which lays down minimum rules for European Championship rides. In 1984 an attempt was made to harmonise these rules with those of the International Equestrian Federation (FEI) which

had only recently involved itself with endurance competitions. This meeting generated some very lively discussion, but the decisions arrived at are unlikely to prove final.

The different types of long distance riding events currently being organised are described in detail by Kydd (1979). These can be generally divided into three categories:

1. **Competitive Trail Rides (CTR)**

In these classes a time target is set and competitors are penalised both for being too fast and for being too slow. All those who finish inside the time bracket and who gain maximum marks at veterinary inspection are given an award and so in one sense these events are not competitive. Golden Horseshoe Rides run by the BHS are all of this type.

2. **Endurance Rides (ER)**

These classes are genuinely competitive with the winner being the first to finish subject to passing a veterinary inspection as "fit to continue".

3. **Long Distance Races**

These are relatively uncommon and are the same as endurance rides except that the requirement on veterinary inspection is merely to show that the horse is "not ill-used".

The unusual time bracket nature of the CTR rules makes these rides very useful as introductory competitions for less experienced riders, but most ambitious competitors progress eventually to the endurance ride proper as these offer a greater challenge and a greater sense of satisfaction on winning.

The major difference between equine endurance events and human long distance races is the involvement of veterinary surgeons in the capacity of judges, and this has far-reaching consequences. The immediate reason for this involvement is the history of ill-use of

horses discussed above, which is also the reason for the low value of the prizes in most endurance rides - cups, rosettes and belt buckles are the most usual rewards. Unlike marathon races, where anyone who can limp, stagger or crawl across the finish line is deemed to have completed the race and receives the appropriate awards, horses can be (and often are) eliminated from the competition even half-an-hour after having crossed the finish. This half-hour veterinary inspection (F+30) is in fact the most important of all, although the veterinary judges can eliminate a horse showing signs of lameness, exhaustion or injury at any time. At the F+30 examination horses are checked for lameness, injury, dehydration (using the "pinch test" where the return time of a fold of skin lifted from the neck can be related to skin turgor and hence state of hydration) and exhaustion, and a failure in any one of these which the veterinary surgeon considers to be sufficient to render the horse unfit to continue will lead to elimination. In addition heart and respiratory rates are recorded and an F+30 heart rate of over a certain figure, usually 60 beats/min, also means automatic elimination. The exact cut-off point for the heart rate and the role of the respiratory rate in assessing stress were the major points of debate at the ELDRIC meeting in 1984. In events run under CTR rules the veterinary control is even more rigorous as the veterinary score-sheet is used to determine the competitor's placing or the class of prize which will be awarded, in addition to eliminating horses who fail under the "fit to continue" rule.

The effect of all this on the tactics adopted by the rider is obviously considerable. There is nothing to be gained by attempting to continue on a lame horse or by pushing a tired horse to exhaustion. Some injustices do arise but these are more than counterbalanced by the constant concern for the horse's well-being which the rules encourage in the competitors. The whole concept of "fit to continue" is in

direct contrast to human athletics where the stated objective of the long distance runner is to pass the finish exactly at the limit of one's capabilities (Kuscsik, 1977), and some of the heroes of the modern people's marathons would not even pass under the "not ill-used" category. This means that direct comparisons of performance between human and equine endurance athletics are meaningless.

The application and interpretation of these rules can present problems for the veterinary surgeon. Arguments over the severity of saddle sores and mild lamenesses are not uncommon, but it is the measurement of heart rate which can be the most misleading parameter. The cut-off point of 60 beats/min can be easily exceeded for a minute or two in a perfectly unstressed horse due to some minor excitement such as separation from a stable companion or the sound of a heavy vehicle moving nearby. There are tales of "gamesmanship" among competitors whereby suitable disturbances have been engineered in order to eliminate rival horses. When the particular ride rules involve the requirement for a return in heart rate to the value measured pre-ride, or to below a certain percentage of the immediate post-ride value, there are obvious advantages in arranging for the earlier value to be as high as possible. When the difficulties are considered of ensuring that each horse's heart rate is measured exactly on time, due to queues forming when a group of riders has finished together, it is clear that the rules as they stand are not infallible. The addition of respiratory rate measurements, with the problems introduced by sniffing or panting horses, provides further complications.

Several studies have been conducted in an attempt to devise the most effective assessment of post-ride fatigue. Littlewort and Hickman (1969) studied the "30% drop test", which requires the sum of the heart and respiratory rates to fall by at least 30% during the first 8 minutes of rest, and concluded that it was of no value in identifying

fatigued horses. Aitken et al (1973) suggested that the rate of deceleration of heart rate after exercise might be inherently different in different breeds of horse, although it is possible that the heavy hunters used were simply less fit than the Thoroughbreds. A later study (Aitken et al, 1975) indicated that the time taken for a horse's heart rate to fall to below 50 beats/min was reduced by a period of training, and suggested that the percentage increase in plasma creatine kinase activity during exercise might also be reduced by training. Rose et al (1977) found a number of significant differences in the post-ride plasma biochemistry of horses with F+30 heart rates of over and under 60 beats/min, which suggests that the system in use at present has some validity. However, that does not solve the problem of the fair administration of the system, although reports indicate that the siting and handling of the veterinary inspections are more strictly organised in Australia (where Rose et al carried out their study) compared to this country. In this present study the subjective assessment of fatigue and the classification of horses into a few broad categories was found to be most satisfactory for the purposes of discussion.

Choice of breed for endurance competition

It is a curious fact that neither of the two countries in which modern endurance riding initially developed has any truly indigenous breeds of horse or pony. All horses at present in America are descended from stock brought across the Atlantic by European settlers, and according to legend the first American horses were actually introduced by Christopher Columbus. These horses were naturally mostly of the hot-blooded type, mainly of Arab, Barb and particularly Andalusian origin, and while it is no doubt the case that some draught horses were also introduced, America as a whole lacks the variety of native pony types to be found in Europe. The introduction of the horse

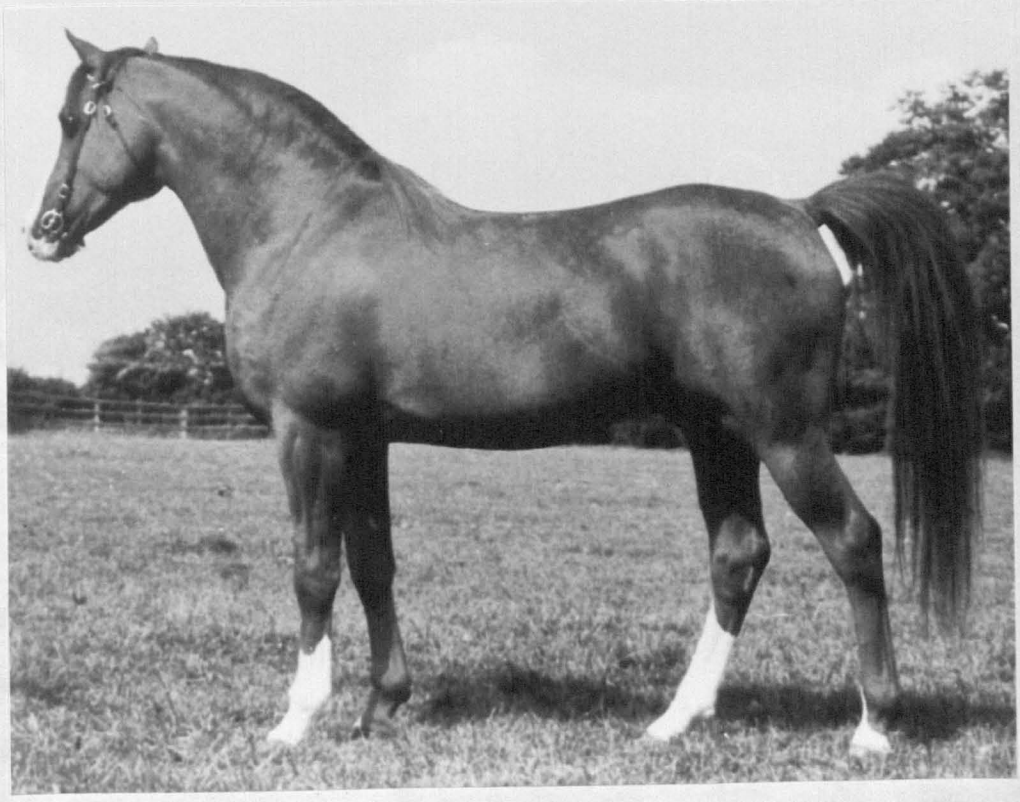
to Australia is even more recent, having occurred only 200 years ago, and again the majority of the animals have been of the light, hot-blooded riding type.

Endurance exercise in the horse has been shown to be primarily aerobic in nature, with increases in plasma lactate concentration being small (Lindholm et al, 1974a; Lucke and Hall, 1980a,b) and acid/base disturbances being virtually absent (Rose et al, 1979). This is in marked contrast to the situation occurring in sprint exercise, which is primarily anaerobic with marked increases in plasma lactate concentration being reported (Snow and Mackenzie, 1977a). Snow (1983) has demonstrated that the Arab horse has a significantly higher proportion of slow twitch (oxidative) muscle fibres compared to the Thoroughbred and Quarterhorse breeds, which have been selectively bred for sprint racing over many generations, and it appears that this higher oxidative capacity of the muscle is advantageous to the endurance horse (Snow et al, 1981). It is therefore not surprising that of the available hot-blooded breeds in Australia and the USA the Arab is the one which has generally found most favour among long distance riders.

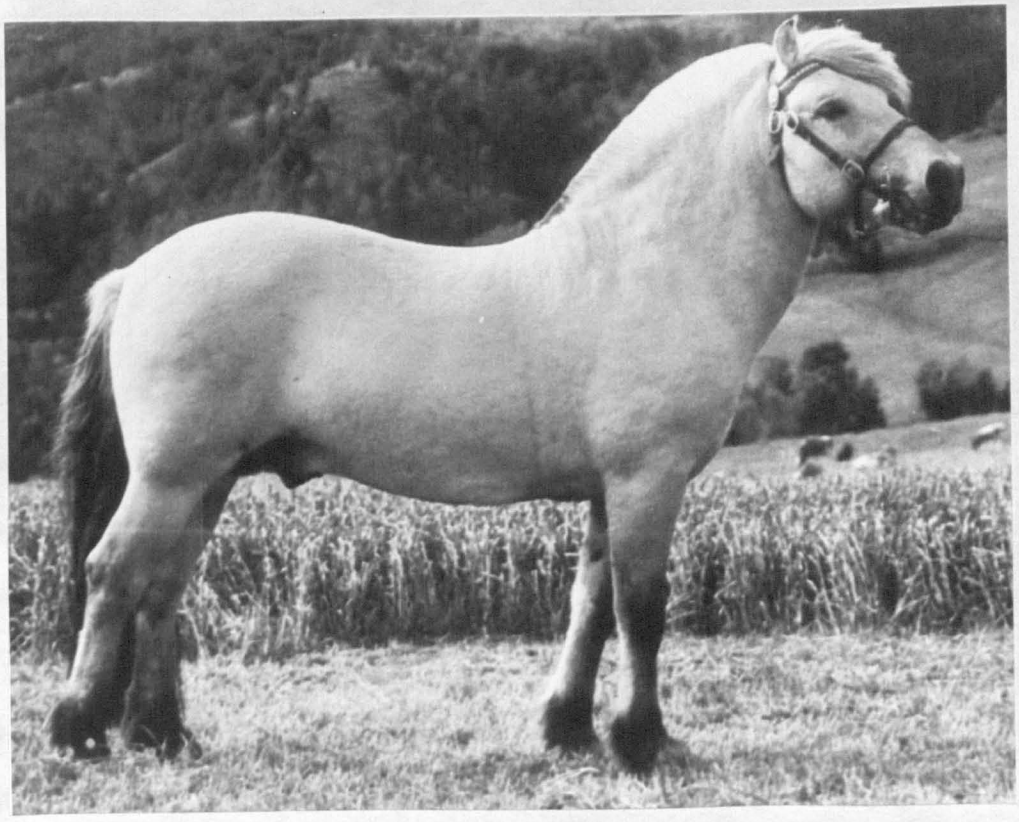
It is possible, however, that this set of circumstances has led to a too uncritical acclamation of the Arab as the supreme endurance machine. Hyland (1975) strongly recommends the Arab to prospective endurance competitors, but the grounds for this recommendation are largely anecdotal (it is of little use to cite the percentage of horses finishing a ride which were Arab unless the percentage of the starters which were Arab is also known) and in some cases erroneous ("they (Arabs) have fewer vertebrae than other breeds"). There is no doubt that the Arab's track record in endurance events is impressive, particularly in Australia and the USA, but this type of publicity tends to become self-perpetuating as it encourages keen and committed riders to acquire Arab horses.

It is in fact just as easy to cite anecdotal evidence to the contrary. For example, of the ten starters in the 100-mile ride reported in Section 2 of this thesis six were Arab. Only one of these Arabs finished - four were eliminated lame and one was withdrawn extremely tired. Meanwhile a long-backed, heavily-built mare had won by a margin of several hours, in excellent condition, while an even heavier, cold-blooded 17-year old Irish hunter gelding was placed second. Only one of the non-Arabs failed to finish. Extensive details of the breeding and achievements of horses active in long distance riding in Germany in 1983 are given by Puppe (1984), and in the list of horses having completed two or more 100-mile rides the Arab takes second place to the Norwegian Fjord pony in terms of overall numbers represented. Fjord ponies also occupy the two top places in terms of numbers of rides completed by the individual. In general the number of animals of European and British pony breeds (particularly the Fjord) and their crosses which appear in European endurance statistics is remarkable. The Fjord pony, a stocky, cold-blooded Asiatic Wild Horse type, is extremely dissimilar to the Arab in appearance and temperament (Figure 3), and its consistent success in Germany would suggest that it is unwise to dismiss the endurance potential of the native pony breeds. In fact the cold-blooded breeds studied by Snow (1983) (Shetland pony, "pony" and heavy hunter) all proved to have a greater proportion of oxidative muscle fibres even than the Arab, and so if it is indeed the case that this aspect is important in the suitability of a horse for endurance work then the success of the native ponies is not really surprising.

In Britain it appears to be the general case that most serious endurance competitors ride Arab horses while the native ponies are occasionally entered in the shorter events by less committed riders, often with very little preparation. There do not seem to be any



Arabian



Fjord pony

Figure 3. Contrasting appearance of Arab horse and Fjord pony.

representatives of British mountain or moorland breeds in training for serious competitive endurance riding in Britain, probably because until very recently both organising bodies imposed a minimum height limit of 14 hh (142 cm) on their more taxing competitions, which excluded most of the native pony breeds. This may represent a waste of a valuable national resource.

Another interesting point regarding successful endurance horses is the apparent lack of any differences attributable to sex, with stallions, geldings and mares competing on equal terms and with seemingly equal measures of success. This is in marked contrast to the situation in human athletics where female performance is consistently poorer than contemporary male performance in all types of competition. The lack of any sex difference in equine athletes suggests that this is more than a simple hormonal effect, as is sometimes suggested.

Scientific studies of endurance horses

The existing literature regarding equine endurance athletics is reviewed in detail in Sections 2 and 5 of the thesis. It is noteworthy, however, that compared to the enormous body of work which has been published in relation to human marathon races and similar events, only about 15 papers are available dealing with endurance horses. Each of these papers describes one or two endurance rides at which blood samples have been collected at intervals from a number of competitors and analysed for various biochemical parameters. Several interesting findings have been made, but the completely uncontrolled nature of the events makes precise discussion of the results very difficult in many cases. Only three papers have been published describing investigations into long distance exercise in horses under controlled conditions. These studies have not been very extensive in scope, covering only muscle glycogen depletion patterns (Lindholm et al, 1974a) and the effect of the level of dietary fat on performance (Hintz et al, 1978;

Hambleton et al, 1980). It appears, therefore, that there is a need for a more systematic investigation into the metabolic changes which occur in horses involved in endurance exercise. The species has evolved over millions of years to occupy an ecological niche in which exercising ability, both sprint and endurance, was of primary importance. Controlled breeding has been practised over many generations to enhance this exercise performance even further. It is therefore very probable that an investigation into the biochemical changes which occur during endurance exercise in the horse will reveal particular adaptations in this species which will add to our general understanding of exercise physiology.

GENERAL MATERIALS AND METHODS

GENERAL MATERIALS AND METHODS

All the experimental animals and techniques of sampling and analysis which are common to all or several of the Sections of the thesis are described under this heading. Details of particular techniques and procedures which apply only to one Section are described within that Section.

A. Animals

A group of 14 experimental animals, nine horses and five ponies, was used in this study. Physical details of these animals are given in Table 1. All animals were clinically healthy at the time of the investigations.

In addition, samples were collected from a group of 50 privately-owned horses and ponies competing in long distance riding competitions organised by the Endurance Horse and Pony Society of Great Britain. Details of these animals are included in Section 2.

B. Sampling Techniques

1. Blood

All samples were collected by jugular venepuncture using a 20G 1" hypodermic needle. Collection was into plastic syringes (Monovette, Sarstedt Ltd) containing fluoride oxalate for glucose estimation and lithium heparin for all other biochemical estimations. After collection the samples were placed in ice until processed. Before centrifugation the heparinised samples were mixed well and capillary tubes were filled with whole blood for packed cell volume estimation. All samples were centrifuged at 700g for 10 minutes; those collected at Glasgow at 4°C and those collected elsewhere at room temperature.

TABLE 1

Experimental animals used in Sections 1, 3, 4 and 5 of this study

Name	Designation	Breed	Sex	Age (years)	Approximate weight (kg)	Height (cm [hands])
Adam	A	Thoroughbred	Castrate	8	470	163 [16.0]
Astryl	B	Thoroughbred	Castrate	12	500	170 [16.3]
Ben	C	Thoroughbred	Castrate	10	530	168 [16.2]
Dirk	D	Thoroughbred	Castrate	9	500	165 [16.1]
Johnny Walker	E	Thoroughbred	Castrate	5	425	160 [15.3]
Smokey	F	Thoroughbred	Castrate	5	480	163 [16.0]
Bewitched	G	Thoroughbred x Hunter	Female	6	500	163 [16.0]
Pearl	H	Thoroughbred	Female	12	510	168 [16.2]
Sunny	I	Thoroughbred	Female	10	490	160 [15.3]
Brigadier	V	Welsh	Castrate	4	250	122 [12.0]
Bianca	W	Shetland	Female	4	160	107 [10.2]
Chance	X	Shetland	Male	2	120	107 [10.2]
Meenie	Y	Shetland	Female	4	135	102 [10.0]
Mo	Z	Shetland	Female	4	130	102 [10.0]

Plasma was removed, aliquoted and stored in plastic tubes at -20°C until analysis. Samples collected at Glasgow and Ayr were processed within an hour of collection. The procedure regarding samples collected in England and Wales is described in Section 2.

2. Urine

Urine samples were collected by fitting the horse with a plastic bag which was suspended below the prepuce by means of a harness, as described by Weir and Giffard (1971). As soon as possible after the horse had urinated the entire volume of urine was drained into a plastic bucket by means of a drain plug fitted to the lowest point of the plastic bag. The total volume of the urine, its pH and specific gravity were measured immediately. A well-mixed aliquot was then transferred to a plastic tube and stored at -20°C until analysis.

3. Sweat

It was necessary to devise suitable means of collecting horse sweat as the occasion arose during this investigation and these methods were modified and improved upon as the work progressed. Because of this, sweat collection methods were not constant throughout the investigation and are described and discussed as they occur in Sections 2, 3, 4 and 5 of the thesis.

C. Analysis

1. Haematology

Packed cell volume (PCV) was the only haematological parameter measured. This was determined on heparinised blood by the micro-haematocrit method using a Hawksley microhaematocrit centrifuge. Each result is a mean of at least two determinations.

2. Plasma biochemistry

(a) Autoanalyser methods

Total protein, albumin, inorganic phosphate, bilirubin, urea and creatinine were all measured by continuous flow analysis on a Technicon Autoanalyser II system using standard AAII methods as follows:

total protein - biuret
albumin - bromo-cresol green
inorganic phosphate - phosphomolybdate
bilirubin - diazo/caffeine benzoate
urea - diacetyl monoxime
creatinine - Jaffe's alkaline picrate reaction.

Uric acid estimations were performed on a Technicon SMA 6/60 Autoanalyser System.

In addition during the early part of the study alkaline phosphatase was also measured on the AAII system using a single point p-nitrophenyl phosphate method with AMP buffer.

(b) Reaction rate methods

The enzymes alkaline phosphatase (ALP; EC 3.1.3.1.), aspartate aminotransferase (AST; EC 2.6.1.1.) and creatine kinase (CK; EC 2.7.3.2.) were determined using a LKB 8600 automatic reaction rate analyser. Enzyme activities were measured at 37°C using Boehringer kits nos. 123 862 (p-nitrophenyl phosphate with DEA buffer) for ALP, 124 443 for AST and 124 184 (glutathione activated) for CK (BCL Ltd). The reaction rate and continuous flow methods did not give comparable results for ALP activity.

Glycerol and triglycerides were also measured on the reaction rate analyser using a modification of Boehringer kit no. 244 473 (BCL Ltd). This kit is designed to measure total glycerol in a sample by enzymatic hydrolysis of triglycerides to glycerol and free fatty acids.

A constant assumed 0.11 mmol/l free glycerol is then subtracted from the total glycerol concentration to obtain a value for triglyceride concentration. The sequence of reactions is as follows:

$$\text{Triglycerides} \xrightarrow{\text{lipase/esterase}} \text{Glycerol} + \text{FFA}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{glycerol kinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{ADP} + \text{PEP} \xrightarrow{\text{PK}} \text{Pyruvate} + \text{ATP}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+$$

It was considered that the assumption that plasma free glycerol concentrations are constant at 0.11 mmol/l was almost certainly invalid in the context of this investigation and so the kit was modified to allow the measurement of free glycerol. The lipase, esterase, pyruvate kinase (PK) and lactate dehydrogenase (LDH) are normally supplied as a mixture in one bottle. A separate solution of PK and LDH was therefore prepared to the same concentrations as those supplied with the kit using rabbit muscle enzymes (BCL Ltd) from the same source as those used in the preparation of the kit. The substitution of this mixture for the lipase/esterase/PK/LDH mixture allowed the first reaction step, hydrolysis of triglycerides, to be omitted so that only plasma free glycerol was measured. In order to measure true triglyceride concentration samples were analysed twice, once with lipase/esterase in the reaction mixture (giving total glycerol concentration) and once without (giving free glycerol concentration). Free glycerol was subtracted from total glycerol to give triglyceride concentration.

(c) **Flame photometry**

Sodium and potassium were measured using an EEL 227 integrating flame photometer with a lithium internal standard. A small number of samples towards the end of the study were analysed by the same method

using an IL 543 flame photometer. There was no significant difference between the two instruments in a group of 20 samples which was analysed on both.

(d) Chloride

Chloride was measured by coulometry (silver nitrate titration) using an EEL 920 chloride meter.

(e) Atomic absorption spectrophotometry

Calcium and magnesium were measured using an IL 257 atomic absorption spectrophotometer with a lanthanum internal standard.

(f) Glucose

Glucose was measured using a Beckman Glucose Analyser 2. This method utilises the reaction:

$$\beta\text{-D-glucose} + O_2 \xrightarrow[H_2O]{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

and measures O_2 consumed in the reaction directly by oxygen electrode.

In the single case of the two adrenaline infusion experiments in Section 4 where sweat glucose was measured, plasma glucose was also determined using the continuous flow method described for sweat glucose.

(g) Free fatty acids

To determine free fatty acids (FFA) plasma samples were extracted by the method of Folch *et al* (1957) after the addition of known amounts of margaric (heptadecanoic) acid as an internal standard. FFA were then isolated by thin layer chromatography, transesterified using 2% v/v sulphuric methanol (Stoffel *et al*, 1959) and analysed by gas liquid chromatography. The areas under the peaks on the g.l.c. trace were measured using a planimeter attachment to a Wang 2200 desk-top computer and the concentrations of the individual free fatty acids calculated by referring to the internal standard.

(h) Radioimmunoassay

Cortisol was determined using the Immophase kit (Corning Ltd). Antibody bound tracer was counted using a Packard 5230 auto-gamma scintillation spectrometer. Counts were recorded on paper tape and results calculated using the RIADS 22 programme in a Wang 2200 desk-top computer.

Insulin was determined using the Insulin RIA kit (Eurotrope Services Ltd).

3. Urine biochemistry

Specific gravity (SG) was measured using a hand-held refractometer (Uricon, Atago) and pH was measured by EIL 7015 pH meter.

Urea and creatinine were measured by continuous flow analysis on a Technicon Autoanalyser I system using standard AAI methods (diacetyl monoxime for urea and Jaffe's alkaline picrate reaction for creatinine). All urine samples were centrifuged at 700g before analysis on any autoanalyser system to prevent damage to the equipment by precipitated calcium carbonate crystals.

Sodium, potassium and chloride were measured by the methods described above for plasma. All urine samples were analysed using the IL 543 flame photometer.

4. Sweat composition

Sodium, potassium, chloride, calcium, magnesium and urea were measured by the methods described above for plasma. All calcium estimations were performed within 24 hours of sample collection as it was found that calcium salts precipitated from solution in the sweat after a short period of storage.

Total protein was determined by the phenol-Folin-Ciocalteu method of Lowry et al (1951) and the absorbence of the reaction complex was measured at 625 nm using a Pye Unicam SP 800 spectrophotometer.

Glucose was measured by continuous flow analysis using the Technicon Autoanalyser II system and Technicon method AAII-2 (reduction). All glucose estimations were performed within 24 hours of sample collection as no inhibitor of glycolysis was added to the sample.

pH was measured by EIL 7015 pH meter.

D. Quality Control

For all methods (except PCV, FFA, SG and pH) at least one quality control sample was included with each assay. Commercially produced lyophilised freeze-dried plasma or serum with pre-assayed results provided by the manufacturer was used for nearly all methods, however these were not suitable for urine urea and creatinine or sweat protein and for these methods one sample collected during preliminary trials was assayed several times, stored, and used as a quality control for all subsequent assays.

Any assay in which the quality control result lay outside pre-determined values, generally less than 5% from the designated result, was rejected and repeated. In addition the quality control sample was inserted at every tenth place on all Autoanalyser sample trays and any assay in which this result drifted by more than 3% of its initial value was also rejected and repeated.

Sources of the quality control samples used were as follows:

creatine kinase - Precepath E (BCL Ltd)
glycerol and triglycerides - Precelip (BCL Ltd)
cortisol - supplied in RIA kit (Corning Ltd)
insulin - supplied in RIA kit (Eurotrope Services Ltd)
All other methods - bovine plasma, prepared and supplied by the Greater Glasgow Area Health Board Group Quality Control Programme.

Wellcomtrol 2 (Wellcome Ltd) was also used in some assays as an extra control.

E. Statistical Analysis

Conventional statistical methods were used for calculating means ($\bar{x}$), standard deviation (SD), standard error of the mean (SEM) and correlation coefficients (r). Results are presented as mean ± SEM unless otherwise indicated.

Intraindividual differences were tested for significance using the paired *t* test and interindividual differences using the Student's *t* test (Snedecor, 1956).

Coefficients of variation (c.v.) were calculated from the formula:

$$\text{c.v.} = \frac{SD}{\bar{x}} \times 100$$ (Steel and Torrie, 1976)

All *t* tests (paired and unpaired) and correlation coefficients were calculated using a Wang 2200 desk-top computer, and all other statistics were performed on a Casio fx-2500 scientific calculator.

SECTION ONE

STUDIES OF CHANGES IN BLOOD AND URINE COMPOSITION IN NORMAL RESTING HORSES OVER 24 HOURS

Introduction

Timing of sample collection in endurance events

Endurance rides have, of course, a much longer duration than Flat or National Hunt races, and one of the consequences of this is that samples from competing endurance horses are taken at widely separated times of day. As a consequence any regular daily variations which might occur in blood or urine biochemistry could erroneously be attributed to the effects of the ride. Almost all of the publications which give data collected from competing endurance horses report some alterations in plasma parameters which are statistically significant but which are small in magnitude and within what would generally be considered the normal range (Carlson and Mansmann, 1974; Rose et al, 1977, 1980; Lucke and Hall, 1978, 1980a,b; Fregin, 1980; Grosskopf et al, 1983). These are difficult to interpret without control data collected at similar times of day.

Another complicating factor concerns the starting times which vary between events. In Britain, 8.00 to 9.00 a.m. is the usual starting time, with the exception of hundred-mile events which usually start around 4.00 a.m. Events in Australia and the U.S.A. often start around midnight in order to finish before the midday heat becomes too severe. These differences in timing could conceivably lead to differences in interpretation of results from events held in different countries.

These factors suggest that it is important to establish what, if any, regular daily variations occur in the blood and urine of unexercised horses.

Diurnal variations in man

It has been known for at least 60 years that urine excretion in man exhibits a regular diurnal variation (Simpson, 1924). This was found to

be connected to a regular variation in blood concentrations of steroid hormones (Laidlaw et al, 1954; Doe et al, 1956), and similar variations have been found in a number of blood and urinary parameters (Bartter et al, 1962, 1979). In recent years, a large volume of work has been published on this subject, detailing the existence of diurnal rhythms in many biological processes in a wide variety of species. The goal of much of this research is the identification of the physiological basis for circadian time measurement, as reviewed by Moore-Ede and Sulzman (1977).

Plasma biochemistry in the horse

Earlier publications which report "normal" or "reference" values for plasma biochemistry in horses (Stewart and Holman, 1940; Jennings and Mulligan, 1953) are generally inadequate for present-day purposes due to the many changes which have taken place in clinical chemistry methods during the past 30 years. In addition, these early studies often utilised a mixture of breeds and types of horse at random, which also renders them less valid as reference publications.

More recent publications have generally referred almost exclusively to Thoroughbred or Standardbred horses (Tasker, 1966; Simesen, 1972; Blackmore, 1975; Ekman et al, 1975; Lumsden et al, 1980; Egan et al, 1980) and have presented data grouped according to age, sex, breed, state of training, etc. With a few exceptions the applicability of these collections of data as "reference values" is somewhat limited by the numbers of animals used, which is generally around 30, and by the absence of any comparable data for other types of horse such as pony breeds or heavy horses. These publications give no information on any effect of time of sampling on the results obtained.

Only a few studies have been carried out in the horse with regard to changes with time of day, and most of these have concentrated on steroid hormones. Cox et al (1973) studied plasma testosterone in a

Welsh Mountain Pony stallion over three separate periods of eight hours (9.00 to 17.00 hrs in each case) and reported a "smoothly fluctuating diurnal variation". A number of authors have followed plasma glucocorticoid concentrations (cortisol, corticosterone and cortisone) over a full 24-hour period. The extremely high concentrations of up to 7000 nmol cortisol/l first reported by Zolovick et al (1966) have been contradicted by all subsequent studies and give rise to some suspicion of a typographical error (µg/100 ml for ng/ml?). Only Flisinska-Bojanowska et al (1974) have reported the absence of a diurnal rhythm in plasma cortisol concentration in the horse. Other authors agree that such a rhythm is present but the time of peak concentration is variously reported as 6.00 hrs (Larsson et al, 1979), 8.00 hrs (Hoffsis et al, 1970; Bottoms et al, 1972), 10.00 hrs (Zolovick et al, 1966; James et al, 1970) and 12.00 hrs (Evans et al, 1977). There is some disagreement regarding the actual peak concentrations present (from a mean of 77 nmol/l, Bottoms et al, 1972, to up to 900 nmol/l, Evans et al, 1977) and the amplitude of the variation, which may be due to differences in analytical methods. Only Evans et al (1977) kept their horses undisturbed and on a strictly regulated light/dark schedule, and under these conditions they also demonstrated ultradian rhythms superimposed on the main circadian rhythm. They also reported, however, that there were periods of days during which no rhythm was distinguishable.

Using the same undisturbed conditions and controlled light/dark schedule the same group has investigated the biorhythms of plasma glucose and insulin concentrations in the horse (Evans et al, 1975). Significant circadian rhythms were demonstrated in both glucose and insulin, with varying responses to changes in the light/dark schedule. A significant ultradian rhythm was also demonstrated for glucose but not for insulin. The authors were unable to determine whether these changes were due to light or to feeding patterns, as behaviour was not observed.

One study of seasonal variations in blood parameters has been carried out in grazing ponies (Owen et al, 1978). Significant seasonal changes were demonstrated in haemoglobin, plasma urea and serum triglyceride concentrations.

Urine biochemistry in the horse

The composition of pooled 24-hour urine samples in the horse was first studied almost 100 years ago by Smith (1889) who reported that equine urine was a fluid of very varying composition, and that much less sodium was present than potassium or chloride. He also reported a decrease in excretion of all three electrolytes in horses at work compared to horses at rest. Knudsen (1959) reported urea and creatinine concentrations of serum and spot urine samples obtained during various degrees of diuresis, and calculated renal clearances of these substances.

Since 1889 only two investigations into the complete 24-hour excretion of water and electrolytes in horses have been reported. Tasker (1967a) also remarked on the extremely low urine sodium excretion compared to man (mean of 7.1 mmoles/day in his horses), however sodium excretion reported by Rumbaugh et al (1982) was much higher than this (mean of 1,001 mmoles/day). The contrasting responses of the two groups of horses to food and water deprivation were also noteworthy. Tasker's horses increased their daily urine sodium excretion (Tasker, 1967b), and yet the highest levels in his study were still below the lowest reported by Rumbaugh et al (1982), whose horses demonstrated a decrease in sodium excretion when starved. These differences appear to reflect the differences in dietary sodium between the two groups. Tasker's horses were fed nothing but hay (total daily sodium intake 329 mmoles) and appeared to be conserving sodium when normally hydrated. However, when both food and water were withheld and progressive dehydration developed they presumably had to excrete more sodium to maintain normal body fluid

tonicity. In contrast the horses used by Rumbaugh et al initially had free access to supplementary salt, and so it is not surprising that urinary sodium excretion decreased when this was withheld.

This difference in dietary sodium may also be the reason for the much higher 24-hour urine volumes reported by Rumbaugh et al (1982) in the normally fed horses (15.6 l compared to 4.9 l reported by Tasker, 1967a). Both studies demonstrated marked reductions in urine volume during food and water deprivation. Urinary potassium excretion was similar in the two studies, and, as Smith (1889) suggested, this was much higher than the excretion rates reported in man (Bartter, 1979). This also decreased markedly during food and water deprivation (and during experimentally-induced diarrhoea, Tasker, 1967c).

Urinary electrolyte and creatinine excretion were also studied by Rawlings and Biscard (1975), who also measured simultaneous plasma concentrations and calculated renal creatinine and electrolyte clearances. However, the "24-hour" urinary excretion figures in this paper were derived by doubling the results of 12-hour urine collection periods. Gelså (1979) also used renal creatinine clearance to calculate glomerular filtration rate in horses in connection with studies on the clearance of antibacterial drugs, but no data on urine electrolyte concentrations or excretion were presented.

Due to its large size, the collection of volumetric urine samples from the horse has always been a problem, particularly with clinical cases. Even when the total volume of urine passed at one urination can be measured, the time of the previous urination is often not known. In order to alleviate this problem Traver et al (1977b) have attempted to eliminate the necessity for employing the rate of urine production to calculate renal clearance of a substance by deriving the concept of "clearance ratios", where the clearance of any substance is expressed as a percentage of creatinine clearance which is assumed to be constant.

This paper also presents data for "24-hour" urinary excretion of creatinine, sodium, potassium and phosphorus which are obtained by extrapolation from urine collections over periods of less than 24 hours, in this case 7 to 10.5 hours.

Considering that almost all studies which have been made of diurnal variations in plasma hormones in the horse have demonstrated that significant variations do occur similar to those seen in man, it would seem probable that urine electrolyte concentrations might also vary significantly over 24 hours. It would therefore appear that the practice of extrapolating 24-hour electrolyte excretion figures from urine collections made over periods of substantially less than 24 hours is open to question. In addition, Traver et al (1977b) have assumed that electrolyte and creatinine clearances will always remain in proportion in the normal horse, which has not in fact been demonstrated. The clearance ratio method has been investigated further with respect to phosphorus alone (Lane and Merritt, 1983). In this study hourly urine samples were collected for a full 24 hours and the conclusion was reached that the clearance ratio (fractional excretion) method is valid to 95% confidence limits for phosphorus. Unfortunately the data are presented only as mean clearance ratios over 24 hours and no details of any diurnal variation in either urinary phosphorus or creatinine concentrations are shown.

Purpose of this section

Chronobiology as a whole involves not only the documentation of regular diurnal changes but also the study of the precise effects of regular cycles of sleeping/waking, light/darkness, eating/fasting, postural changes, etc. on these variations. Detailed investigation of equine chronobiology along these lines is obviously outwith the scope of this present study. The results from this section are also not proposed

as reference values for any horses involved in endurance exercise other than those same individuals, when applicable.

The purpose of this section is to establish whether significant daily variations do occur in the resting horse in plasma and urine parameters which will be measured during endurance exercise, and the magnitude of these variations. These results may then be used as a base for interpretation of the clinical significance of statistically significant alterations seen during exercise. This is particularly relevant to urine biochemistry and to plasma parameters where the significant changes are small in magnitude.

It was therefore decided to study the changes in composition in blood and urine of stabled, resting horses over two or three 24-hour periods. After this had been completed it was noted that certain changes had occurred which appeared to be related to the consumption of a feed of hay. In order to investigate this finding, a further experiment was carried out specifically to study the changes occurring in a limited number of plasma parameters in association with eating hay.

Materials and Methods

A. 24-hour experiment

1. Animals

Blood sampling - horses A, B, C, D and E were used.

Urine sampling - horses A, B, D, E and F were used.

2. Management

The horses were stabled in loose boxes on a peat bed throughout this investigation. At the time of the investigation they were receiving walking exercise only and on the days when sampling took place they were not exercised at all. They had free access to water via automatic drinking fountains. Feeding protocol was as follows:

9.15 hrs 1.8 kg commercial cube diet (Spillers Complete Cubes)

12.15 hrs 1.8 kg commercial cube diet

16.30 hrs 2.7 kg commercial cube diet + 5.5 kg hay.

3. Procedure

(a) **Blood sampling.**

This was carried out on two separate occasions. Blood was collected at 9.00 hrs and every three hours thereafter until 9.00 hrs the following morning. PCV analysis was performed at once, all samples were then immediately centrifuged and the plasma stored at -20°C until analysis. Plasma samples were analysed for total protein, sodium, potassium, chloride, calcium, magnesium, phosphate, creatinine, urea, glucose, bilirubin, ALP and AST by the methods described in the General Materials and Methods section. The AAII method was used for the ALP analysis.

(b) **Urine sampling.**

This was carried out on three separate occasions. Urine bags were fitted at 9.00 hrs and inspected regularly every 30 to 45 minutes until 9.00 hrs the following morning, when they were removed. The time of

each urination was noted as nearly as possible and urine was immediately drained from the bags. For each urination the total volume, specific gravity and pH were measured immediately and a well-mixed aliquot of urine was then frozen at -20°C until analysis. These samples were analysed for sodium, potassium, chloride, urea and creatinine by the methods described.

4. Statistical analysis

(a) **Blood samples.**

The results from the two occasions of sampling were considered together, i.e. for each sampling time n = 10. Comparisons between different times were made using the paired *t* test.

(b) **Urine samples.**

Analysis of these results was complicated by the variation in times of urination between the five individuals. The results from all three occasions of sampling were considered together, i.e. for each time spot n = 15. Mean results were obtained by considering each result to represent the period between the previous urination and the measured urination, i.e. the period of time during which that urine sample was secreted. A mean value was then calculated at every hour. This calculation leads to the inclusion of certain results in more than one mean, depending on the length of time between urinations. Comparisons between different times were made using the paired *t* test and comparisons between two extended periods of day were made using the unpaired *t* test to compare groups of mean results. Correlation coefficients were calculated between parameters showing similar patterns of change.

B. Hay feeding experiment

1. Animals

Horses A, C, D, E, F, G, H and J were used in this Section.

2. Management

The management system was exactly the same as that used for the 24-hour experiment, except that the bedding was not peat but wood shavings. On the days when this experiment was carried out, the horses were given no hay for at least 20 hours before the first blood sample and no concentrate feed for at least four hours before the first blood sample.

3. Procedure

On the "test" day a blood sample was collected from each horse at 14.00 hrs, and 5.5 kg hay was immediately given. Between 2 and 3.5 hours were required for the consumption of this amount. Further blood samples were collected at 15.00, 16.00 and 17.00 hrs. A control experiment was also carried out in which the procedure was exactly the same but no hay was fed. In horses A, C, D and E the control day was two days after the test day and in horses F, G, H and J the control day was two days before the test day. Samples were processed exactly as described for the 24-hour experiment except that the analyses carried out were PCV, total plasma protein, albumin, sodium, potassium, chloride, bilirubin, ALP and AST. On this occasion the reaction rate method was used for the ALP analysis.

4. Statistical analysis

All comparisons were made using the paired *t* test.

Results

A. Blood samples, 24-hour experiment

The changes which occurred in PCV and plasma constituents measured are presented in Figure 4.

PCV and Total plasma protein

Throughout the 24-hour period these two parameters changed in parallel and there was close correlation between them ($r = 0.966$). Both were unchanged until 15.00 hrs, then between 15.00 and 18.00 hrs a significant increase occurred: PCV increased from 0.352 ± 0.002 to 0.414 ± 0.002 ($p < 0.001$) and total protein concentration increased from 66.1 ± 1.5 g/l to 74.0 ± 2.1 g/l ($p < 0.001$). Subsequently a gradual fall was noted in both parameters until 9.00 hrs the following morning, when the values were not significantly different from those at the start of the experiment.

Sodium

Plasma sodium concentration fluctuated slightly throughout the day with the lowest point (133.8 ± 1.6 mmol/l) at 12.00 hrs and the highest point (139.0 ± 1.6 mmol/l) at 3.00 hrs. The difference between these points was just significant ($p < 0.05$).

Potassium

The greatest changes in plasma potassium concentration occurred between 15.00 and 21.00 hrs. There was a significant fall from 15.00 hrs (4.02 ± 0.13 mmol/l) to 18.00 hrs (3.64 ± 0.15 mmol/l) ($p < 0.05$) followed by a significant rise from 18.00 to 21.00 hrs (4.14 ± 0.17 mmol/l) ($p < 0.001$).

Chloride

There was a gradual rise in plasma chloride concentration from the lowest point of 95.0 ± 0.9 mmol/l at 9.00 hrs to the highest point of 99.9 ± 1.0 mmol/l at 18.00 hrs ($p < 0.01$). Following this there was a

gradual decrease so that by 9.00 hrs the following morning the concentration was not significantly different from at the start of the experiment. This decrease was also significant at $p < 0.01$.

Calcium

The lowest plasma calcium concentrations (2.61 ± 0.15 mmol/l) occurred at 12.00 hrs and the highest (3.06 ± 0.12 mmol/l) at 6.00 hrs but none of the changes observed was significant.

Magnesium

The lowest plasma magnesium concentrations (0.67 ± 0.013 mmol/l) occurred at the start of the experiment and the highest (0.76 ± 0.012 mmol/l) at 18.00 hrs. The difference between these points was significant ($p < 0.001$).

Inorganic Phosphate

Plasma phosphate concentration varied during the day in a pattern approximating to a shallow sine curve. The lowest point (0.85 ± 0.03 mmol/l) was at 15.00 hrs and the highest (1.08 ± 0.04 mmol/l) at 6.00 hrs. The difference between these points was significant ($p < 0.001$).

Creatinine

There did not appear to be any regular pattern of variation in plasma creatinine concentration. However, the difference between the lowest point (142 ± 9 µmol/l at 18.00 hrs) and the highest one (164 ± 13 µmol/l at the end of the experiment) was a significant one ($p < 0.01$).

Urea

Plasma urea concentration increased significantly ($p < 0.01$) between 9.00 hrs (5.22 ± 0.21 mmol/l) and its highest point at 12.00 hrs (5.53 ± 0.28 mmol/l). There was then a significant decrease ($p < 0.02$) to the lowest point (4.98 ± 0.18 mmol/l) at 24.00 hrs followed by a

significant increase ($p < 0.001$) throughout the night until 9.00 hrs the following morning.

Glucose

The most striking feature of the changes in plasma glucose concentration was the sharp peak which occurred in the morning/early afternoon. First there was a significant increase ($p < 0.001$) between 9.00 hrs (4.63 ± 0.07 mmol/l) and the highest point (5.84 ± 0.24 mmol/l) at 12.00 hrs. There was then a significant decrease ($p < 0.01$) to the lowest point (4.08 ± 0.11 mmol/l) at 18.00 hrs, followed by a small but significant increase ($p < 0.01$) between 18.00 and 24.00 hrs (4.46 ± 0.9 mmol/l). Thereafter there was no change until 9.00 hrs the following morning.

Bilirubin, ALP and AST

None of these parameters altered a great deal during the experiment. However, as with PCV and total plasma protein, all three showed a significant increase between 15.00 hrs and 18.00 hrs. Bilirubin concentration rose from 35.0 ± 3.0 µmol/l to 39.2 ± 3.5 µmol/l ($p < 0.001$), ALP activity rose from 135 ± 7 iu/l to 153 ± 8 iu/l ($p < 0.001$) and AST activity rose from 308 ± 38 iu/l to 339 ± 41 iu/l ($p < 0.01$).

Although the variation in these three parameters within animals was not great, variation between animals was fairly marked. In particular the mean value of AST activity for horse A (516 ± 10 iu/l) was significantly higher ($p < 0.001$) than for the other four horses (270 ± 25 iu/l).

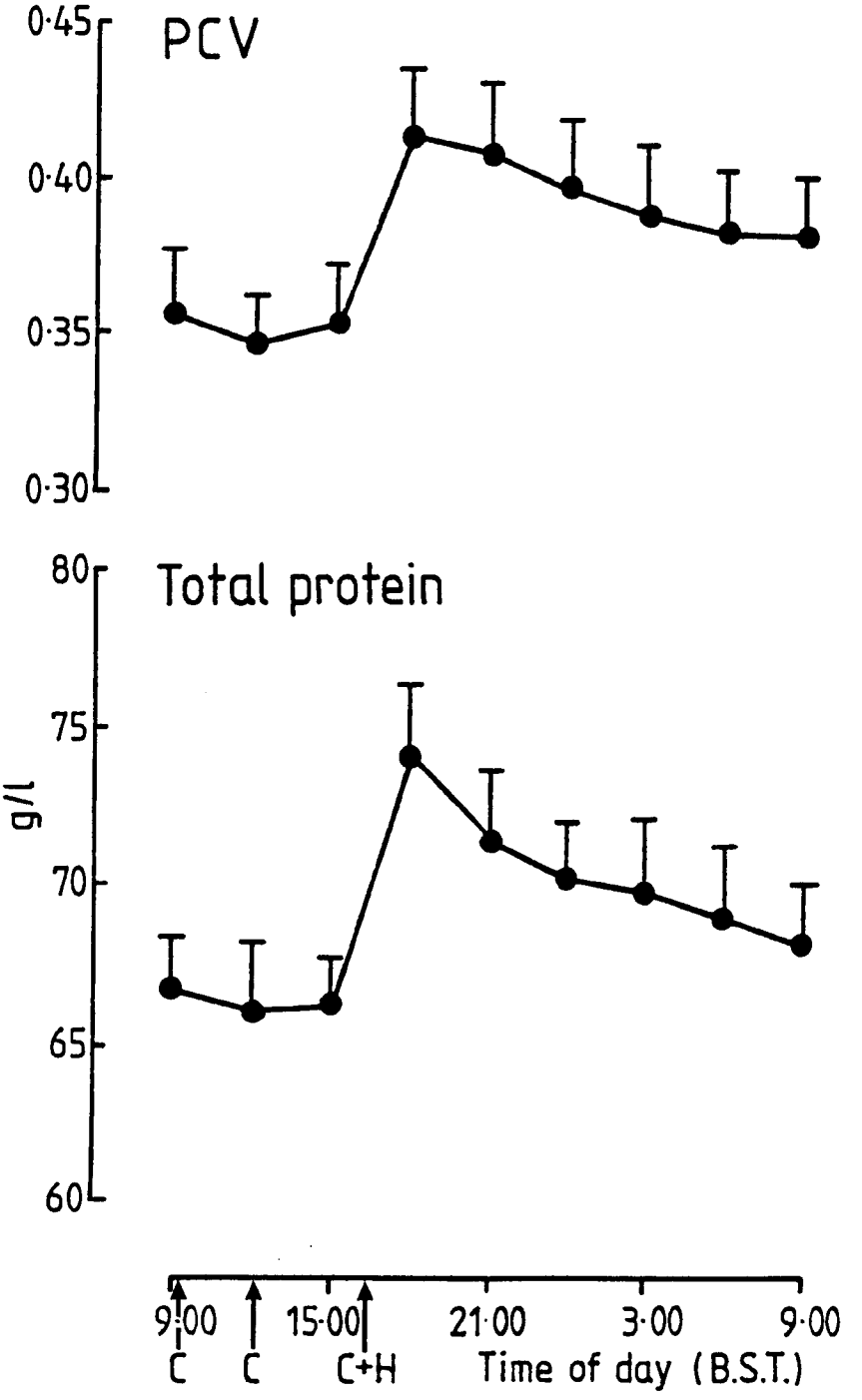


Figure 4(a). Changes in PCV and total plasma protein concentration in resting horses over 24 hours. Feeds given at times indicated:
C, 1.8 kg cubes
C+H, 2.7kg cubes + 5.5 kg hay.

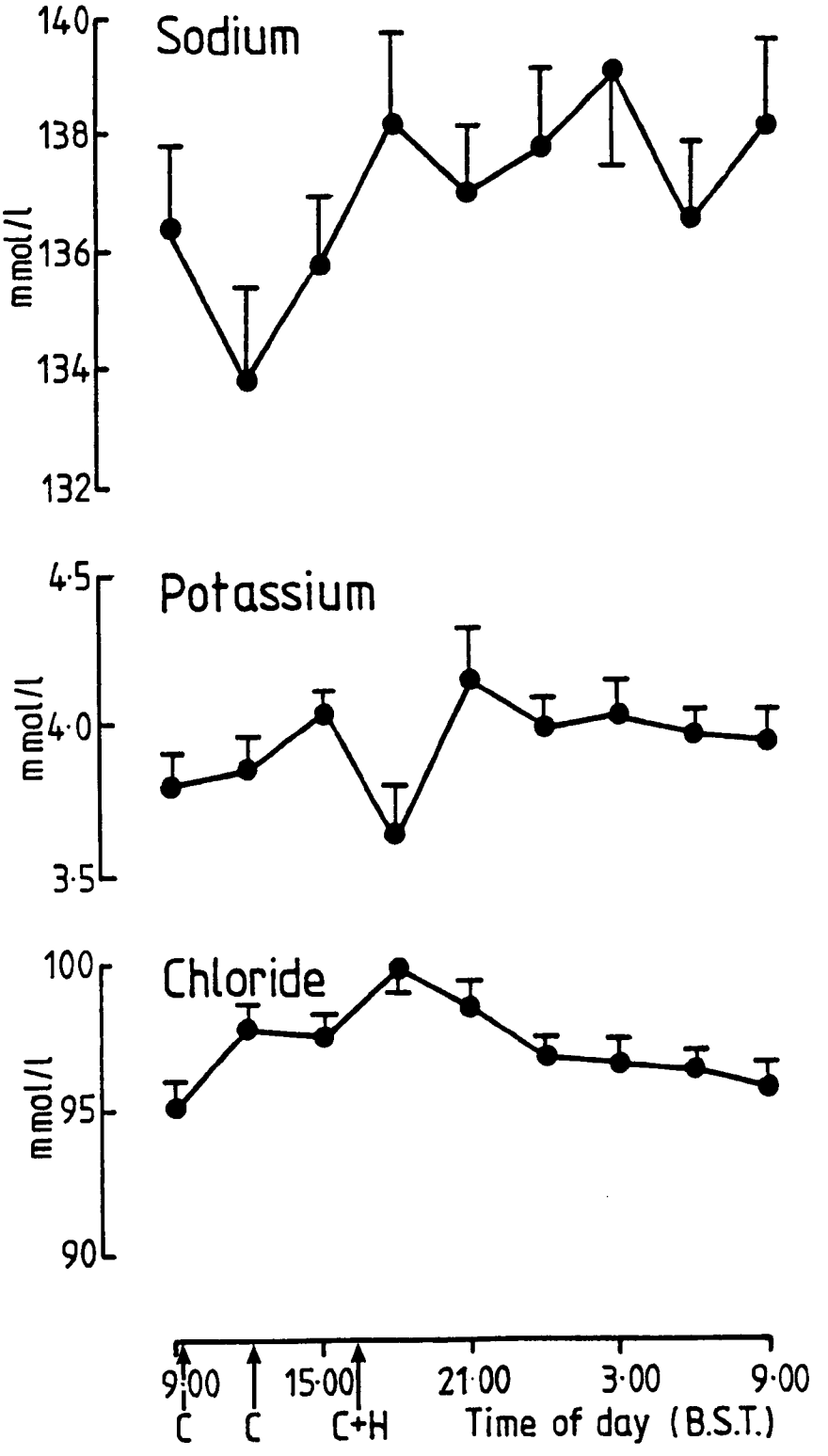


Figure 4(b). Changes in plasma electrolyte concentrations in resting horses over 24 hours. Feeding protocol as in Figure 4(a).

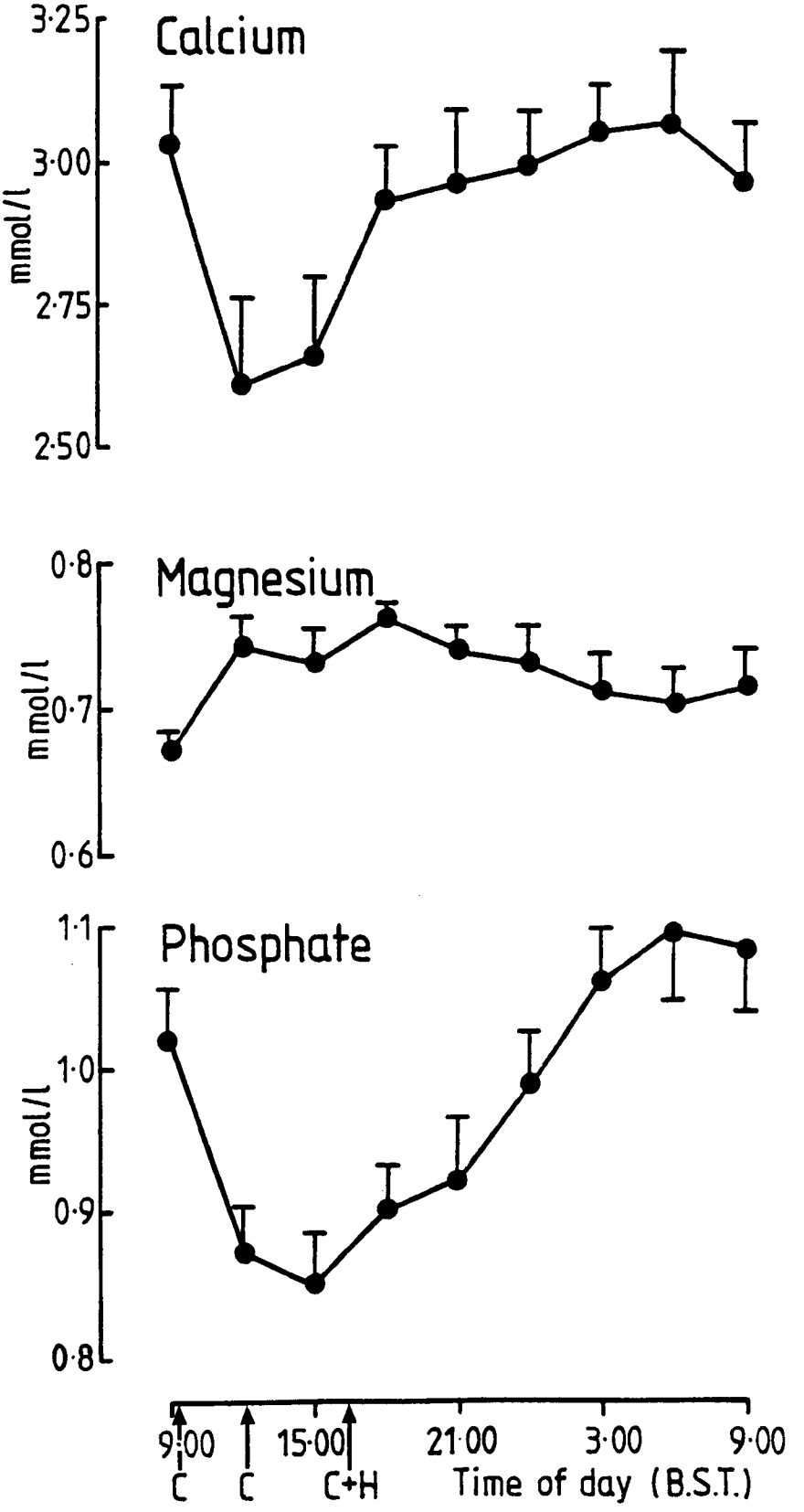


Figure 4(c). Changes in plasma mineral concentrations in resting horses over 24 hours. Feeding protocol as in Figure 4(a).

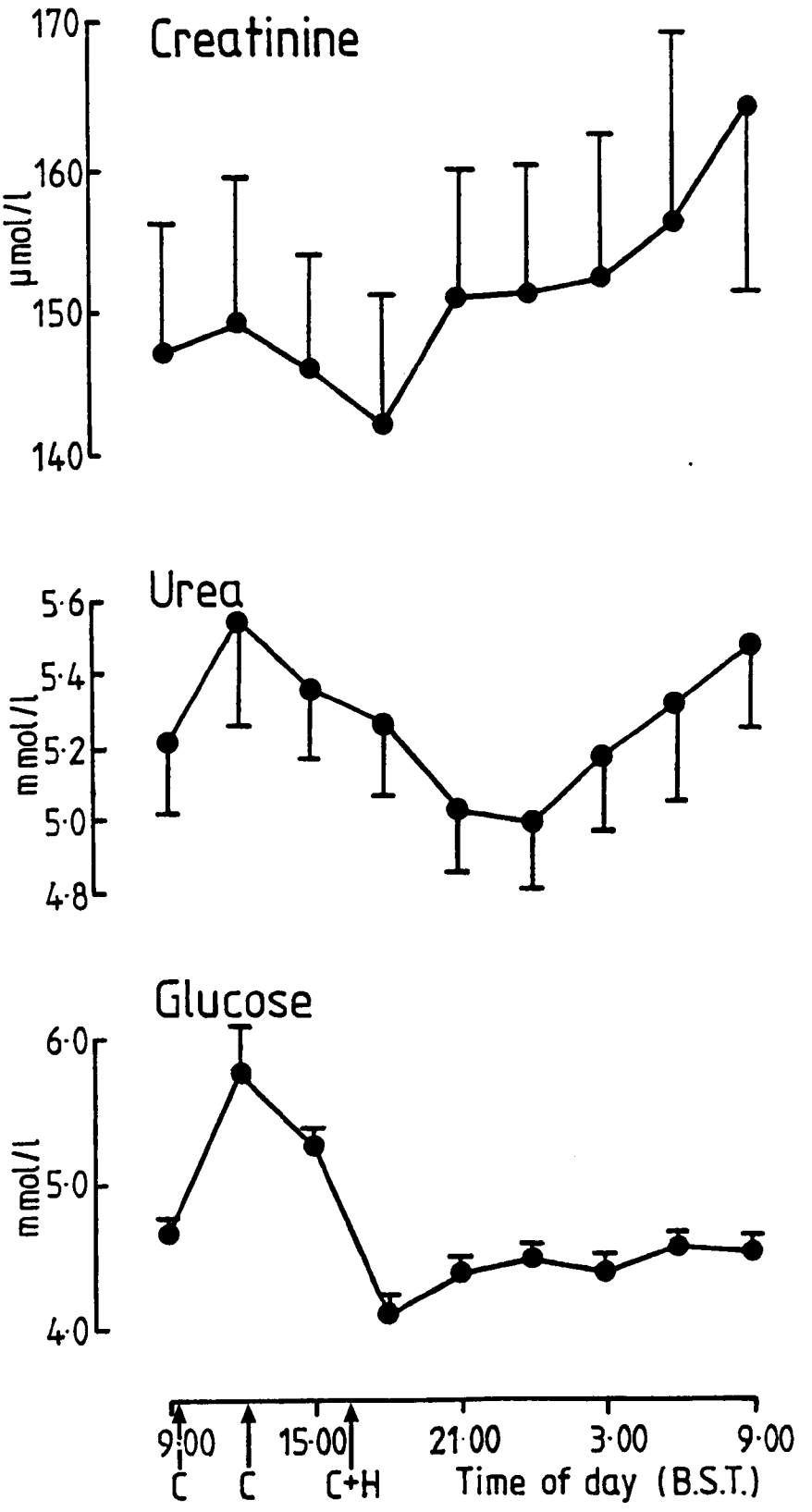


Figure 4(d). Changes in plasma creatinine, urea and glucose concentrations in resting horses over 24 hours. Feeding protocol as in Figure 4(a).

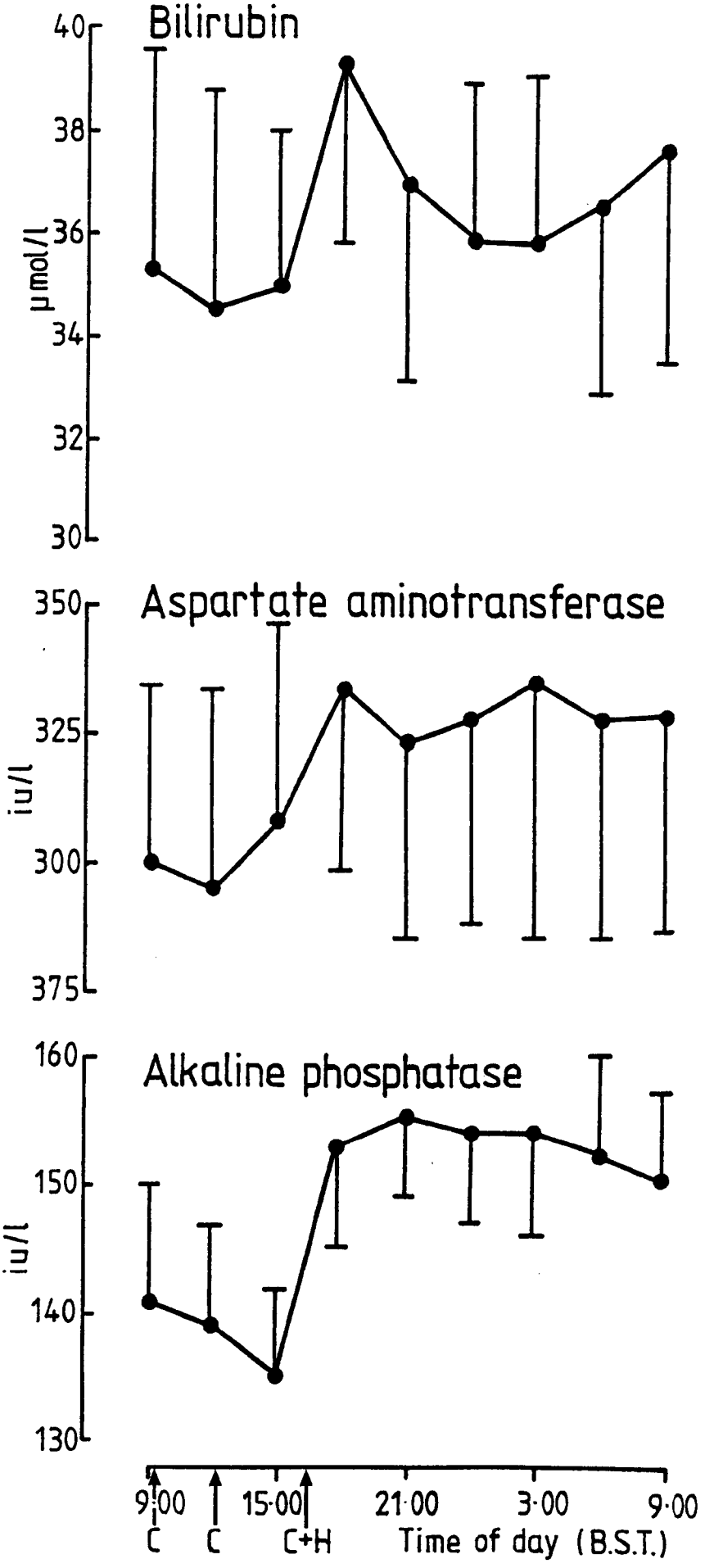


Figure 4(e). Changes in plasma bilirubin concentration and enzyme activities in resting horses over 24 hours. Feeding protocol as in Figure 4(a).

B. Urine samples, 24-hour experiment

The urination patterns of six horses (including horse C whose results were otherwise omitted) during the second 24-hour period studied are shown in Figure 5. In general, urination was more frequent during the day than during the night, with the exception of horse C who urinated only once per day while wearing the urine collection harness. In total 121 samples were collected over the three 24-hour periods.

The total 24-hour urine volume and excretion of creatinine, urea, sodium, potassium and chloride are given in Table 2.

The rate of urine production was calculated as:

$$\text{flow rate (ml/min)} = \frac{\text{volume of urine passed (ml)}}{\text{time elapsed since previous urination (min)}}$$

The changes in urine flow rate, specific gravity, creatinine concentration and urea concentration are presented in Figure 6. The most striking feature of these results is the marked difference between urine excreted up to 22.00 hrs and that excreted from 23.00 hrs on, which was significant for all four parameters (Table 3). The changes in all four parameters between 22.00 and 24.00 hrs were also significant ($p < 0.001$ for flow rate, $p < 0.01$ for specific gravity, creatinine and urea). A significant increase occurred in the urine flow rate between 11.00 and 14.00 hrs ($p < 0.01$) but the increase seen between 17.00 and 20.00 hrs was not significant.

There was a close negative correlation between urine flow rate and creatinine concentration ($r = 0.886$), but the equivalent correlation between flow rate and urea concentration was not significant. Rates of excretion of creatinine and urea were calculated as:

$$\text{excretion rate (mmol/min)} =$$

$$\text{flow rate (ml/min)} \times \text{concentration (mmol/l)} \times 1000$$

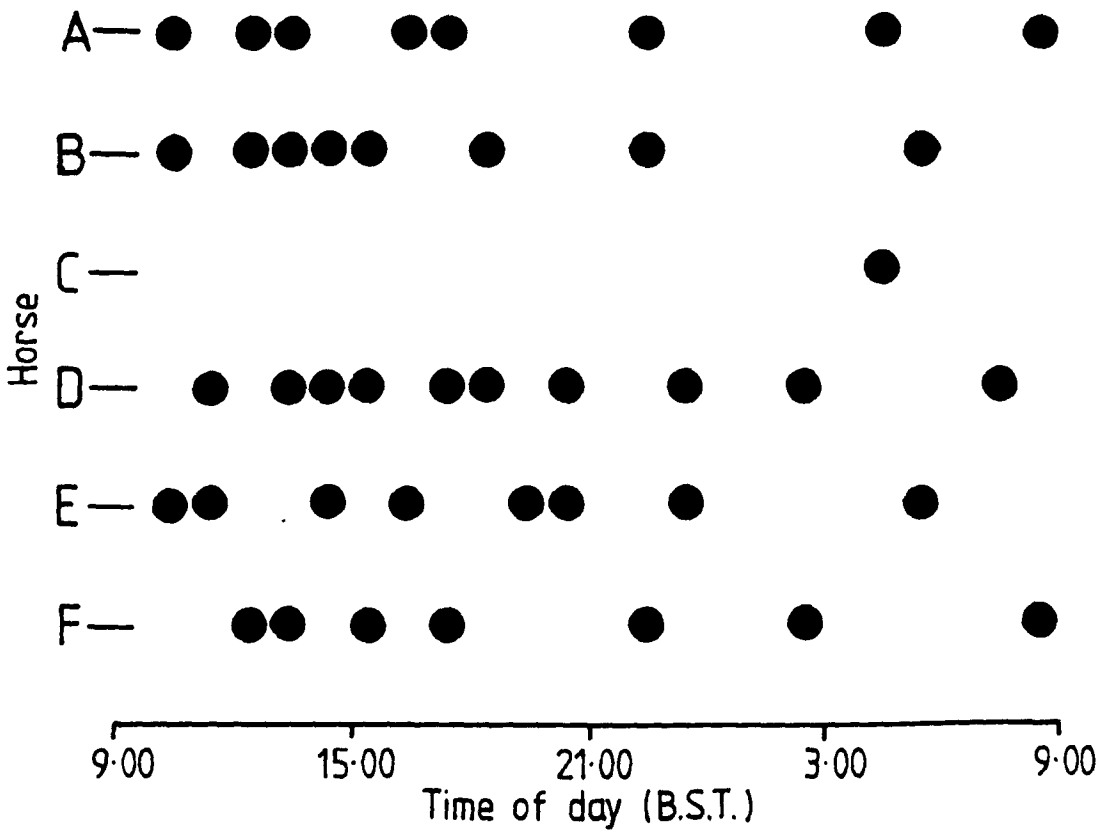


Figure 5. Patterns of urination in all six horses during the second 24-hour period studied.

TABLE 2

24-hour urine volume and excretion of electrolytes and nitrogenous substances

	Absolute values	After correction for bodyweight
Volume	6.46 ± 0.37 litres	13.2 ± 0.8 ml/kg
Creatinine	0.128 ± 0.004 moles	0.26 ± 0.008 mmol/kg
Urea	2.14 ± 0.19 moles	4.37 ± 0.39 mmol/kg
Sodium	0.18 ± 0.04 moles	0.37 ± 0.09 mmol/kg
Potassium	2.19 ± 0.09 moles	4.48 ± 0.20 mmol/kg
Chloride	1.77 ± 0.13 moles	3.62 ± 0.27 mmol/kg

TABLE 3

Comparison between urine excreted before 22.00 hrs and after 23.00 hrs

	Overall mean 9.00-22.00 hrs	Overall mean 23.00-8.00 hrs	Level of significance
Flow rate (ml/min)	5.33 ± 0.26	3.34 ± 0.09	$p < 0.001$
Specific gravity	1.037 ± 0.0003	1.041 ± 0.0003	$p < 0.001$
Creatinine (mmol/l)	19.5 ± 0.7	26.7 ± 0.4	$p < 0.001$
Urea (mmol/l)	324 ± 4	377 ± 3	$p < 0.001$

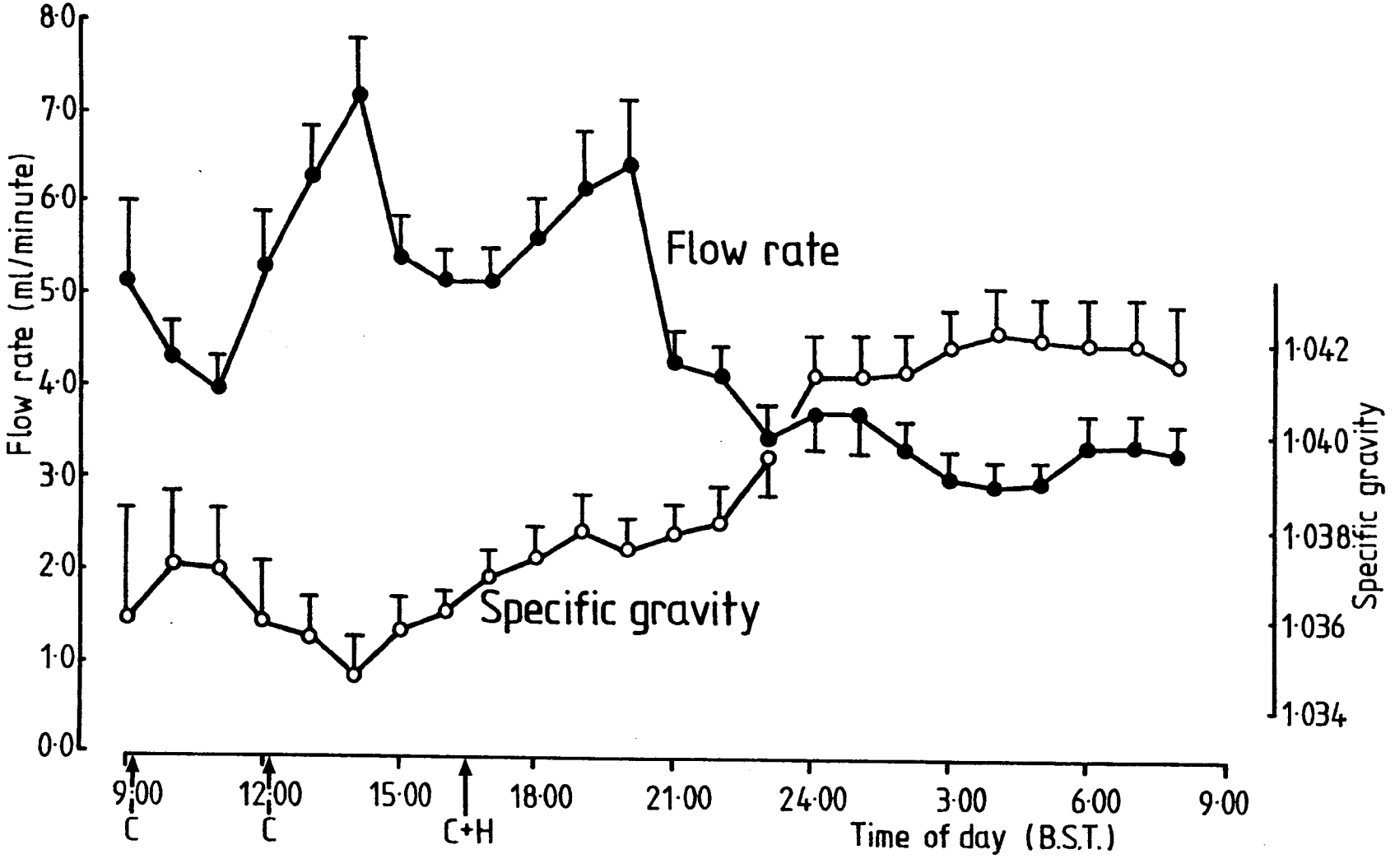


Figure 6(a). Changes in urine flow rate and specific gravity in resting horses over 24 hours. Feeding protocol as in Figure 4(c).

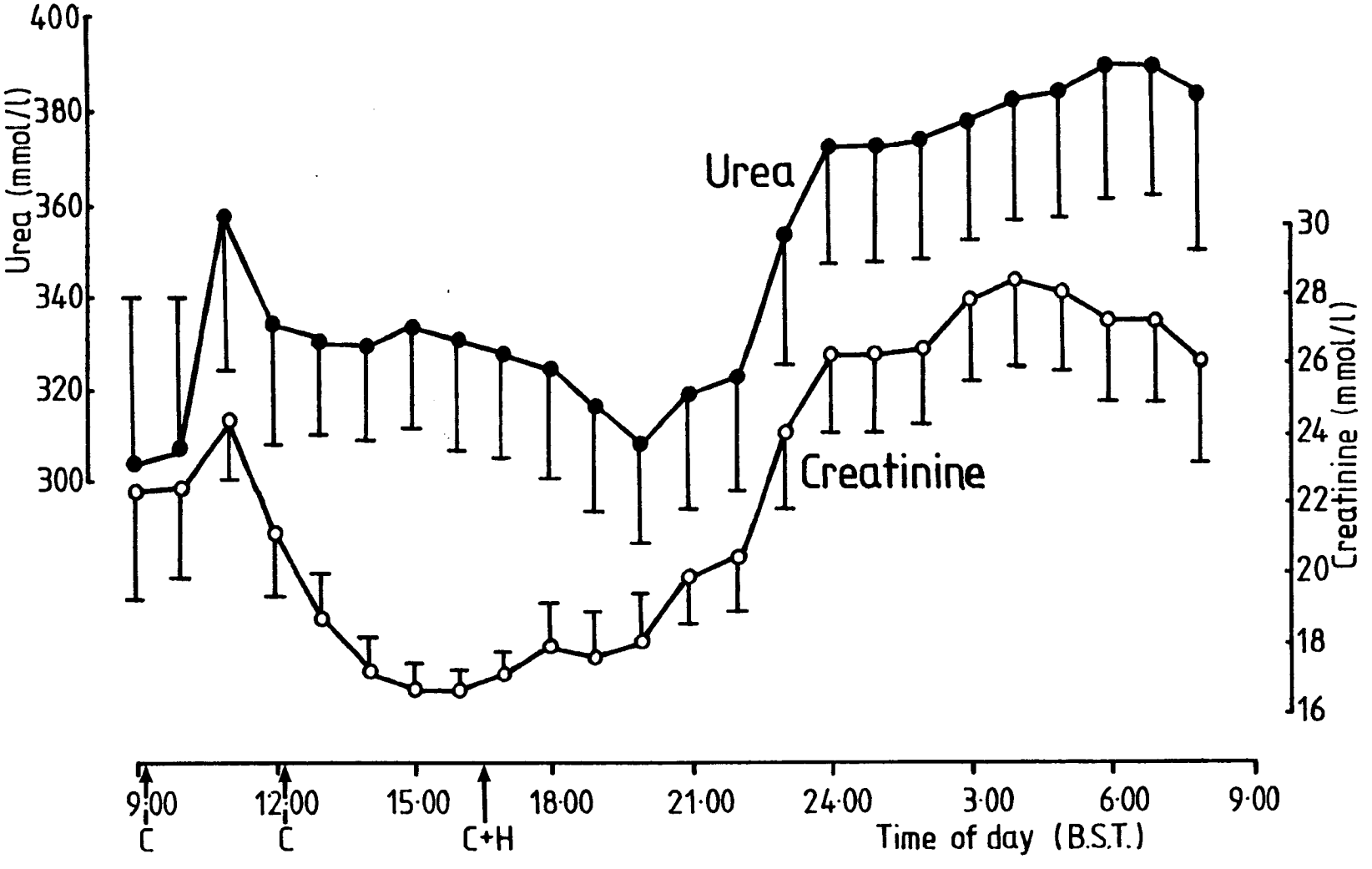


Figure 6(b). Changes in urine urea and creatinine concentrations in resting horses over 24 hours. Feeding protocol as in Figure 4(a).

and are presented in Figure 7. The changes in the rate of creatinine excretion were not large, but slightly higher rates were seen corresponding with the periods of apparent diuresis, and overall the excretion rate between 9.00 and 20.00 hrs (mean 97.0 ± 2.7 μmol/min) was slightly higher than between 21.00 and 8.00 hrs (81.6 ± 1.2 μmol/min) ($p < 0.001$). The urea excretion rate showed a similar pattern to creatinine, but in this case the difference between the daytime and night-time rates was greater: 1.75 mmol/min from 9.00 to 20.00 hrs compared to 1.25 ± 0.02 mmol/min from 21.00 to 8.00 hrs ($p < 0.001$).

The changes in urine electrolyte concentrations are shown in Figure 8. These followed patterns markedly different from the nitrogenous constituents.

Sodium concentration was highest early in the experiment (84 ± 23 mmol/l at 10.00 hrs). Thereafter there was a steady decrease until by 23.00 hrs concentrations were almost negligible. No increase was seen before 8.00 hrs when sampling was discontinued.

Concentrations of potassium and chloride were much higher than sodium. The lowest concentration of potassium was at the beginning of the experiment (274 ± 42 mmol/l). There was then a gradual significant rise ($p < 0.001$) until 22.00 hrs which was the highest point (403 ± 24 mmol/l). The slight decrease between 22.00 and 8.00 hrs was not significant.

Chloride concentration was also at its lowest at the beginning of the experiment (183 ± 26 mmol/l). There was then a significant increase ($p < 0.001$) to 15.00 hrs which was the highest point (323 ± 13 mmol/l). The gradual decrease between 15.00 and 8.00 hrs was significant ($p < 0.02$).

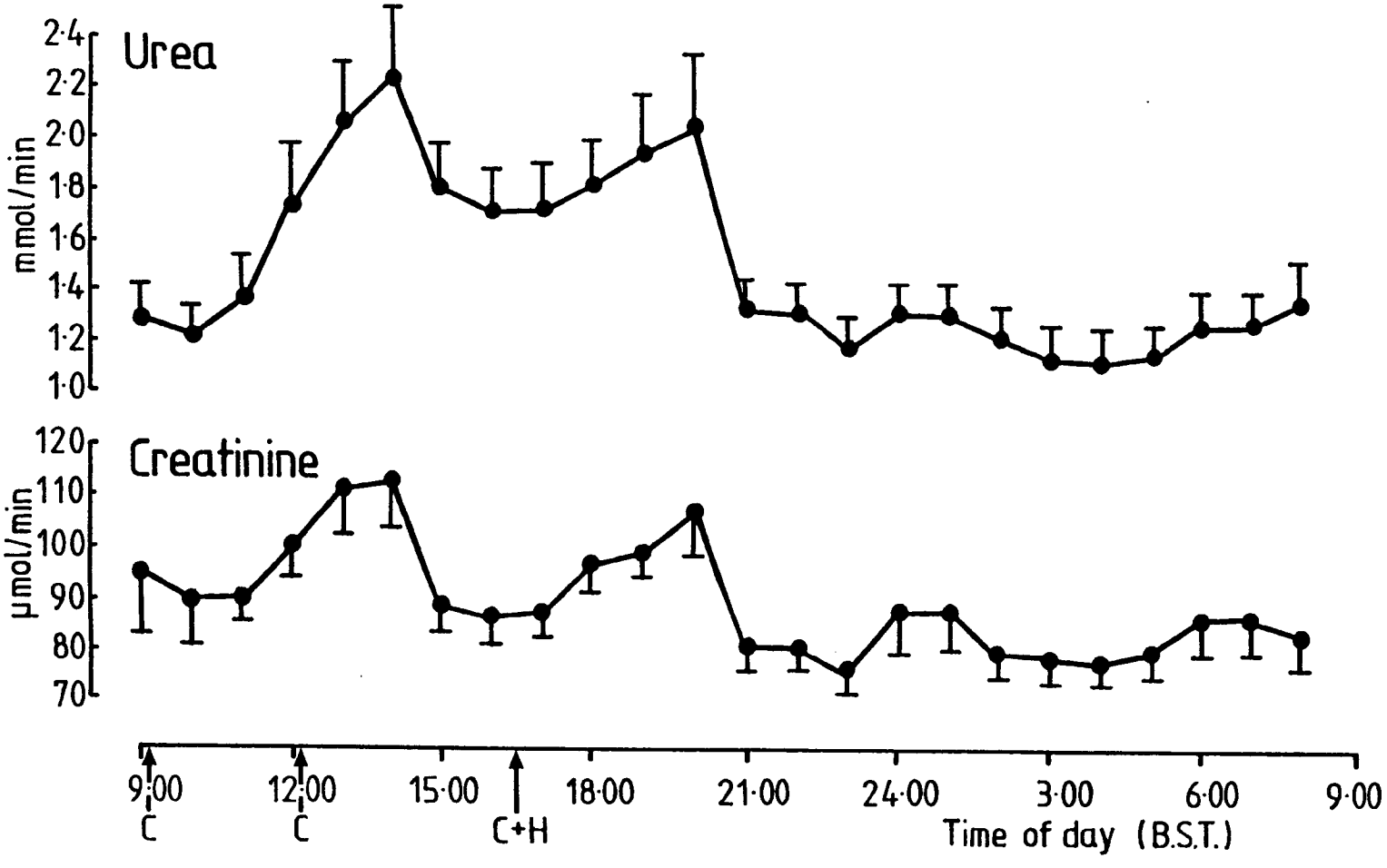


Figure 7. Changes in urinary excretion rates of urea and creatinine in resting horses over 24 hours. Feeding protocol as in Figure 4(a).

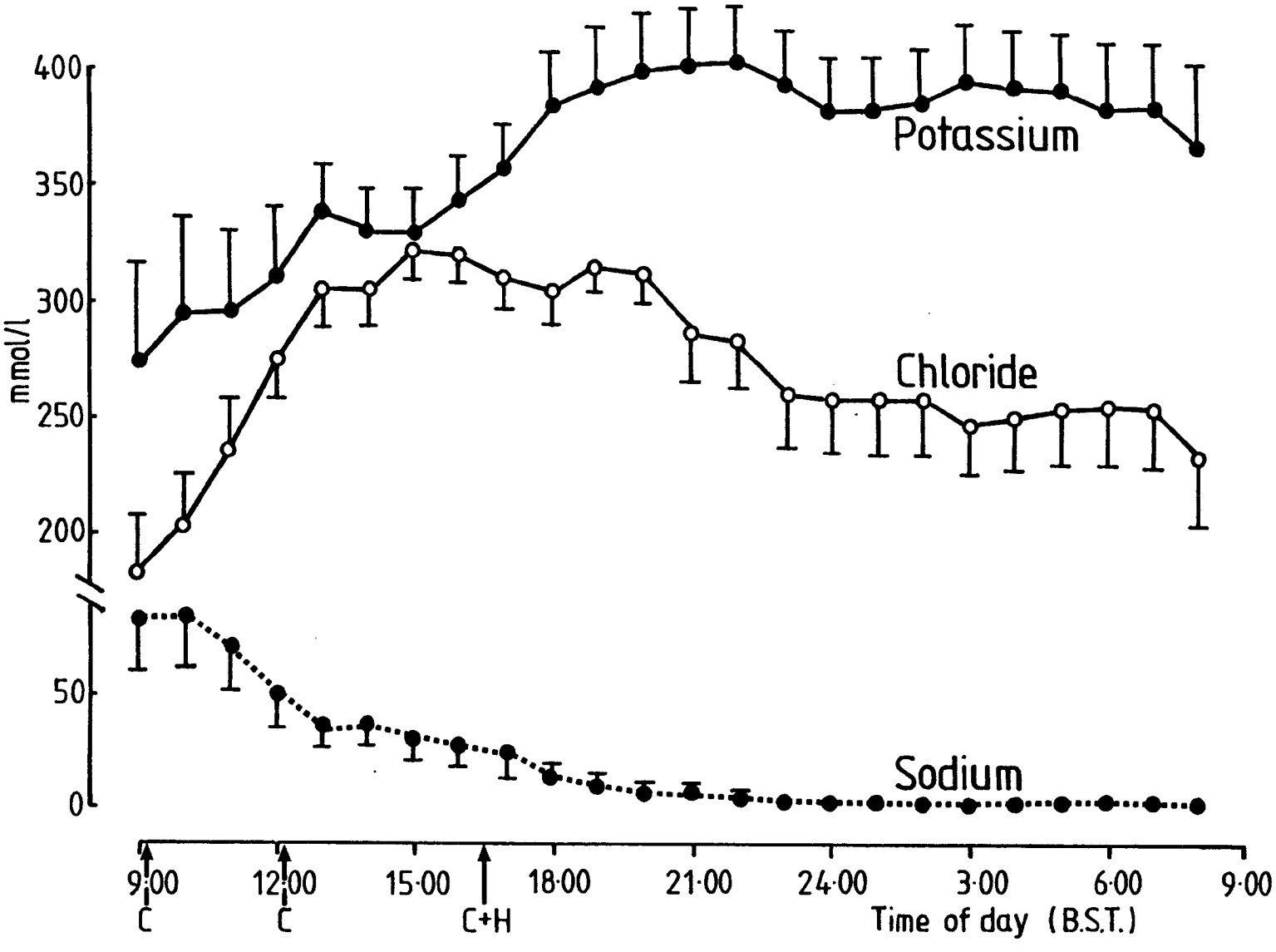


Figure 8. Changes in urine electrolyte concentrations in resting horses over 24 hours. Feeding protocol as in Figure 4(a).

Electrolyte excretion rates were calculated as for urea and creatinine and are presented in Figure 9 together with the urine pH.

Urine pH was at its highest at the beginning of the experiment (7.78 ± 0.12). There was then a significant decrease ($p < 0.01$) to 12.00 hrs. The lowest point was at 15.00 hrs (7.24 ± 0.05); thereafter pH rose slightly ($p < 0.05$) until 21.00 hrs, and remained constant from then until the end of the experiment.

Sodium excretion rate followed the pattern of the sodium concentration, with the highest value of 0.41 mmol/min at 9.00 hrs, followed by a gradual decrease. During the night the excretion rate was very low (under 0.01 mmol/min) and there was no significant increase before 8.00 hrs.

Potassium and chloride excretion rates were much higher than sodium and varied in parallel, with potassium always slightly higher than chloride. As with the nitrogenous constituents, peaks were noted coinciding with the peaks in urine flow rate. Between 12.00 and 22.00 hrs excretion rates of both electrolytes were significantly higher than during the rest of the experiment ($p < 0.001$ in both cases). Mean excretion rates were: 12.00 - 22.00 hrs potassium 1.94 ± 0.1 mmol/min, chloride 1.69 ± 0.09 mmol/min; 9.00 - 11.00 and 23.00 - 8.00 hrs potassium 1.21 ± 0.03 mmol/min, chloride 0.85 ± 0.03 mmol/min.

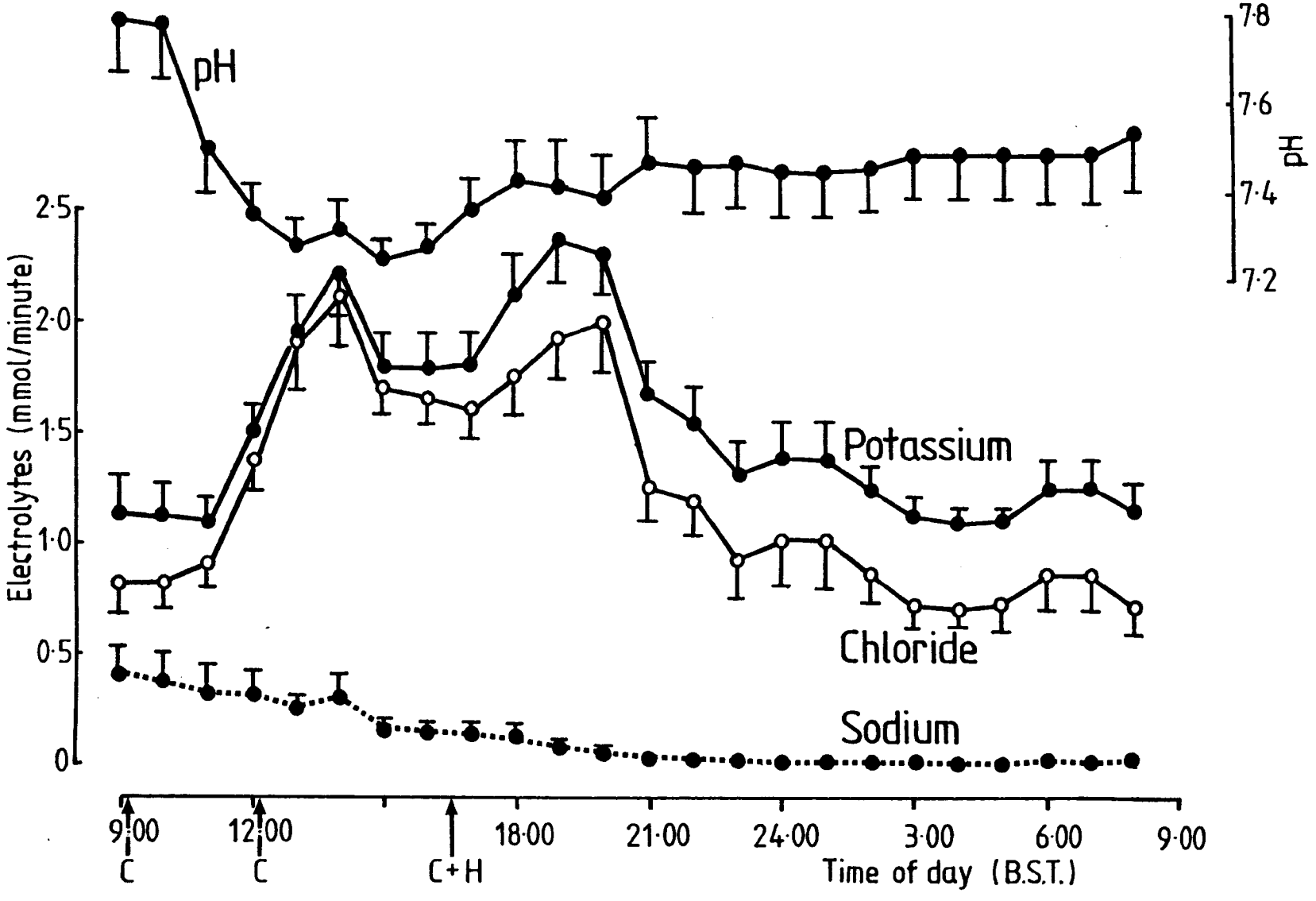


Figure 9. Changes in urine pH and in urinary electrolyte excretion rates in resting horses over 24 hours. Feeding protocol as in Figure 4(a).

C. Hay feeding experiment

The changes which occurred in PCV and total protein, albumin, sodium, potassium, chloride and bilirubin concentrations after the feeding of hay are compared to the control period in Figure 10. Although the horses took at least two hours to finish the hay, the greatest changes in all parameters were seen in the sample taken one hour after feeding. Table 4 shows the mean concentrations of all parameters in the first two samples of both the control and test days.

PCV and plasma proteins

During the first hour of hay feeding there was a significant rise in PCV and total protein and albumin concentrations ($p < 0.001$ in all cases) which was followed by a gradual return towards normal. The two enzymes measured, AST and ALP, also increased significantly in the first hour of hay feeding (AST, $p < 0.001$; ALP, $p < 0.01$). There was no change in any of these parameters during the control period.

Electrolytes

Again there was no change during the control period. The most marked change during hay feeding occurred in potassium concentration, which decreased considerably in the first hour ($p < 0.001$) then returned to normal by the end of the experiment. The increase in chloride concentration during the first hour of hay feeding was just significant ($p < 0.05$) but was very small in magnitude. Sodium concentration appeared to vary in the opposite direction to potassium, increasing during the first hour and then returning to the pre-feeding value. However, although the decrease between one and three hours was significant ($p < 0.01$) the initial increase was not significant.

Bilirubin

During the test period changes in bilirubin concentration were very similar to the changes in PCV and plasma proteins, with a significant increase in the first hour ($p < 0.001$) followed by a return to the initial value. However, bilirubin also exhibited a significant change during the control period, increasing from 28.2 ± 1.3 µmol/l in the first sample to 33.6 ± 2.6 µmol/l in the last ($p < 0.01$).

TABLE 4

Changes occurring in PCV, plasma proteins and electrolytes during the first hour of hay feeding (test) compared to a control period

	Test		Control	
	Pre-feeding	1 hour after	Pre-feeding	1 hour after
PCV	0.342 ± 0.01	0.396 ± 0.009***	0.347 ± 0.006	0.350 ± 0.007
Total protein (g/l)	73.2 ± 1.9	82.1 ± 2.3***	73.1 ± 1.6	73.2 ± 1.7
Albumin (g/l)	34.1 ± 0.7	38.1 ± 0.8***	34.4 ± 0.8	34.5 ± 0.9
ALP (iu/l)	656 ± 96	743 ± 104**	620 ± 70	621 ± 76
AST (iu/l)	259 ± 37	311 ± 35***	279 ± 48	277 ± 44
Sodium (mmol/l)	138 ± 0.6	139.9 ± 0.7	137.4 ± 0.3	137.7 ± 0.6
Potassium (mmol/l)	3.82 ± 0.08	3.19 ± 0.13***	3.89 ± 0.07	3.87 ± 0.09
Chloride (mmol/l)	98.5 ± 0.9	99.5 ± 0.7*	99.0 ± 0.7	98.5 ± 0.7
Bilirubin (µmol/l)	26.2 ± 2.4	30.1 ± 2.8***	28.1 ± 1.3	29.1 ± 1.8

Significantly different from pre-feeding values: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

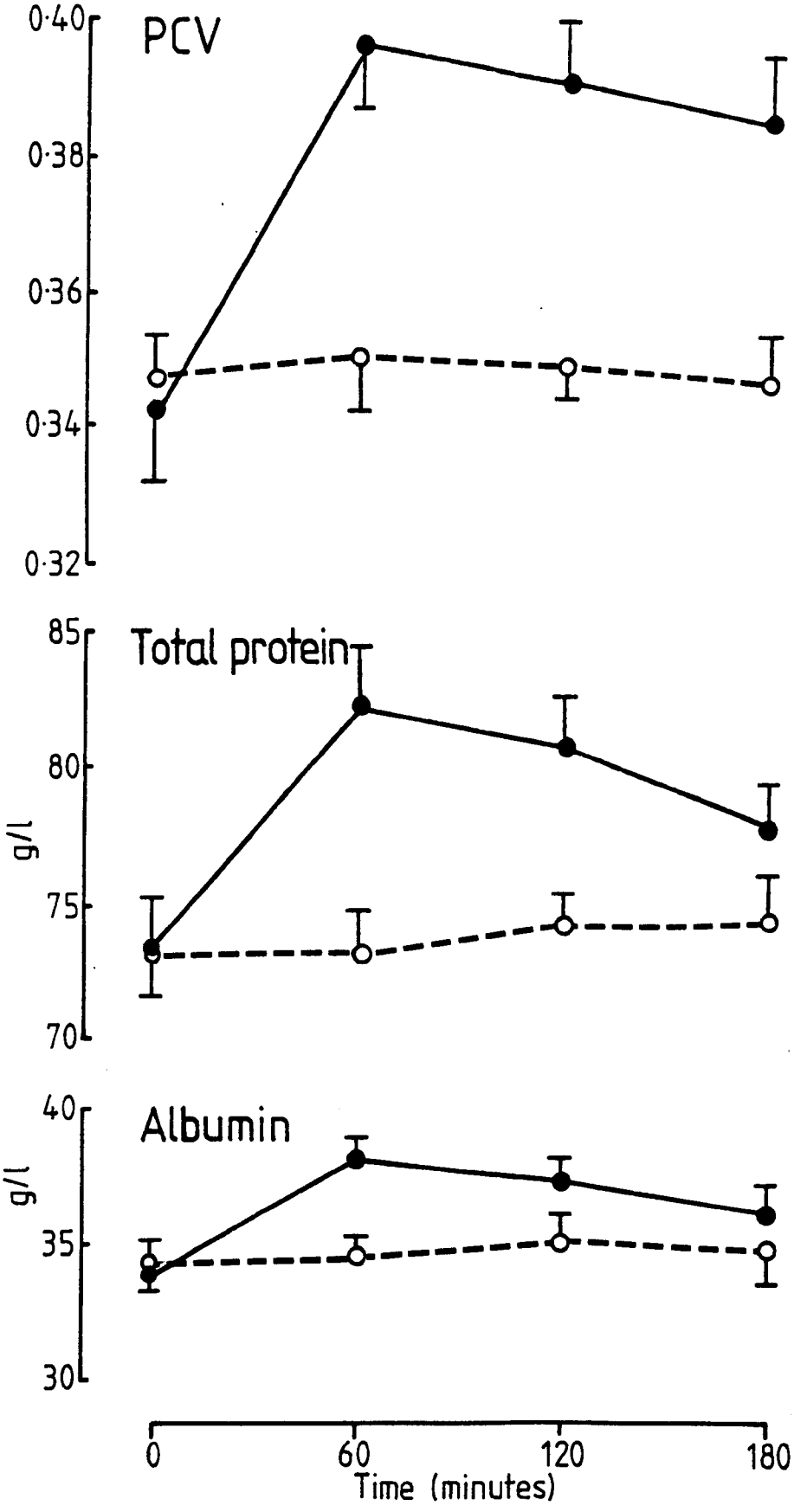


Figure 10(a). Changes in PCV and plasma protein concentrations following the feeding of hay (solid line, 5.5 kg hay given immediately after the collection of the first blood sample) compared to a control period (broken line).

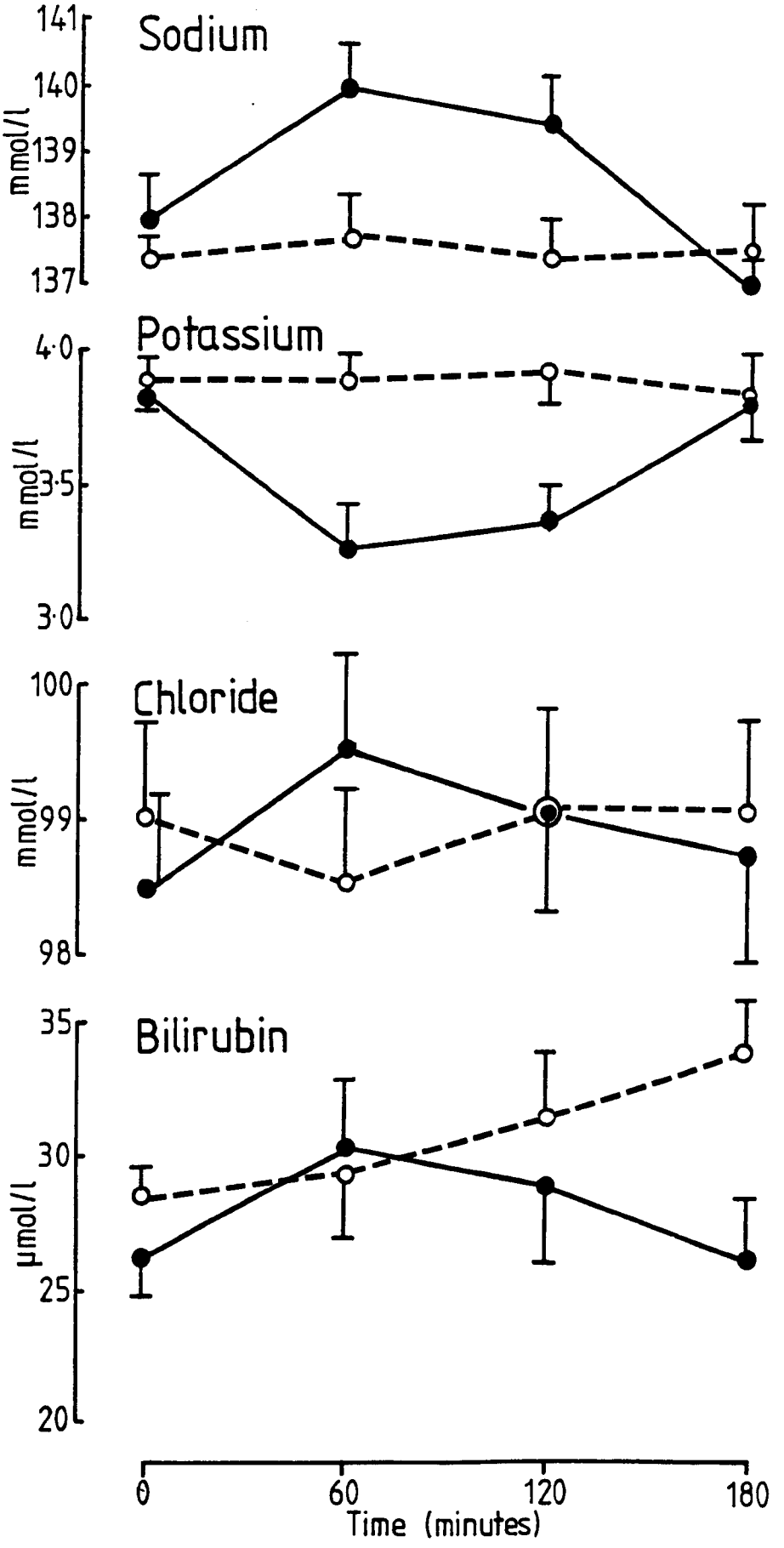


Figure 10(b). Changes in plasma electrolyte and bilirubin concentrations following the feeding of hay (solid line, 5.5 kg hay given immediately after the collection of the first blood sample) compared to a control period (broken line).

Discussion

Experimental design

Most horses involved in competitive endurance rides are not Thoroughbreds, but Arabs, Arab crosses and various pony breeds. It might therefore seem less than ideal to use a group of Thoroughbreds in a study designed to provide a baseline for the investigation of endurance horses. However, the use of such a homogeneous group of animals for this purpose was advantageous when analysing the results, because the number of animals in the group was, of necessity, small. Although these horses could not be considered to be a reference population for competing endurance horses, the intention was to study changes in concentrations rather than absolute values, and there is no reason to believe that there is a great deal of difference between breeds so far as the dynamic situation is concerned. In fact, although the horses used were not competing endurance horses, they were the same individuals as were used in Sections 3, 4 and 5 of this thesis, and the data from this Section therefore provided a valid reference base for a large part of the entire study.

Blood samples in this study were collected by repeated venepuncture. A number of other investigators have preferred to use indwelling intravenous cannulae when collecting repeated blood samples (Knudsen, 1959; James *et al*, 1970), presumably because the simple act of performing a venepuncture can significantly alter haematological findings in the horse (Seckington, 1969; Stewart and Steel, 1975). Other authors have, however, performed similar studies using repeated venepuncture and given no indication that this caused any problems (Hoffsis *et al*, 1970; Cox *et al*, 1973; Larsson *et al*, 1979). The horses in this present study were well accustomed to the blood-sampling procedure and did not show evidence of being disturbed by it. Many samples were collected with no

restraint being used. It was therefore concluded that the effect of this procedure was unlikely to be greater than the effect of the more time-consuming routine required when collecting from and flushing out an indwelling intravenous cannula.

Urine samples in this study were collected following natural urination rather than by bladder catheterisation, which introduced a number of drawbacks. The timing of the collections was random and varied between horses, which made statistical evaluation of the results more difficult, and all useful data from horse C was lost because of his failure to urinate more frequently than once in 24 hours. It was not always possible to ascertain the exact time of each urination because the design of the stable block and the necessity for processing blood and urine samples during the experiment meant that a constant watch could not be kept. This means that the urine flow rate figures are subject to a degree of error. Another source of error in the flow rates is the fact that bladder emptying could not always be proved to be complete, in fact it obviously was not complete on a few occasions. It is noteworthy, however, that bladder catheterisation does not always remove this problem (Knudsen, 1959).

The most serious problem caused by the urine collection protocol concerns the discontinuity of results between 8.00 and 9.00 hrs. The fact that the urine bags were only in place for exactly 24 hours meant that there were no data for the period after the last urination in the early morning, around 8.00 hrs. Considering the marked discontinuity seen in a few parameters, particularly sodium concentration, it would have been preferable with hindsight to have continued the urine collection periods for 30 hours rather than 24. In fact the protocol as followed means that the total 24-hour urine excretion figures given in Table 2 are the total amounts present in urine passed during a 24-hour period, which are not necessarily the same as the amounts secreted by

the kidney in that time (Zilva and Pannall, 1975). However, the horses habitually urinated at about 8.00 to 8.30 hrs every morning and so the error in these figures is probably not great. True 24-hour urine excretion figures have never been published for the horse.

In spite of these drawbacks, it is considered that meaningful results were obtained by the procedure used. With the exception of horse C, the horses all urinated frequently enough to provide a good indication of changes occurring in urine composition, and the use of urine bags for collection allowed the management regime during the experiment to be kept as close to normal as possible. The use of indwelling bladder catheters requires horses to be kept in closely-confining standing stalls (Knudsen, 1959; Rawlings and Biscard, 1975; Lane and Merritt, 1983) which were not available for the present study. It is impossible to perform this type of investigation in horses without disturbing them to some degree, but the individuals used in this study were well accustomed to wearing the urine bags and the entire study was carried out without removing them from their normal environment.

Blood samples

Most of the changes in blood parameters measured can be related directly or indirectly to the feeding regime, both concentrate feed and, most particularly, the hay ration.

Effect of concentrate feeds

Evans et al (1975) described a diurnal variation in plasma glucose almost identical to the variation seen in this study. However, it is difficult to compare the two situations as the horses used by Evans et al were kept undisturbed on a constant light/dark cycle and fed a complete ration ad lib, while in the present study the light/dark situation was not strictly controlled but the feeding times were known. As a result, Evans et al were unable to state with certainty whether the changes they observed were due to the light/dark cycle or to times of

feeding, while in the present study the results can be related to the feeding times.

The effects of feeding on plasma glucose concentration in the horse have been studied by Anderson (1973) who reported that a glucose peak occurred two hours after a concentrate feed but that there was no change in plasma glucose following a hay feed. The results of this study agree closely with these observations. In this present study, three concentrate feeds were given, but only one peak was seen in plasma glucose, after the first feed. There was also a temporary slight fall below the fasting level following the peak. These findings are well-recognised in man (Zilva and Pannall, 1975) and are explained by the effects of insulin concentrations. The normal peak in plasma glucose is only seen when the meal follows a period of fasting of sufficient length to give rise to basal insulin concentration. If the meal is eaten when plasma insulin is still elevated after a previous meal, as would be the case with the second and third concentrate feeds, a second peak in plasma glucose will not occur. As plasma glucose returns to fasting levels a slight "overswing" when insulin concentration is still falling is a common finding.

The small but significant changes seen in plasma urea concentration also appear to be related to the diet. An identical pattern to that observed in this investigation has been reported in the sheep (Parkins, 1972) and the reasons for the changes seen are discussed. The daytime peak is considered to be due to the absorption of protein in the concentrate feed, some of which is deaminated. The gradual increase during the night is attributed to fasting which leads to the mobilisation and deamination of labile protein stores which are a source of body fuel (Munro, 1964). This explanation is equally valid for the findings in this present study. However, the night-time increase could

also be connected to the observation that the rate of urinary urea excretion was 29% lower during the night.

The significantly lower plasma sodium concentration in the 12.00 hrs sample may be a secondary effect of the high glucose and urea concentrations at that time. Berl and Schrier (1978) have pointed out that, under normal physiological conditions, plasma osmolality remains constant according to the following formula:

$$\text{Osmolality} = 2\ Na^{+}(mEq/l) + \frac{\text{Glucose (mg\%)}}{18} + \frac{\text{Urea (mg\%)}}{2.8}$$

Therefore when glucose and urea are high, sodium concentration will be decreased.

Effect of hay feed

During the 24-hour experiment, significant changes occurred in several parameters between 15.00 and 18.00 hrs, particularly PCV and total protein and potassium concentrations. This time bracket included the evening feed of concentrates and hay, and the fact that no such changes were seen earlier when concentrates alone were fed suggests that the alterations were primarily connected with the hay feed. The results of the second experiment confirm that marked changes in PCV and a number of plasma constituents occur during the consumption of a feed of hay. This observation has not been reported previously.

In both experiments PCV increased by about 17% while total protein (and albumin in the second experiment) increased by about 12%. In the horse a relative polycythaemia may be caused either by loss of plasma water or by mobilisation of splenic erythrocyte stores. Total plasma protein, however, is not affected by splenic contraction and a simultaneous increase in both parameters is a strong indication of a loss of plasma water. (These points are discussed in greater detail in Section 2.) The accompanying fall in plasma potassium suggests that the

fluid lost had a potassium concentration markedly higher than that of plasma. These changes occurred very soon after the start of feeding and the return to normal began while the horses were still eating, which suggests that they are a reflection of the secretion of fluid into the upper part of the gastro-intestinal tract, possibly saliva.

The horse lacks the grinding reticulum and omasum of the ruminant and must therefore render coarse feed down into a consistency suitable for passage into the small intestine by mastication alone. Alexander (1966) has shown that horses only secrete parotid saliva while masticating and that during this time large volumes are produced. He has also shown how essential this saliva is to the consumption of hay, by demonstrating that ponies deprived of a normal salivary flow must dip each mouthful in water to lubricate it before eating (Alexander, 1969). These same publications report that horse saliva has a potassium concentration of about 15 mmol/l while being hypotonic compared to plasma for both sodium and chloride.

These findings support the hypothesis that salivary secretion was responsible for the changes in blood parameters demonstrated in association with the hay feed. Although the saliva is not lost from the body, it appears from Figure 4 that 12 hours or more may elapse before all the water is returned to the circulation. Plasma potassium recovered much more quickly, probably due to the fact that the hay itself contains a very high level of potassium (1.6 - 2.0%; Cunha, 1980).

The small increases seen in both experiments in plasma sodium and chloride concentrations may also be due to the fluid shifts associated with the hypotonic saliva, but the small magnitude of the changes renders firm interpretation somewhat questionable.

The increases which were noted in the enzyme activities underlines the fact that enzymes behave in the same way as other plasma proteins in response to dehydration, but it is interesting to note that such a minor

occurrence as beginning a hay feed can cause changes in enzyme activities significant at the $p < 0.001$ level, however small the magnitude of the change may be.

The changes in plasma bilirubin concentration over the 24-hour experiment and during the test day of the hay feeding experiment would suggest that the transient dehydration had a concentrating effect on bilirubin similar to the effect on the plasma proteins. However, the significant increase during the control period of the hay feeding experiment was unexpected. Normal plasma bilirubin is well known to be higher in the horse than in other domestic species, and to increase very readily during fasting or anorexia (Cornelius, 1980). Benign fasting hyperbilirubinaemia (Gilbert's disease) is well characterised in man and is thought to be due to defective transport of bilirubin into the liver cell (Barrett et al, 1968). The characteristic 20 - 35 µmol/l unconjugated bilirubin in these patients is similar to normal equine values. Recently it has been shown that the Bolivian Squirrel Monkey normally exhibits most of the criteria used to characterise Gilbert's disease in man (Cornelius, 1983). However, studies of fasting horses by Naylor et al (1980) have suggested that the mechanism of the hyperbilirubinaemia in this species is not the same as in Gilbert's disease but is related to a simultaneous increase in plasma free fatty acids. It is suggested that competitive inhibition by free fatty acids of the binding of bilirubin to ligandin could restrict hepatic uptake of bilirubin and allow it to accumulate in the plasma. The reason for the significant increase in plasma bilirubin during the control period of the hay feeding experiment compared to the absence of any significant change during the overnight fast of the 24-hour experiment is not immediately apparent, as plasma free fatty acids were not measured.

Plasma mineral concentrations

Plasma inorganic phosphate concentration exhibited a particularly smooth and regular diurnal variation. There is no immediately obvious dietary or other explanation for this, and it may be that it reflects a regular diurnal hormonal change which was not monitored in this investigation. It is interesting to note that the plasma calcium concentration appeared to show a pattern of change very similar to that of phosphate, although in this case it was not statistically significant. In man, circadian rhythms have been demonstrated in both plasma and urinary phosphate (Stanbury, 1958) and in parathyroid hormone (Arnaud et al, 1971), but whereas in this present study the peak phosphate concentration was at 6.00 hrs, the peak time in these reports is about 20.00 hrs. No investigation of parathyroid hormone (or calcitonin) appears to have been carried out in the horse.

The significant increase in plasma magnesium concentration between 9.00 and 12.00 hrs is again not easily explained. It is possible that there is some association with the absorption of magnesium contained in the concentrate feed.

Plasma creatinine

The slight rise in plasma creatinine concentration noted towards the end of the 24-hour period may well be a consequence of the 16% decrease in the rate of urinary creatinine excretion which occurred during the night.

Urine Samples

Patterns of urination

The patterns of urination seen in this study are almost certainly not representative of the habits of horses when undisturbed, and are very different from the patterns observed when the horses were working. The act of walking round the stables to check the urine bags was seen to stimulate urination in a number of cases, and it does appear unlikely

that healthy horses habitually void volumes of urine of under 500 ml when the bladder is capable of containing several litres. Horse C's habit of urinating only once per day while wearing the urine bag was a long-established one, and he was observed to urinate more frequently when the bag was not in place.

Urine flow rate, specific gravity and concentrations of nitrogenous constituents

Total 24-hour urine volumes recorded in this study were much closer to those reported by Tasker (1967a) and Fonnesbeck (1968) than to the 15.6 l reported by Rumbaugh et al (1982). It may be that the ad lib salt supplementation offered to the horses in that last study encouraged a higher water intake.

Stanbury and Thompson (1951) have reported a pattern of change in urine flow rates in man which is remarkably similar to that seen up to 23.00 hrs in this present study. In that experiment, the periods of diuresis occurred immediately after the ingestion of a litre of water. In this study the horses had free access to water at all times and so their drinking habits were not known. It seems probable, however, that the pattern of urine flow rates seen was due to the horses habitually drinking at the times of the morning and evening feeds. This is in accordance with the findings of Sufit et al (1985), who showed that almost 90% of the water intake of ponies kept in a similar management regime occurred periprandially (from 10 minutes before to 30 minutes after feeding). If this is the case, it implies that the horses drank either during or immediately after the consumption of the hay feed, which makes the very gradual rate of decrease in PCV and total plasma protein after that feed quite remarkable.

It has been known for many years that, in man, urine flow rate is higher during the day than during the night and that this difference persists even in the absence of any meals or daytime activity (Simpson,

1924). It is, however, difficult to assess the comparable significance of the higher daytime flow rates seen in this present study, as it seems probable for the reasons discussed above that the drinking patterns of the horses were not regular throughout the 24 hours. Almost all the studies of urinary excretion patterns in man have been carried out under conditions of complete bed rest and regular "meals" at short intervals throughout the day and night. The horses in this study were confined to their loose-boxes throughout and did not lie down at any time because of the urine bags, but it seems very probable that many of the changes seen in urine excretion have been influenced by irregular drinking habits.

Hendry et al (1964) studied the changes in the amount of urinary solutes excreted during various degrees of diuresis and reported that although urine concentration (expressed as osmolality) decreased as flow rate increased, these changes did not cancel out exactly and there was a direct relationship between solute excretion and flow rate. This agrees with the findings in this study of higher rates of creatinine and urea excretion during the periods of diuresis. Again this effect of the occurrence of periods of diuresis during the day makes comparison with findings in human studies difficult. It has been reported many times that, under constant experimental conditions, urinary creatinine excretion rate is slightly lower during the night (Simpson, 1924; Sirota et al, 1950; Stanbury and Thompson, 1951; Bartter et al, 1962) and this is attributed to a reduction in glomerular filtration rate. Simpson (1924) reported similar findings with regard to urea excretion rates. It is impossible to determine with certainty whether a similar effect would be present in this study if the effects of the periods of diuresis could be excluded. It is, however, interesting to note that the creatinine excretion rate varied comparatively little and returned to basal levels between the peaks of diuresis while the urea excretion rate, which

varied by a greater percentage, did not return to basal levels between the peaks.

Urine pH and electrolyte excretion

Total 24 hour urinary potassium excretion in this study was very similar to that reported by Tasker (1967a) and Rumbaugh et al (1982). Tasker (1967a) has pointed out that this is very much greater than the level seen in man, while urinary sodium excretion is very much less (both on a body weight basis), and he considers that this is due to the hay diet being fed which is high in potassium and low in sodium. 24-hour urinary sodium excretion in this present study was somewhat higher than that reported by Tasker (180 mmoles compared to 7.1 mmoles), but still markedly below the 1,001 mmoles reported by Rumbaugh et al (1982). These differences are certainly due in part to differences in diet. The horses used by Rumbaugh et al were given free access to supplementary salt while the hay diet fed by Tasker provided a total daily sodium intake of only 329 mmoles; in this present study the concentrate feed alone (excluding the hay) provided approximately 540 mmoles of sodium per day. It is clear, however, that both Tasker's horses and those in this present study excreted only a small percentage of their daily sodium intake in the urine, and that some other route of loss must play an important role in sodium homeostasis in this species. This is in marked contrast to the situation in man where urinary sodium excretion rates are much higher (Bartter et al, 1979), and this subject is considered further in the General Discussion.

When considering the changes in excretion rates over 24 hours, it is clear from Figure 9 that the two periods of diuresis have had a similar effect on potassium and chloride excretion rates to that discussed above in relation to creatinine and urea, but there is no obvious effect on sodium excretion rate. Again this makes comparison with findings in man more difficult.

Studies of urinary electrolyte excretion in man (Stanbury and Thompson, 1951; Doe et al, 1956, 1960; Bartter et al, 1962, 1979) all agree that sodium and potassium excretion vary in parallel and that sodium excretion is much higher than potassium. Where chloride is measured, it varies in parallel with the other two electrolytes and has an excretion rate very close to that of sodium. Urine pH also varies in parallel with the electrolytes and although it was once believed that the daytime increase in pH was due to the secretion of gastric acid leading to alkalosis, the pattern has been shown to occur irrespective of the timing of meals (Simpson, 1924; Stanbury and Thompson, 1951).

The hormonal basis of these changes has been discussed at length by Bartter et al (1962, 1979). Briefly, plasma renin activity, aldosterone and 17-hydroxycorticosterone show a circadian rhythm with a peak at around 6.00 hrs. (Studies in rats (Hilfenhaus, 1976) have shown a similar result and have indicated that the peak occurs at the beginning of the activity period whether the animal is diurnal or nocturnal.) The aldosterone peak is considered to be the cause of the subsequent daytime increase in urine pH and potassium excretion. However, the sodium and chloride excretion rates, which increase at the same time, are 180° out of phase with what would be expected if aldosterone were the controlling factor in these parameters. It is therefore concluded that these are controlled by some factor other than steroid hormones, and the fact that chloride excretion peaks slightly in advance of sodium has led to the conclusion that active resorption of chloride in the thick ascending limb of the loop of Henlé is the controlled process with sodium resorption being secondary to this.

In this present study, potassium and chloride excretion rates varied in close parallel, with potassium always slightly higher than chloride. Excretion rates of both these electrolytes were markedly higher during the period from 12.00 to 22.00 hrs. This could simply be

an effect of the diuresis already discussed, but considering that potassium and chloride excretion increased to 2.5 times their baseline levels, while at the same time creatinine excretion only increased by about 25%, it appears that some other factor may well be involved.

The marked discontinuity seen between sodium excretion rates at 8.00 and 9.00 hrs, which was also present to a lesser degree in the concentrations (but not excretion rates) of most of the other parameters, was certainly to some extent an effect of the experimental design as discussed above. In spite of this, it is clear that sodium excretion must have increased fairly sharply at some time between 8.00 and 10.00 hrs and that there was then a more gradual decrease during the day. This pattern in no way resembled that of potassium and chloride.

Horse urine was first demonstrated to be alkaline by Smith (1889) and this is a finding common to all herbivores. In this present study, urine pH did not vary in parallel with any of the electrolytes, in fact the lowest pH was close to the time of the highest chloride excretion rate. The pattern was one of a sharp drop between 10.00 and 12.00 hrs, a gradual rise until about 21.00 hrs and then a period of no change. Subjectively this pattern appears to be almost an inverse of the variation in sodium excretion rate but with the sharp decrease occurring about two hours later than the apparent sharp increase in sodium excretion. However, due to the discontinuity in the sodium excretion, this could not be verified statistically.

Plasma aldosterone concentrations in the horse have been studied by Guthrie et al (1980, 1982) with respect to spot morning samples and the effects of exercise and frusemide administration. These authors suggest that the horse has a functioning renin-aldosterone system which is characterised by unusually low levels of plasma renin activity and an unusual response to exogenous ACTH. However, no investigation has been made of any diurnal variation in this system. Studies of plasma

glucocorticoids in the horse have indicated, as with man and the rat, a peak concentration near the beginning of the activity period (day) (Zolovick et al, 1966; James et al, 1970; Hoffsis et al, 1970; Bottoms et al, 1972; Evans et al, 1977; Larsson et al, 1979). If plasma aldosterone concentration follows a similar pattern it could perhaps explain the increasing rate of potassium excretion seen in the horses during the late morning, as is postulated with regard to similar findings in man (Bartter et al, 1979). However, urine pH in the horses varied in the opposite direction to potassium excretion at this time, which is in complete contrast to the situation in man, and this effect is not consistent with such a pattern of plasma aldosterone concentration.

Urine chloride excretion varied in parallel with potassium and in the opposite direction to that which would be expected if aldosterone were controlling both parameters, as is the case in man. However, unlike man, there was no relationship between chloride and sodium excretion rates.

It is clear from these findings that patterns of electrolyte excretion in the horse are very different from those in man. It seems probable that the controlling factors involved in the changes over the 24-hour period are not the same as those postulated for man, at least insofar as urine pH and sodium excretion are concerned. It is likely that these differences are related to the different diets of the two species, particularly concerning the relative amounts of sodium and potassium in the diet, as discussed by Tasker (1967a). Michell (1977) has reported an inverse relationship between urine sodium and potassium concentrations in the sheep, which, although a ruminant, has a similar diet to the horse. However, as no plasma hormones were measured in this study and the horses were not on a constant regime of food and water intake throughout the 24 hours, it is difficult to speculate on the

precise hormonal or other factors involved in the changes which were seen.

"Clearance ratios"

It has been suggested (Traver et al, 1977a,b) that it is possible to diagnose a number of mineral and electrolyte disorders, including hypoaldosteronism and renal dysfunction, from a single, non-volumetric urine sample. This procedure is recommended in order to avoid the considerable inconvenience involved in collecting timed volumetric urine samples in horses, which are necessary for the traditional calculation of renal clearances from the formula:

$$\text{clearance} = \frac{\text{urine concentration x urine flow rate}}{\text{plasma concentration}}$$

The basis of the clearance ratio calculation is that the clearance of the substance under consideration is expressed as a percentage of the clearance of creatinine, thus allowing the urine flow rate to be cancelled from the equation. The formula thus derived is:

$$\% \text{ clearance of x} = \frac{[x] \text{ urine}}{[x] \text{ plasma}} \times \frac{[\text{creatinine}] \text{ plasma}}{[\text{creatinine}] \text{ urine}} \times 100$$

It is claimed that this investigation is of particular value in the early stages of disease when plasma electrolyte concentrations are normal. "Normal values" for clearance ratios are quoted (Table 5) and data from seven urine samples are used to demonstrate a significant correlation between clearance ratio figures and traditionally calculated clearances. The validity of this procedure depends on the assumption that electrolyte clearances bear a constant relationship to creatinine clearance.

In this present study, the variations in plasma electrolyte concentrations were insignificant in comparison to the variations in urine concentrations. This implies that the "clearance ratio" will vary

according to the urine concentrations and it can be seen from the equation above that it will only remain constant if the urine creatinine and electrolyte concentrations vary in parallel. Figures 6 and 8 above demonstrate that none of the three electrolytes studied varied in parallel with creatinine, and when the clearance ratios were calculated for each of the 121 urine samples in this experiment (using the plasma sample nearest to the time of collection of each urine sample) there were marked discrepancies from the "normal values" quoted by Traver et al (1977a) (Table 5). Overall only 13 samples (11%) mostly collected during the morning or early afternoon, had "normal" clearance ratios for all three electrolytes, while 19 samples (16%), mostly collected during the late afternoon, evening and night, had "abnormal" clearance ratios for all three electrolytes. In two horses the sodium clearance ratios moved from above "normal" to "normal" to below "normal" within the space of about seven hours in the middle of the day.

TABLE 5

Electrolyte "clearance ratios" (%C) compared to the normal values of Traver et al (1977a). n = 121.

	"Normal %C"	Below normal	Normal	Above normal
Sodium	0.02-1	44 (36%)	73 (60%)	4 (3%)
Potassium	15-65	0	56 (46%)	65 (54%)
Chloride	0.04-1.6	0	31 (26%)	90 (74%)

Conclusions

This study has demonstrated significant variations in 13 out of 14 plasma parameters and all of the eight urine parameters which were measured over 24 hours. The changes in the blood could almost all be related to the effects of feeding and the changes following the eating of hay were particularly marked. Alterations in urine composition were much greater in magnitude than the plasma changes. Findings with regard to flow rate and nitrogenous constituents were similar to those in man, while findings relating to pH and electrolytes were very different.

This suggests that significant changes in plasma parameters seen over a period of exercise should be interpreted with great care, particularly when the magnitude of the changes is no greater than those seen in this study. Due allowance must be made for any feed consumed and the possibility of the occurrence of regular circadian rhythms, particularly in plasma phosphate concentration, cannot be dismissed.

Changes in urinary composition during the day are so marked that it is obviously quite impossible to compare the results of a urine sample collected before exercise with one collected after exercise to identify alterations due to the effects of the exercise. It will be necessary to compare urine results collected during any experiment with results from samples taken at the same time on a control day. It is also clear that the practice of calculating total 24-hour urinary excretion values by collecting urine for a portion of the 24-hour period and multiplying up the results is quite invalid. It is often assumed that the rate of urinary creatinine excretion is a constant irrespective of the degree of diuresis. That this is not entirely so, even under constant 24-hour experimental conditions, has been known for some time (Simpson, 1924) and the marked changes caused by changes in the degree of diuresis have also been pointed out (Hendry *et al*, 1964). This present study emphasises the difficulty of interpreting data from

subjects on a normal routine of daily meals and nightly fasting in comparison with work done on subjects placed on a controlled, unvarying routine over a 24-hour period.

Considering the particularly large variations seen in urine electrolyte concentrations over 24 hours, it is probably very unwise to read a great deal of clinical significance into electrolyte results from a single spot urine sample. This applies whether the concentration itself, its ratio to creatinine or its renal clearance is used for interpretation. There is still a great deal to be learned about urinary electrolyte excretion in the horse, particularly concerning the effects of diet, feeding and management regimes, and the factors involved in the control of basic circadian rhythms.

SECTION TWO

STUDIES OF COMPETING ENDURANCE HORSES

Introduction

Biochemical studies of endurance horses

The overall background to modern competitive endurance riding, as discussed in the General Introduction, has led to very close involvement of the veterinary profession in the running of the events. Veterinary surgeons attend all equine endurance competitions not simply as a precaution against accident or illness but in the capacity of judges. The reason for this, as already discussed, is to ensure professional application of sets of rules which are designed to protect horses from ill-use or over-stress in the pursuit of a prize. Certain criteria for penalisation or elimination such as saddle sores or lameness do not often give rise to serious uncertainty or disagreement. However, certain other criteria admit of only approximate assessment on clinical examination, for example dehydration or exhaustion. The close involvement of veterinary surgeons in the competitions has made it reasonably easy to obtain blood samples from the competing horses and a number of investigators have taken advantage of this opportunity. The aims have been generally to study further the normal biochemical changes which occur in horses during these competitions and to attempt to identify the particular sources of stress, sometimes in the hope of being able to apply more scientific criteria to the judging of the events.

Fluid and electrolyte studies

The horse, like man, relies heavily on the secretion of profuse quantities of watery sweat for thermoregulation during exercise or in a hot environment. This can of course lead to marked losses of fluid and disturbances in plasma electrolytes. A number of authors have reported

changes in plasma protein and electrolyte concentrations in competing endurance horses covering distances of 50 miles (80 km) or more and their results are summarised in Table 6.

Packed cell volume, total plasma protein and albumin results suggest that while plasma water loss is a common occurrence in endurance rides it is by no means invariable. The position with regard to plasma chloride, calcium and magnesium concentrations is similar, with most authors reporting a decrease but some studies finding no change in these parameters. Plasma sodium and potassium results are more complex with increases, decreases and no change being variously reported. Interestingly, all papers which have investigated further changes after a period of rest have reported a substantial drop in plasma potassium concentration compared to the finishing value (Rose et al, 1977, 1980; Lucke and Hall, 1980a). In both studies involving 160 kilometre rides changes in many parameters tended to be most marked at an intermediate stage and to show a partial return to normal towards the end. On both occasions significantly elevated total protein concentrations were noted at some point during the rides (Carlson and Mansmann, 1974; Fregin, 1980).

Rose et al (1980) have suggested that these variations seen in the reports of endurance rides are due to local factors, particularly terrain and temperature. The only ride showing no evidence of plasma water loss was that studied by Lucke and Hall (1978), which was held in unusually cold weather, and rides over similar terrain in higher temperatures showed marked changes (Lucke and Hall 1980a,b). The terrain covered in rides studied by Rose et al (1977, 1980) was much more severe and alterations in most parameters, particularly total plasma protein, were very much greater on these occasions.

TABLE 6

Summary of previous publications relating to fluid and electrolyte changes during endurance competitions

Authors	Date	Distance Covered (km)	PCV	Total Protein	Albumin	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}
Carlson & Mansmann	1974	160	↑	NC	-	↓	↓	↓	↓	↓	↑
Rose et al	1977	100	-	↑	-	↑	↑	↓	NC	-	↑
Lucke & Hall	1978	80	NC	NC	-	NC	↓	NC	↓	↓	↑
Fregin	1980	160	↑	NC	-	↑	↑	↓	↓	-	↑
Lucke & Hall	1980a	80	↑	-	↑	NC	NC	↓	↓	-	↑
Lucke & Hall	1980b	80/40 (2-day ride)	↑	-	↑	NC	↓	-	↓	-	-
Rose et al	1980	100	-	↑	-	↓	↓	↓	-	-	-
Grosskopf et al	1983	80/48/48 (3-day ride)	↑	↑	-	NC	↑?	-	NC	NC	-

↑ Significant increase reported.

↓ Significant decrease reported.

NC No significant change reported.

Fuel metabolism and related studies

Prolonged exercise makes enormous metabolic demands as the amount of energy required to move the horse (and rider) over an endurance course is very considerable. The availability of fuel substrates to the exercising muscle has been summarised by Newsholme (1977) and Hultman and Sjöholm (1983), concentrating mainly on work carried out with human athletes and experimental animals, particularly rats. Briefly, the primary substrate for energy production is ATP, which is continually renewed from a variety of other sources, both within the muscle cell and transported to the muscle in the blood. Phosphocreatine, which is easily converted to ATP by the following reaction,

$$\text{Phosphocreatine} + \text{ADP} \xleftrightarrow{\text{creatine kinase}} \text{ATP} + \text{creatine}$$

serves as an immediate energy store which acts as a buffer against sudden changes in energy demand. Glycogen is the largest locally available store of energy in the muscle and during aerobic exercise its availability appears to be the limiting factor to endurance performance, with exhaustion of muscle glycogen corresponding with the onset of serious fatigue. (During anaerobic exercise intracellular accumulation of lactic acid may induce fatigue while some muscle glycogen still remains. However, although plasma lactate does increase significantly in horses during endurance rides (Lucke and Hall, 1980a,b) the magnitude of the increase is very small compared to that seen during sprint exercise (Snow and Mackenzie, 1977a), which indicates that in endurance rides the exercise is essentially aerobic. Rose et al (1979) believe that trained endurance horses can sustain aerobic metabolism at speeds of up to 18 km/hr.) Muscle glycogen is spared by the additional utilisation of other substrates, particularly blood glucose and fatty acids, and the proportion of glycogen to other substrates used varies directly with the intensity of the exercise and inversely with the fitness of the

individual. The maintenance of blood glucose concentration depends mainly on the utilisation of the comparatively labile liver glycogen store and partly on hepatic gluconeogenesis from glycerol, pyruvate and other substrates. Fat is the body's largest energy reserve, and can only be used aerobically. Lipids are stored both in the muscle itself and more distantly in adipose tissue, and free fatty acids from adipose tissue may account for up to 60% of total energy utilisation in endurance exercise. However, the mechanisms governing the rate of utilisation of lipid fuels and the relationship to free fatty acid uptake by the muscle are still relatively unknown. The exact role of protein as a fuel source during exercise is also not fully understood.

A number of authors have measured plasma levels of various substrates and related by-products in competing endurance horses (Rose et al, 1977; Lucke and Hall, 1978, 1980a,b; Dybdal et al, 1980; Rose and Sampson, 1982). A significant decrease in plasma glucose concentration has been reported by most authors, which indicates that carbohydrate reserves, particularly liver glycogen, were becoming depleted. However, in the 80 km ride in which the fastest times were recorded (Rose and Sampson, 1982) no change in plasma glucose was seen. Very marked increases in plasma free fatty acid and glycerol concentrations have been reported in all events and it is generally agreed that fat provides a major source of body fuel in horses during long distance exercise as originally suggested by Carlson et al (1965). Significant increases in plasma acetoacetate and β-hydroxybutyrate concentrations have occasionally been reported but these are small in magnitude and it appears that in spite of the development of hypoglycaemia the ketone pathway of fat metabolism is relatively unimportant in the horse. In man, an important substrate for gluconeogenesis is provided by alanine which is released from the exercising muscle and metabolised in the liver to pyruvate - the glucose-alanine cycle. The only study which has investigated this

subject in the horse (Lucke and Hall, 1980a) has reported a significant decrease in plasma alanine concentration, an anomalous result which suggests that other gluconeogenic amino acids may be more important in the horse. Glycerol is also an excellent substrate for gluconeogenesis and as its availability as a by-product of lipolysis is high it is probable that it contributes significantly to the maintenance of blood glucose concentrations under these conditions.

Increases reported in plasma inorganic phosphate and creatinine concentrations may well reflect utilisation of high energy stores in the muscle (phosphocreatine and ATP), but it is not clear whether the increased urea concentration reported by Lucke and Hall (1980a,b) is a reflection of reduced excretion or of increased protein catabolism.

The effect of the fat content of the diet on fuel metabolism has been investigated under more controlled conditions by Hintz et al (1978) and Hambleton et al (1980). In the former study the horses were ridden at 9.7 km/hr for 60 km and in the latter they were trotted without riders at 11.5 km/hr for about 65 km. It appears that dietary fat level may influence the changes seen in a number of plasma parameters during exercise, but due to the comparatively short distances and slow exercising speeds used in these studies it is not clear whether there is any effect on actual performance.

Utilisation of intramuscular fuels, particularly glycogen, in endurance horses has been studied by Lindholm et al (1974a), Snow et al (1981) and Hodgson et al (1983). It appears that in the horse, as in man, exhaustion of intramuscular glycogen stores is directly related to the development of fatigue, with the type I (slow twitch) and type IIA (fast twitch high oxidative) muscle fibres being most important in endurance exercise while the type IIB (fast twitch low oxidative) fibres may be selectively recruited when the speed is increased for a short period during the event. Hodgson et al (1983) have demonstrated a

direct correlation between the extent of muscle glycogen depletion and the duration of exercise and have suggested that muscle fibres are progressively recruited in the order type I - type IIA - type IIB as exercise continues. It was found that repletion of muscle glycogen took up to 48 hours to complete and that the fibres were repleted in the reverse order to their depletion. It is not clear whether or not intramuscular lipid content decreases during endurance exercise in horses (Snow et al, 1981).

Plasma hormone studies

Changes in plasma hormone concentrations in endurance horses have been investigated by several authors (Dybdal et al, 1980; Lucke and Hall, 1980a,b,c; Snow and Rose, 1981). Significant increases in plasma cortisol (and glucagon) concentrations have been reported in all events studied, however while plasma insulin concentration decreased significantly in all rides of 80 km or over, no change was seen in the horses competing in a 42 km "equine marathon" event (Lucke and Hall, 1980c). Lucke and Hall (1980a,b) consider that these changes are primarily aimed at the stimulation of lipolysis. However, the effect of cortisol on blood glucose concentration is primarily achieved through increased gluconeogenesis, and gluconeogenesis (and glycogenolysis) are also favoured by the increase in glucagon. It is therefore possible that these effects on carbohydrate metabolism may be more important than any effect on fat metabolism. In addition, an increase in plasma cortisol concentration is often associated with non-specific "stress", for example as a result of hyperthermia or hypoglycaemia (Myles and Daly, 1974), which are common occurrences in competing endurance horses.

The fall in plasma insulin during endurance exercise in man has been the subject of debate for many years. It appears that glucose is able to enter the muscle cell during exercise in spite of very low plasma insulin concentrations, and even in Roman times exercise of

moderate intensity was known to be beneficial to patients suffering from diabetes mellitus. At one time another hormone (Goldstein's factor) was postulated as being responsible, but this theory later fell into disfavour (Berger and Berchtold, 1981). More recently the concept has been revived in the suggestion that the kinins (bradykinin and kallidin) may act locally to control glucose uptake by the muscle during exercise (Dietze et al, 1981). The mechanism involved is not yet fully understood, but it is interesting to note that the same effect occurs in the horse.

The only study which has investigated plasma catecholamine concentrations in competing endurance horses (Snow and Rose, 1981) demonstrated significant increases in both adrenaline and noradrenaline. It seems very probable that it is this increased level of adrenergic activity which is primarily responsible for the observed increase in lipolysis in these horses, and that the consequent release of glycerol provides a substrate for cortisol- and glucagon-induced gluconeogenesis, as discussed above.

Hall et al (1982, 1983) have studied the changes in gut-related hormones in more depth, again comparing an 80 km ride, where horses were hypoglycaemic at the finish, to an equine "marathon" (42 km) where the horses finished in a hyperglycaemic condition. In addition to the findings detailed above they investigated somatostasin, pancreatic polypeptide, vasoactive intestinal polypeptide, gastrin and gastric inhibitory peptide. A number of marked alterations were found which differed slightly between the two distances but the exact functions of the responses seen were not entirely apparent.

Plasma enzyme studies

Changes in the activity of enzymes in plasma are generally ascribed to a deficit in the integrity of the membrane of the cell containing the enzyme thus allowing its escape into the plasma. Increased membrane

permeability or complete disruption of the cell may be involved. A number of plasma enzymes have been investigated by various authors, but in general the concern has been with enzymes characteristic of the muscle cell in an attempt to quantify the muscle stress which occurs during endurance rides.

Tissue levels of various enzymes in the horse have been studied by Cardinet et al (1967) and Gerber (1969) who agree that the enzyme which is most specific for muscle tissue (skeletal and cardiac) is creatine kinase (CK), whose role in the metabolism of the muscle cell is described above. Aspartate aminotransferase (AST) activity is also high in muscle but as it is also found at similar levels in liver tissue it is less specific. In spite of this its use in the investigation of muscular disorders is recommended because plasma levels remain elevated for up to 14 days after the initial injury while CK, which has a plasma half-life of only 108 minutes, may return to normal in under two days.

A marked increase in plasma CK activity is a universal finding in endurance rides (Rose et al, 1977; Lucke and Hall, 1978, 1980a; Fregin, 1980; Grosskopf et al, 1983). Rose et al (1977) found no significant increase in AST activity, but increased activity of alkaline phosphatase (ALP) has been reported on two occasions (Rose et al, 1977; Lucke and Hall, 1980b). Where clinical cases of exhaustion and rhabdomyolysis have been encountered at endurance rides markedly elevated plasma CK activities have invariably been reported (Rose et al, 1977; Hinton, 1976; Lucke and Hall, 1978, 1980a; Grosskopf et al, 1983). CK isoenzymes were investigated in one horse which collapsed near the end of an 80 km ride and proved to be derived largely from cardiac muscle (Lucke and Hall, 1978). However, it was considered that this was a comparatively unusual occurrence and it is generally believed that the skeletal muscle is the normal source of the elevated plasma CK activities to be found in endurance horses, as has been shown to be the

case in human athletes (Fowler et al, 1962; Rose et al, 1970a; Riley et al, 1975). It is interesting to note that in two of the foreign rides studied two horses on each occasion demonstrated post-ride plasma CK activities of a similar magnitude to those associated with rhabdomyolysis, without, however, developing any clinical symptoms of muscle problems (Fregin, 1980; Grosskopf and van Rensburg, 1983).

Several authors have studied plasma enzyme changes in horses in more detail. A regular pattern of change in AST activity as training progresses has been reported (Cornelius et al, 1963; Cardinet et al, 1963; Mullen, 1969) which consists of a sharp rise in the very early stages of training followed by a more gradual decrease to a level somewhat higher than the original pre-training value, which is then maintained. This has led to claims that an individual horse's level of fitness can be accurately assessed by measuring plasma AST activity, but it seems extremely unlikely that any useful information of this kind could be gained from a single estimation. More recently Rose and Hodgson (1982) studied a number of endurance horses during training and found no significant changes in plasma AST or CK (or in 15 other biochemical parameters).

It appears that the magnitude of the increases in enzyme activities over a standard exercise test might be a better guide to degree of fitness. Edqvist et al (1971) considered that pre- and post-exercise estimation of both CK and AST could give information about the condition of the skeletal muscle while Murakami and Takagi (1974) suggested that the magnitude of the increase in CK activity during exercise may be related to the degree of fitness while elevated AST activity might be an indication of over-training. Aitken et al (1975) used the magnitude of increase in CK during exercise as an index of fitness and reported that the peak activity occurred five hours after the end of exercise. More recently Hambleton et al (1980) demonstrated a significant negative

correlation between the duration of training and the changes in plasma levels of both CK and AST in a group of four horses during a standard 65 km exercise test.

Codazza et al (1975) have shown that a number of plasma biochemical changes which are associated with exercise also occur after a journey (of 300 km) in a horsebox. In particular CK activity showed a marked increase while AST activity did not, in fact a slight decrease in AST activity was noted.

Plasma enzyme activities in horses suffering from rhabdomyolysis (equine paralytic myoglobinuria, azoturia, "tying up" or "set-fast") have been studied by Cardinet et al (1963, 1967), Gerber (1969) and Lindholm et al (1974b). Extremely large increases in CK and AST (also LDH and aldolase) were reported and these are believed to be disease-specific. The peak CK activity appears to occur 0.5 - 4 hours after the onset of symptoms while the peak AST activity is not seen until 24 - 48 hours. This may suggest that the finding of a peak CK activity as late as 5 hours after the end of exercise (Aitken et al, 1975) could be due to some continuing leakage of the enzyme from the muscle following exercise. A similar suggestion has been made by Riley et al (1975), who found "dramatic" rises in plasma activities of both CK and AST in human subjects in the 20 - 30 hours following a marathon run.

Clinical problems of endurance horses

The range of clinical disorders encountered during endurance rides, their diagnosis and treatment have been discussed by Fowler (1980a). The most commonly reported metabolic problems, as distinct from lamenesses and other injuries, appear to be exhaustion with or without dehydration, synchronous diaphragmatic flutter (SDF, a condition in which the diaphragm contracts spasmodically in time with the heartbeat, also known as "the thumps") and rhabdomyolysis. Several authors have suggested that the basic cause of all of these problems is fluid and

electrolyte loss as a result of prolonged sweating. Mansmann et al (1974) found a relationship between hypokalaemia and hypocalcaemia and cases of SDF while Carlson and Nelson (1977) have suggested that hypokalaemia may be a major contributory factor in cases of rhabdomyolysis which occur in the later stages of endurance rides. Hinton (1976) found evidence of severe dehydration in a horse which developed both SDF and rhabdomyolysis during a ride in England. It is however very difficult to prove that the dehydration and electrolyte disturbances are actually involved in the pathogenesis of the conditions and not simply coincidental findings, as many endurance horses, particularly the poor performers, tend to become quite markedly dehydrated towards the end of a competition.

Purpose of this section

This work was carried out in order to amass data regarding fluid and electrolyte losses, fuel utilisation and plasma enzyme changes from as large a number of horses as possible competing under the rules of the Endurance Horse and Pony Society of Great Britain. These rules, in certain classes, involve direct competition between horses, as opposed to simple time trials.

The inclusion of a number of different events held in different weather conditions and over different terrain was intended to allow some assessment of the effects of these variables on biochemical changes. It was hoped that the number of animals would be large enough to allow valid statistical comparisons between groups of horses to examine effects of breed of horse, feeding and training regimes and ride tactics. In addition it was intended that the results should not only be presented as mean figures but that where appropriate individual performance would be related to individual biochemical findings.

Materials and Methods

1. Animals

The horses and ponies used in this section were all competing in long distance riding events organised by the Endurance Horse and Pony Society of Great Britain (EHPS). Fifty separate individuals were sampled, 10 of them on more than one occasion, so that a total of 70 sets of samples was collected. The distribution of the animals according to breed (or type), sex and age is given in Table 7. The level of training varied widely among the group according to the degree of involvement of the rider in the sport, and a subjective assessment of each horse's standard is also included in Table 7. This was based on the animal's performance at the ride when sampling took place according to the following grading:

Good: top class performer, little sign of fatigue.

Moderate: average performer, not unduly fatigued.

Poor: insufficiently fit horse showing obvious signs of fatigue.

Horses sampled on more than one occasion are classed according to their best performance.

2. Management

The feeding and management regimes also varied considerably and in many cases no information was available. Most of the animals were stabled, but a number, particularly those competing in the shorter (40 km) events, were kept at grass.

3. Competitions

A total of seven long distance events was attended. At four of these events horses entered for two competitions were sampled, making 11 competitions in all. Details of the distances covered, terrain, weather conditions, etc. are given in Table 8.

TABLE 7

Competing endurance horses taking part in this study

Designation	Name	Breed or type	Sex	Age (years)	Ride(s) entered	Standard of performance
1	Badger	Pony	Castrate	(9)	A*	Poor
2	Bethan	Arab cross	Female	4	J*	Poor
3	Boston Bay	Thoroughbred cross	Castrate	8	F	Moderate
4	Bracken	Pony	Castrate	12	J	Moderate
5	Brig o'Doon	Anglo-Arab	Castrate	9	BGH	Good
6	Cairo	Arab	Male	11	CEG	Good
7	Danny	Pony/cob	Castrate	10	E	Poor
8	Darren's Pal	Thoroughbred	Female	6	G	Moderate
9	Dido	Hunter/cob	Female	(8)	F*	Moderate
10	Dorocina Kiss-Kiss	Arab	Male	13	H	Moderate
11	Dresden	Pony	Castrate	10	AF	Moderate
12	Gay William	Thoroughbred	Castrate	10	B	Poor
13	Gelert	Pony	Castrate	9	J	Moderate
14	Glas-mynydd Honey	Welsh cob cross	Female	8	F	Moderate
15	Gloss	Cob	Castrate	12	F	Poor
16	Harvey	TB x Suffolk Punch	Castrate	10	E	Moderate
17	Holly	Shire x cob	Female	11	G	Poor
18	Jason	Appaloosa	Castrate	12	B	Moderate
19	Kazmahal	Arab	Male	7	CGJ	Good
20	Kushti-bok	Welsh pony cross	Castrate	7	J	Moderate
21	Leewood Isolde	Arab cross	Female	5	H	Moderate
22	Linnet	New Forest x TB	Female	(7)	H	Moderate
23	Louchi	Welsh pony	Castrate	6	E	Poor
24	Little Soula II	Thoroughbred x cob	Castrate	5	F*	Poor
25	Maelor	Thoroughbred cross	Female	(7)	A*I	Good

TABLE 7 (cont'd)

Designation	Name	Breed or type	Sex	Age (years)	Ride(s) entered	Standard of performance
26	Magnus	Arab	Male	11	AD	Moderate
27	Minnie	New Forest	Female	7	H	Moderate
28	Myebon	Arab	Castrate	10	B	Moderate
29	Nizar	Arab x Standardbred	Castrate	6	A	Good
30	Nizette	Arab x Standardbred	Female	8	B	Moderate
31	Nizzolan	Arab	Male	11	B	Good
32	Pal-o-mine	Palomino	Castrate	(6)	H	Moderate
33	Phille	Thoroughbred	Female	9	E	Good
34	Pixie	Appaloosa cross	Female	16	J	Moderate
35	Polly	Cob	Female	8	J	Moderate
36	Roxena	Arab	Female	8	AFJ	Good
37	Royal Lord	Arab cross	Castrate	9	J	Moderate
38	Rusty	Welsh cob	Female	11	J	Moderate
39	Sandpiper	Hunter/cob	Castrate	10	G	Moderate
40	Shaaban	Arab	Castrate	6	BCG	Good
41	Silver Sabre	Arab	Castrate	6	E	Poor
42	Stefano	Arab	Castrate	(8)	A*	Moderate
43	Tarquin	Irish hunter	Castrate	16	ABCDEFG	Good
44	The Stork	Thoroughbred	Female	7	B	Moderate
45	Washington Proud Sir	Cob	Castrate	(6)	D*	Poor
46	Wootton Jasper	Arab cross	Castrate	15	H	Good
47	Wyere Lad	Arab cross	Castrate	6	FJ	Good
48	Zayir	Arab cross	Male	5	J	Moderate
49	Zodiac	Arab cross	Castrate	11	B	Poor
50	(unknown)	Arab	Castrate	5	K	Moderate

*failed to complete distance.

TABLE 8

Characteristics of long distance rides attended

Ride Designation	Location	Number of Animals Sampled	Type of Event*	Distance (km)	Terrain	Time of Year	Weather
A	Sussex	8	ER	160	South Downs Way - stony tracks, moderately hilly	June	Fairly warm, cloudy, little sun, no rain
B	Hampshire	10	ER	95	New Forest - only slightly hilly	June	Fairly warm, sunny intervals, no rain
C	Hampshire	4	ER	80	As B	June	Fairly warm, cloudy, humid but no rain
D	Hampshire	3	ER	80	As B	June	Warm, light cloud, sunny intervals, no rain
E	Dyfed	7	ER	80	Welsh mountains - rough and hilly	Sept.	Wet and misty, cold wind
F	Dyfed	9	ER	80	As E	Sept.	Cool, occasional light showers
G	Gloucestershire	8	CTR	65	Undulating, not very severe	April	Cloudy, occasional rain, fairly cool
H	Hampshire	7	CTR	40	As B	June	As C
I	Hampshire	1	CTR	40	As B	June	As D
J	Dyfed	12	CTR	40	As E	Sept.	As E
K	Gloucestershire	1	CTR	40	As G	April	As G

* ER = endurance ride, CTR = competitive trail ride.

4. Procedure

Blood samples were collected before the start of each ride at the time of the pre-ride veterinary inspection, as soon as possible after the horses had crossed the finish line and 30 minutes after finishing (F+30), again at the time of the veterinary inspections. In addition, samples were collected at the halfway points of the 65 - 95 km rides (B - G) and after 40, 80 and 120 km, and 12 hours after the finish of the 160 km ride (A). At the 160 km ride the pre-ride veterinary inspection was carried out on the afternoon of the day before the ride, at all other events it was immediately before the start. For a variety of reasons a number of samples were not collected; these are not listed here in detail but are evident in Appendix II.

5. Sample processing

All samples were placed in ice immediately after collection. Processing was usually carried out as soon as possible after the end of the event at the laboratory of a local veterinary surgeon. First, capillary tubes were filled and sealed for subsequent PCV analysis. The samples were then centrifuged and the separated plasma was placed in a vacuum flask containing solid carbon dioxide for transport back to Glasgow. Due to variation in facilities between ride venues this procedure was departed from on two occasions. At ride G/K lack of a centrifuge necessitated the separation of the samples by sedimentation followed by centrifugation at Glasgow the following day, after transport unfrozen in ice. At ride F the availability of suitable equipment on site allowed all the samples to be processed immediately after collection. Plasma was stored at -20°C until analysis.

Samples from all rides were analysed for packed cell volume, total plasma protein, sodium, potassium, chloride, calcium, magnesium, phosphate, creatinine, urea, glucose, cortisol, bilirubin, CK, AST and ALP. Samples from certain rides were also analysed for albumin,

glycerol, triglycerides and insulin. The methods used are described in the General Materials and Methods section. The AAII method of ALP analyses was used for rides B, C, E, G, H and K while the reaction rate method was used for rides A, D, F, I and J.

6. Urine samples

On a number of occasions several of the riders were requested to take note of the last time their horse urinated before the ride and then to collect the entire amount of the first urination after the ride in a plastic bucket provided. However, the majority of the horses urinated while the rider was busy with some other task and some of the horses resented the procedure. As a result only one volumetric sample (i.e. total volume and time elapsed since previous urination known) and four spot urine samples were collected. These were transported back to Glasgow in ice and stored at -20°C until analysis. Analyses performed were pH, specific gravity, creatinine, urea, sodium, potassium and chloride by the methods described.

7. Sweat samples

Attempts were made to collect sweat samples from a number of horses involved in rides A and F by the method described in detail in Section 5. Exceptions were that the horses' backs were not washed and the plastic-lined saddle cloths were not used. In many horses sweating was insufficient to allow any sweat to be squeezed from the pads, and in several cases the pads were lost from beneath the saddle. As a result only five random samples were obtained. These were processed as described in Section 5 and analysed for sodium, potassium, chloride and magnesium.

8. Statistical analysis

Comparisons were made between start and finish values and between finish and F+30 values using the paired *t* test. Certain horses with markedly elevated resting CK and AST results were omitted from all means

and *t* tests (Tables 24 and 25). Where there was a missing value for any reason that animal was omitted from that particular *t* test which means that in a few instances the *t* test does not exactly relate to the mean results listed.

In order to determine whether or not the changes in plasma parameters were consistent among a group of rides of approximately the same distance (65 - 95 km, rides B,C,E,F and G), paired *t* tests were also carried out on the mean results from rides in this group, again comparing start and finish values, and finish and F+30 values.

Results

A. Blood samples

Mean results are not presented for rides D, I and K due to the very small number of horses sampled. The remaining rides have been divided into three groups as follows: group 1, 160 km ride (A); group 2, rides of 65 - 95 km (B,C,E,F and G); group 3, 40 km rides (H and J).

PCV and plasma proteins

Changes in PCV and total plasma protein concentration associated with the rides are presented in Figure 11 and in Tables 9 and 10. It can be seen that these changes were most marked in the group 2 rides, and that while PCV generally increased throughout the rides in this group, increases in total plasma protein concentration were mainly confined to the first 40 km. During the 160 km ride changes in PCV were slight and confined to the early parts of the ride while total plasma protein did not change at all. Plasma albumin, however, showed its highest level at the 120 km point. More detailed study of one horse in an 80 km ride (Figure 12) suggested that PCV may continue to decrease for at least an hour after the finish.

The percentage increases in PCV, total plasma protein and plasma albumin concentration in individual horses competing in one 80 km ride are presented in Table 11. Increases in PCV were always greater than in either total protein or albumin, and while increases in the latter two parameters were generally similar in magnitude it appeared that overall there was a tendency for albumin to increase by a greater percentage than the other plasma protein fractions.

TABLE 9

Packed cell volume before, immediately after and 30 minutes after long distance rides

Ride	Start	Finish	Finish + 30 min
A	0.43 ± 0.01	0.44 ± 0.02	0.40 ± 0.01**
B	0.38 ± 0.01	0.44 ± 0.01**	0.42 ± 0.02**
C	0.37 ± 0.01	0.53 ± 0.03**	0.44 ± 0.03*
E	0.38 ± 0.01	0.49 ± 0.03*	0.43 ± 0.02*
F	0.34 ± 0.01	0.46 ± 0.02***	0.42 ± 0.01*
G	0.38 ± 0.01	0.47 ± 0.01**	0.46 ± 0.02
H	0.38 ± 0.01	0.45 ± 0.01***	0.44 ± 0.01
J	0.38 ± 0.01	0.42 ± 0.01**	0.39 ± 0.01*

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Values at the finish were compared to the start values and values 30 minutes after the finish were compared to the finish values.

TABLE 10

Total plasma protein concentration (g/l) before, immediately after and 30 minutes after long distance rides. Statistics as Table 9.

Ride	Start	Finish	Finish + 30 min
A	74 ± 2	73 ± 1	72 ± 2
B	73 ± 1	74 ± 2	76 ± 2
C	70 ± 2	82 ± 1*	78 ± 1**
E	75 ± 2	79 ± 2*	78 ± 2
F	71 ± 1	79 ± 3**	77 ± 2
G	66 ± 1	70 ± 2	71 ± 2
H	77 ± 3	81 ± 2*	82 ± 3
J	73 ± 2	76 ± 2	78 ± 2

TABLE 11

Percentage increase in PCV and total plasma protein and albumin concentration in horses in ride F

Horse	PCV (% increase)	Total protein (% increase)	Albumin (% increase)
3	26.8	15.7	23.5
9	29.0	1.4	0
11	32.2	5.9	5.9
14	28.1	5.6	10.3
15	22.2	4.3	9.4
24	66.7	27.4	30.0
36	22.2	2.7	13.3
43	41.2	10.4	17.1
47	40.5	26.8	23.5
Mean ± SEM	34.3 ± 4.6	11.1 ± 3.3	14.8 ± 3.2

Key to Figures 11, 13, 14, 16 and 17

Group 1 (160 km ride)

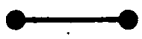
Pre: sample collected the afternoon before the ride

40, 80, 120, 160: distance covered (km)

F+30: sample collected 30 minutes after the finish

F+12hr: sample collected 12 hours after the finish

* $p<0.05$; ** $p<0.01$; *** $p<0.001$: indicates result significantly different from pre sample except for F+30, when it indicates result significantly different from 160 km sample.

 ride A, mean ± SEM

Groups 2 and 3 (65-95 km and 40 km rides)

S: sample collected immediately before the start

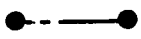
½way: sample collected at the half-way halt (group 2 rides only)

F: sample collected immediately after the finish

F+30: sample collected 30 minutes after the finish

* $p<0.05$; ** $p<0.01$; *** $p<0.001$: indicates that the group 2 rides as a whole show a significant difference, finish results tested against start, F+30 results tested against finish

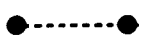
Group 2

 ride B

 ride C

 ride E

 ride F

 ride G

Mean results only

Group 3

 ride H

 ride J

mean ± SEM

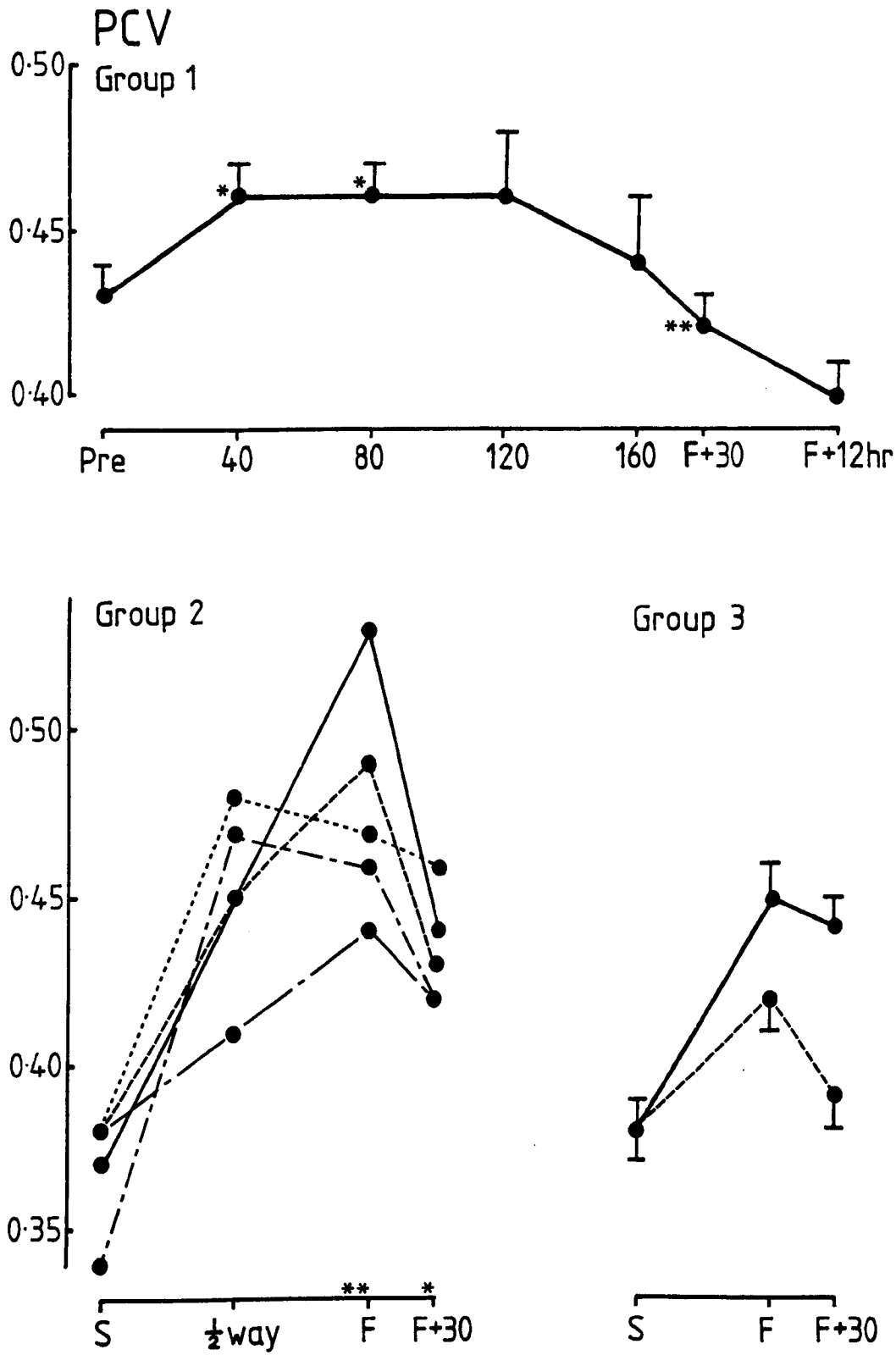


Figure 11(a). Changes in packed cell volume during and after the competitive long distance rides. Key on previous page.

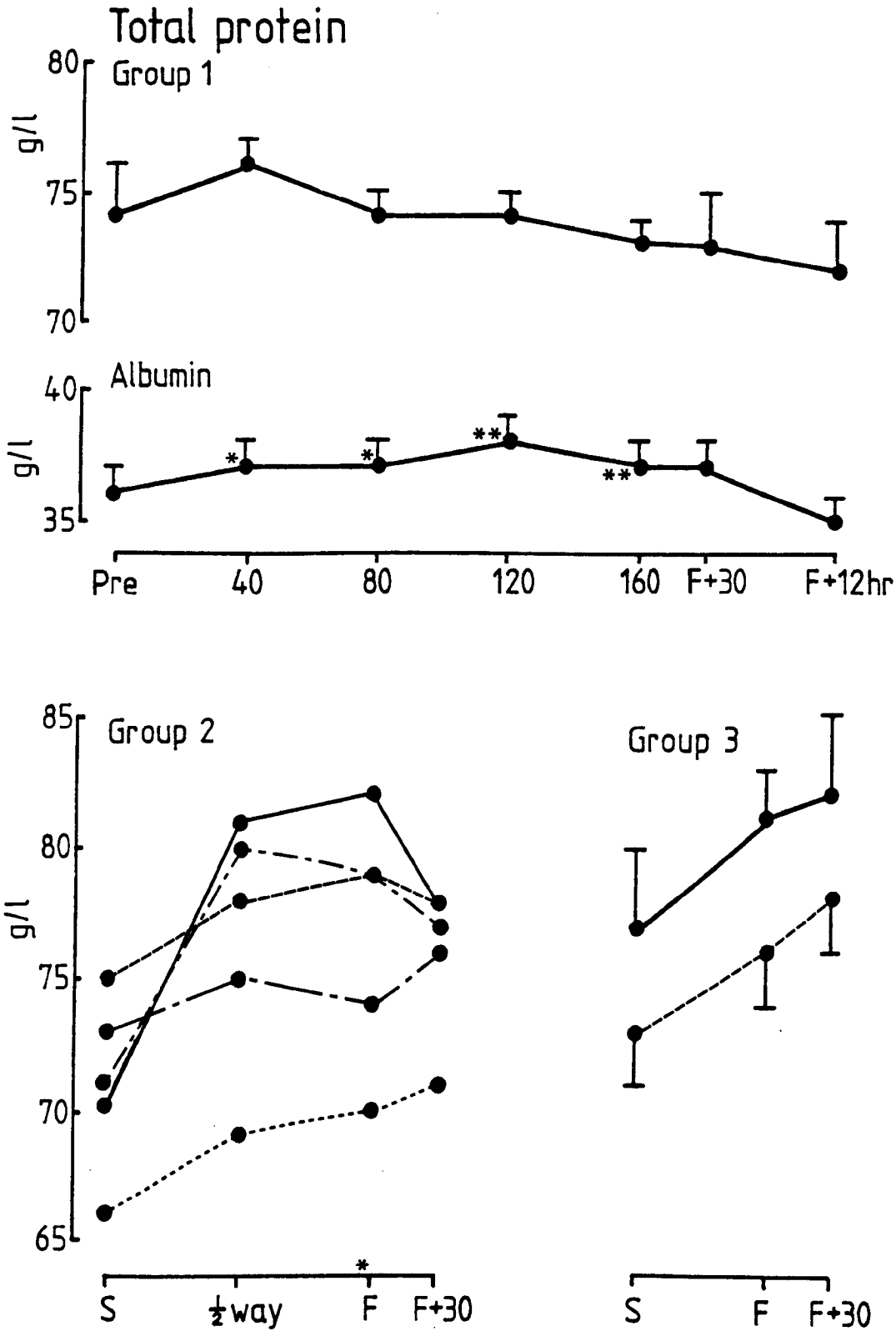


Figure 11(b). Changes in total plasma protein concentration (and albumin concentration in the 160 km ride) during and after the competitive long distance rides. Key preceding Figure 11(a).

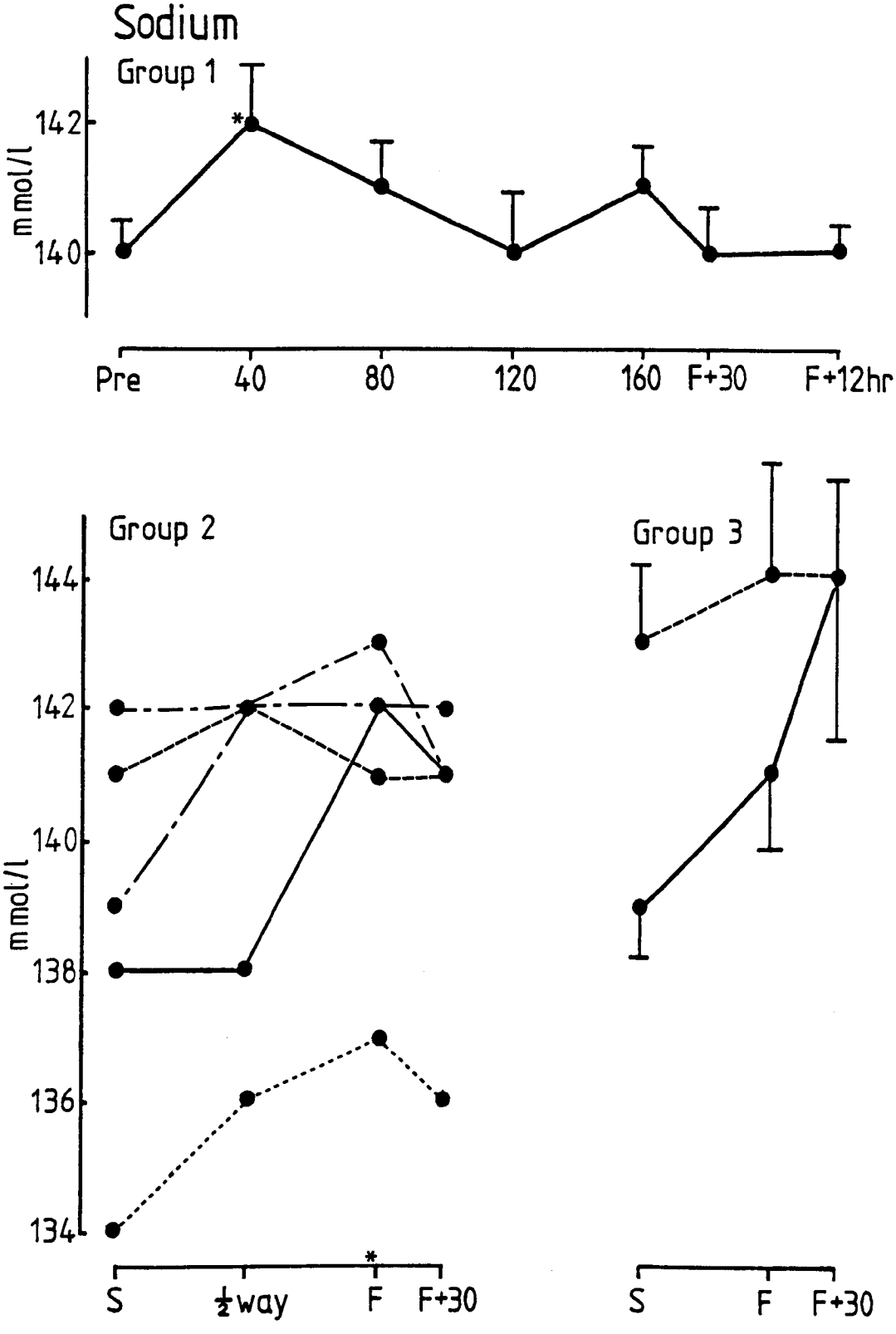


Figure 11(c). Changes in plasma sodium concentration during and after the competitive long distance rides. Key preceding Figure 11(a).

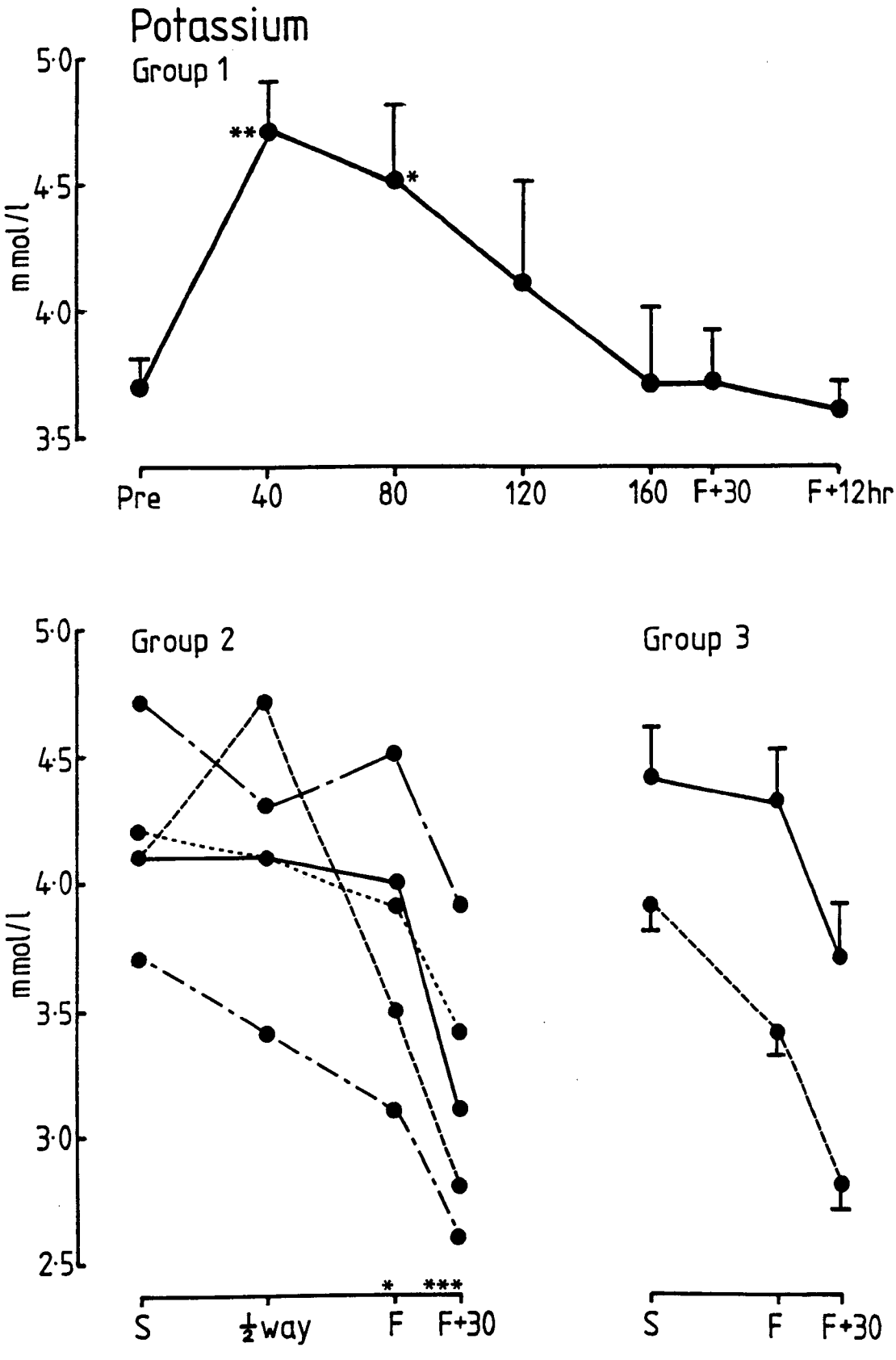


Figure 11(d). Changes in plasma potassium concentration during and after the competitive long distance rides. Key preceding Figure 11(a).

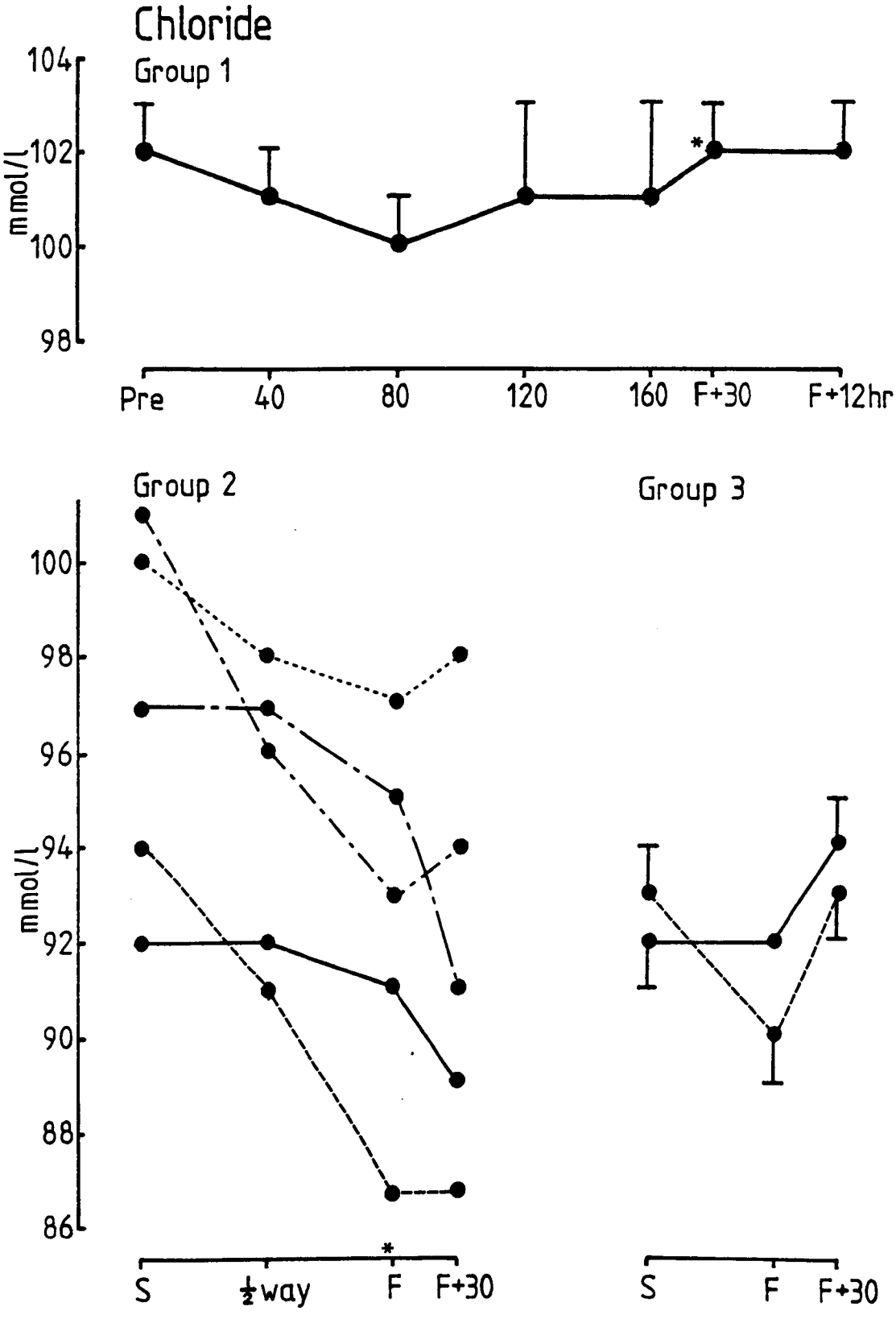


Figure 11(e). Changes in plasma chloride concentration during and after the competitive long distance rides. Key preceding Figure 11(a).

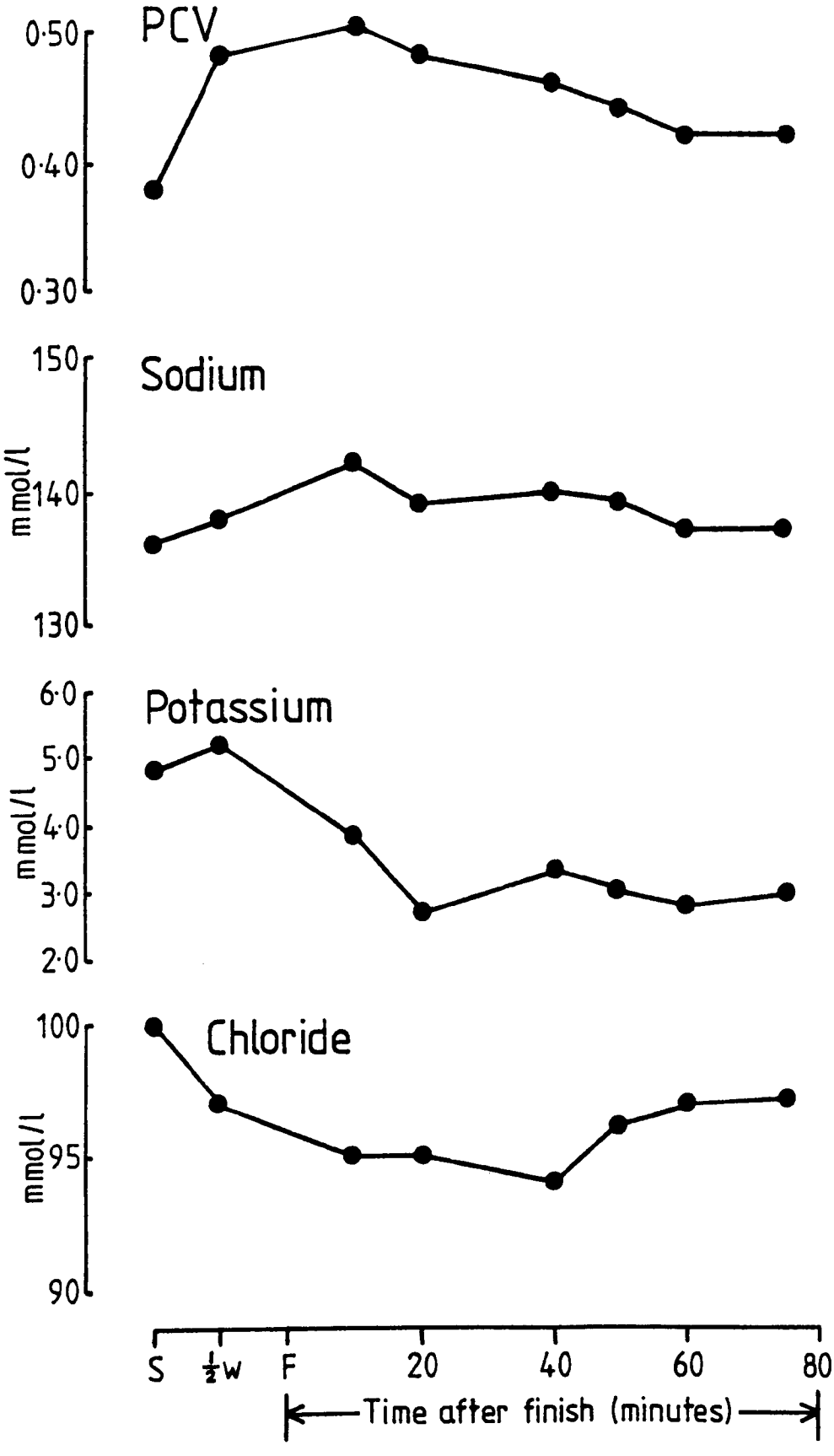


Figure 12. Changes in PCV and plasma electrolyte concentrations in horse 43 during and after ride D.

Sodium, potassium and chloride

Changes in plasma electrolyte concentrations associated with the rides are also presented in Figure 11 and in Tables 12, 13 and 14. Alterations in plasma sodium concentration were very small in magnitude but overall in the group 2 rides there was a general tendency towards a slight increase ($p < 0.05$).

Plasma potassium concentration also changed very little during the rides but in this case the general tendency in the group 2 rides was towards a decrease ($p < 0.05$). In the 160 km ride, as with sodium, significant changes were confined to the early stages of the ride. In the first 30 minutes after the finish of all but the 160 km ride a marked decrease in plasma potassium concentration was evident, and although this was only statistically significant in three of the seven rides the general tendency among all the five rides in group 2 was highly significant ($p < 0.001$).

Plasma chloride concentration decreased quite markedly in three of the group 2 rides and overall the general tendency towards a decrease in this group was significant ($p < 0.05$). However, no change was evident in either the 160 km or the 40 km rides.

More detailed study of one horse in an 80 km ride (Figure 12) indicated that while plasma electrolyte concentrations appeared to fluctuate somewhat following the ride, all three were beginning to return towards pre-ride levels by at least an hour after the finish.

Calcium, magnesium and phosphate

Changes in plasma mineral concentrations associated with the rides are presented in Figure 13 and in Tables 15, 16 and 17. Plasma calcium concentration decreased significantly during several rides and a significant increase was seen after the finish of several others. However, the overall tendency towards a decrease in the group 2 rides was not statistically significant.

TABLE 12

Plasma sodium concentration (mmol/l) before, immediately after and 30 minutes after long distance rides. Statistics as Table 9.

Ride	Start	Finish	Finish + 30 min
A	140 ± 1	141 ± 1	140 ± 1
B	139 ± 1	142 ± 1	142 ± 2
C	138 ± 3	142 ± 1	141 ± 2
E	141 ± 3	141 ± 2	141 ± 3
F	142 ± 1	143 ± 1	141 ± 1*
G	134 ± 1	137 ± 1*	136 ± 1
H	139 ± 1	141 ± 1	144 ± 1**
J	143 ± 1	144 ± 2	144 ± 2

TABLE 13

Plasma potassium concentration (mmol/l) before, immediately after and 30 minutes after long distance rides. Statistics as Table 9.

Ride	Start	Finish	Finish + 30 min
A	3.7 ± 0.1	3.7 ± 0.3	3.7 ± 0.2
B	4.7 ± 0.1	4.5 ± 0.2	3.9 ± 0.2
C	4.1 ± 0.3	4.0 ± 0.1	3.1 ± 0.3
E	4.1 ± 0.1	3.5 ± 0.3	2.8 ± 0.2
F	3.7 ± 0.1	3.1 ± 0.1*	2.6 ± 0.1*
G	4.2 ± 0.1	3.9 ± 0.1**	3.4 ± 0.1**
H	4.4 ± 0.2	4.3 ± 0.2	3.7 ± 0.2
J	3.9 ± 0.1	3.4 ± 0.1**	2.8 ± 0.1***

TABLE 14

Plasma chloride concentration (mmol/l) before, immediately after and 30 minutes after long distance rides. Statistics as Table 9.

Ride	Start	Finish	Finish + 30 min
A	102 ± 1	101 ± 2	102 ± 1*
B	97 ± 3	95 ± 3	91 ± 4
C	92 + 2	91 ± 1	89 ± 2
E	94 ± 2	86 ± 1**	86 ± 1
F	101 ± 1	93 ± 1**	94 ± 1
G	100 ± 1	97 ± 1**	98 ± 1
H	92 ± 1	92 + 1	94 ± 1
J	93 ± 1	90 ± 1	93 ± 1*

TABLE 15

Plasma calcium concentration (mmol/l) before, immediately after and 30 minutes after long distance rides. Statistics as Table 9.

Ride	Start	Finish	Finish + 30 min
A	3.12 ± 0.03	2.81 ± 0.05***	3.02 ± 0.06
B	3.02 ± 0.04	2.96 ± 0.05	3.14 ± 0.05*
C	3.32 ± 0.13	3.39 ± 0.05	3.49 ± 0.08
E	3.09 ± 0.04	2.69 ± 0.08**	2.87 ± 0.08*
F	2.87 ± 0.05	2.66 ± 0.10*	2.75 ± 0.11
G	2.80 ± 0.06	2.79 ± 0.07	2.89 ± 0.06
H	3.43 ± 0.04	3.41 ± 0.10	3.63 ± 0.07***
J	2.98 ± 0.05	2.86 ± 0.06*	2.92 ± 0.06

TABLE 16

Plasma magnesium concentration (mmol/l) before, immediately after and 30 minutes after long distance rides. Statistics as Table 9.

Ride	Start	Finish	Finish + 30 min
A	0.73 ± 0.01	0.59 ± 0.02**	0.67 ± 0.02
B	0.69 ± 0.02	0.64 ± 0.05	0.68 ± 0.08
C	0.67 ± 0.03	0.64 ± 0.01	0.61 ± 0.04
E	0.71 ± 0.02	0.64 ± 0.02*	0.66 ± 0.05
F	0.73 ± 0.01	0.69 ± 0.04	0.68 ± 0.04
G	0.70 ± 0.02	0.50 ± 0.03***	0.53 ± 0.02
H	0.76 ± 0.02	0.75 ± 0.03	0.78 ± 0.03
J	0.72 ± 0.02	0.64 ± 0.02**	0.68 ± 0.02*

TABLE 17

Plasma inorganic phosphate concentration (mmol/l) before, immediately after and 30 minutes after long distance rides. Statistics as Table 9.

Ride	Start	Finish	Finish + 30 min
A	0.89 ± 0.05	1.09 ± 0.07	0.94 ± 0.07*
B	1.04 ± 0.07	1.49 ± 0.16***	1.60 ± 0.13
C	1.32 ± 0.21	2.26 ± 0.24*	1.93 ± 0.36
E	0.99 ± 0.10	1.39 ± 0.11*	1.19 ± 0.09
F	1.03 ± 0.11	1.55 ± 0.09***	1.34 ± 0.19
G	1.03 ± 0.05	1.29 ± 0.10	1.19 ± 0.10
H	2.00 ± 0.10	2.28 ± 0.12*	2.26 ± 0.12
J	1.03 ± 0.06	1.23 ± 0.06**	1.08 ± 0.07***

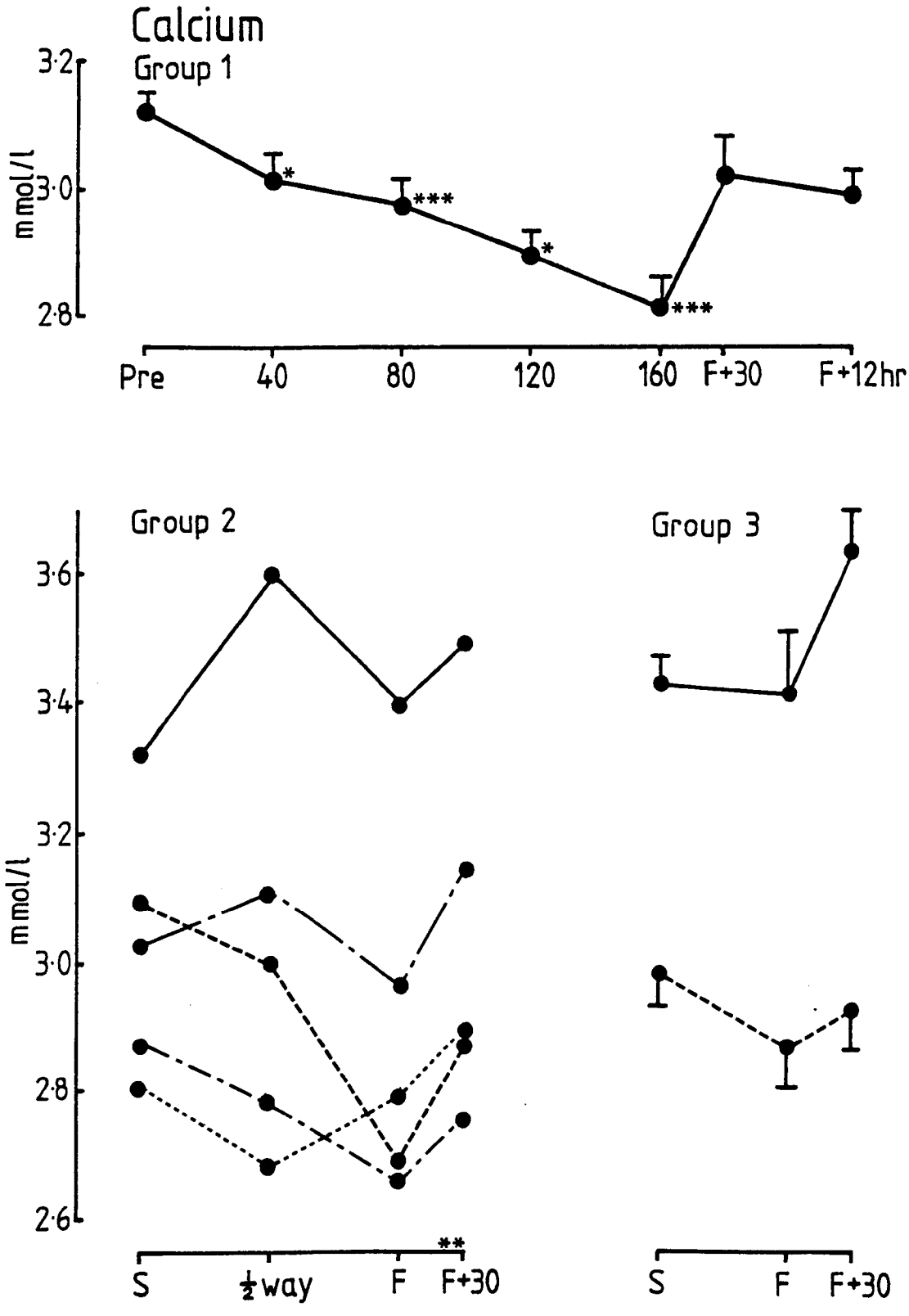


Figure 13(a). Changes in plasma calcium concentration during and after the competitive long distance rides. Key preceding Figure 11(a).

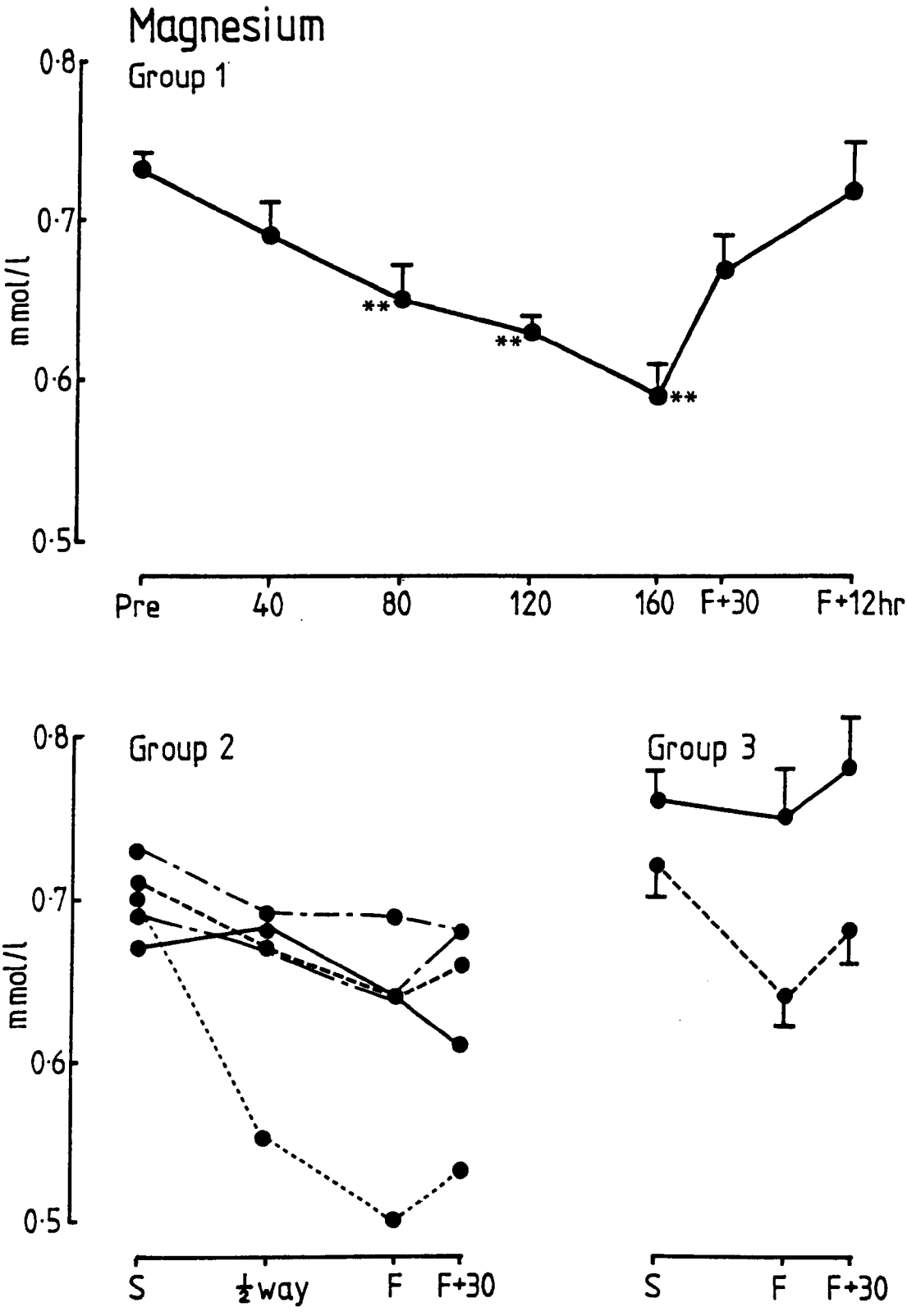


Figure 13(b). Changes in plasma magnesium concentration during and after the competitive long distance rides. Key preceding Figure 11(a).

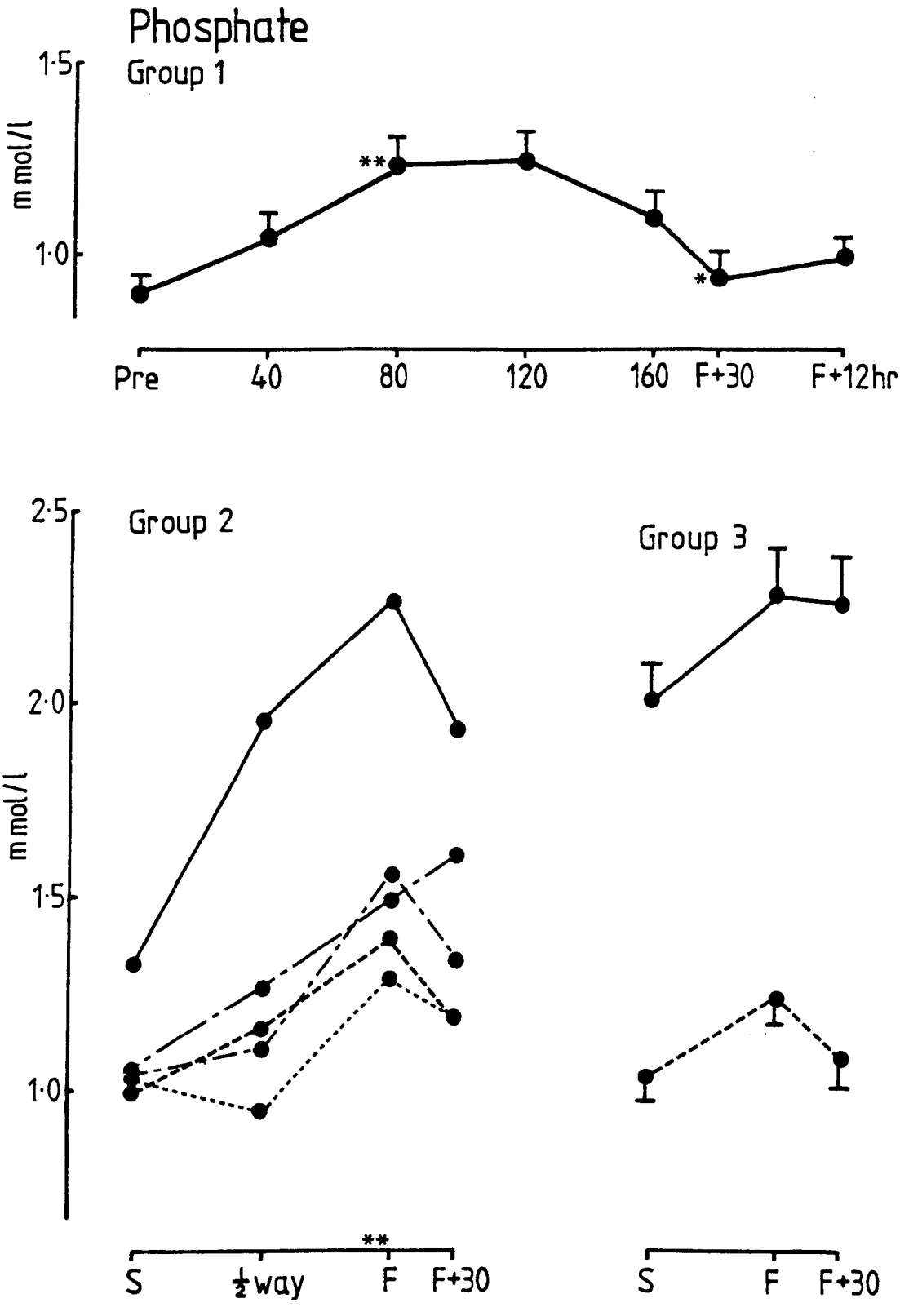


Figure 13(c). Changes in plasma inorganic phosphate concentration during and after the competitive long distance rides. Key preceding Figure 11(a).

Plasma magnesium concentration also decreased significantly during several rides, but again the overall changes in the group 2 rides were not statistically significant.

A significant increase in plasma inorganic phosphate concentration occurred during seven of the eight rides, although in the case of the 160 km ride the change was again most pronounced at the 80 km stage.

Glucose, glycerol, triglycerides, bilirubin, creatinine and urea

Changes in plasma concentrations of these parameters associated with the rides are presented in Figure 14 and in Tables 18, 19, 20, 21 and 22. During the 160 km ride plasma glucose concentration did not change. In contrast, while there was little regular change during the first half of the group 2 rides, there was a very consistent decrease during the second half of these rides ($p < 0.001$) which had begun to return towards normal by 30 min after the finish ($p < 0.01$). More detailed study of one horse in an 80 km ride (Figure 15) suggested that plasma glucose concentration may return to its pre-ride value by about one hour after the finish.

A significant increase in plasma glycerol concentration was seen in all three rides in which it was measured, and concentrations had always fallen significantly by half-an-hour after the finish. The highest plasma glycerol concentrations occurred not in the 160 km ride but in ride F, an 80 km ride. In contrast plasma triglyceride concentration appeared to decrease significantly on both occasions on which it was measured.

Significant increases in plasma bilirubin, creatinine and urea concentrations were a universal finding in all rides studied, and urea concentration in particular tended to continue rising in the first half-hour after the finish. More detailed study of one horse in an 80 km ride (Figure 15) suggested that concentrations of all three

TABLE 18

Plasma glucose concentration (mmol/l) before, immediately after and 30 minutes after long distance rides. Statistics as Table 9.

Ride	Start	Finish	Finish + 30 min
A	5.2 ± 0.9	4.2 ± 0.4	4.8 ± 0.2*
B	6.1 ± 0.5	3.5 ± 0.3**	4.7 ± 0.5*
C	5.5 ± 0.7	3.5 ± 0.3	5.1 ± 0.4*
E	5.5 ± 0.3	3.5 ± 0.4**	3.8 ± 0.5
F	5.2 ± 0.1	3.2 ± 0.5**	4.3 ± 0.4*
G	5.8 ± 0.2	3.5 ± 0.2***	4.2 ± 0.2**
H	5.5 ± 0.3	5.3 ± 0.1	5.1 ± 0.2
J	6.5 ± 0.5	5.3 ± 0.4*	4.8 ± 0.3*

TABLE 19

Plasma glycerol and triglyceride concentrations (μmol/l) before, immediately after and 30 minutes after certain long distance rides. Statistics as Table 9.

	Ride	Start	Finish	Finish + 30 min
Glycerol	A	19 ± 2	302 ± 58**	141 ± 30*
	B	7 ± 0.1	171 ± 48**	74 ± 24*
	F	47 ± 9	537 ± 92**	204 ± 26**
Triglycerides	A	120 ± 24	40 ± 14*	87 ± 17***
	B	178 ± 24	102 ± 10*	85 ± 10

TABLE 20

Plasma bilirubin concentration (μmol/l) before, immediately after and 30 minutes after long distance rides. Statistics as Table 9.

Ride	Start	Finish	Finish + 30 min
A	19 ± 2	71 ± 5***	72 ± 7*
B	18 ± 2	39 ± 3***	41 ± 4
C	24 ± 4	56 ± 9**	54 ± 7
E	20 ± 2	40 ± 4***	46 ± 5
F	19 ± 2	48 ± 8**	52 ± 2*
G	27 ± 1	52 ± 5***	54 ± 6
H	18 ± 3	26 ± 4***	26 ± 4
J	18 ± 2	27 ± 4*	29 ± 5

TABLE 21

Plasma creatinine concentration (μmol/l) before, immediately after and 30 minutes after long distance rides. Statistics as Table 9.

Ride	Start	Finish	Finish + 30 min
A	120 ± 7	160 ± 11**	152 ± 14***
B	134 ± 6	149 ± 9*	150 ± 10
C	163 ± 12	221 ± 13*	225 ± 15
E	120 ± 5	175 ± 16**	182 ± 20*
F	145 ± 11	219 ± 17**	202 ± 15
G	87 ± 6	136 ± 9***	136 ± 8
H	149 ± 8	171 ± 8***	180 ± 9
J	129 ± 6	169 ± 9***	164 ± 7

TABLE 22

Plasma urea concentration (mmol/l) before, immediately after and 30 minutes after long distance rides. Statistics as Table 9.

Ride	Start	Finish	Finish + 30 min
A	6.6 ± 0.4	11.1 ± 1.0***	11.2 ± 1.0**
B	6.5 ± 0.3	9.3 ± 0.6***	10.0 ± 0.6*
C	6.5 ± 1.0	9.1 ± 1.4*	9.4 ± 1.6
E	7.3 ± 0.1	10.9 ± 0.9***	10.9 ± 0.9**
F	6.8 ± 0.5	11.0 ± 0.8***	11.3 ± 0.8***
G	7.0 ± 0.4	8.6 ± 0.6***	9.0 ± 0.5**
H	6.6 ± 0.3	8.1 ± 0.3***	8.4 ± 0.4*
J	7.3 ± 0.4	8.4 ± 0.3***	8.7 ± 0.4

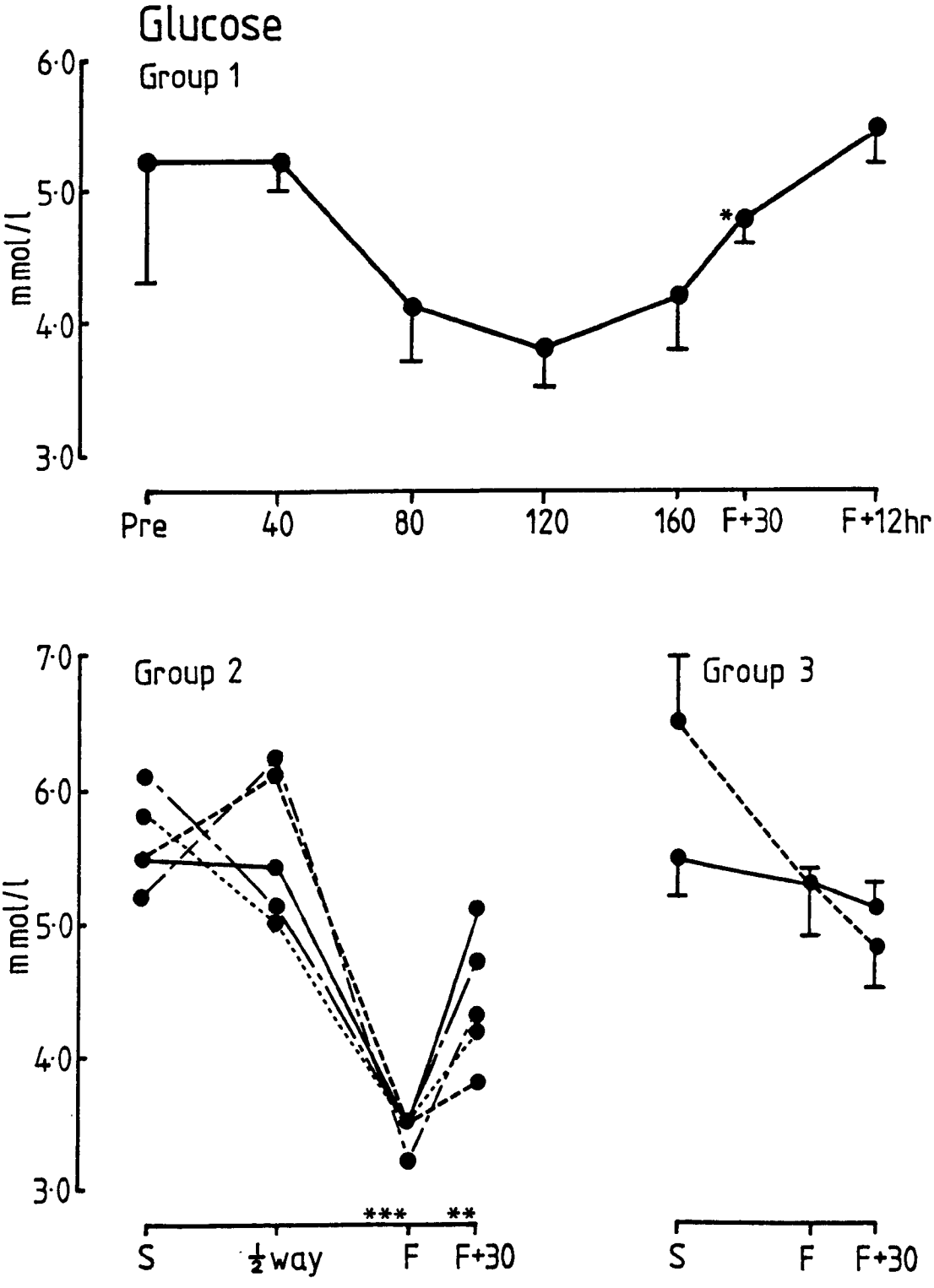


Figure 14(a). Changes in plasma glucose concentration during and after the competitive long distance rides. Key preceding Figure 11(a).

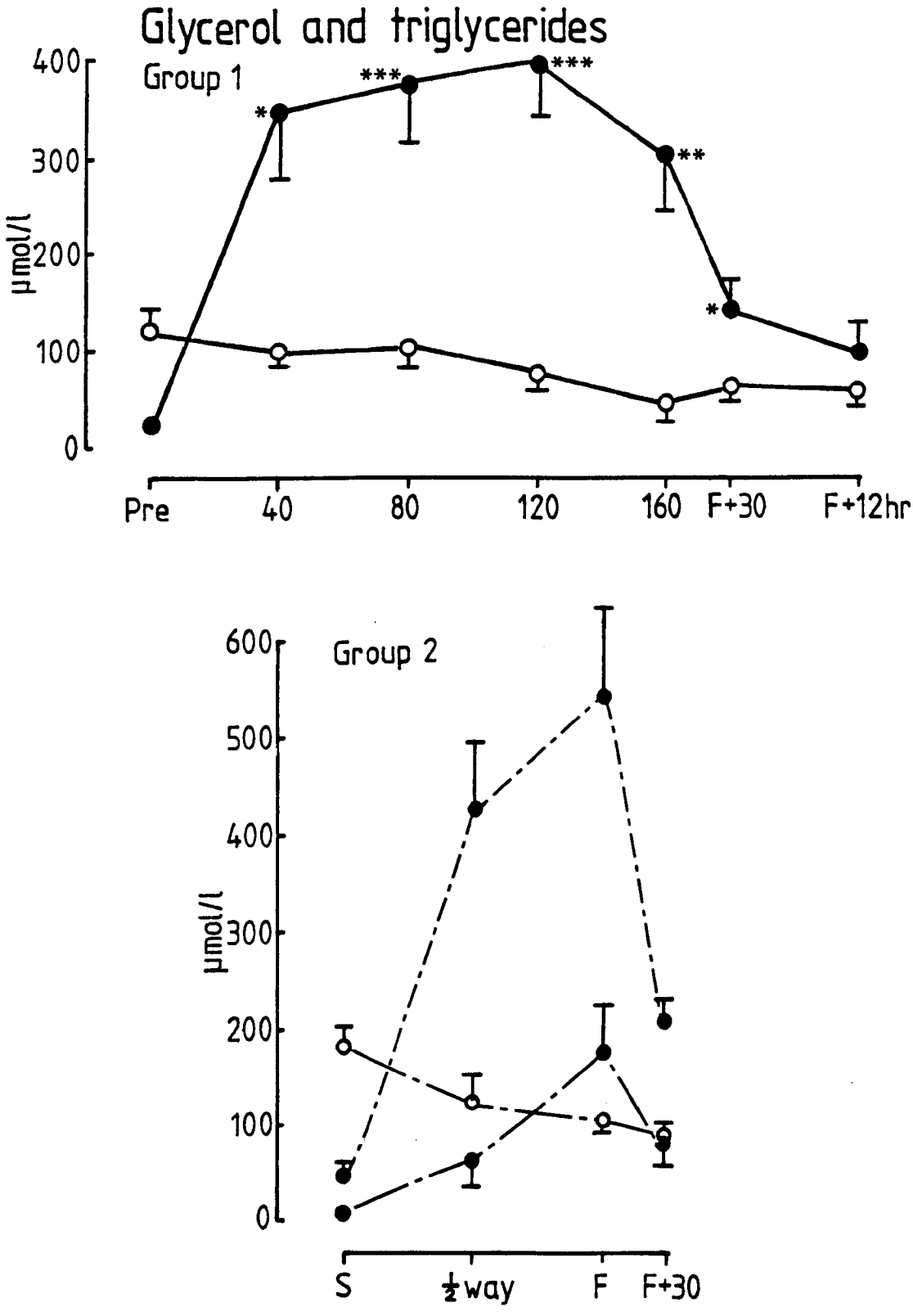


Figure 14(b). Changes in plasma glycerol concentration (and triglyceride concentration in rides A and B) during and after three of the competitive long distance rides (A, B and F). Key preceding Figure 11(a),

● glycerol, ○ triglycerides.

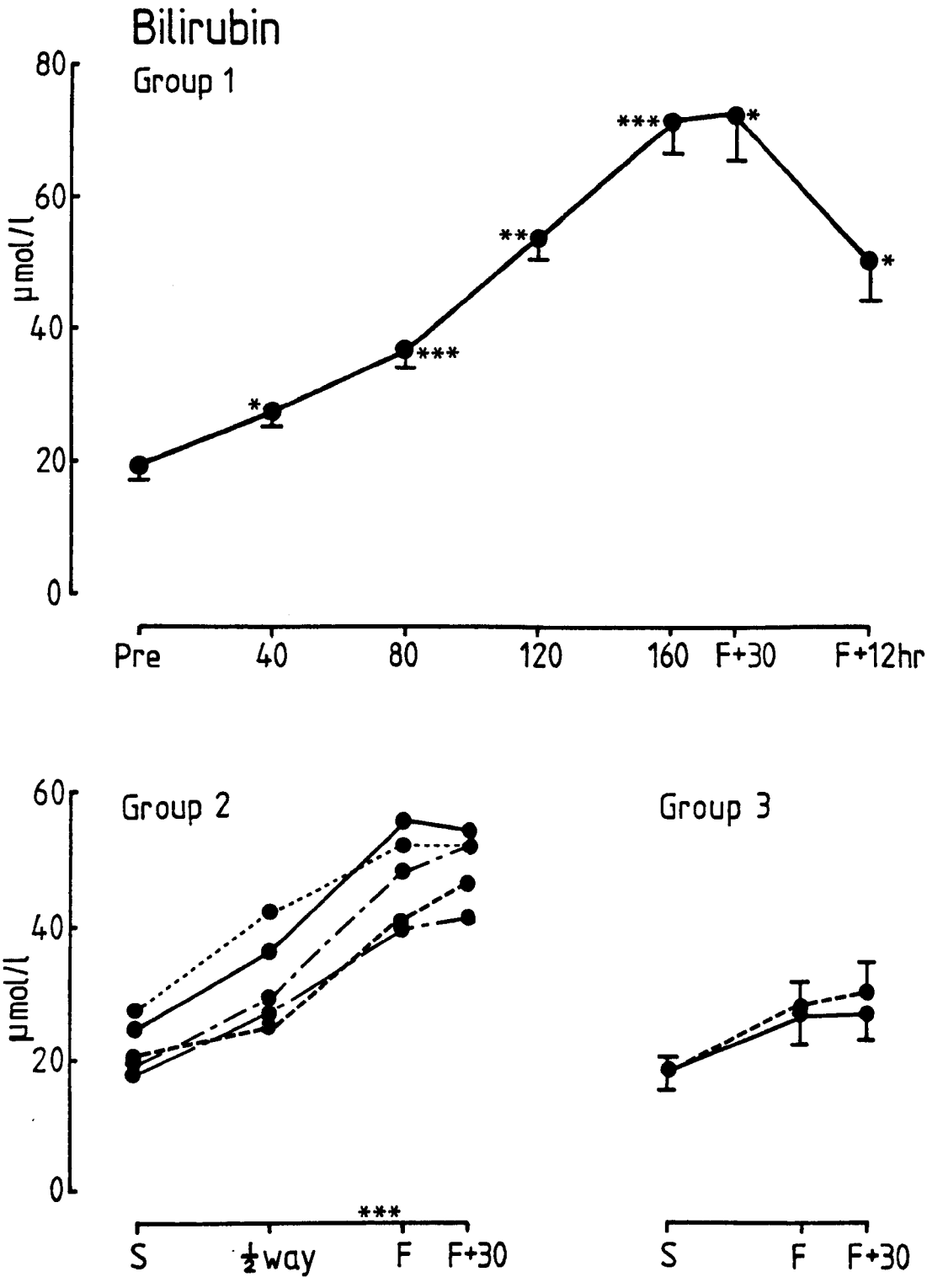


Figure 14(c). Changes in plasma bilirubin concentration during and after the competitive long distance rides. Key preceding Figure 11(a).

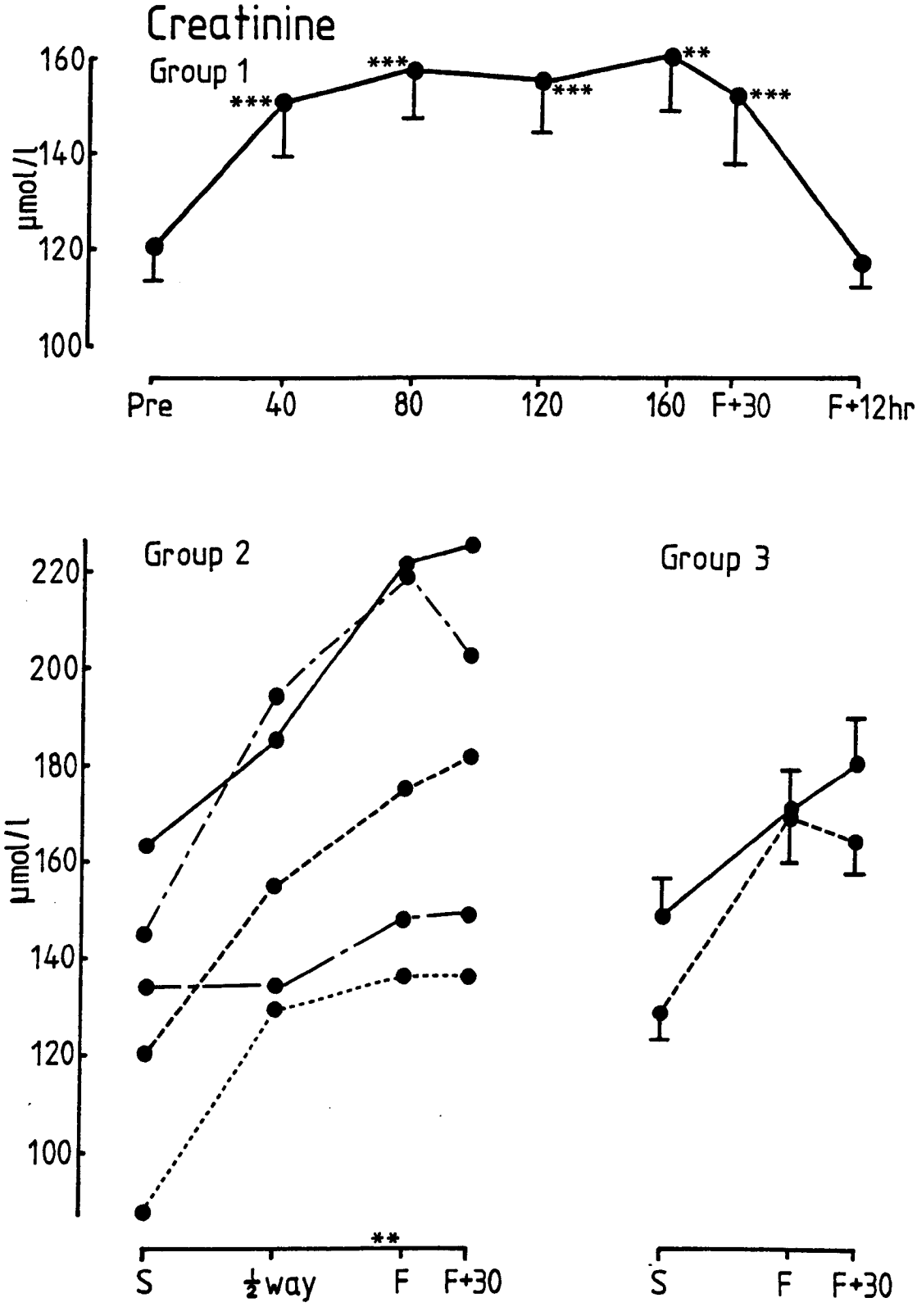


Figure 14(d). Changes in plasma creatinine concentration during and after the competitive long distance rides. Key preceding Figure 11(a).

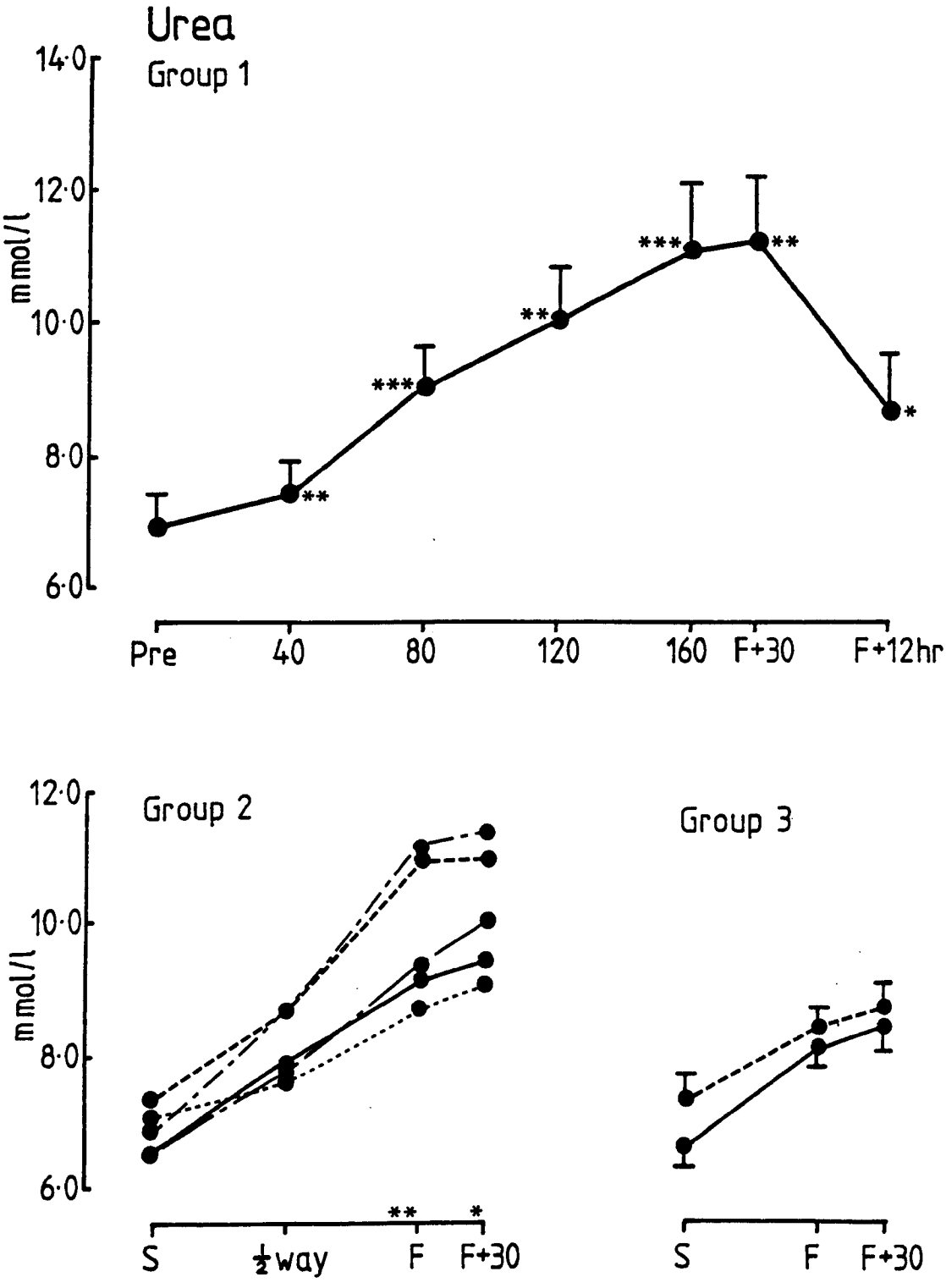


Figure 14(e). Changes in plasma urea concentration during and after the competitive long distance rides. Key preceding Figure 11(a).

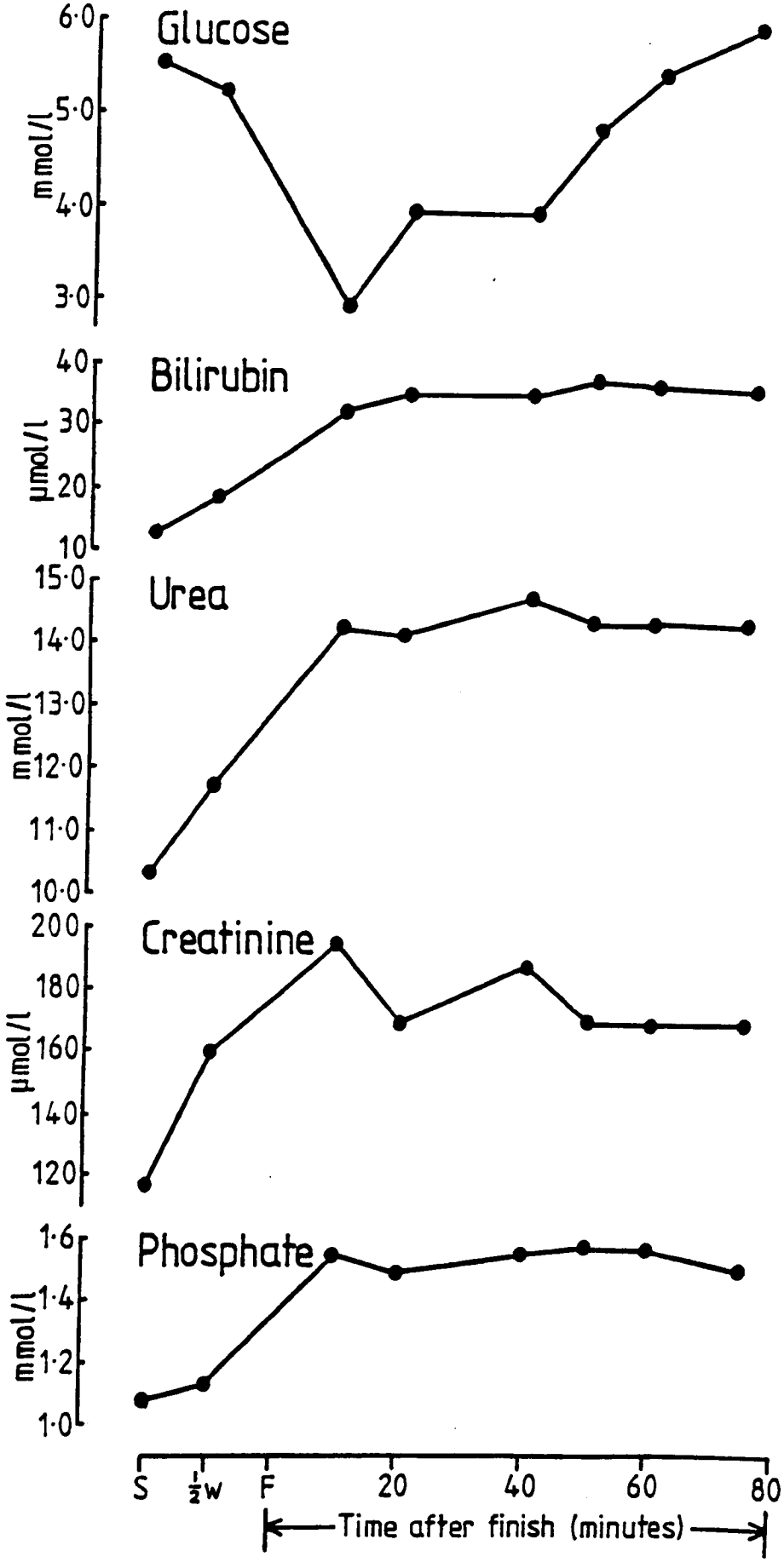


Figure 15. Changes in five plasma parameters in horse 43 during and after ride D.

parameters (and inorganic phosphate) were slow to return towards pre-ride levels and were still very much above these levels more than an hour after the finish.

Plasma hormones

Changes in plasma cortisol concentration associated with the rides are presented in Figure 16 and in Table 23, and changes in plasma insulin concentration in the only ride in which it was measured (ride B) are also included in Figure 16. A significant increase in plasma cortisol concentration was an almost universal finding during the rides. However, while concentrations increased fairly steadily throughout the group 2 rides the highest concentrations in the 160 km ride were seen at the 40 km stage with a steady decrease thereafter. Even at this highest point plasma cortisol concentrations in the 160 km ride were lower than at the finish of all of the group 2 rides (and one 40 km ride).

By the half-way point of ride B plasma insulin concentrations had fallen to below the level of sensitivity of the assay (< 5 mU/l) in several horses, and by the finish concentrations had fallen below this level in all but one horse.

Plasma enzymes

Changes in plasma activities of CK, AST and ALP associated with the rides are presented in Figure 17 and in Tables 24, 25 and 26. Several horses with particularly high pre-ride CK and AST activities were excluded from the mean figures and the statistics, but in spite of this the changes in plasma activities of these two enzymes were so variable between individual horses that the overall changes in several of the rides were not statistically significant. However, there was a clear tendency towards increases in both of these enzymes during the rides - fairly large in magnitude in the case of CK, rather more modest in the

TABLE 23

Plasma cortisol concentration (nmol/l) before, immediately after and 30 minutes after long distance rides. Statistics as Table 9.

Ride	Start	Finish	Finish + 30 min
A	118 + 19	330 ± 43**	289 ± 32
B	305 ± 40	552 ± 61**	485 ± 66**
C	303 ± 54	572 ± 54*	477 ± 34
E	358 ± 39	752 ± 70**	779 ± 67
F	251 ± 27	692 ± 67***	592 ± 51*
G	222 ± 32	506 ± 48**	379 ± 43**
H	329 ± 37	396 ± 85	283 ± 75**
J	298 ± 24	543 ± 35***	460 ± 23*

TABLE 24

Plasma creatine kinase activity (iu/l) before, immediately after and 30 minutes after long distance rides. Statistics as Table 9.

Ride	Start	Finish	Finish + 30 min
A[2]	181 ± 28	1548 ± 492	1580 ± 545
B	278 ± 60	580 ± 77**	568 ± 102
C[1]	231 ± 69	572 ± 54	477 ± 34
E	279 ± 51	1074 ± 243*	1180 ± 335
F[1]	206 ± 27	828 ± 90***	912 ± 98*
G[1]	170 ± 25	571 ± 111	485 ± 66
H	242 ± 23	380 ± 71**	326 ± 26
J[1]	266 ± 40	354 ± 39***	400 ± 43*

[1] One horse excluded [2] Two horses excluded

TABLE 25

Plasma aspartate aminotransferase activity (iu/l) before, immediately after and 30 minutes after long distance rides. Statistics as Table 9.

Ride	Start	Finish	Finish + 30 min
A[3]	376 ± 37	532 ± 62*	560 ± 62**
B[1]	446 ± 32	545 ± 52*	558 ± 76
C[1]	423 ± 77	551 ± 100*	556 ± 111
E	376 ± 27	491 ± 34***	510 ± 41
F[1]	296 ± 14	400 ± 17***	391 ± 25
G[1]	281 ± 29	382 ± 51	416 ± 117
H[1]	403 ± 42	446 ± 53*	448 ± 59
J[1]	366 ± 37	388 ± 41	395 ± 42

[1] One horse excluded [3] Three horses excluded

TABLE 26

Plasma alkaline phosphatase activity (iu/l) before, immediately after and 30 minutes after long distance rides. Statistics as Table 9.

Ride	Start	Finish	Finish + 30 min
A	391 ± 65	495 ± 41**	536 ± 36*
B	301 ± 43	305 ± 45	301 ± 38
C	179 ± 17	243 ± 20	222 ± 18**
E	186 ± 26	220 ± 35	215 ± 42
F	509 ± 54	600 ± 59***	581 ± 62*
G	265 ± 40	292 ± 52**	303 ± 46
H	327 ± 43	372 ± 45**	384 ± 48
J	379 ± 42	436 ± 26**	445 ± 37

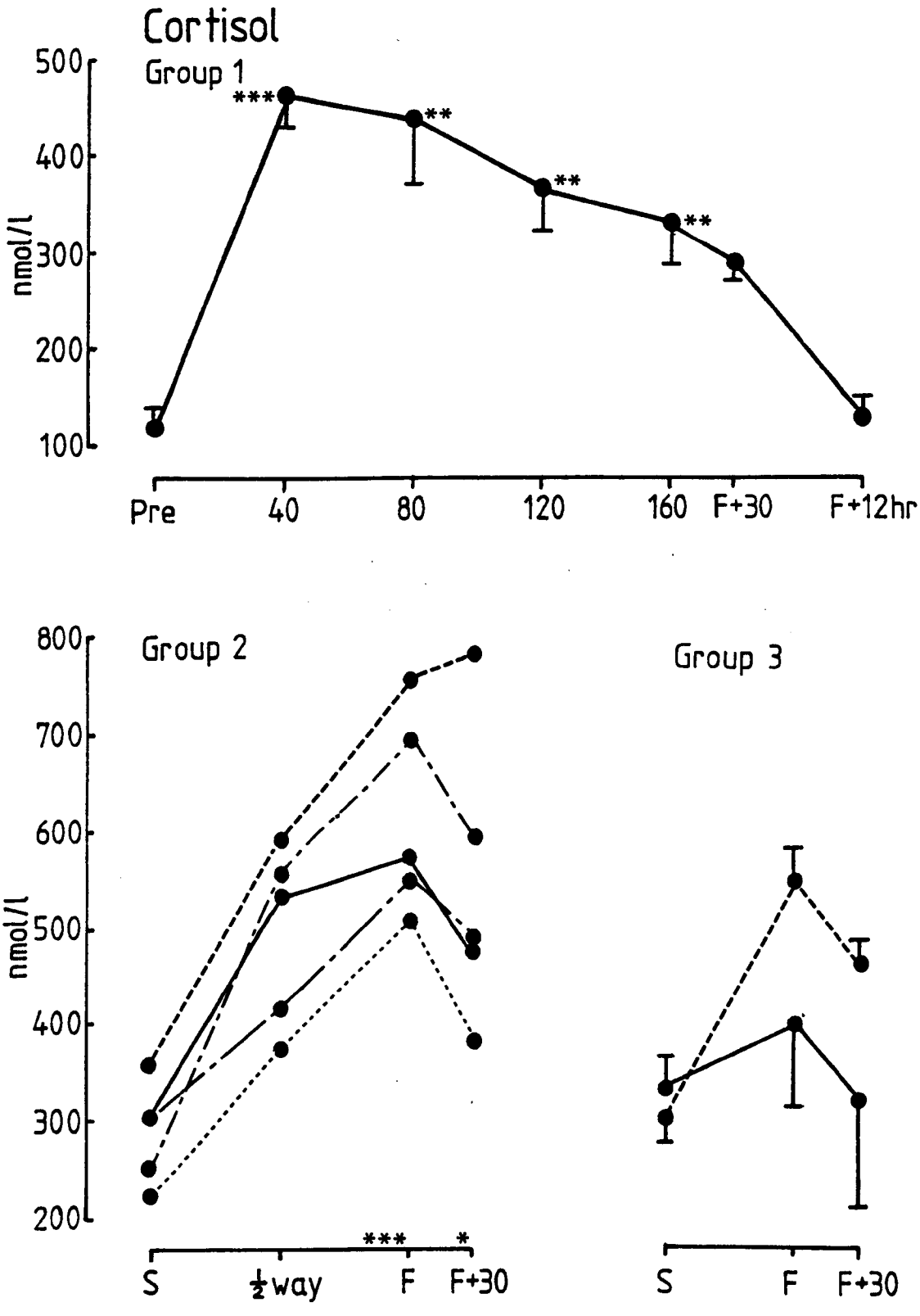


Figure 16(a). Changes in plasma cortisol concentration during and after the competitive long distance rides. Key preceding Figure 11(a).

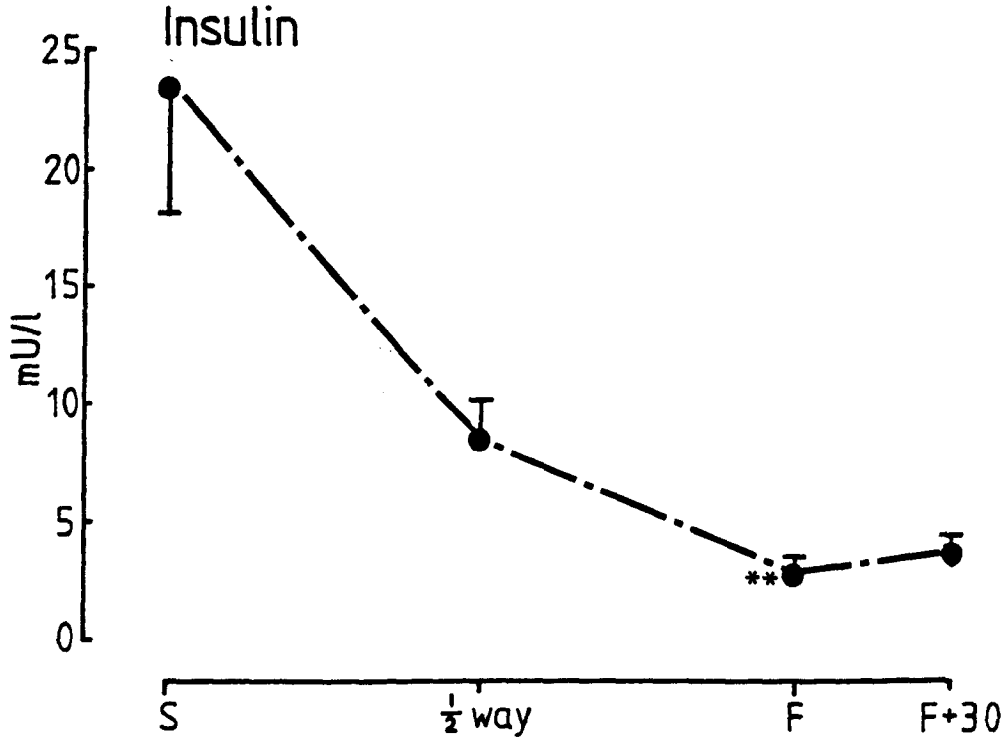


Figure 16(b). Changes in plasma insulin concentration (mean ± SEM) during and after ride B. Key preceding Figure 11(a).

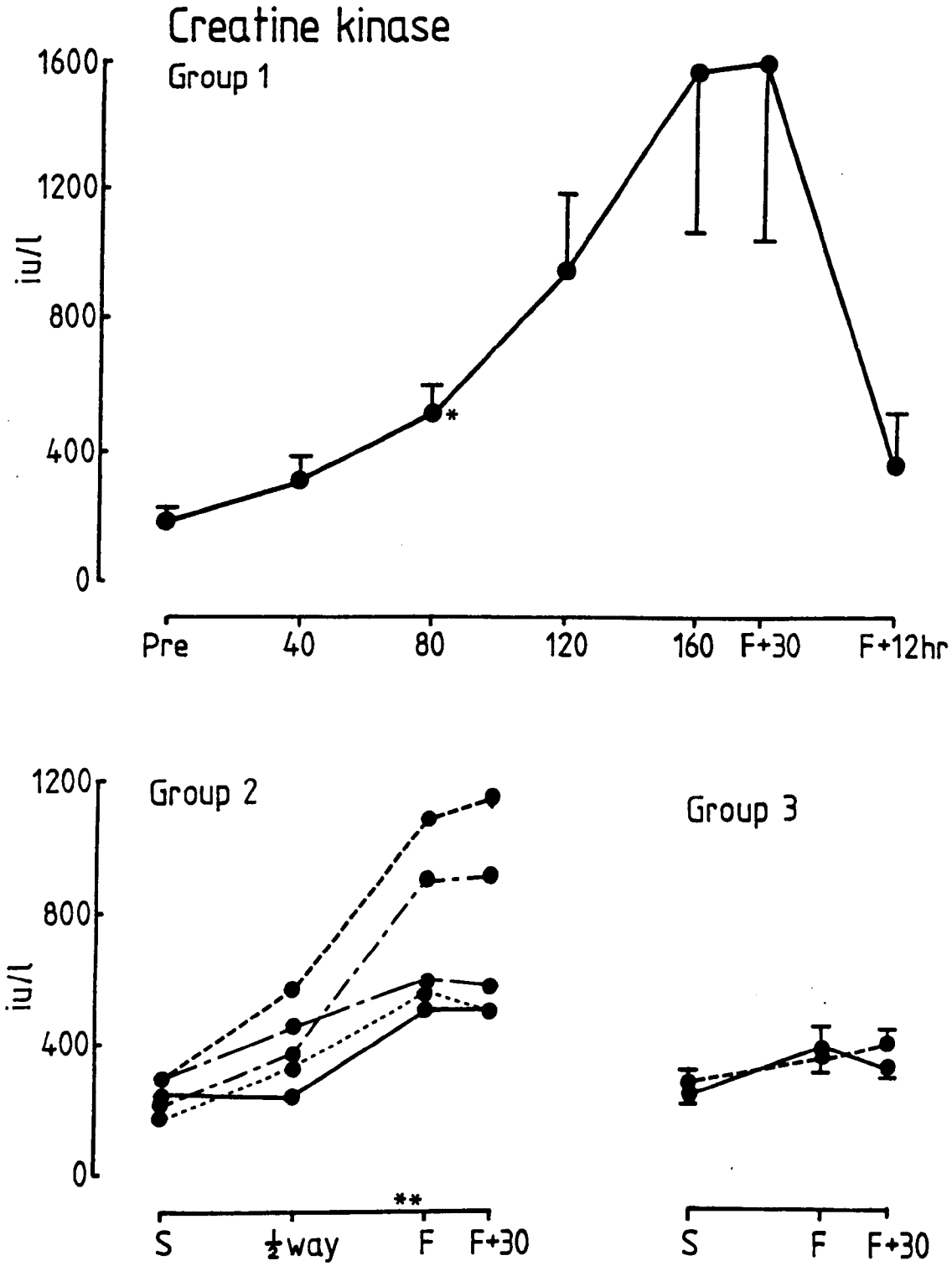


Figure 17(a). Changes in plasma creatine kinase activity during and after the competitive long distance rides. Key preceding Figure 11(a). One horse was excluded from all calculations in each of rides C, F, G and J, and two horses were excluded in ride A.

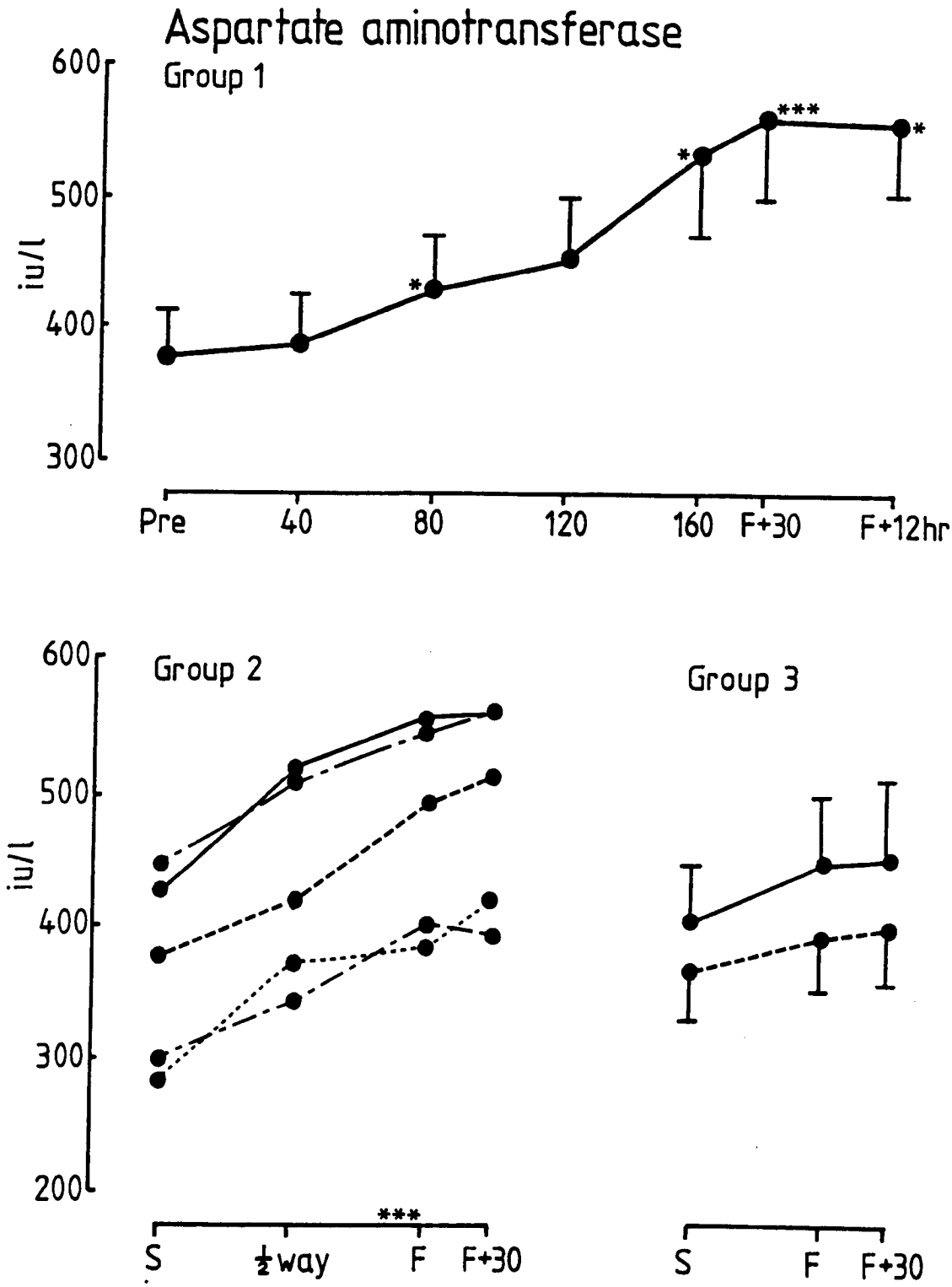


Figure 17(b). Changes in plasma aspartate aminotransferase activity during and after the competitive long distance rides. Key preceding Figure 11(a). One horse was excluded from all calculations in each of rides B, C, F, G, H and J, and three horses were excluded in ride A.

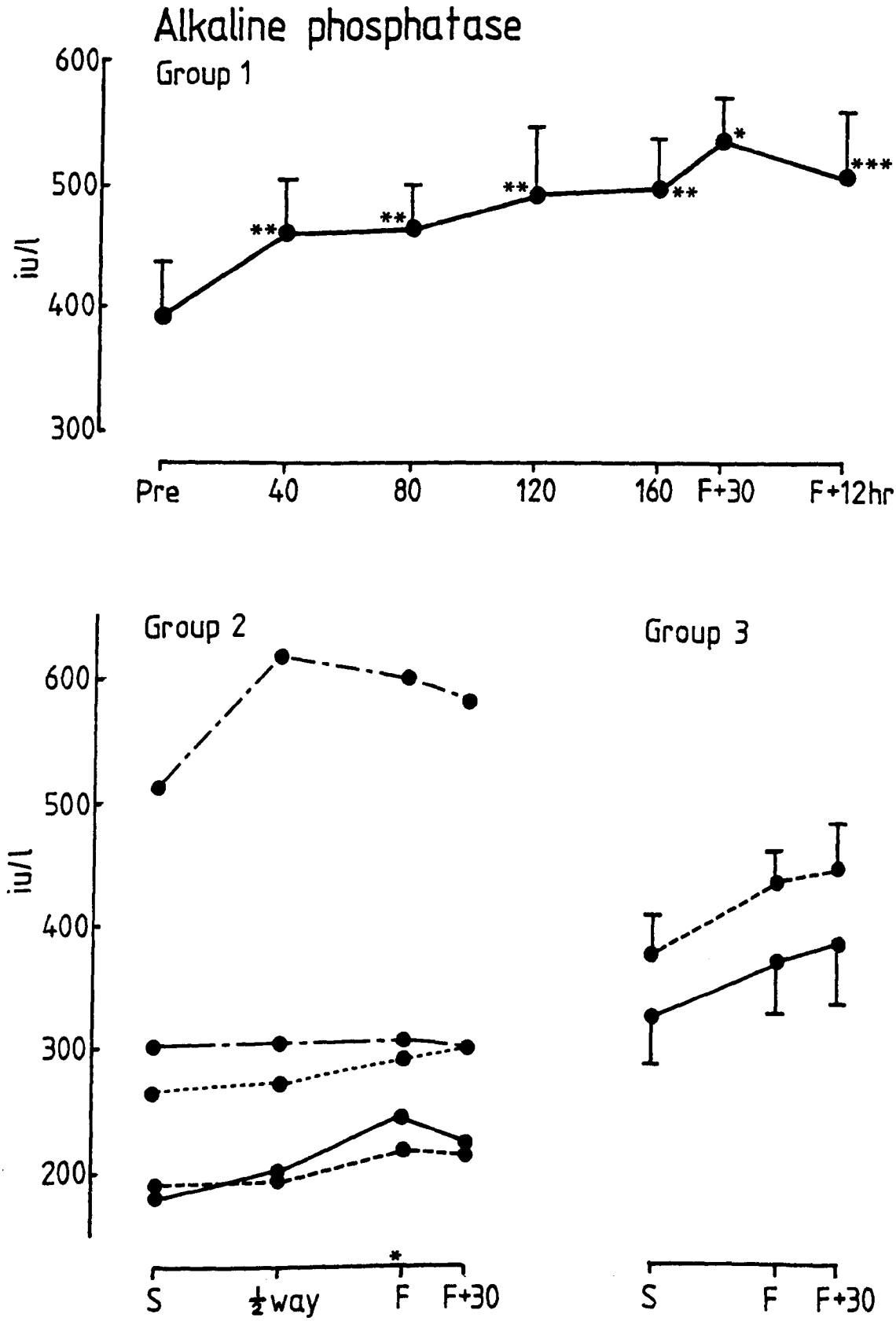


Figure 17(c). Changes in plasma alkaline phosphatase activity during and after the competitive long distance rides. Key preceding Figure 11(a).

case of AST. Considering group 2 rides in general the overall increase in CK activity was significant at $p < 0.001$, and in AST activity at $p < 0.01$.

Increases in plasma ALP activity were generally smaller in magnitude than the increases in AST, and were only statistically significant in five of the eight rides. However, in the group 2 rides overall the general tendency towards an increase was significant ($p < 0.05$).

Pre-ride activities of CK and AST in the 68 cases where data is available were very variable and showed a distribution which was markedly skewed (Figure 18). There appeared to be no relationship between pre-ride activities of either enzyme and subsequent ride performance, based on subjectively assessed performance criteria. High pre-ride activities of one enzyme were sometimes but not always associated with high pre-ride activities of the other, and most of the horses sampled on more than one occasion did not demonstrate any obvious consistency in enzyme activities. Comparison of Figure 18 with Table 7 revealed no factor such as age or breed which is generally common to all horses showing high pre-ride enzyme activities.

The magnitude of the increase in plasma CK activity during the ride is presented in Figure 19 for all 37 cases in which data is available for horses which completed a ride in group 2. Again there appeared to be no relationship between increase in CK activity and subjectively assessed performance. The horses which showed particularly large increases were not always those with high pre-ride enzyme activities, and again there was no obvious common factor such as age or breed linking those horses with large increases in CK activity during exercise. In addition, the finding of an increase or a decrease in plasma CK or AST activity in the 30 minutes after the finish appeared to be virtually random.

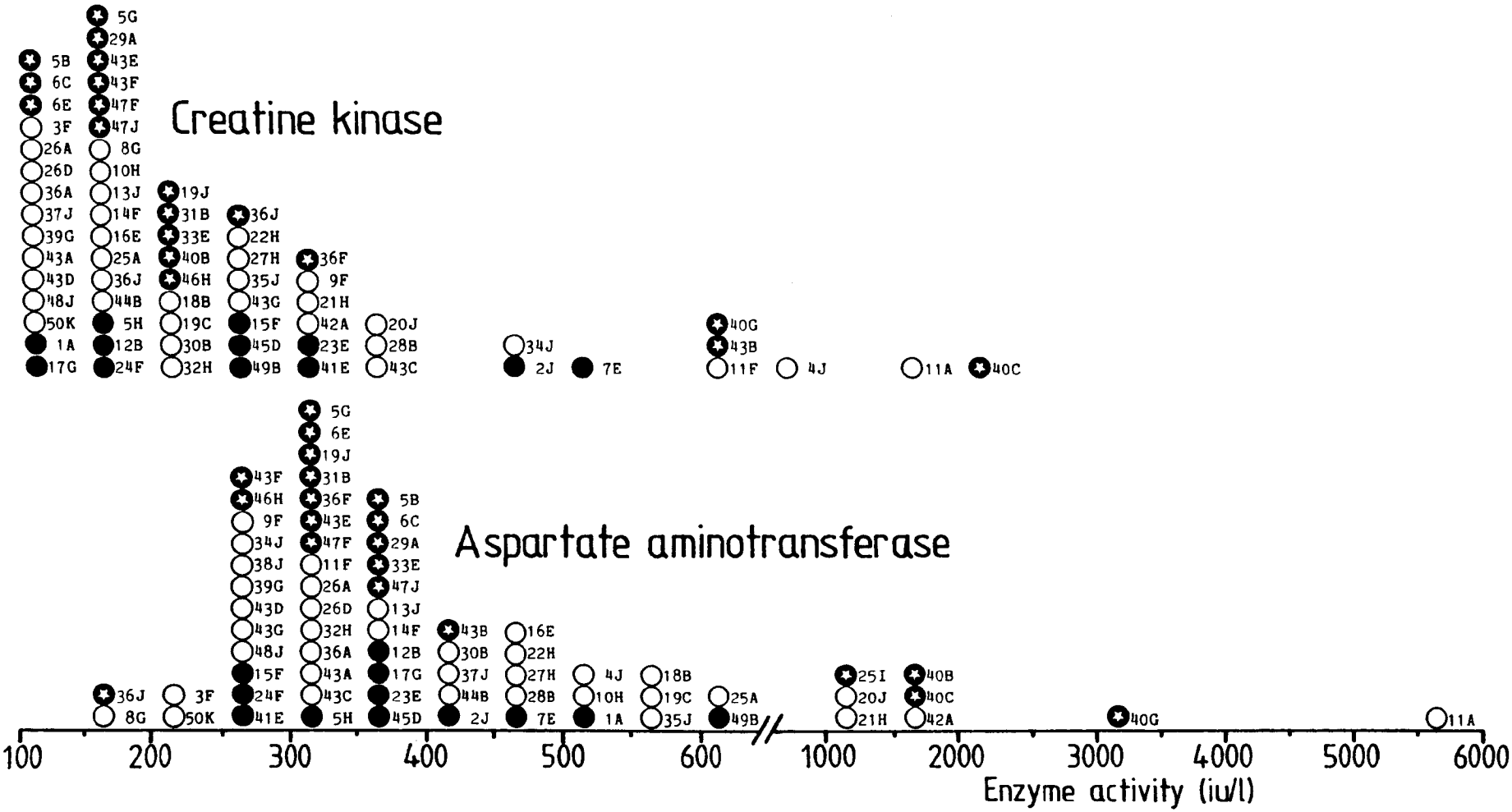


Figure 18. Pre-ride plasma CK and AST activities of competing endurance horses compared to their subjectively assessed level of performance in the subsequent event. Horses and competitions identified as in Tables 7 and 8.

✪ Good performers ○ Moderate performers ● Poor performers

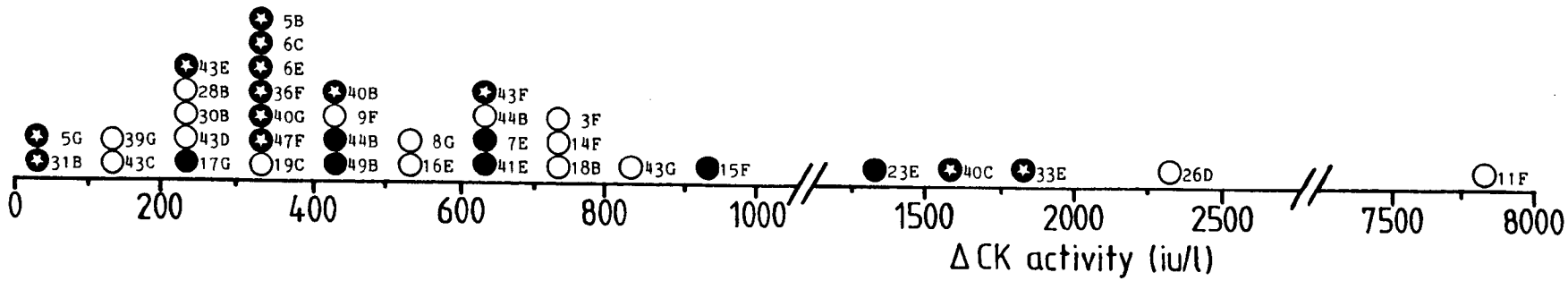


Figure 19. Increase in plasma CK activity in all horses which completed a ride in group 2 (65-95 km) compared to their subjectively assessed level of performance in that event. Horses and competitions identified as in Tables 7 and 8.

Good performers ○ Moderate performers ● Poor performers

Changes in plasma CK and AST activities in horse 11 in the 160 km ride were quite remarkable and are presented separately in Figure 20. The peak CK activity of over 35,000 iu/l was reached at 80 km and a steady decrease occurred in the second half of the ride. In contrast the increase in AST activity occurred entirely during the second half of the ride and the level had reached almost 9,000 iu/l by the finish. This horse showed no evidence of any muscular problems during this time and on the morning after the ride he was awarded the prize for the horse in best condition by the veterinary judges. This horse also competed successfully in ride F which was held about 12 weeks after ride A. His pre-ride CK activity on that occasion (686 iu/l) was again the highest of the group but his corresponding AST activity (322 iu/l) was little more than average. Again he showed the greatest enzyme increases of all the horses entered, but the finishing values (CK 8,500 iu/l, AST 930 iu/l) were modest in comparison with the findings at the 160 km ride. This horse is still competing successfully in EHPS long distance rides at the time of writing.

General observations, 160 km ride

In the 160 km ride a clear distinction was evident between those parameters which exhibited a steady consistent increase or decrease throughout the entire distance (calcium, magnesium, bilirubin, creatinine, urea and all three enzymes) and those parameters in which the greatest deviation from the resting levels was seen at an intermediate stage and in which this was followed by a return towards the pre-ride value (PCV, albumin, sodium, potassium, phosphate, glucose, glycerol and cortisol). This was in marked contrast to the situation in the shorter (65 - 95 km) events in which almost all parameters either increased or decreased steadily throughout the period of the rides.

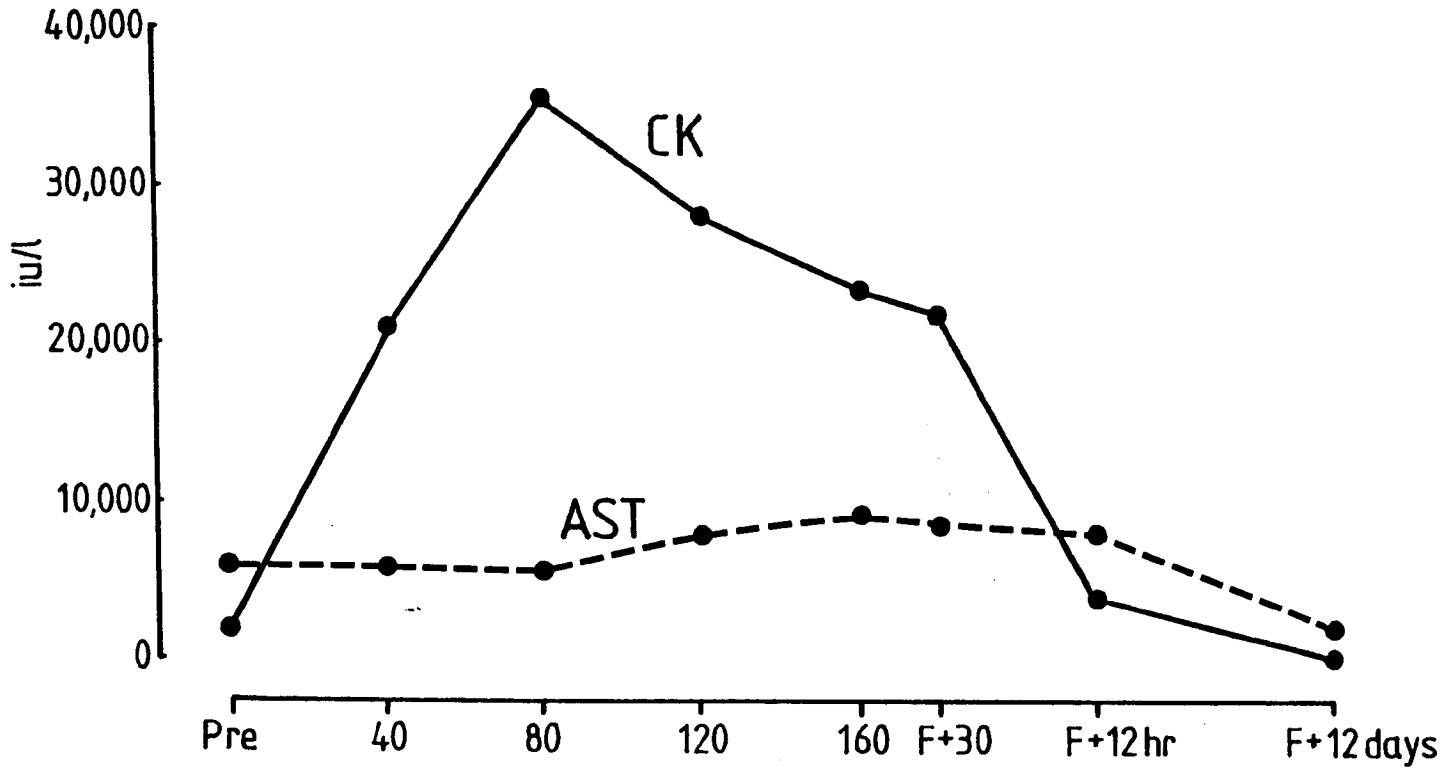


Figure 20. Changes in plasma CK and AST activities in horse 11 during and after the 160 km ride. Key to distance/time axis as in Figure 11.

In spite of the much greater distance involved, the highest (or lowest) concentrations of many parameters in the 160 km ride were in fact not so high (or low) as the levels reached in several of the group 2 rides. This was particularly noticeable in the case of total plasma protein, chloride, phosphate, glucose and cortisol.

B. Urine samples

Specific gravity, pH and concentrations of creatinine, urea, sodium, potassium and chloride of the five urine samples which were obtained from horses which had just completed an 80 km ride are presented in Table 27. The total volume of urine passed and the time since the previous urination were only known for one of these samples, that from horse 39 at ride G. Excretion rates calculated from this sample were as follows:

Urine flow rate	7.65 ml/min
Creatinine excretion rate	155 µmol/min
Urea excretion rate	2.91 mmol/min
Sodium excretion rate	1.22 mmol/min
Potassium excretion rate	1.93 mmol/min
Chloride excretion rate	0.59 mmol/min

TABLE 27

Composition of urine samples collected from five horses after 80 km rides

Horse	Ride	Specific Gravity	pH	Creatinine (mmol/l)	Urea (mmol/l)	Sodium (mmol/l)	Potassium (mmol/l)	Chloride (mmol/l)
5	G	1.032	8.5	16.4	391	13	258	122
11	F	1.038	8.8	24.7	502	42	351	83
15	F	1.027	8.9	12.4	334	82	145	16
39	G	1.035	8.6	20.2	380	160	252	77
43	D	1.037	8.6	16.3	510	5	281	25
Mean ± SEM		1.034 ± 0.002	8.7 ± 0.1	18.0 ± 2.1	423 ± 35	60 ± 28	257 ± 33	65 ± 20

C. Sweat samples

Only five sweat samples were obtained from horses competing in rides A and F, and concentrations of chloride, sodium, potassium and magnesium in these samples are presented in Table 28.

TABLE 28

Composition of sweat samples collected from five horses during endurance rides

Horse	Ride	Chloride (mmol/l)	Sodium (mmol/l)	Potassium (mmol/l)	Magnesium (mmol/l)
1	A	298	213	85	2.4
15	F	220	198	44	1.0
24	F	234	209	38	1.3
42	A	272	192	90	5.7
47	F	204	183	39	1.1
Mean ± SEM		246 ± 17	199 ± 5	59 ± 12	2.3 ± 0.9

Discussion

Physical performance of the horses

The degree of involvement of the riders in the sport and hence, usually, the degree of fitness of the horses varied widely within the group sampled. Some horses (such as 5, 6, 19, 29, 40 and 43) were kept with the primary aim of competitive endurance riding in mind and were subjected to a carefully planned regime of training and competition throughout the spring and summer. Others (such as 4, 7, 15, 23, 24 and 27) were kept for general riding purposes and were entered for the long distance event because it was being held near their home base. In general these former horses performed consistently well while those in the latter category were usually good performers only in the shorter (40 km) events and were often obviously fatigued when longer events were attempted. However, fatigue of the horse was only involved in three of the seven withdrawals listed, with lameness, and fatigue of the rider also leading to withdrawal on a number of occasions.

It is difficult to give a meaningful figure for the speeds of the horses involved in the endurance rides. In the CTR events all the horses finished within the target time bracket (9.7 - 12.9 km/hr) but in fact some horses were known to have been rested for some time just before crossing the finish line in order to extend the recovery period before the crucial post-ride examination by more than the alotted 30 minutes. This means that the "finish" blood samples did not in fact represent true finishing values. In the endurance rides the speeds of the winning horses were usually about 12 - 14 km/hr, however there were a few competitors whose speeds were considerably slower than this (notably horse 7 in ride E) and often these were not actually recorded as there was no question of eligibility for a prize. In general it appeared to be the case that such competitors completed the first half

of the ride at a similar speed to the others then lost touch with their group and slowed down considerably in the second half. In the 160 km ride the average speed of the winning horse was 11.9 km/hr, however this animal was not sampled and the speeds of the five finishers which were sampled were between 9.7 and 10 km/hr.

The difficulties of administering the rules relating to heart and respiratory rates have been discussed in the General Introduction, and in fact it proved quite impossible to include statistics on these parameters in this study. In addition to the problems already discussed it was found that judges frequently failed to record these measurements when horses were eliminated due to lameness or injury after completing the distance, and when such elimination occurred immediately after the finish these horses were never presented for an F+30 examination. Eliminations under the heart rate rules were in fact very infrequent, with lamenesses (usually fairly minor) being by far the most common cause of elimination. It was clear, however, that the existence of these rules had a very salutory effect on the general tactics of those riders of a more tearaway disposition.

That being said, however, it was also clear that the elite horses in the EHPS rides, and indeed in British rides in general, were not usually performing to the limits of their capabilities. In contrast to the usual speeds of 12 - 14 km/hr or less recorded in this study and in other British studies (Lucke and Hall, 1978, 1980a,b) speeds recorded abroad are generally much higher, for example winning speeds of 24.3 km/hr over a 3-day 210 km course in South Africa (Grosskopf et al, 1983) and 22.1 km/hr over 80 km in Australia (Snow and Rose, 1981; Rose and Sampson, 1982). When the generally hotter weather and more severe terrain in these countries is also considered it is clear that there is considerable room for improvement in the British performances. The reason for this discrepancy is not clear. It may be that British riders

are afraid of veterinary penalisation or elimination under the heart rate rules, or of causing their horses to become lame, but in fact the rules and the level of veterinary supervision in Australia and South Africa are equally strict if not more so. It is more likely that the lower level of competition in Britain is a self-perpetuating phenomenon exacerbated by the comparatively small number of entrants for each ride, and that this will continue to be the case until a number of riders deliberately set out to emulate the foreign performances. It is noticeable, however, that endurance riders and indeed those concerned with equine sport in general are much less concerned with times and record-breaking than are human athletes, and it is quite difficult to extract actual times or speeds from many compilations of equine statistics. There has been some suggestion since the work reported in this thesis was undertaken that a few British riders are indeed aiming for faster times, but it is not yet apparent whether or not the general level of the sport will approach that seen in some foreign countries.

Many publications relating to competitive long distance rides have presented case studies of horses which became ill during or shortly after the rides with a variety of metabolic problems, rhabdomyolysis being the most common (Mansmann et al, 1974; Hinton, 1976; Rose et al, 1977; Fregin, 1980; Lucke and Hall, 1978, 1980a,b; Grosskopf and van Rensburg, 1983). In contrast to this comprehensive tale of disaster and death, it was noteworthy that there was not one single case of medical illness associated with any of the events documented in this Section, even among horses which were not part of the study. One of the reasons for this must be the more temperate climate and generally easier terrain in England and Wales compared to Australia, South Africa and the USA. However, four of the reports listed above (Hinton, 1976; Lucke and Hall, 1978, 1980a,b) concern events in Britain, all Golden Horseshoe Rides organised by the BHS, and while most of the horses concerned in these

reports were certainly less seriously ill than the others on the list the question arises as to why these events seem to be more accident-prone than the EHPS events. In fact it would be expected that the situation would be the reverse, as the BHS events are all of the time trial or CTR type and so no direct competition between horses is involved. As the target time bracket in CTR's is certainly no faster than the usual riding speeds of endurance rides it is difficult to account for the apparently higher casualty rate in the former type of event. Many EHPS members maintain that the ride rules and general attitudes of this Society encourage better preparation and hence fitter entrants, but it is very difficult to substantiate this view from an examination of the rule books of the two societies and a certain amount of chauvinism would appear to be involved. It was noteworthy that while most other publications cite hot and/or humid weather as being associated with the greatest risk of fatigue or metabolic problems the event in this present study which seemed to lead to the greatest fatigue, and in which many of the greatest changes in plasma biochemical parameters were seen, was ride E/J, where the weather was unpleasantly cold with a high wind and a good deal of rain.

Blood samples

One of the major problems encountered with the blood sampling in this Section was, as reported above, that of missing samples. There were a number of reasons for this, apart from the obvious one of early elimination or withdrawal, such as the departure of a rider from a checkpoint before the sample was collected, the horse resenting the procedure, the rider resenting the procedure, or a horse eliminated after the finish being taken home before the time for the F+30 sample had arrived. When these missing values were added to the initially small number of entrants for each ride (not more than 10 - 15 and sometimes as low as 4) and the fact that the riders of a number of

entrants refused permission for any blood samples to be taken, the problems inherent in statistical analysis of the results become apparent. Some of the mean results were quite noticeably biased by the appearance of a particular horse in one group but not in the next; this problem was eliminated in the analysis of significance by the use of the paired *t* test, but at the cost of reducing the number of horses still further.

An even bigger problem with the statistical interpretation of the results was the amount of individual variation between animals. Horses sampled varied in breed, sex, age, degree of fitness, diet, management regime and distance travelled to the ride, while the riders varied very much in experience and so in ride tactics employed. In ER events some horses were involved in racing (galloping) finishes while others were not, and in CTR events some horses may have been deliberately rested before officially crossing the finish. Because of this, results from horses in the same event often varied enormously, particularly with regard to the magnitude of the changes seen. This had the effect of rendering some quite spectacular alterations non-significant statistically even when the direction of change was the same for all horses - particular examples of this were the decrease in plasma potassium concentration after the finish of ride C and the increase in plasma CK activity during ride A. In addition, all these variable factors combined to render it almost impossible to find even a pair of horses which could really be considered to be comparable in most respects. Because of this any meaningful comparison between groups of horses to examine the effects of such things as feeding or training regimes on the biochemical findings, as was originally intended, proved quite out of the question. In spite of these drawbacks, however, the total number of horses sampled was sufficient to allow some useful conclusions to be drawn.

PCV and plasma proteins

The assessment of changes in the amount of plasma water present by measuring changes in the concentration in constituents of the blood or plasma is notoriously unreliable. In man the measurement most often used is the packed cell volume, as the erythrocyte is almost entirely confined to the blood with minimal escape into other fluid compartments. This means that in the short term changes in PCV will generally mirror changes in plasma volume (van Beaumont, 1972). However, it has been suggested that whole blood haemoglobin concentration in fact gives a better estimate as it allows for the decrease in mean corpuscular volume (MCV) and the consequent increase in mean corpuscular haemoglobin concentration (MCHC) which occur during dehydration (Costill and Fink, 1974).

This interpretation is, however, invalid in the horse. In common with most other domestic mammals the horse possesses a smooth muscle layer in the splenic capsule which is absent in man. Contraction of this muscle is mediated by α-adrenoceptors (Snow, 1979) and the net effect of the system is to transfer a proportion of the splenic erythrocyte store into circulation whenever the animal is excited, frightened or exercised (Torten and Schalm, 1964). Even a minor stress such as the collection of a blood sample can lead to a measurable PCV increase (Seckington, 1969; Stewart and Steel, 1975) and a short burst of anaerobic exercise can result in a PCV of almost double the resting value (Sreter, 1959). In this present study the combination of horses in various states of pre-competition excitement when the "start" samples were collected, with horses subjected to various degrees of anaerobic exercise during the last few minutes of a ride just before the "finish" sample was collected (this latter depending on whether or not a racing

finish developed in a particular event and which horses were actually involved in this) makes the interpretation of PCV changes in relation to plasma volume changes extremely questionable.

Apart from the erythrocytes the only other blood constituents suitable for the assessment of plasma volume changes are the plasma proteins. These are, however, much smaller than the erythrocytes, which means that only those with exceptionally large molecular weights are genuinely confined to the plasma and the majority may enter the interstitial fluid by leakage from the capillary bed (van Beaumont et al, 1973; Dill and Costill, 1974). In a situation where there has been a substantial loss of body fluid it is very probable that the permeability of the capillaries to protein molecules and/or the efficiency of lymphatic return to the circulation will be altered. This implies that the percentage distribution not only of the extracellular fluid but also of the proteins contained in that fluid may be altered, not necessarily to the same extent in each case. Because of the number of variables involved, assessment of plasma volume reduction in horses is probably best done by considering both PCV and plasma protein changes together, but of the two parameters plasma protein concentration is probably by far the more reliable, particularly where exercise is involved.

The demonstration of increases in both total plasma protein concentration and PCV in most of the rides studied indicates that a decrease in plasma water content was a general occurrence. It is difficult to quantify this, as the generally larger percentage increases in PCV compared to plasma proteins may represent augmentation of the circulating erythrocyte numbers by red cells from the spleen, or else may be due to escape of the smaller proteins from the capillary bed. In addition, the apparent occurrence in ride F of a larger percentage increase in plasma albumin concentration compared to the other protein fractions is inconsistent, as it would be expected that the smaller

molecular weight albumin would escape more easily from the circulation and so plasma concentrations would increase by a smaller amount. It is clear, however, that the extent of the apparent fluid loss varied between events. The greatest loss seemed to occur at ride C, which was held on the warmest day of all the group 2 rides. The almost complete absence of any appreciable fluid losses in the horses on the 160 km ride was surprising, as although the riding speeds were slower, the weather was pleasantly warm and the terrain quite demanding.

Plasma electrolyte concentrations

Studies of human marathon runners have generally demonstrated increases in plasma sodium and potassium concentrations, and either an increase or no significant change in plasma chloride concentration (Rose et al, 1970b; Riley et al, 1975). This is consistent with the accepted view that the sweat is by far the most important route of fluid losses in long distance exercise, as human sweat has been shown to be hypotonic compared to plasma for these three electrolytes (Robinson and Robinson, 1954; Kuno, 1956). In contrast, the general pattern of electrolyte changes seen in this study was of increases in plasma sodium concentrations together with decreases in potassium and chloride concentrations.

It appears, as discussed below, that concentrations of electrolytes in equine sweat may actually be hypertonic compared to plasma. If this is so then the general tendency of plasma potassium and chloride concentrations to decrease is explicable, but it is not clear why the rides in which the greatest fluid losses appeared to occur, in particular ride C, should show comparatively small potassium and chloride decreases. It is also not clear why plasma potassium concentrations should consistently decrease by such a large amount during the immediate post-ride period - in almost all events the mean decrease between the finish and the F+30 samples was greater than the decrease

seen over the entire period of exercise. In addition, the significant increases in plasma sodium concentration appear to be inconsistent with the production of hypertonic sweat, in particular when in this case the largest increase did in fact coincide with the greatest apparent fluid loss (ride C).

Plasma mineral concentrations

On initial inspection it might be thought that the changes in plasma calcium and magnesium concentrations in the endurance horses were not very different from those seen in the resting horses in Section 1, which, unlike the changes in plasma protein and electrolyte concentrations, could not be positively related to any feed consumed. However, although the ranges of both parameters were almost identical in both groups of horses, the changes in plasma calcium concentration reported in Section 1 were not in fact statistically significant, while the plasma magnesium concentration actually showed an increase, not a decrease, at the time of day in Section 1 which corresponded to the time of the rides. It therefore seems most probable that the significant decreases in the concentrations of both parameters seen in the endurance horses were in fact a consequence of the exercise.

Rose et al (1970b) have demonstrated a decrease in plasma magnesium concentration in human marathon runners and have suggested that this is a consequence of the high magnesium concentration of human sweat. The same explanation may account for the fall in plasma magnesium seen during this present study, as sweat magnesium concentrations, although very variable, were consistently hypertonic compared to plasma. It appears, however, that plasma calcium concentration may actually increase in human athletes during endurance exercise (Riley et al, 1975), and it is believed that this is an effect of the increase in plasma albumin concentration. As plasma albumin concentration also increased in the endurance horses it is difficult to account for the

significant decrease in plasma calcium concentration in these animals, which was also seen in all the reports summarised in Table 6. These authors attribute this decrease to sweat calcium losses, however reports of sweat calcium concentration vary enormously, as discussed in Section 3 below, and the most extensive study (Carlson and Ocen, 1979) suggests that it may in fact be lower than normal plasma calcium concentrations.

It has been observed that endurance horses suffering from synchronous diaphragmatic flutter can often be successfully treated by the intravenous administration of calcium borogluconate, and this has led a number of authors to suggest that this condition may be a result of loss of calcium in the sweat (Mansmann et al, 1974; Carlson, 1980; Fowler, 1980a,b). However, it appears that horses with evidence of SDF are not usually hypocalcaemic and many of the horses in this present study, none of whom developed SDF, had lower plasma calcium concentrations at the end of the rides than the cases of SDF reported by Mansmann et al (1974). Regular exercise per se appears to lead to profound alterations in calcium metabolism in the horse, in particular to decreased urinary excretion and to an increase in the turnover rate of skeletal calcium (Schryver et al, 1978). These authors consider that sweat losses, while not negligible, are not sufficient to account for the increased calcium retention measured in exercising horses, and it appears that the factors involved in the consistent decreases seen in plasma calcium in this present study and the response of clinical SDF to calcium therapy may be complex.

Previous studies of competing endurance horses have been unanimous in reporting a significant increase in plasma inorganic phosphate concentrations during the rides (Table 6), and the same finding has also been reported in marathon runners (Riley et al, 1975). The authors of this last publication consider that this is due to a general release of

intracellular ions to the extracellular compartment, however if that were the case in the horses then plasma potassium and magnesium concentrations would be expected to increase also, whereas in fact these ions decreased in concentration. Carlson and Mansmann (1974) have suggested that this increase in plasma phosphate concentration is a consequence of general haemoconcentration as the concentration of phosphate in sweat is said to be relatively low. However, the percentage increase in phosphate concentration was invariably much greater than the percentage increases in either PCV or total plasma protein in this present study, which strongly indicates that some factor other than haemoconcentration was involved. Transfer of phosphate groups is an integral part of nearly all metabolic pathways, in particular those concerned with body fuel catabolism. While uptake and release of phosphate are evenly balanced in glycogenolysis and most other metabolic pathways, it is possible that the extensive depletion of intramuscular stores of phosphocreatine (rather than ATP as postulated by Rose et al, 1977) may lead to a net production of phosphate and hence an increase in plasma phosphate concentration.

Body fuel utilisation

Changes in many of the parameters relevant to a discussion of fuel metabolism were much more clear-cut and consistent than those relating to fluid/electrolyte balance. One factor which did appear to upset the interpretation of the plasma glucose concentrations was the finding of particularly high pre-ride levels (> 6 mmol/l) in a few horses. It appeared that this was probably a consequence of these animals having eaten a high carbohydrate feed before the samples were collected, and the return towards fasting levels in these cases seemed to account for the decrease in plasma glucose concentrations noted in the first 40 km of some of the rides. In two of the rides (E and F) plasma glucose concentration actually increased during the first 40 km, presumably due

to glucose production (gluconeogenesis or hepatic glycogenolysis) occurring more quickly than was necessary to maintain the current rate of glycolysis. These two rides took place over the most taxing terrain of all the group 2 events and plasma cortisol concentrations rose to their highest levels at these rides. It is possible that "stress"-induced increases in plasma cortisol (and possibly adrenaline) led to a greater than usual rate of glucose production in these events.

Lucke and Hall (1980c) have theorised that the liver glycogen of a horse travelling at 27.4 km/hr would be exhausted after 60 km. The horses in this present study were travelling at only half that speed, but the general decrease in plasma glucose concentration in the second half of the group 2 rides suggests that before 80 km hepatic glycogen stores were exhausted and the rate of gluconeogenesis could not match the rate of glycolysis. The absence of any significant decrease in plasma glucose concentration during the 160 km ride suggests either that liver glycogen was not completely depleted in this event, possibly because the slower speeds involved allowed a greater proportion of fat utilisation relative to carbohydrate, or that gluconeogenesis was occurring rapidly enough to supply all the necessary substrate for glycolysis. It is very difficult to separate these factors on the evidence available, and it is quite probable that a combination of both possibilities was in operation. In contrast to this present study Fregin (1980) and Dybdal et al (1980), who investigated comparable events with very much larger numbers of entrants, demonstrated steady decreases in plasma glucose concentrations throughout the 160 km. It appears, therefore, that the exact balance of carbohydrate to fat metabolism may vary quite considerably between different events at this distance.

Variation in the degree of increase in plasma glycerol concentrations between the three events in which this was measured was considerable, and a similar finding was noted by Lucke and Hall (1978, 1980a,b) in three Golden Horseshoe rides. Circulating free glycerol concentrations represent a balance between glycerol production as a side-effect of lipolysis and glycerol utilisation as a substrate for gluconeogenesis. It is obviously difficult to separate these two factors, but there is some evidence that the highest plasma glycerol concentrations occur at the most stressful rides. It has already been noted that in terms of terrain and speed ride F was one of the most stressful studied, certainly more so than rides A and B, while among the three rides studied by Lucke and Hall the one with the highest end of ride glycerol concentrations was the 1979 event, which was characterised by unusually hot weather and a drop-out rate of over 75% of horses. It appears that a particularly stressful competition may result in hormonal changes which favour a high rate of lipolysis, although whether or not this mobilised fat is actually utilised is difficult to assess. The consistent decrease in plasma glycerol concentrations after the finish presumably reflects the usefulness of this substance as a substrate for gluconeogenesis (Terblanche et al, 1981) and its availability is probably important in the significant increase seen in plasma glucose concentrations in the first 30 minutes after the finish, before most of the horses were fed.

The apparently significant fall in plasma triglyceride concentrations during rides A and B may be considered to be yet another indication of increased fat metabolism, however it seems more probable that this was in fact an artefact of the methodology used. Triglyceride concentration represents the difference between the measured values of total glycerol and free glycerol. Where free glycerol was a comparatively small value (i.e. in the pre-ride samples) the error in this

estimation was only small. However, when a comparatively small triglyceride value was calculated by the subtraction of two much larger numbers, each with its own 3 - 5% error, the percentage error in the resulting triglyceride figure was very large. This appeared to manifest itself in the finding of very low triglyceride concentrations whenever the free glycerol concentration was high, i.e. in the end of ride samples. In the few cases where horses finished with low free glycerol concentrations the corresponding triglyceride concentrations were usually almost identical to the pre-ride values, and it seems quite probable that plasma triglyceride concentration does not in fact change during endurance exercise in horses, as reported by Lucke and Hall (1980a).

The very striking increase in plasma bilirubin concentrations seen in all the rides in this study has also been reported in other investigations of endurance horses (Rose et al, 1977; Lucke and Hall, 1980a,b) and in marathon runners (Riley et al, 1975). This finding has generally been related to a presumed increase in intravascular haemolysis due either to the forcing of erythrocytes through small muscle capillaries at high pressure or to physical stresses of running on hard road surfaces. Those authors studying endurance horses have also suggested that reduced liver or biliary tract function may be involved, because of the simultaneous increase in plasma alkaline phosphatase activity. This enzyme is however also present in muscle (Gerber, 1969) and it does not seem necessary to postulate hepato-biliary insufficiency in order to explain its increase in plasma during exercise. It may be, though, that the increase in plasma bilirubin concentration in the endurance horses is connected to an elevation in plasma free fatty acid concentrations, suggested in this study by the increase in plasma glycerol concentration and shown in other events by Fregin (1980), Lucke and Hall (1978, 1980a,b) and Rose and Sampson (1982). It has been suggested by

Naylor et al (1980) that high plasma FFA concentrations can restrict hepatic uptake of bilirubin in the horse. This possibility, which was discussed in detail in Section 1, may well explain the much larger increases in plasma bilirubin concentrations seen in horses compared to human athletes.

The increase in plasma urea concentration seen in this study has also been reported previously in endurance horses (Lucke and Hall, 1980a,b) and in marathon runners (Riley et al, 1975). The former believe this increase to be due to a reduction in renal function during exercise, however the latter consider that the magnitude of the changes, which are similar in both species, can better be explained as a consequence of increased protein catabolism. The very limited data available from urine samples in this study show no evidence that urea excretion is actually reduced in horses involved in endurance events, while there is some evidence from studies of human athletes (which is discussed at greater length in Section 5) that amino acids may in fact be a significant source of energy after about the first hour of exercise (Decombaz et al, 1979; Lemon and Nagle, 1981). It appears, however, that it will be necessary to obtain more reliable data on changes in urinary urea excretion associated with endurance exercise before any conclusions can be drawn on this matter.

The increase in plasma creatinine concentration seen in endurance horses has also been generally considered as evidence of reduced renal function (Rose et al, 1977; Lucke and Hall, 1980a,b). However, there was again no evidence of reduced urinary creatinine excretion in the few urine samples obtained in this present study. The possibility of large scale breakdown of intramuscular stores of phosphocreatine has been discussed above with reference to increases in plasma phosphate

concentration, and it would appear quite possible that this occurrence might also lead to an increase in the rate of creatinine production from creatine.

Plasma hormones

The association of large increases in plasma cortisol concentration with the apparent degree of "stress" involved in a ride has been discussed above, and a very similar observation has also been made by Dybdal et al (1980). However, the end of ride plasma cortisol concentrations at the particularly hot Golden Horseshoe Ride studied by Lucke and Hall (1980b) were in fact lower than those seen at the previous year's event (Lucke and Hall, 1980a).

An increase in plasma cortisol concentration as a consequence of non-specific stress is well established, and excitement, hyperthermia and hypoglycaemia, all common occurrences in endurance horses, are particularly implicated in this phenomenon (Myles and Daly, 1974). Apparently high pre-ride corticosteroid levels in endurance horses were attributed by Dybdal et al (1980) to "psychogenic stress". It may be that the higher pre-ride cortisol levels in the group 2 and 3 rides in this present study (when samples were collected from "warmed up" horses just about to set off) compared to the 160 km ride (when samples were collected from resting horses the previous afternoon) represent a similar occurrence, but it is more likely that this was an effect of the diurnal variation discussed in Section 1, as the group 2 and 3 pre-ride samples were collected at 8.00 - 9.00 a.m., close to the time of maximum plasma cortisol concentrations. Whatever may be the actual trigger involved in the particularly large increases in plasma cortisol seen in two of the rides in this study, there is some evidence as discussed above that the metabolic effects of the hormone may have been in excess of the actual requirements of the animal in these cases.

The marked decrease in plasma insulin concentrations seen in ride B is in accordance with the findings of other authors who have investigated endurance rides of 80 km or more (Dybdal et al, 1980; Lucke and Hall, 1980a,b; Snow and Rose, 1981; Hall et al, 1982, 1983). It appears that in the horse, as in man, some non-insulin-mediated mechanism is involved in the uptake of glucose by the muscle cell during prolonged exercise, as was discussed in the introduction to this Section.

Plasma enzyme activities

The most striking feature of the plasma CK and AST activities measured in this study was their extreme variability, both in absolute terms in the pre-ride samples and in terms of the magnitude of the increases observed in different horses during the rides. In a number of cases this led to the change in the enzyme activity during a ride being statistically non-significant even although an increase occurred in all the animals, and even although any animals with extremely high pre-ride activities were omitted from the calculations. Very similar observations have been made previously by Fregin (1980), who omitted three horses with elevated CK levels from his mean results although only one of these showed clinical signs of rhabdomyolysis, Grosskopf et al (1983), who reported "extremely high" CK values in seven horses only one of which developed rhabdomyolysis, and Grosskopf and van Rensburg (1983), who omitted two horses from their mean results because of plasma CK activities of over 4000 iu/l.

Because of the association of elevated plasma CK and AST activities with rhabdomyolysis it is usually assumed by most authors that horses which demonstrate such particularly marked elevations in these enzymes during or after a ride without showing any clinical symptoms of the disease are actually suffering from it in a mild or subclinical form (Fregin, 1980). The inference is then drawn that such horses are more stressed or fatigued than the others and it is sometimes implied that

the magnitude of the increase in plasma CK activity during the ride will tend to be in inverse proportion to the animal's fitness (Lucke and Hall, 1980a). However, it must be questioned as to how many animals with "mild" or "subclinical" rhabdomyolysis can complete endurance rides in good physical condition, pass the veterinary inspection, and be awarded prizes, before this interpretation is reconsidered.

In this present study the enzyme analyses were not carried out for at least one or two days after the events, and in every case the identity of the horse with the four-figure pre-ride plasma AST activity came as a complete surprise. Careful examination of the known information regarding all horses with high pre-ride CK or AST activities, or with particularly large increases in CK activity during the rides, revealed no common factor such as age, experience, distance travelled to the ride or time since last competition which linked all the animals. In only two horses was there any suggestion of a muscle problem which could be linked to these findings. Horse 25 had had a mild attack of rhabdomyolysis several months before demonstrating a plasma AST activity of 1410 iu/l at the start of ride I, however this time gap was much greater than the 2 - 4 weeks usually associated with elevated AST activities following rhabdomyolysis (Cardinet et al, 1967). Horse 40, who had plasma AST activities of over 1000 iu/l before all three rides in which he was sampled, was reported by his owner to suffer from intermittent muscular cramps which might be mistaken for rhabdomyolysis but which invariably passed off quickly if he was encouraged to walk on. One of these attacks occurred only a few days before ride C, which this horse won in a close-fought galloping finish. Both this horse and horse 11, whose remarkable plasma CK and AST activities during the 160 km ride are presented separately, are still competing regularly and successfully in EHPS rides at the time of writing. In the case of

horse 11 the owner and her veterinary surgeon were advised of the enzyme findings, but in spite of close vigilance no adverse clinical signs were detected.

There is no reason to doubt the assumption that the source of these plasma enzymes is the skeletal muscle, and the conclusion seems inevitable that the particularly high values, whether seen pre-ride or post-ride, are a consequence of some alteration in integrity of the muscle cell membrane. The actual underlying condition behind these findings is something of a mystery. However, there is no evidence that this occurrence either before or during a ride is associated with poor performance or the risk of injury, and there is no justification for attempting to use these enzyme results as a retrospective index of a horse's degree of fitness or fatigue. It would seem to be important that veterinary surgeons in practice are aware of this phenomenon, as it is not unusual to find plasma AST activity used as the only biochemical index of liver "function" in the horse, and the possibility of a mis-diagnosis in an endurance horse which becomes ill through some other cause is far from remote.

Increases in plasma ALP activities during the endurance rides studied were comparatively modest and were not seen in all horses. It has been suggested that increases in this enzyme in endurance horses may be indicative of biliary or hepatic dysfunction (Rose et al, 1977; Lucke and Hall, 1980b). However, there are other explanations for the observed increases in plasma bilirubin, as discussed above, and as ALP is present in equine muscle (Gerber, 1969) it may not be necessary to seek further reasons to explain its increase in plasma beyond the muscle changes discussed above. On the other hand, the particularly large increases in CK and AST activities were not in fact accompanied by any particularly large ALP increases, which argues against muscle as the source of this enzyme. Rather than liver, however, it seems much more probable that bony tissue was responsible for the increase in ALP activity, as stress to the limb bones clearly increases during long distance riding.

160 km ride

The greatest alterations in many parameters were seen at an intermediate point in this event. While parameters indicative of fluid/electrolyte changes were returning to pre-ride values by the end of the ride, calcium and magnesium concentrations continued to decrease, which may suggest that the changes in these minerals were dependent on something other than sweat losses. The major increases in phosphate and creatinine were seen before the 80 km point, while urea increased steadily throughout the ride. If reduced urinary excretion was not a significant factor, this suggests that breakdown of high energy stores occurred mainly in the early stages while protein breakdown was a continuing occurrence. The recovery of plasma glucose concentration after the half-way point together with the progressive decrease in glycerol concentration suggests that in this ride, unlike the group 2 rides, glycogenolysis and gluconeogenesis were able to keep pace with the requirement for glycolysis. Assessment of the continuing rate of lipolysis is impossible, but the steadily increasing concentration of bilirubin suggests that it may have been maintained. The plasma cortisol fluctuations could in fact be explained by diurnal variation, as the highest concentration was seen around 9.00 a.m., about the time of the natural peak, and in any case the decrease seen after this point suggests that the general level of stress was less than on most of the group 2 rides. Excluding the particularly aberrant individuals discussed above, the general pattern of enzyme changes was of a steady increase throughout, which indicates that disruption of muscle cell integrity tended to continue over the entire distance.

These observations all tend to suggest that the intensity of exercise was relaxed somewhat as the ride progressed so that less sweating was necessary and maximum utilisation of the products of lipolysis was possible. Some slowing down was evident in the second

half of the ride, but as the reduction in speed was at the most 2 - 3 km/hr it is surprising that the effects on electrolyte conservation and fuel metabolism were so marked. In this context it was particularly unfortunate that the winning horse could not be sampled, as she maintained the same speed throughout the ride and would have provided a useful comparison.

The findings of other studies of 160 km rides have been somewhat variable, as already discussed, but all three publications have reported that at least some of the parameters under discussion began to return towards their pre-ride values at some point before the finish (Carlson and Mansmann, 1974; Fregin, 1980; Dybdal et al, 1980). However, it is not really clear whether this is a result of a general tendency to slow down as these rides progress or of some other adaptive mechanism.

Urine composition

The very limited number of urine samples collected from competing horses makes firm interpretation of the results impossible, however certain provisional suggestions can be made. The mean specific gravity of the samples was similar to that of the resting horse urine (Section 1) during the morning and early afternoon, which indicates that general urinary concentration had not increased. In contrast the urine from the endurance horses was much more alkaline, but it is highly probable that this was an artefact due to the impossibility of measuring the pH until the following day, as stale urine tends to become alkaline due to the breakdown of urea to ammonia.

The dependence of the concentrations of urinary solutes on the degree of diuresis occurring at the time, and the consequent inadvisability of interpreting concentration results of spot urine samples, has been discussed in Section 1. However, comparison of the concentrations of creatinine, urea and the three electrolytes with the values seen at the same time in the resting horses in Section 1 tends to suggest that

only chloride concentration was considerably lower in the endurance horses, with the ranges of concentrations of the other parameters being fairly close to the resting values.

Only one single volumetric urine sample was obtained and it is impossible to draw any conclusions from this sample alone. However, it seemed that excretion rates of water, urea and creatinine in this animal were higher than were generally seen in the resting horses, which again suggests that a decrease in renal function did not occur and that reduced urinary excretion would not account for the increases seen in plasma urea and creatinine concentrations. In addition, while the rate of potassium excretion was almost the same as in the resting horses the sodium excretion rate was higher and the chloride excretion rate lower.

These suggestions are in direct contrast to those of Carlson (1980), who states that urine output and concentrations of sodium, potassium and chloride all decrease markedly during endurance exercise, but without giving any figures in support of these assertions. It is clear that the collection of an adequate number of volumetric urine samples under controlled conditions will be necessary to elucidate this matter further.

Sweat composition

The electrolyte concentrations of the five sweat samples collected in this investigation were intermediate between those reported by Carlson and Ocen (1979) and Rose *et al* (1980), who also collected sweat from endurance horses by a similar method to that used in this study. The suggestion that equine sweat is hypertonic compared to plasma appears to be unavoidable, and this theory has been discussed in relation to the plasma electrolyte results presented above. However, both Carlson and Ocen (1979) and Rose *et al* (1980) point out the difficulties of ensuring that sweat electrolyte concentrations have not been affected either by evaporation or by contamination either from the

hair or from the leather saddle, and it is not at all certain that such effects were not present in this study. The possibly hypertonic sweat of the horse and its consequences in terms of fluid-electrolyte homeostasis raise a number of interesting questions, and it seems that the further study of equine sweat composition might be a worthwhile exercise. This subject and the literature relating to it are therefore discussed at greater length in Section 3 below.

Conclusions

This study has generally tended to confirm the results of previous studies in which samples have been taken from competing endurance horses, but in a number of cases the discussion has been carried further and additional conclusions suggested.

It seems clear that fluid losses in horses competing in endurance rides can be fairly extensive, amounting at a conservative estimate to over 10% of extracellular fluid volume in some events. However, the relationship of these fluid losses to electrolyte losses (particularly sodium) and the composition of the sweat is not at all clear, and considering the degree of importance placed on sweat losses by authors discussing clinical problems of endurance horses (Mansmann et al, 1974; Hinton, 1976; Carlson and Nelson, 1977; Carlson, 1980; Fowler, 1980a,b) it appears that this subject warrants further investigation.

Body fuel metabolism in the endurance horse appears to be similar to that of most other mammalian species during exercise, including man. The general pattern in this study was of exhaustion of liver glycogen stores at some stage between 40 and 80 km, with plasma glucose concentration then maintained to some extent by gluconeogenesis, apparently utilising the glyerol produced by lipolysis as a major substrate. Increased protein catabolism may also have provided a significant source of carbohydrate.

It was impossible to relate the plasma enzyme findings, either pre-ride AST or CK activities or the magnitude of the increase in CK activity during the ride, to the level of performance or the degree of fatigue of the individual horses. A number of horses demonstrated plasma AST and/or CK activities which were remarkably high and in some cases comparable to those reported by other authors in cases of clinical rhabdomyolysis. However, in this study these findings were not associated with any medical illness, or even with poorer performance, and it is possible that they are a reflection of some non-pathological alteration in the integrity of the muscle cell membrane which may be peculiar to a proportion of horses involved in long distance riding events.

The number of urine and sweat samples collected was insufficient to allow any firm conclusions to be drawn, however it appeared that chloride might be the only substance being positively conserved by the kidney during exercise. The sweat samples collected were hypertonic compared to plasma for sodium, potassium, chloride and magnesium, but the possibility of evaporation or contamination of the samples cannot be dismissed.

In general this study was restricted by the extremely large variability among the horses sampled in terms of age, breed, training and so on and by the fact that most of the elite horses were not performing to the limits of their ability. In addition the small number of horses entered for each ride and the absence of a number of particular samples made statistical interpretation very difficult. It was concluded that further investigation of a more restricted aspect of endurance exercise under controlled conditions would be necessary in order to advance this study further, and from the possible alternative subjects detailed above that of the conflicting requirements of evaporative thermoregulation and fluid/electrolyte balance was chosen.

SECTION THREE

STUDIES OF BLOOD AND SWEAT COMPOSITION DURING HEAT EXPOSURE

Introduction

Fluid and electrolyte changes during endurance rides

Although it was evident from the results of Section 2 that changes in blood biochemistry indicative of fluid and electrolyte losses are not the most consistent finding in competitive endurance rides nor, usually, are they the most dramatic, it appears that this subject may be one of considerable interest. In a number of other studies which have shown broadly similar results, these fluid and electrolyte changes have been attributed to loss of water and electrolytes in the thermoregulatory sweat produced by the exercising horse, and it has been noted that these changes are more marked when the endurance event has been held in hot weather (Carlson and Mansmann, 1974; Rose et al, 1977, 1980; Fregin, 1980; Lucke and Hall, 1980a,b). It has been suggested that such fluid and electrolyte disturbances are a major factor in the development of certain clinical problems encountered in endurance horses such as rhabdomyolysis and synchronous diaphragmatic flutter (Mansmann et al, 1974; Hinton, 1976). However, there are a number of apparent inconsistencies which have not yet been investigated. What volume of sweat is actually lost by a horse during the course of an endurance ride? If it is indeed true that electrolyte concentrations of equine sweat are generally hypertonic compared to plasma, what is the advantage of this massive electrolyte loss to the horse? Why should the secretion of a hypertonic sweat be accompanied on almost all occasions by an increase in plasma sodium concentration?

One of the major problems involved in the interpretation of results from exercising horses is that of differentiation between effects which are exclusively due to sweating, and other metabolic changes which are seen during exercise, such as depletion of body fuels and effects of

muscular activity. In order to investigate the importance of sweat losses of water and electrolytes to the endurance horse in further detail it would seem to be necessary to study the electrolyte composition of equine sweat, a subject about which there is little information at present, and to examine the effects on horses of prolonged sweating unassociated with exercise.

Classification of sweat glands

The horse and man are the only two species which produce large quantities of watery sweat as a major thermoregulatory process during both heat exposure and exercise, while discharges of sweat from other species are generally much smaller in volume (Jenkinson, 1973). However, the basic type of gland which secretes this watery, thermoregulatory sweat is not the same in the two species. The subject of the nomenclature of sweat glands is a contentious one and is summarised by Jenkinson (1967), Sato (1977) and Robertshaw (1981). The labels "apocrine" and "eccrine" were originally attached to equine and human sweat glands respectively on the basis of their assumed modes of secretion (Schieferdicker, 1917). More recently, the applicability of the term "apocrine" (i.e. implying necrobiosis, decapitation or reverse pinocytosis) to the secretion of equine sweat has been questioned (Jenkinson, 1967) and a morphological classification into epitrichal (horse) and atrichal (human) was suggested, based on the association of the gland with a hair follicle (Allen and Bligh, 1969). This classification has not, however, been adopted into general use. The terms "apocrine" and "eccrine" have conventionally been retained but with the understanding that this is a matter of convenience rather than a true description of the modes of secretion of the glands.

The major differences between the sweat glands of the two species, excluding the contentious area of mode of secretion, are that the human gland is primarily cholinergically stimulated and has a duct which opens

directly on to the surface of the skin, while the horse gland is primarily adrenergically stimulated and the duct opens into a hair follicle. The two types of gland have different embryological origins (Sato, 1977).

Apocrine sweat glands have been described in man, but their function is odoriferous rather than thermoregulatory (Shelley, 1951), while eccrine sweat glands have not been described in the horse.

Considering these morphological and physiological differences between the sweat glands of the two species, it is not surprising that the composition of the sweat also varies.

Sweat composition in man

The normal composition of human sweat has been extensively studied and the findings have been reviewed by Robinson and Robinson (1954), Kuno (1956) and Sato (1977). Briefly, the initial precursor fluid produced in the secretory coil is an isotonic ultrafiltrate which is modified as it flows down the sweat duct by the absorption of sodium and chloride in excess of water so that the final secretion at the skin surface is hypotonic compared to plasma for these electrolytes.

Actual sweat sodium and chloride concentrations vary considerably and chloride concentration is generally about 10 - 30 mmol/l lower than sodium at any one time. There is a close direct relationship between sweating rate and sweat sodium and chloride concentration, and the concentration of both electrolytes decreases as a result of acclimatisation to exercise and heat, due to the sodium-conserving action of aldosterone on the sweat gland (Streeten et al, 1960).

Potassium concentration in the precursor sweat is also isotonic, but in contrast to sodium and chloride it is always hypertonic in the final secretion at concentrations which vary from 5 - 35 mmol/l

inversely with sweating rate (and sodium concentration). Sweat potassium concentration, unlike sodium, is higher in heat-acclimatised individuals (Sato, 1977).

Many other substances have been measured in human sweat, but of these only calcium, magnesium, protein, glucose, urea and pH are relevant to this present study.

Calcium is important in the secretory processes of human sweat and appears in the secreted sweat in hypotonic or isotonic concentrations (up to 2.4 mmol/l, Verde et al, 1983). Magnesium is also hypotonic and shows a marked decrease with time during exercise-induced sweating from an initial 0.1 - 0.4 mmol/l to 0.02 - 0.04 mmol/l after one hour (Strømme et al, 1975). Verde et al (1983) have demonstrated sweat calcium and magnesium concentrations in heat-induced sweat to be approximately double those of exercise-induced sweat.

Page and Remington (1967) have recorded a number of proteins in human sweat, including albumin and immunoglobulins, and have reported that the total sweat protein concentration is less than 0.8 g/l.

Sweat glucose has not been studied recently but Lobitz and Osterberg (1947) reported very low concentrations of less than 0.5 mmol/l in both normal subjects and hyperglycaemic individuals (blood glucose up to 14 mmol/l).

In contrast, urea concentrations in sweat are higher than those in plasma, with the sweat urea:plasma urea ratio varying from two to four at low sweating rates to just over one at higher sweating rates (Schwartz et al, 1953; Sato, 1977).

Human sweat is slightly acid at a pH which is dependent on sweating rate and Sato (1977) has suggested that the pH is primarily regulated by the carbonate/bicarbonate buffer system.

Sweat composition in the horse

Some of the earliest investigations into sweat composition were performed in horses (Leclerc, 1888; Smith, 1890), but since then only a very few studies have been carried out compared to the enormous body of work published concerning human sweat.

The original study by Leclerc (1888) reported that horse sweat was alkaline, and recorded protein concentrations of two samples as 11.2 and 15.6 g/l. This protein was assumed to be albumin. Smith (1890) reported very similar findings and suggested that the protein consisted of two "albumins" and two "globulins". He also expressed the opinion that the "debility" seen following excessive sweating in the horse could not be explained by loss of water and electrolytes and must be due to loss of protein. Electrolytes and minerals were also studied in this report and, although the data are hard to interpret, they suggest that fairly high levels were found.

Jirka and Kotas (1959) also identified the protein of horse sweat as mainly albumin with traces of a pre-albumin and four globulin fractions, using an electrophoretic technique. A mucoprotein was also demonstrated. The range of total sweat protein reported in five samples was 7.6 - 34.0 g/l. These authors also analysed two sweat samples (one exercise-induced, the other "emotionally"-induced) for sodium and potassium, and the former also for chloride, and reported values which were markedly hypertonic compared to plasma: sodium 382 - 432 mmol/l, potassium 48 - 141 mmol/l and chloride 432 mmol/l.

Soliman and Nadim (1967) confirmed that horse sweat was alkaline (pH 8.4) and reported mean electrolyte concentrations from 22 samples of: sodium 593 mmol/l, potassium 48 mmol/l and calcium 28.8 mmol/l.

Early studies of human sweat composition also reported hypertonic electrolyte concentrations, but Robinson and Robinson (1954) demonstrated that these were due to faulty sample collection which allowed

sweat to evaporate from the skin. In all of the above studies (except Leclerc, 1888), sweat was collected by scraping it from the exposed skin of horses after exercise. It is therefore not surprising that the suggestion that horse sweat was hypertonic compared to plasma was generally dismissed (Sato, 1977).

More recently, Meyer et al (1978) collected sweat from ponies by absorption into filter paper and reported mean electrolyte concentrations of: sodium 183 mmol/l, potassium 74 mmol/l, chloride 155 mmol/l, calcium 4.74 mmol/l, magnesium 6.99 mmol/l. They also reported that chloride concentration increased with rate of sweating, while calcium and magnesium decreased, and that a high potassium diet resulted in lower sweat sodium concentrations. A high protein diet was shown to increase the total nitrogen concentration of the sweat, but it was not reported whether this nitrogen was protein, urea or other non-protein nitrogen.

Dill et al (1979) used a body washdown sweat collection method in the donkey (Equus asinus) and reported a slightly acid pH (6.5 - 6.9) and electrolyte concentrations similar to those seen in man (sodium 9 - 21 mmol/l, chloride 17 - 47 mmol/l and potassium 3 - 8 mmol/l).

Carlson and Ocen (1979) collected exercise-induced sweat from horses by absorption into gauze pads placed under the saddle and sweat induced by adrenaline infusion from beneath an impermeable plastic barrier. Electrolyte concentrations reported were: exercise-induced sweat, sodium 132 mmol/l, chloride 174 mmol/l, potassium 53 mmol/l, calcium 3.1 mmol/l, magnesium 2.3 mmol/l; adrenaline-induced sweat, sodium 130 mmol/l, chloride 148 mmol/l, potassium 25 mmol/l, calcium 1.5 mmol/l and magnesium 2.1 mmol/l.

Rose et al (1980) also used absorbent pads placed under the saddle to collect exercise-induced sweat and reported electrolyte concentrations of: sodium 249 mmol/l, chloride 301 mmol/l and potassium 78 mmol/l.

With the exception of the study involving the donkey (Dill et al, 1979), there is general agreement among recent investigators that equine sweat is genuinely hypertonic compared to plasma for chloride, potassium, magnesium and possibly sodium and calcium.

The protein content of equine sweat has also been investigated more recently by Jenkinson et al (1974) who reported that cutaneous protein loss during exercise was much greater in the horse compared to man. Electrophoretic studies demonstrated three protein bands in both species, but the mobility of the bands was markedly different: greater than bovine serum albumin in the case of the horse, less in the case of man.

Effect of prolonged sweating on plasma electrolytes

The hypotonicity of human sweat implies that prolonged sweating will lead to water loss in excess of electrolyte loss, and therefore it would be expected that plasma sodium and chloride would tend to rise. Itoh (1952) studied human subjects who did not drink during prolonged periods of heat exposure (with walking exercise) and found that in spite of dehydration and the loss of up to 3.7 litres of sweat, plasma chloride was unchanged. He demonstrated a significant increase in urinary chloride excretion during the experiments and concluded that renal chloride loss increased to prevent hypertonicity of the extracellular fluid. This subject was discussed further by Kuno (1956) who demonstrated that when sweat loss was over four litres, plasma chloride concentration did, in fact, increase. The situation is different when sweat losses are replaced by drinking water, when plasma electrolytes will tend to decrease. Under these conditions there is a decrease in

urinary chloride excretion (Itoh, 1952), and it appears that it requires both the drinking of large amounts of pure water and a significant reduction in renal perfusion to produce the attacks of muscular cramps typically associated with excessive sweat electrolyte losses (Kuno, 1956). Later studies have indicated that fluid shifts between body compartments also play an important part in minimising the disruption of fluid/electrolyte balance caused by sweating (Kozlowski and Saltin, 1964; Costill, 1977).

If horse sweat is hypertonic then it would be expected that plasma electrolytes would tend to decrease in concentration during prolonged sweating whether or not any water is drunk. However, reports of plasma electrolyte changes during endurance rides (Table 6) indicate that while chloride concentration almost always decreases significantly, this may be accompanied by either an increase or a decrease or no significant change in plasma sodium and potassium. Similarly inconsistent findings were also demonstrated in Section 2 of this thesis. Until very recently, the practice of adding electrolyte supplements to the drinking water of competing endurance horses was not widespread and it is known that such additives were not used in the horses involved in the rides reported in Section 2. The reasons for the occurrence of significant increases in plasma sodium (and occasionally potassium) concentrations are therefore not immediately apparent.

Purpose of this section

Costill (1977) has pointed out that in man, muscular activity *per se* causes marked fluid and electrolyte shifts between body compartments, in particular movement of water into active musculature may result in a decrease of up to 13% in plasma volume. It would seem to be desirable in the first instance to study the effects of prolonged sweating in the horse in the absence of any strenuous exercise. In addition, the collection of sweat during exercise is difficult and very susceptible to

errors due to evaporation and contamination. Collection of repeated frequent samples from the same area of the body is almost impossible, as is the collection of comparable samples from different areas of the body. However, when the animal is stationary, the chance of evaporation and contamination of the sample is obviously less and the collection of multiple samples from different parts of the body and at different times becomes possible.

The purpose of this section is therefore to study the alterations in the biochemistry of plasma in horses during a period of several hours' exposure to high environmental temperatures, with particular reference to parameters indicative of fluid and electrolyte status. It is also intended to determine whether thermally-induced sweat in the horse is indeed hypertonic for all the major electrolytes and minerals, whether the composition varies according to the area of the body sampled, and whether the composition remains constant during a prolonged period of sweating. This experiment was carried out both on Thoroughbred horses and on small ponies to determine whether there are any significant differences in sweating responses between breeds, as has been suggested by a previous study (Langley and Bennett, 1923).

Materials and Methods

1. Animals

Horses B, C, D and E and ponies V, W, Y and Z were used.

2. Management

For the horses the diet, exercise and management routine was as described in Section 1. The ponies were stabled similarly to the horses, were fed 3 kg hay daily with no concentrate feed and were not exercised. This experiment was carried out from May to early June after a very cold, late winter and so none of the animals was acclimatised to heat.

3. Procedure

(a) **Heat exposure**

This experiment was carried out at the Hannah Research Institute, Ayr, in a climatic room described by Findlay et al (1959). The temperature was maintained at 41°C, 33°C wet bulb. The animals were exposed to this environment in pairs and were restrained in stocks constructed in the climatic chamber. Immediately the animal entered the room, a ventilated capsule as described by McLean (1963) was fixed to the back in the saddle region and a thermistor probe was inserted into the rectum. The ventilated capsules proved ineffective for continuous monitoring of the sweating rates as the rate of air passage through the capsules was insufficient to evaporate all the sweat formed within that area. They did, however, allow the exact time of onset of sweating to be determined for each animal. Heart rate was monitored by auscultation, respiratory rate by observation and rectal temperature by thermistor probe. The animals were weighed immediately before entering the chamber and again immediately after leaving, and all faeces passed during the experiment were added to the second weighing. None of the animals urinated while in the climatic room and so this bodyweight loss

could be regarded as approximately equal to sweat loss plus respiratory water loss. The duration of the heat exposure experiment was six hours for horses C and E and ponies V and W, and five hours for horses A and D and ponies Y and Z.

Blood samples were collected from each animal immediately before entering the climatic chamber and hourly thereafter. PCV analysis was performed at once, all samples were then immediately centrifuged and the plasma stored at -20°C until analysis. Plasma samples were analysed for total protein, sodium, potassium, chloride, calcium, magnesium, phosphate, urea, creatinine and cortisol by the methods described.

No urine samples were obtained in this experiment.

Sweat samples were collected as described below as soon as possible after the onset of sweating and at regular intervals thereafter. These were immediately centrifuged at 700 g to remove dust and epithelial debris and stored at -20°C until analysis. Sweat samples were analysed for sodium, potassium, chloride, magnesium, glucose and total protein by the methods described. Sweat calcium concentrations were not determined in this experiment as it was found that calcium salts had precipitated from solution in the sweat after a short period of storage. It appears that sweat calcium analysis must be performed within 24 hours of sample collection.

(b) **Control experiments**

These were carried out one to four days before the heat exposure in the horses and in ponies V and W. The heat exposure experiment was delayed, however, until four weeks after the control experiment in ponies Y and Z. The procedure was exactly as described above (apart from the sweat samples) but with the temperature of the climatic room maintained at 15°C, 10°C wet bulb. The duration of the control experiments was six hours in all animals.

4. Sweat sample collection

The ponies still retained a significant amount of their thick winter coats at the time of the experiment and they were therefore trace-clipped a week before the experiment took place. The horses' finer summer coats were not clipped and none of the animals was washed. All animals were regularly and carefully groomed and all items which came in contact with the sweat samples were carefully rinsed in deionised water and dried before use. Sweat was collected from the neck by gently scraping it into a beaker with a plastic spatula and from the body by holding a plastic tray under the abdomen to collect the falling drops.

5. Skin biopsies

Skin biopsies were taken by power-driven rotary punch from the thoraco-lumbar region of one of the horses and all four ponies at the beginning and end of both the control and the heat exposure periods, and also immediately after the onset of sweating on the latter occasion. The findings relating to these biopsies are not included in this thesis but have been reported elsewhere (Montgomery et al, 1982).

6. Statistical analysis

(a) **Blood samples**

Marked differences between the horses and the ponies were noted in certain parameters and so the mean results are presented separately, i.e. $n = 4$ for both horses and ponies. All comparisons are made using the paired t test.

(b) **Sweat samples**

Again horses and ponies are presented separately, as are samples from the two body areas, i.e. $n = 4$ for each point. Comparisons between body areas are made using the paired t test and between horses and ponies using the unpaired t test.

Results

A. Clinical measurements

The control experiments lasted six hours in all cases. During this period there was no significant change in rectal temperature, heart rate or respiratory rate in either the horses or the ponies and these resting clinical parameters are presented in Table 29, together with the percentage bodyweight decrease during the control period.

The period of heat exposure was six hours for horses C and E and for ponies V and W. However, due to the adverse clinical responses recorded below, horse D and pony Y were removed from the chamber after only five hours, together with horse B and pony Z who were in the chamber at the same time.

In all animals except horse D and pony Y, rectal temperature, heart rate and respiratory rate increased initially to a maximum after two hours' heat exposure, and subsequently remained steady or decreased slightly. In horse D and pony Y these parameters all continued to increase throughout the experiment. This was accompanied by signs of agitation and increasing discomfort until after five hours, when the heart rates of both animals were over 100 beats/min, they were removed from the hot environment. Both animals regained clinical normality soon afterwards.

The maximum increases in rectal temperature and heart rate, maximum respiratory rate (all at two hours in animals B, C, E, V, W and Z and at five hours in animals D and Y), together with percentage bodyweight decrease during the experiment and the delay in onset of sweating after entering the hot environment, are presented for each animal in Table 30.

TABLE 29

Clinical parameters measured during control experiment (15°C, 10°C wet bulb for 6 hours)

	Rectal temperature (°C)	Heart rate (beats/min)	Respiratory rate (breaths/min)	Body weight lost (%)
Horses	37.5 ± 0.03	39 ± 0.7	12 - 15	0.37 ± 0.05
Ponies	37.8 ± 0.08	48 ± 1.2	12 - 15	0.56 ± 0.03

TABLE 30

Clinical changes in the animals during exposure to 41°C (33°C wet bulb)

Animal	Duration of exposure (hours)	Delay in onset of sweating (min)	% Body weight lost	Max. increase in rectal temperature (°C)	Max. increase in heart rate (beats/min)	Max. respiratory rate (respirations/min)
Horse						
B	5	0	2.64	1.2	10	140
C	6	18	2.64	1.0	15	140
D	5	0	4.94	1.9	65	210
E	6	0	5.21	0.4	15	140
Pony						
V	6	18	3.01	0.7	45	140
W	6	93	1.73	1.4	30	200
Y	5	8	8.95	1.8	80	230
Z	5	14	3.10	1.5	20	200

B. Blood samples

Changes in PCV and plasma parameters measured during heat exposure in both the horses and the ponies are compared to the control period in Table 31 and in Figure 21. Because the experimental period lasted only five hours in half of the animals, only the first five hours of all experiments are presented. There was, in any case, little or no change in these parameters in the sixth hour.

The individual differences in the reaction to the hot environment were reflected to a very large extent in the results of the blood samples. In several instances one or two animals showed large changes, while in the other animals little or no change was seen. Together with the small number of animals used, this made strict statistical interpretation of the results very difficult, and in many instances it is more informative to consider the animals as individuals (Table 31).

PCV

During the control period, PCV tended to decrease but the changes were very small. During the first hour of heat exposure, PCV of the horses also decreased similarly to the control period. After that there was a general increase but with the exception of horse D it was of small magnitude and the change was not statistically significant. In the ponies, PCV also showed a small, non-significant increase during heat exposure.

Total plasma protein

During the control period plasma protein concentration did not change significantly. During heat exposure there was a significant increase ($p < 0.05$ for both horses and ponies) which was greater in the horses than the ponies. Again individual differences were very marked, with horse D showing a very large rise (44% of the pre-experiment value) while the increases seen in horse B and all of the ponies were no greater than some of the changes seen during the control period.

The horses had significantly higher PCV's and significantly lower total plasma protein concentrations than the ponies ($p < 0.001$ for both parameters comparing the first samples on the control days).

Sodium

Plasma sodium concentration also showed no significant change during the control period or in the horses during the heat exposure. The slight decrease seen in the ponies during heat exposure was significant ($p < 0.02$). Individual differences in the horses during heat exposure were again marked, with horse E showing a substantial increase.

Potassium

The marked fall in plasma potassium during heat exposure was significant in both the horses ($p < 0.01$) and the ponies ($p < 0.001$). There was, however, also a significant fall during the control period in the horses ($p < 0.02$). This decrease was of a lesser magnitude and the plasma potassium after five hours' heat exposure was significantly lower than after five hours of the control experiment ($p < 0.01$).

Chloride

Plasma chloride concentration did not change during the control period, and during heat exposure there was a decrease of similar magnitude in both the horses and the ponies, but in the ponies this decrease was significant ($p < 0.001$) while in the horses it was not, again due to individual variations. Once more horse D showed the greatest change and horse B the least.

Calcium

There was no significant change in plasma calcium concentration during either the control or the heat exposure experiments.

The plasma calcium in the ponies at the time of the control experiments was significantly lower than that of the horses ($p < 0.001$, comparing the first samples on the control days). The apparently higher

values seen in the ponies during heat exposure were entirely due to ponies Y and Z who underwent the heat exposure period four weeks later than all the other pony experiments.

Magnesium

The mean decreases seen in plasma magnesium concentration in both horses and ponies were almost identical in magnitude in the control and the heat exposure periods. Although the concentration decreased consistently in all animals during heat exposure, the wide variation in the magnitude of the changes resulted in a lack of statistical significance. During the control period the decrease in magnesium concentration in the horses was significant ($p < 0.02$).

Inorganic phosphate

During the period of heat exposure changes in plasma phosphate concentration varied between animals and were not statistically significant. During the control experiment there was a significant increase in phosphate in the horses ($p < 0.01$) while in the ponies there was a general (non-significant) tendency for it to decrease.

Creatinine

Plasma creatinine concentration did not change significantly during the control period. During the heat exposure there was a significant increase in the horses ($p < 0.05$), but the consistent increase seen in the ponies was not statistically significant. Again there were marked individual differences between animals, and the increases seen in five animals during heat exposure were no greater than increases which occurred in two (other) animals during the control period.

The apparently higher plasma creatinine concentrations seen in the ponies at the beginning of the heat exposure period compared to the control experiment were due, as for calcium, entirely to higher initial values for ponies Y and Z in the later experiment.

Urea

Plasma urea concentration also did not change significantly during the control period and increased significantly during heat exposure in the ponies ($p < 0.02$). The consistent increase seen in the horses during heat exposure was not statistically significant. Individual differences between animals were again evident, with horse D showing a marked rise while the increases in horse E and pony Z were no more than that seen in horse E during the control period.

Cortisol

This was the parameter in which individual differences were most obvious. During the control period concentrations showed a general tendency to increase in the horses and to decrease in the ponies, but neither change was statistically significant. The results from pony Z were always very variable, ranging from 560 - 947 nmol/l on the control day and from 443 - 928 nmol/l during the heat exposure period. During heat exposure, changes in plasma cortisol varied from a decrease of 89 nmol/l (pony V) to an increase of 1259 nmol/l (horse D). These variations again led to the changes not being statistically significant for either horses or ponies. However, the two animals which showed the adverse clinical response to the heat exposure (horse D and pony Y) also had much the largest increases in plasma cortisol.

TABLE 31 (a)

Changes in PCV and plasma parameters during five hours in environmental chamber at control temperature (15°C, 10°C wet bulb)

Animal	ΔPCV	ΔTotal protein (g/l)	ΔNa^+ (mmol/l)	ΔK^+ (mmol/l)	ΔCl^- (mmol/l)	ΔCa^{2+} (mmol/l)	ΔMg^{2+} (mmol/l)	ΔPO_4^{2-} (mmol/l)	ΔCreatinine (μmol/l)	ΔUrea (mmol/l)	ΔCortisol (nmol/l)
Horse											
B	-0.04	-3	-1	-1.2	-3	-0.78	-0.13	+0.30	-9	-0.7	+90
C	-0.03	+8	+1	-0.5	-1	+0.36	-0.18	+0.43	+35	+0.3	+68
D	+0.01	+6	+1	-0.3	+2	-1.01	-0.31	+0.32	0	-0.6	+10
E	-0.03	-1	0	-0.3	-1	+0.03	-0.15	+0.40	+26	+0.4	+197
Mean	-0.02	+2.5	0	-0.6	-1	-0.35	-0.19	+0.36	+13	-0.15	+91
±SEM	±0.01	±2.7	±0.5	±0.2	±1	±0.33	±0.04	±0.03	±10	±0.29	±39
Pony											
V	-0.04	+5	+1	-0.7	-2	+0.11	-0.25	-0.41	0	-0.3	0
W	-0.01	+1	+4	-0.7	0	-0.28	-0.23	-0.10	-17	-0.2	-6
Y	0	-4	0	+0.9	+1	-0.81	-0.03	-0.29	-9	+0.2	-136
Z	+0.02	+2	+3	+1.0	+3	+0.25	+0.02	-0.07	0	-0.2	-387
Mean	-0.01	+1.0	+2	+0.1	+0.5	-0.18	-0.12	-0.23	-6.5	0	-132
±SEM	±0.01	±1.9	±0.9	±0.5	±1.0	±0.24	±0.07	±0.08	±4.1	±0.1	±90

TABLE 31 (b)

Changes in PCV and plasma parameters during five hours exposure to a hot environment (41°C, 33°C wet bulb)

Animal	ΔPCV	ΔTotal protein (g/l)	ΔNa^+ (mmol/l)	ΔK^+ (mmol/l)	ΔCl^- (mmol/l)	ΔCa^{2+} (mmol/l)	ΔMg^{2+} (mmol/l)	ΔPO_4^{2-} (mmol/l)	ΔCreatinine (μmol/l)	ΔUrea (mmol/l)	ΔCortisol (nmol/l)
Horse											
B	+0.01	+6	0	-1.1	-1	+0.03	-0.09	+0.10	+35	+0.5	+105
C	+0.02	+10	+3	-1.0	-4	+0.09	-0.08	+0.20	+53	+0.5	+237
D	+0.10	+27	-1	-1.1	-9	+0.25	-0.59	-0.28	+35	+1.8	+1259
E	+0.02	+16	+10	-1.7	-6	+0.27	-0.05	+0.34	+89	+0.4	+209
Mean	+0.04	+15	+3	-1.2	-5	+0.16	-0.20	+0.09	+53	+0.8	+453
±SEM	±0.02	±4.6	±2.5	±0.2	±1.7	±0.06	±0.13	±0.13	±13	±0.33	±270
Pony											
V	+0.03	+7	-1	-1.2	-6	+0.05	-0.06	+0.03	+27	+1.0	-89
W	+0.01	+3	-1	-1.3	-6	+0.41	-0.12	-0.08	+26	+0.5	+91
Y	+0.02	+2	-1	-1.1	-8	-0.53	-0.19	-0.48	+62	+0.7	+521
Z	0	+7	-2	-1.3	-6	-0.38	-0.02	-0.31	+9	+0.4	+126
Mean	+0.015	+5	-1	-1.2	-6.5	-0.11	-0.10	-0.21	+31	+0.65	+162
±SEM	±0.006	±1.3	±0.2	±0.05	±0.5	±0.21	±0.04	±0.11	±11	±0.13	±129

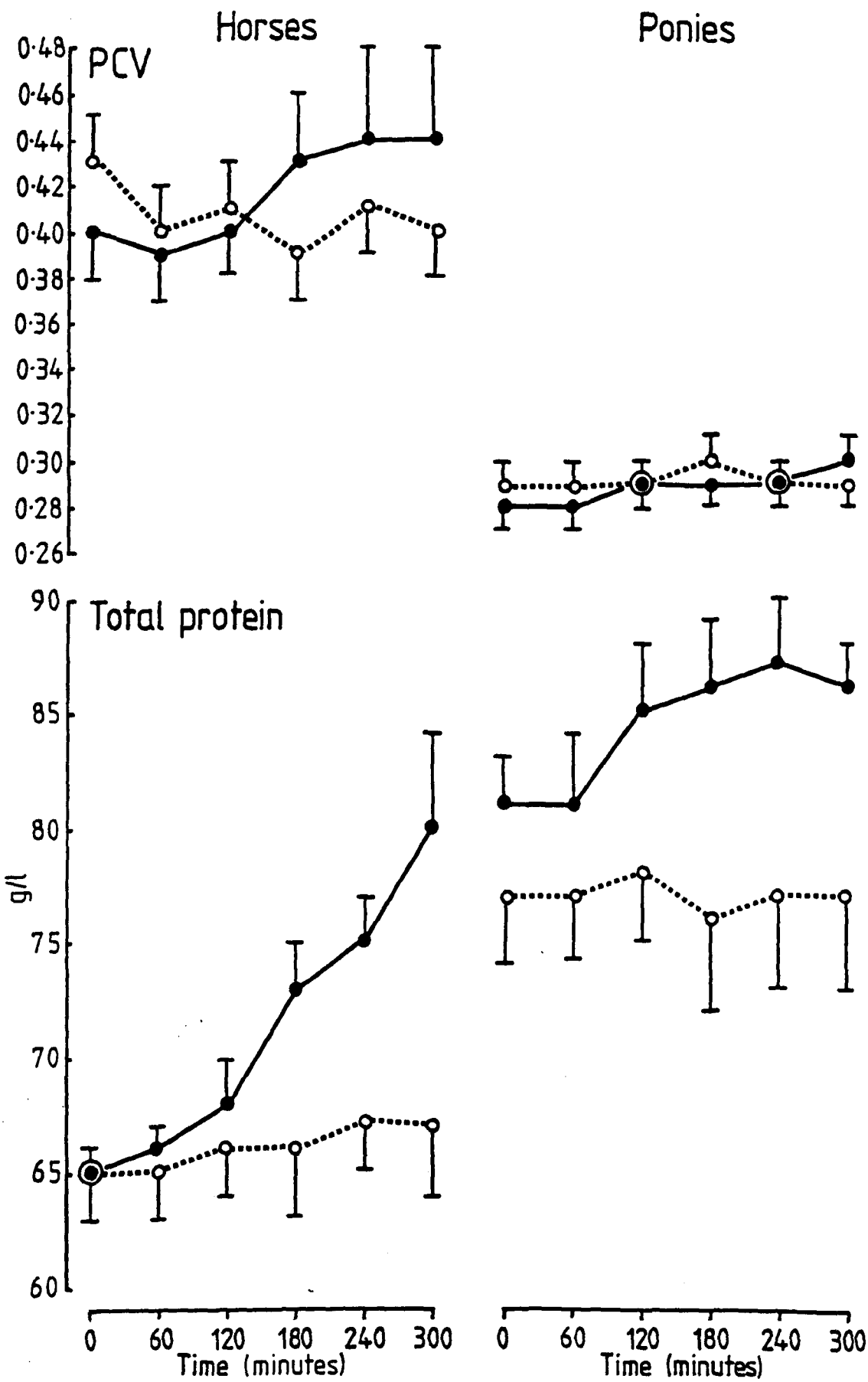


Figure 21(a). Changes in PCV and total plasma protein concentration in horses and ponies during heat exposure (41°C, 33°C wet bulb) (solid line) compared to a control period (15°C, 10°C wet bulb) (broken line).

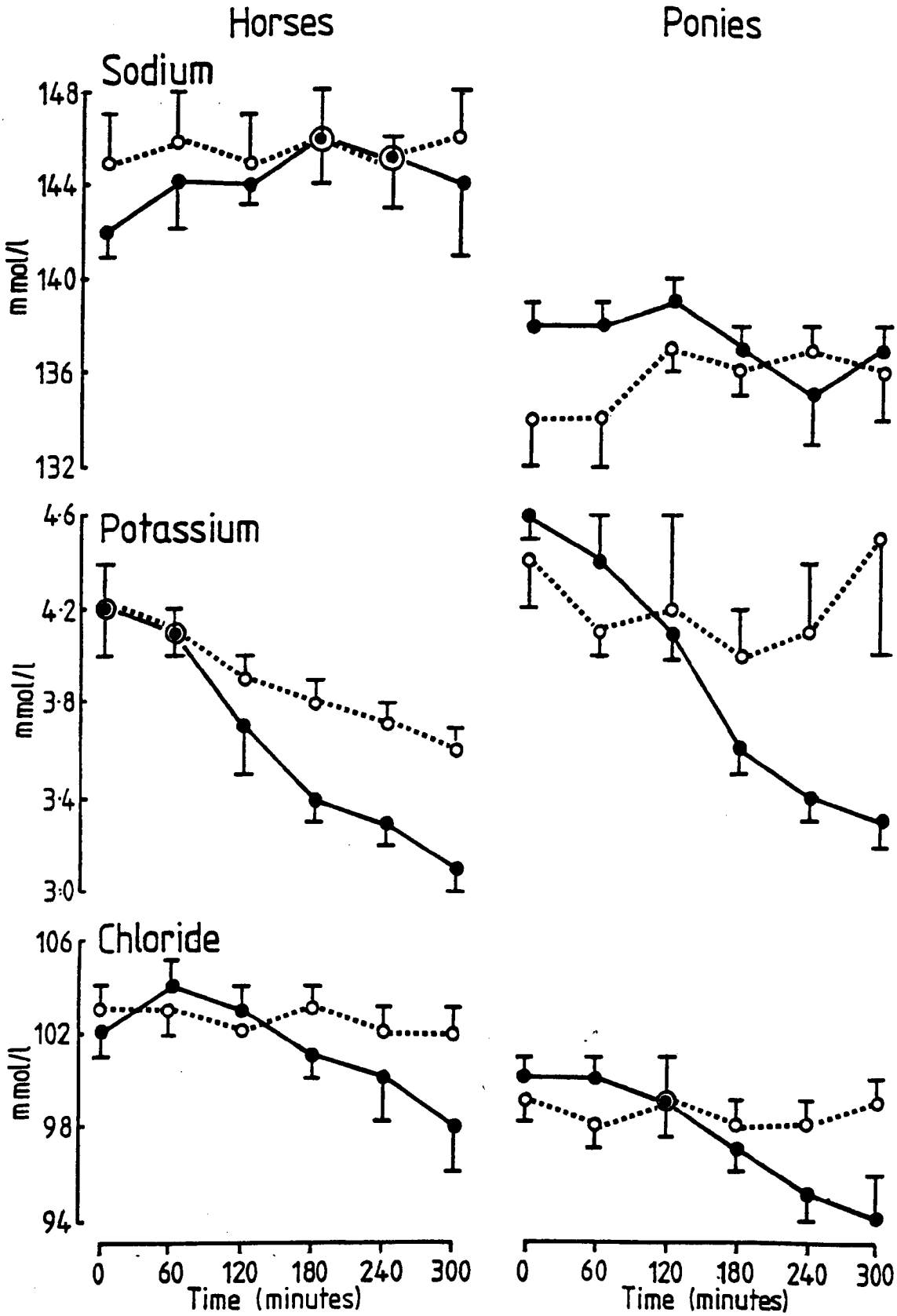


Figure 21(b). Changes in plasma electrolyte concentrations in horses and ponies during heat exposure (41°C, 33°C wet bulb) (solid line), compared to a control period (15°C, 10°C wet bulb) (broken line).

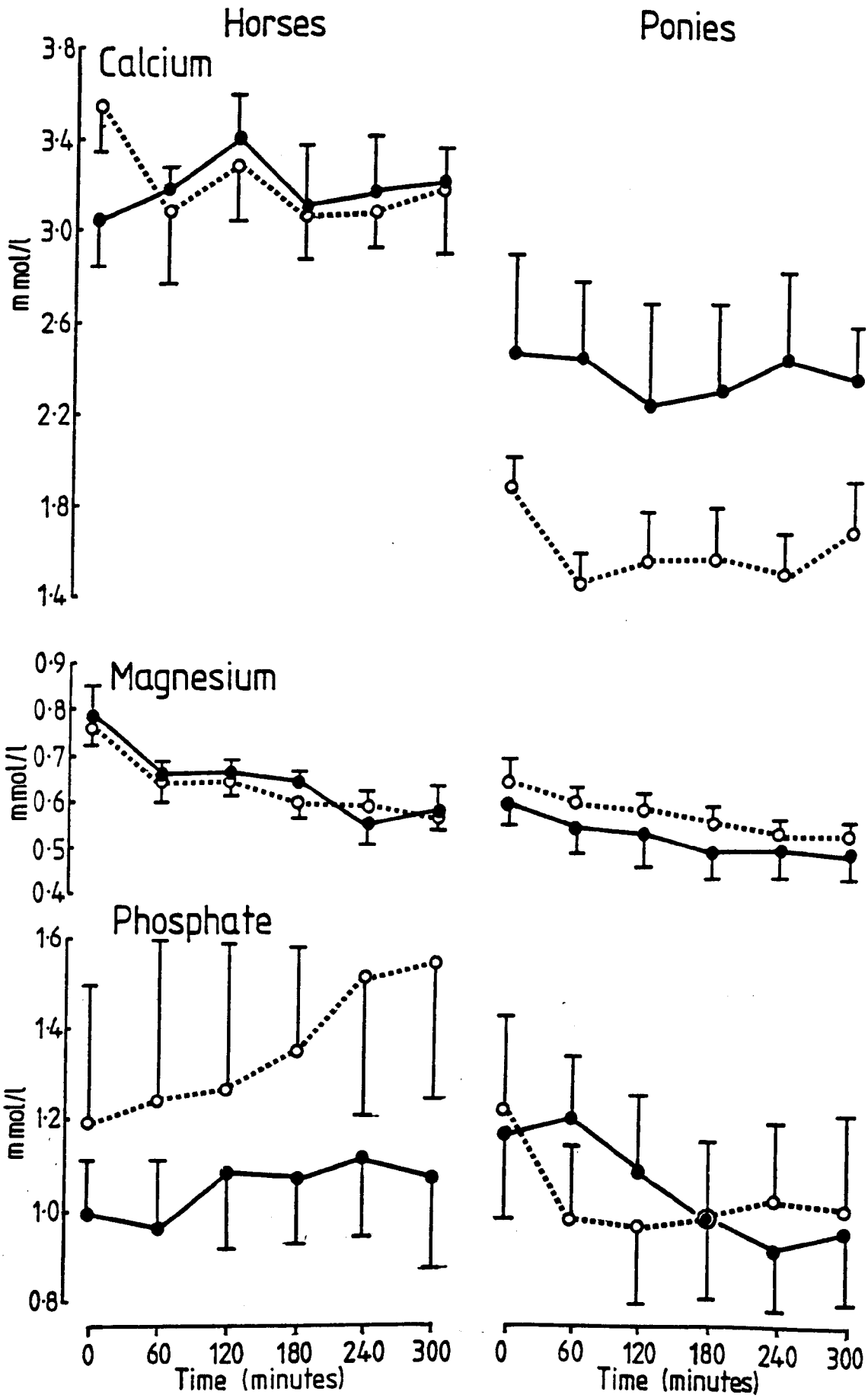


Figure 21(c). Changes in plasma mineral concentrations in horses and ponies during heat exposure (41°C, 33°C wet bulb) (solid line) compared to a control period (15°C, 10°C wet bulb) (broken line).

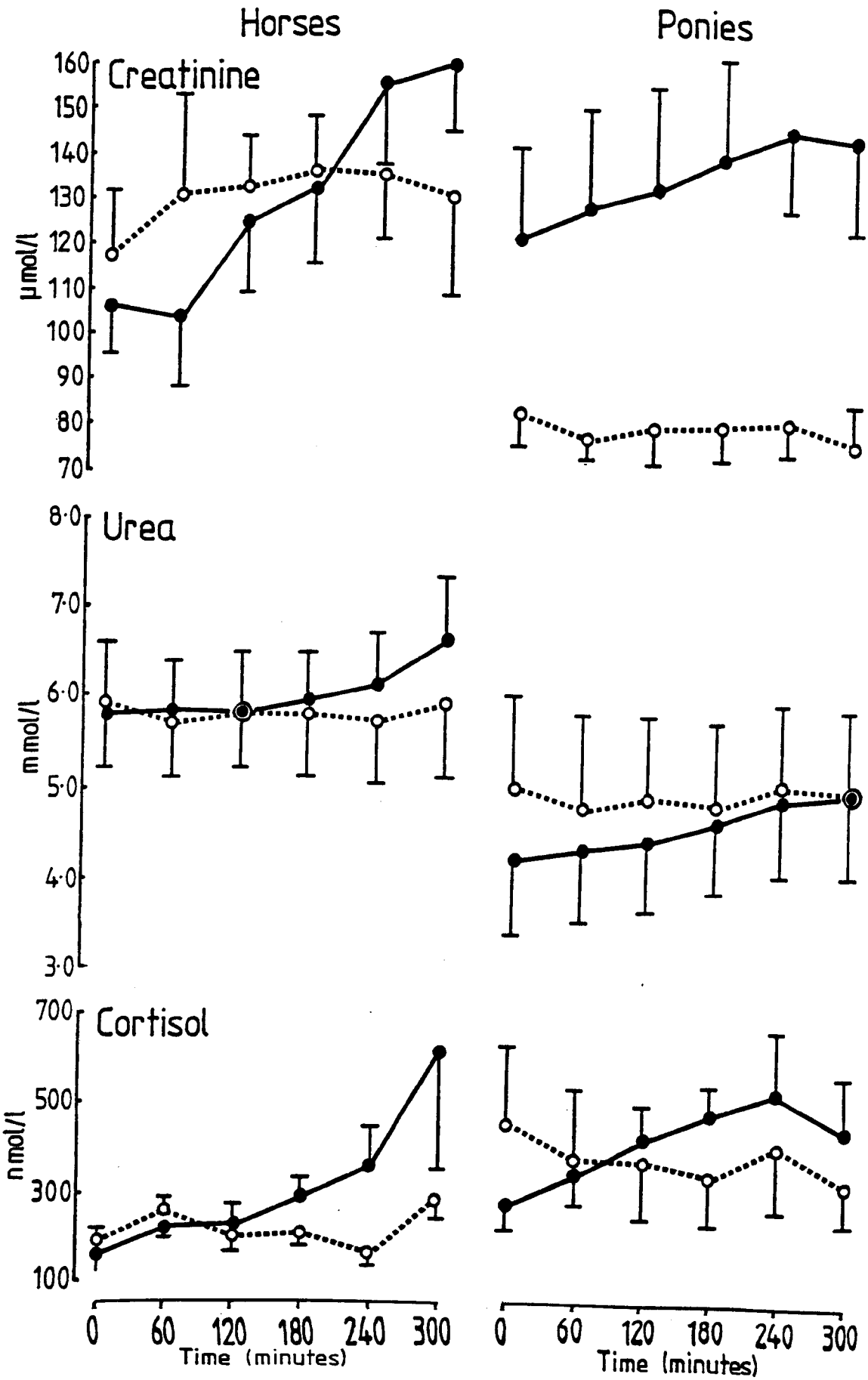


Figure 21(d). Changes in plasma creatinine, urea and cortisol concentrations in horses and ponies during heat exposure (41°C, 33°C wet bulb) (solid line) compared to a control period (15°C, 10°C wet bulb) (broken line).

C. Sweat samples

Delay in onset of sweating after entry into the hot environment was variable (Table 30). Sweating rates could not be determined by the ventilated capsule technique because the rates were so high that sweat ran continually from the body. However, sweating was observed subjectively to increase rapidly to a maximum which was maintained for about four hours. After this time sweating rates appeared to decrease and by the end of the experiment sweating had almost ceased in some animals, so that collection of a final sweat sample was impossible. Sweat samples which were collected after 4.5 hours (270 minutes) were obtained with difficulty and it was apparent that the sweat was drying on the skin in spite of the humid atmosphere. Electrolyte concentrations of these samples were markedly higher than those obtained earlier in the experiment and, as it was considered that this was due to evaporation having occurred, results from these samples are omitted.

Sweat appearance

Sweat was initially opaque with a slight yellow colouration. The yellow colour disappeared gradually as sweating continued, but, although the opacity also decreased, it did not disappear completely.

Sweat chloride, sodium and potassium concentrations

The patterns of change in sweat electrolyte concentrations with time are presented in Figure 22. The only significant change with time was in the sodium concentrations of the samples from the horses' necks which increased from 134 ± 4 mmol/l in the first sample to 161 ± 3 mmol/l in the last ($p < 0.01$).

The first sweat samples collected from the ponies appeared slightly dirty compared to the later ones and, although the difference was not statistically significant, the electrolyte concentrations were in several cases much higher. It was therefore feared that the samples

TABLE 32

Mean electrolyte concentrations (mmol/l) of sweat from the neck and body of horses and ponies during heat exposure

		Chloride		Sodium		Potassium	
		Neck	Body	Neck	Body	Neck	Body
Horse	B	211 ± 5	234 ± 6	149 ± 4	158 ± 3	61 ± 2	71 ± 5
	C	198 ± 7	218 ± 6	138 ± 4	146 ± 3	65 ± 4	67 ± 5
	D	184 ± 3	189 ± 7	143 ± 4	143 ± 4	36 ± 2	44 ± 4
	E	179 ± 5	173 ± 9	151 ± 7	142 ± 2	39 ± 2	40 ± 4
Mean		193 ± 7	203 ± 14	145 ± 3	147 ± 4	50 ± 7	55 ± 8
Pony	V	295 ± 8	259 ± 7	199 ± 1	173 ± 2	73 ± 5	68 ± 3
	W	253 ± 3	249 ± 8	190 ± 2	178 ± 6	58 ± 4	54 ± 2
	Y	288 ± 18	219 ± 9	209 ± 5	151 ± 1	82 ± 8	66 ± 7
	Z	188 ± 7	194 ± 4	168 ± 6	168 ± 3	35 ± 2	39 ± 1
Mean		256 ± 24	230 ± 15	191 ± 9	167 ± 6	62 ± 10	57 ± 7

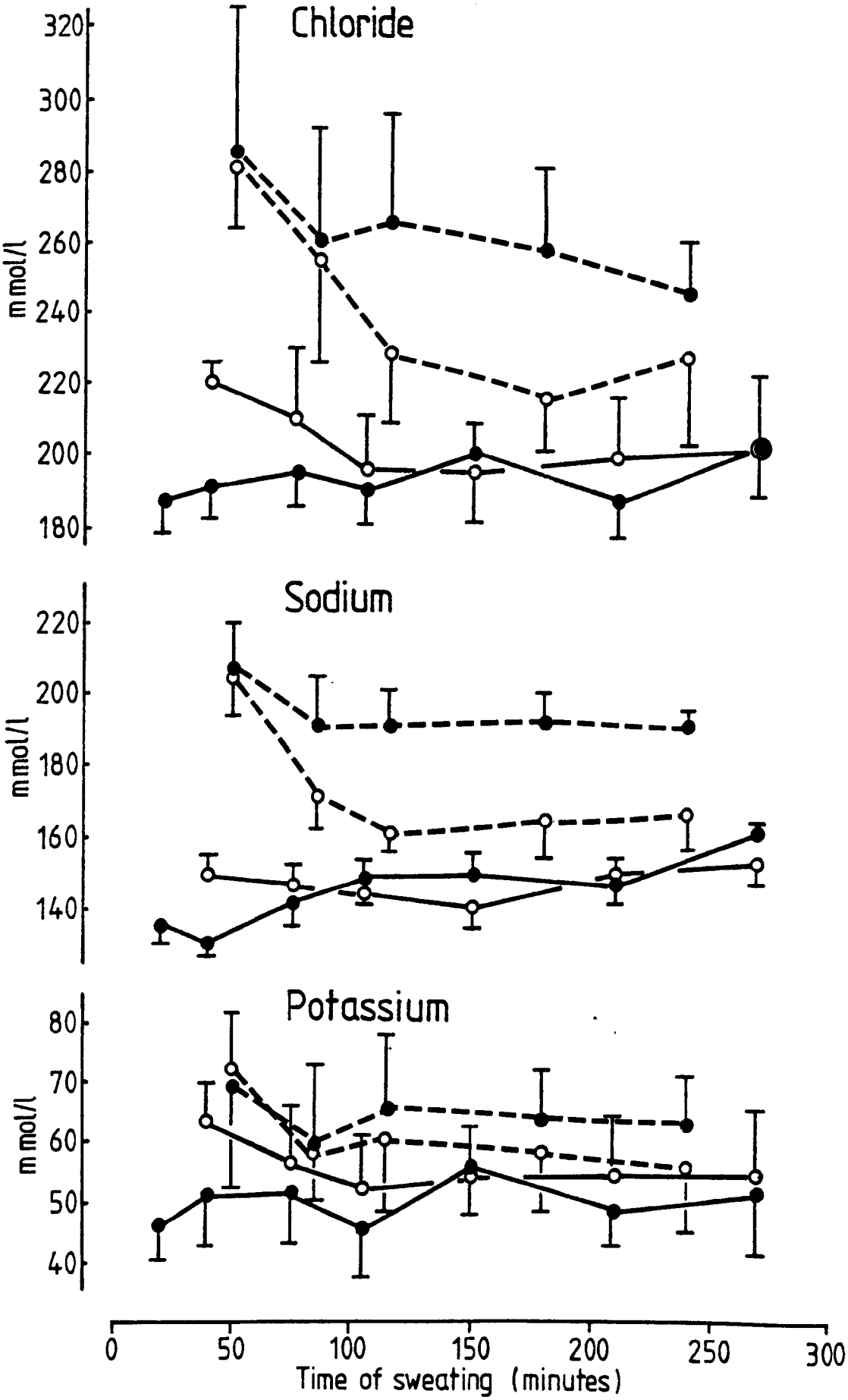


Figure 22. Changes in electrolyte concentrations of heat-induced sweat with time.

——— horses
– – – – ponies

● sweat from neck
○ sweat from body.

might have been contaminated in spite of the grooming procedure, and so they were omitted from the calculation of the mean results described below.

In order to compare sweat electrolyte concentrations between the body areas sampled and between horses and ponies, a mean of all results from each body area of each animal (except the first samples from the ponies) was calculated (Table 32).

No significant differences were found between the two body areas, but the sodium concentration was significantly higher in the ponies than the horses ($p < 0.001$).

Sweat magnesium and protein concentrations

The patterns of change of sweat magnesium and protein concentrations (Figure 23) were markedly different from those of the electrolytes. Concentrations were initially high and decreased in an exponential manner until four hours (240 min) of sweating, after which they remained constant. There was a close correlation ($r = 0.985$) between magnesium and protein in all samples.

There was no significant difference between the horses and the ponies, but concentrations of both parameters appeared to be generally slightly higher in the sweat from the body. It was however considered that this was probably an artefact of the different sweat collection methods, in that the absence of any active removal of sweat from the body resulted in a longer delay between sweat secretion and collection of a sample. The results from all samples were combined to obtain exponential regression equations of the form $y = Ae^{Bx}$ where x is time of sweating (minutes) and y is concentration. For magnesium, $A = 15.03$, $B = -0.0105$ and $r = 0.747$. For protein, $A = 14.94$, $B = -0.0113$ and $r = 0.780$.

Sweat glucose

Glucose concentration of all samples of heat-induced sweat was under 0.5 mmol/l.

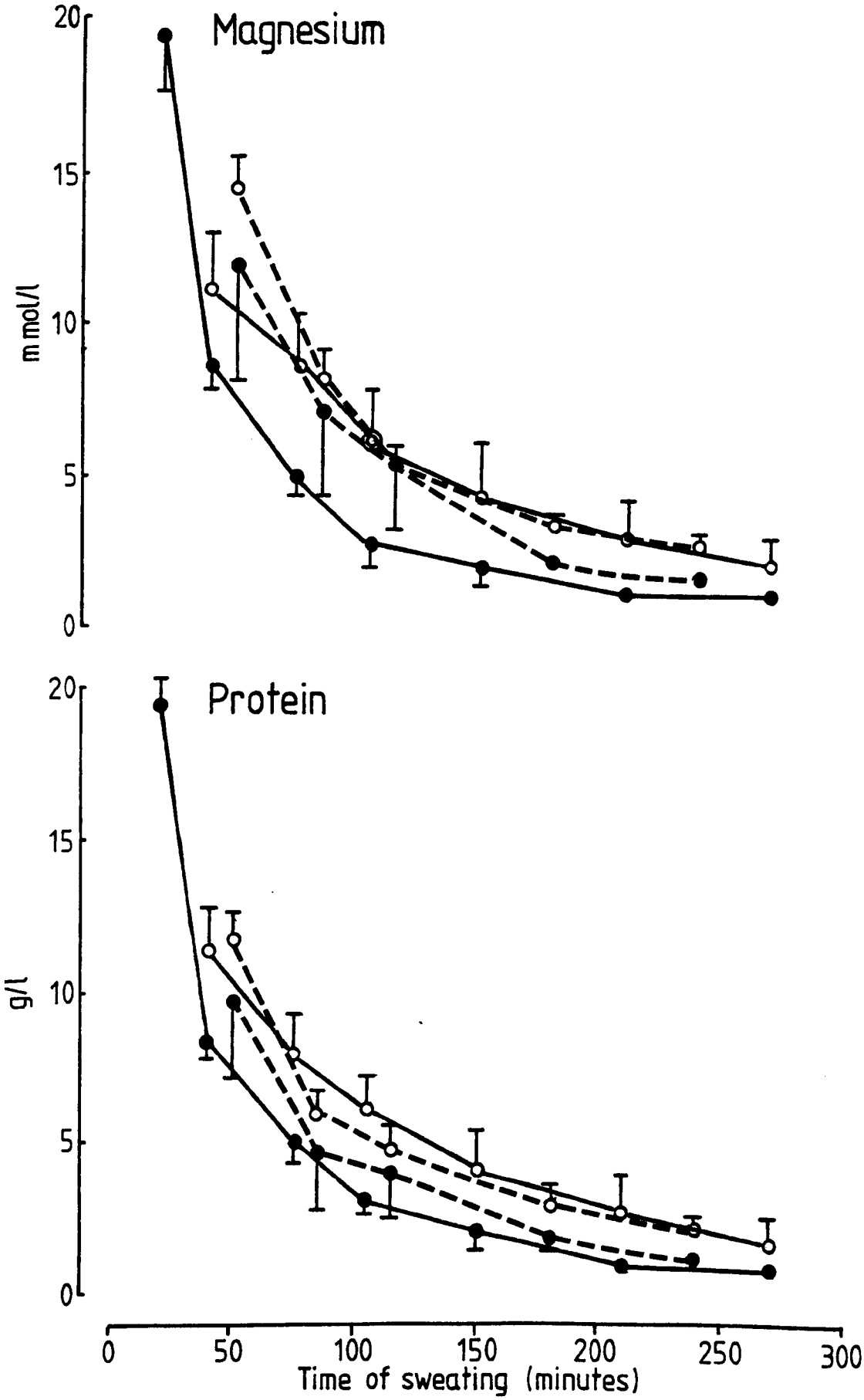


Figure 23. Changes in magnesium and protein concentrations of heat-induced sweat with time. Symbols as Figure 22.

D. Calculation of respiratory water loss

It was not possible to measure sweating rate directly, and therefore the proportion of bodyweight loss which was due to respiratory water loss could not be calculated directly. Indirect calculation of this figure is possible, however. The total fluid loss (bodyweight decrease) is assumed to be borne entirely by the extracellular fluid, and the total (ECF) sodium loss is calculated from this assumption. The total sweat loss which would account for this sodium loss is then calculated from the mean sweat sodium concentration measured in that animal.

Specimen calculation for horse B, assuming total ECF volume (l) is equal to 0.22 x bodyweight (kg) (Carlson et al, 1979a):

Before heat exposure:

Bodyweight = 495.5 kg => ECF volume = 116.4 l.

Plasma $[Na^+]$ = 141 mmol/l => total ECF Na^+ = 16.41 moles.

After heat exposure:

Bodyweight loss = total ECF fluid loss = 13.1 l

=> ECF volume = 103.3 l.

Plasma $[Na^+]$ = 141 mmol/l => total ECF Na^+ = 14.57 moles.

Total ECF Na^+ loss = 1.84 moles.

Mean sweat $[Na^+]$ = 153 mmol/l

=> total sweat loss = 12.0 l

=> respiratory H_2O loss = 1.1 l

= 8.4% of total ECF fluid loss.

This calculation was carried out for all animals and the results are presented in Table 33.

This calculation rests on certain assumptions which are not entirely valid, in particular, any contribution of the intracellular fluid is ignored, and the fact that all animals' coats were wet when they were weighed after the experiment means that the total fluid loss

has been underestimated. It is therefore not surprising that calculated sweat loss appears to equal or even exceed total fluid loss in three animals. However, the mean figure for all eight animals suggests that about 10% of the total fluid loss was due to respiratory evaporation, which is close to the 11 - 15% measured by Dill et al (1979) in donkeys exposed to 38 - 41°C.

TABLE 33

Total bodyweight loss, calculated sweat loss and calculated respiratory water loss for all animals during heat exposure

Animal	Total bwt decrease (kg)	Calculated sweat loss (l)	Respiratory H_2O loss (as % bwt decrease)
B	13.1	12.0	8.4
C	14.0	14.2	0
D	25.2	26.2	0
E	24.5	20.6	15.9
V	7.4	5.8	21.6
W	2.7	2.7	0
Y	12.8	9.9	22.7
Z	4.3	3.8	11.6
Mean ± SEM			10 ± 3%

Discussion

Clinical effects of heat exposure

It was interesting to note the extent to which panting appeared to be important for thermoregulation in this environment. Horses cannot breathe through the mouth under normal circumstances and their "panting" takes the form of rapid, shallow breathing through flared nostrils. They appear to use the extensive area of the turbinate mucous membrane for heat exchange in the same way as a dog uses the oral mucous membrane. The fact that total respiratory water loss was calculated to be only 10% of total body fluid loss (or a little more, due to the error introduced by the animals' wet coats when weighed after the experiment) does not reflect its relative importance in thermoregulation, as all the respiratory water was evaporated while most of the sweat ran on to the floor and was therefore ineffective. In pony W, who did not begin to sweat until 1.5 hours after entering the hot environment, this "panting" at 200 respirations/min was the only active thermoregulatory response noted at first. After sweating began, her respiratory rate fell to 150 respirations/min.

Robertshaw (1974) believed horses to have a poorly-developed panting mechanism, but the ability of the normal horse to pant has been noted previously (Arnold, 1950) and the marked increase in the panting response seen in horses unable to sweat due to anhydrosis has frequently been highlighted (Evans et al, 1957b; Marsh, 1961; Correa and Calderin, 1966). However, endurance horses which are acclimatised to environmental conditions similar to those in the present study, as is the case in southern California, are not observed to pant in this manner (Carlson, personal communication). It is possible that, in horses, the panting response is reserved for acute or emergency situations, particularly when sweating is prevented or, as in the present study, ineffective.

One of the most striking features of this experiment was the wide individual variation in reaction to the hot environment. Horse B appeared quite unperturbed and his heart rate after five hours was the same as at the beginning of the experiment (37 beats/min). This horse was, in fact, observed to be shivering slightly during the control experiment. In contrast, horse D, who was in the environmental chamber at the same time as horse B, became violently agitated and was removed from the hot environment with a heart rate of 105 beats/min. The same range of responses was seen in the ponies. Follenius et al (1979) also noted a poor clinical tolerance to heat exposure in two out of eight human subjects on a low-sodium diet in a similar but shorter experiment. As in this present study, the poor tolerance of the hot environment was accompanied by an exaggerated rise in plasma cortisol. The reason for the poor heat tolerance in these particular individuals was not known, nor is it clear in this present study. Horse D and pony Y showed the two greatest increases in rectal temperatures, but as their respiratory rates were also the highest seen and their bodyweight losses among the greatest, it appears that deficiencies in panting or sweating were not responsible for this. The agitated behaviour was noted in these animals well before the rectal temperatures had risen any higher than those of the other animals, and it seems likely that their higher rectal temperatures at the end of the experiment were a result of this behaviour rather than the cause of it. There was no physical or other factor peculiar to horse D and pony Y which could explain this occurrence.

The high humidity in this experiment produced conditions which were very poor for evaporative heat loss. Most of the sweat secreted ran from the body on to the floor and was therefore ineffective in cooling the animal. The panting was probably more effective due to the movement of air generated, but must also have been hindered by the high humidity. In spite of this, six of the eight animals succeeded in stabilising

their rectal temperatures within two hours and were not distressed even after five or six hours in this environment. This indicates that even completely unacclimatised horses generally have a high tolerance to acute heat exposure.

Blood samples

The individual differences in reaction to the hot environment were reflected in the plasma biochemistry to a very large extent, and this, together with the small number of animals used, makes strict statistical interpretation of the results very difficult. In particular, horse D showed much the greatest increases in PCV, total plasma protein and cortisol, while these parameters changed very little in horse B.

PCV and total plasma protein

The initial decrease seen in the PCV's of the horses in both the control and the heat exposure periods was probably the return to normal of an increase caused by apprehension at entering an unfamiliar environment. The much less marked response of the spleen to excitement in pony breeds is well-documented, as is the general tendency for the resting PCV to be lower (Schalm *et al*, 1975). The lower total plasma protein concentration seen in the Thoroughbreds is similar to that seen in the greyhound compared to other breeds of dog, which is generally considered to be due to the requirement to keep blood viscosity reasonably constant in spite of the higher PCV of the athletic breeds.

In the horse, total plasma protein is generally held to be a better indicator of changes in plasma volume than PCV, due to the possible effects of the contractile spleen on the latter parameter, as discussed in Section 2. Carlson *et al* (1979*b*) studied a three-day period of dehydration with an average bodyweight loss of 10.7% and demonstrated that a decrease in plasma volume of 21% was accompanied by an increase in total protein of only 14.3% and a non-significant increase in PCV of only 12.2%. This suggests that, at least over a period of days, protein

as well as fluid will leave the plasma. However, it also confirms that PCV is even less useful as a measure of change in plasma volume, probably because extra erythrocytes are sequestered into the spleen to prevent an unnecessary polycythaemia. In the present shorter experiment the mean increase in total plasma protein (14.5%) was over twice that of PCV (7.1%) and as in the study of Carlson et al, the increase in PCV was not significant. The most striking point about this present experiment, however, is the wide degree of individual variation which occurred. Table 34 compares the apparent decrease in plasma volume as shown by change in total plasma protein with total fluid loss in each animal.

The variation in the increase in total plasma protein (c.v. = 94%) was much greater than the variations in the increases during the hay feeding experiment in Section 1 (c.v. = 20%) or during the endurance exercise experiment in Section 5 (c.v. = 25%). Part of the explanation for this may be the inclusion of the ponies in this experiment, as they showed a clear tendency to smaller changes in total protein than the horses, but the variation even among the four horses was considerable. This suggests that the percentage of the fluid loss which was borne by the plasma varied widely between animals, from 44% of 25.2 litres in horse D to 1.6% of 12.8 litres in pony Y.

In a similar study of 12 hours' duration in human subjects, Senay and Christensen (1965), unlike Carlson et al (1979b), presented individual data for changes in plasma volume, PCV and total plasma protein compared to loss of body weight. These figures demonstrate correlations between total plasma protein (and PCV) increases and measured decrease in plasma volume (Evans' blue space) which are just as poor as those observed in this present study between these parameters and bodyweight loss. However, the correlation between measured decrease in plasma volume and bodyweight loss in Senay and Christensen's experiment was also equally poor. A decrease in albumin:globulin ratio

TABLE 34

Apparent decrease in plasma volume compared to total fluid loss during heat exposure

Animal	Plasma volume (0.05 l/kg)*	ΔPCV (%)	Δ Total protein (%)	Decrease in plasma vol.(l) θ	Total fluid loss (l)	Decrease in plasma vol. / Total fluid loss (%)
Horse						
B	24.8	2.9	9.2	2.3	13.1	17.6
C	26.5	5.3	14.9	3.9	14.0	27.9
D	25.5	23.4	43.5	11.1	25.2	44.0
E	23.5	4.4	23.9	5.6	24.5	22.9
Pony						
V	12.3	10.3	9.3	1.1	7.4	14.9
W	7.8	3.7	3.6	0.3	2.7	11.1
Y	7.1	6.9	2.5	0.2	12.8	1.6
Z	6.1	0	8.2	0.5	4.3	11.6
Mean ± SEM		7.1 ± 2.6	14.5 ± 4.8			19.0 ± 4.5

*Deavers et al, 1973; Carlson et al, 1979b.

θCalculated from Δ total protein.

was also demonstrated, and this was considered to be due to augmentation of both plasma protein fractions with fractionally larger additions of globulins, rather than to differential escape of albumin. These changes are considered by the authors to invalidate the Evans' blue measure of plasma volume change, and the conclusion is that the rate of plasma water loss is no greater than that of whole-body water loss. This is in contrast to the work of Saltin (1964) and Kozlowski and Saltin (1964) who consider the Evans' blue measure to be valid and suggest that plasma water shows a disproportionately large decrease compared to total body water. Costill (1977) believes that water loss from the extracellular fluid is much greater than from the intracellular fluid, but does not indicate what proportion of this is due to plasma volume changes.

The findings in this present experiment show too great an individual variation to allow any firm conclusions about these matters. The apparent proportional decrease in plasma volume in horse D seems improbably large and the possibility of augmentation of plasma protein must be considered. However, increases in PCV due to excitement or stress in horses are not usually associated with increases in plasma proteins, and pony Y, who also appeared stressed and who showed a smaller increase in PCV, had almost no change in total plasma protein despite a very high total fluid loss. It may be that general conclusions about fluid shifts during thermal dehydration are inappropriate due to the variety of individual response seen, as suggested by Senay and Christensen (1965).

Plasma electrolyte concentrations

The directions of the changes seen during heat exposure in plasma sodium, potassium and chloride concentrations were all consistent with the sweat electrolyte concentrations. In the horses, sweat sodium was almost isotonic and there was no change in plasma sodium, while in the ponies the slightly hypertonic sweat sodium was accompanied by a slight

decrease in plasma sodium. The markedly hypertonic sweat potassium and chloride were accompanied by significant decreases in plasma concentrations of both electrolytes.

This is in contrast to the findings in man, in which markedly hypotonic sweat chloride concentrations do not normally lead to any significant changes in plasma chloride concentration, due to increased renal chloride excretion (Itoh, 1952; Kuno, 1956). Plasma sodium and potassium concentrations both, however, tend to increase (Senay and Christensen, 1965). The possibility of renal regulation of plasma electrolyte concentration in the horses cannot be ruled out, in particular the fall in plasma chloride was of smaller magnitude than would have been expected from the very high sweat chloride concentrations, but as it was not possible to include urinanalysis in this experiment, the extent of such regulation is unknown. Senay and Christensen (1965) consider that the increase in plasma potassium seen during thermal sweating is due to a movement of potassium-rich fluid out of the cells. No such tendency was seen in this present experiment, on the contrary, plasma potassium concentration fell sharply in all animals. This was presumably due to the much higher sweat potassium concentration in the horses.

The significant (but smaller) decrease in plasma potassium concentration seen during the control period is discussed in Section 5.

Plasma mineral concentrations

The lack of any significant change in plasma calcium concentration during heat exposure suggests that the sweat calcium concentration, which unfortunately could not be measured, was probably not much greater than plasma concentration. This is in agreement with the findings of Carlson and Ocen (1979) but in marked contrast to the findings of Soliman and Nadim (1967).

It is now apparent that in early May, when the control experiments on all the ponies and the heat exposure experiments on ponies V and W were carried out, all the ponies were hypocalcaemic (compared to normal values quoted by Simesen, 1980). This was not appreciated at the time as no clinical symptoms were seen, and it appeared to have corrected itself by June when the heat exposure experiments were carried out on ponies Y and Z. The hypocalcaemia was probably due to the poorer diets of the ponies during the winter, when they were stabled and on a hay-only diet. They were turned out to grass for three weeks between the two experimental periods, which is probably the reason for the recovery.

The marked decrease in plasma magnesium concentration seen during this experiment is of much greater magnitude than, and in the opposite direction to, the change seen at the same time of day in the 24-hour experiment in Section 1. It seems very unlikely that this could be an effect of the heat exposure, as the changes seen during the control experiment were virtually identical, and the reason for this occurrence is not known. A change of this magnitude was not seen in any of the other sections of this study, but on this occasion it made comparison of plasma magnesium with sweat magnesium concentrations impossible.

The lack of any significant change in plasma phosphate concentration during heat exposure indicates that thermal sweating has little effect on this parameter. The regular changes noted during the control period were unexpected, and curiously took the form of an increase in the horses and a slight decrease in the ponies. It is conceivable that these observations are related to the possible occurrence of a regular diurnal variation in plasma phosphate concentration, as discussed in Section 1.

Plasma creatinine and urea

The increase in both of these parameters during the period of heat exposure suggests that the decrease in plasma volume was accompanied by a reduction in glomerular filtration rate. However, it is impossible to be certain about this because of the lack of urine samples.

Plasma cortisol

Collins et al (1969) have shown that in man an increase in body temperature to above 38.3°C leads to increased cortisol secretion. It is difficult to relate this to the present study due to the higher normal body temperature of the horse, but it is noticeable that the two animals with the highest increases in rectal temperature also had the highest increases in plasma cortisol concentration. However, one horse with a rectal temperature increase of only 1.0°C showed a rise in plasma cortisol which exceeded that on the control day by 170 nmol/l. Follenius et al (1979) associated particularly large increases in plasma cortisol with poor heat tolerance and a feeling of "annoyance" in the subjects, even though increases in body temperature were only around 0.6°C. This certainly corresponds well with the present study in which the individuals with the greatest increases in plasma cortisol showed very poor heat tolerance. However, as discussed above, the reason for the poor heat tolerance of certain individuals was not apparent. Follenius et al (1979) also demonstrated a decrease in plasma cortisol during heat exposure in one individual, as was seen in the present study, and showed that in spite of this the usual increase in plasma aldosterone concentration still occurred.

The increase seen in the horses during the control day was surprising, as the diurnal rhythm in plasma cortisol reported by several authors (Hoffsis et al, 1970; Larsson et al, 1979) would indicate that a decrease would be expected at that time of day (10.00 ¬ 16.00 hrs), as was in fact seen in the ponies. The reasons for this and for the wide

variations in plasma cortisol seen in pony Z are unknown. However, Evans et al (1977) have shown that patterns of cortisol secretion can vary quite considerably between individuals and even within an individual on different days, and that concentrations of up to about 900 nmol/l can occur in undisturbed, unstressed horses.

Sweat collection techniques

In man, sweat collection techniques are almost as numerous as the publications on the subject. They can, however, be divided into three basic types of technique.

1. Collection of sweat directly from an uncovered skin surface.
2. Enclosure of the body or a limb in a rubber or plastic bag.

   In both of these methods sweat may be collected directly into a collection vessel, or it may be soaked into absorbent material. The sweat may then be squeezed or eluted from the material, or the material may be ashed to recover the electrolytes.
3. Washing down of the whole body and clothing, or of a measured area of skin, with distilled water. The washings are then concentrated and analysed to obtain total sweat losses.

All of these methods have been used in the horse.

The relative advantages of these techniques have been discussed by Robinson and Robinson (1954). They recommend method 3 as the most accurate method for measuring total body losses of materials dissolved in the sweat, but in order to obtain sweat concentrations of these materials it is also necessary to have a very accurate measure of sweating rate or total sweat volume. This method has obvious limitations when applied to the horse. It is extremely difficult, if not impossible, to ensure that a large animal with a hair coat is completely clean before beginning the experiment, or to ensure that it is completely washed down in a reasonable volume of water afterwards. It requires that all sweat evaporates on the body and is therefore not

applicable to conditions such as the present study where sweat may drip from the body, and it cannot normally be used to compare serial results or different body areas. When studying exercise-induced sweat in the horse, it is also very difficult to eliminate contamination from leather tack and from dust or earth kicked up by the horse. This was, however, the original method used to collect sweat from the horse (Leclerc, 1888) and it has also been used recently by Schryver et al (1978) and in the donkey by Dill et al (1979).

Method 2 was used by Carlson and Ocen (1979) to collect adrenaline-induced sweat, and has the advantage of eliminating errors due to evaporation. However, it has been shown in man that the enclosing of part of the body in an impermeable material may alter both sweat composition and rate of sweating (van Heyningen and Weiner, 1952; Collins and Weiner, 1962). In spite of this, it is the only feasible collection method for obtaining exercise-induced sweat from horses (see Section 5).

Method 1 was used by Smith (1890), Jirka and Kotas (1959) and Soliman and Nadim (1967) to collect exercise-induced sweat. It has the disadvantage of allowing the sweat to concentrate on the skin and of allowing dirt or sand kicked up by an exercising horse to contaminate the sample, and this is probably the reason for the extremely high electrolyte concentrations reported by the last two studies. This method has, however, been used with some success in man under conditions where sweating was so heavy that sweat flowed freely from the skin and could be collected before a significant degree of evaporation had occurred (Hancock et al, 1929; Page and Remington, 1967; Strømme et al, 1975).

In the present study the humidity in the environmental chamber and the sweating rate were so great that sweat flowed continually from the skin for most of the experiment. Under these conditions it was believed

that evaporative effects on sweat composition would be minimal, and the results from samples collected after four hours of sweating, when a reduction in sweating rates was observed to be allowing sweat to evaporate on the skin, were omitted. It was also found to be difficult to secure a plastic covering to any part of a horse's body in such a way that it would reliably stay in place for several hours when the hair was wet. It was therefore decided to collect the sweat samples directly from the uncovered skin and so avoid any possible interference from covering the skin with plastic. This method was also found to be convenient in allowing easy collection of serial samples as closely as possible to time of secretion.

It appears from the results of the present study that contamination of the skin and hair may have been the main source of error in this protocol. In spite of the grooming routine, the initial samples from the ponies appeared dirty, and the individual differences between animals could partly be due to differences in the effectiveness of the grooming. It might have been more satisfactory if it had been possible to wash the animals prior to the experiment.

Sweating rates

It was unfortunate that the ventilated capsule apparatus did not allow continuous measurement of the sweating rates, but only of the time of onset of sweating. In general, the ponies appeared to begin sweating later than the horses, but over the period of the experiment there was no significant difference between the percentage weight losses of the horses and the ponies. Langley and Bennett (1923) reported that the sweat glands of a cold-blooded horse ("cart horse") were less sensitive to local adrenaline injection, but the slightly longer delay in the start of sweating seen in the ponies in this experiment is not really sufficient evidence to allow the proposition of a similar difference in sensitivity to thermal stimulus.

The mean rate of bodyweight loss during the experimental period (0.8% /hr) was greater than that noted in human subjects under similar conditions (Senay and Christensen, 1965). No water was drunk or urine passed during the heat exposure period and so this bodyweight loss is equivalent to the total fluid loss over the period. Studies on human subjects have generally assumed that this is equivalent to the total sweat loss, i.e. that respiratory water loss is insignificant (Kozlowski and Saltin, 1964; Beisel et al, 1968; Costill, 1977). This may be valid for heat exposure experiments but its extrapolation to exercise is surely questionable. In this experiment, approximate calculation of actual sweat loss suggested that around 90% of the total fluid loss was due to sweating. This figure is probably a slight overestimate as the animals' coats were wet when they were weighed at the end of the experiment, which means that the total fluid loss was underestimated. These approximate figures indicate that the calculation of total body electrolyte losses from sweat concentration and total bodyweight loss will lead to overestimation of these losses.

The decline in sweating rates observed towards the end of the heat exposure period in all the animals has also been reported in man (Ladell, 1945; Gerking and Robinson, 1946), although in the human experiments the subjects were working in the hot environment. Wyndham et al (1966) have stated that this effect is due to fatigue of the sweat glands "analogous to muscle fatigue". It appears that this explanation may also be applicable to the horse, as Montgomery et al (1982), who studied skin biopsy samples obtained concurrently with the investigations reported here, found substantial amounts of glycogen in the secretory cells of the sweat glands, which became progressively depleted as sweating continued. This suggests that sweat secretion in the horse is an active process dependent on a supply of glycogen, as appears to be the case in man.

It is interesting to speculate on the possible involvement of this factor in the development of anhydrosis ("dry-coat"), an equine disease of uncertain aetiology in which horses exported to the tropics lose the ability to sweat during exercise after having demonstrated unusually profuse exercise-related sweating for several weeks (Correa and Calderin, 1966). However, Evans et al (1957a) made a histological study of the sweat glands of horses suffering from the disease and found glycogen to be present in all cases. In addition, anhydrosis is usually associated with much lower plasma chloride concentrations than were seen in this study (Gilyard, 1944) and only a proportion of exported animals are affected. The suggestion of Evans et al (1957b) that the condition is due to the development of an insensitivity of the sweat glands to adrenaline has yet to be confirmed.

Sweat composition

Sweat electrolyte concentrations

This study is in broad agreement with the findings of Meyer et al (1978) and Carlson and Ocen (1979) that sweat electrolyte concentrations in the horse are much greater than those in man, with potassium and chloride both markedly hypertonic compared to plasma. The results are most comparable to those reported by Carlson and Ocen (1979) for exercise-induced sweat, with the exception that both sodium and chloride concentrations were approximately 20 mmol/l greater in this present experiment. Meyer et al (1978) are the only authors to have reported a (molar) sweat sodium:chloride ratio of greater than one, and the results of this present experiment indicate that in the horse, in contrast to man, sweat chloride concentration is greater than sodium concentration.

These findings suggest that the mechanism of sweat secretion in the horse is quite different from that of man. It appears that chloride and potassium, and possibly to a lesser extent sodium, are actively secreted into the sweat. Due to the lack of a continuous measurement of sweating

rate in this experiment, it is impossible to determine whether sweat sodium and chloride concentrations become closer to the plasma concentrations with increasing sweating rate, as is the case in man. The only significant change in electrolyte concentration with time was the slight increase in sodium noted in the sweat from the horses' necks. This increase was only seen in one set of samples, with no change being seen in the ponies or the body sweat of the horses, and as the change was from hypotonic to hypertonic concentrations it is difficult to relate it to sweat secretory mechanisms. The reason for the hypertonicity of horse sweat is obscure. It would be assumed that such high electrolyte losses would be physiologically disadvantageous to the animal, and it is apparent that high sweat electrolyte concentrations are a serious disadvantage to humans in hot climates (Streeten et al, 1960).

The results presented by Carlson and Ocen (1979) suggest that chloride and potassium concentrations are much lower in equine sweat produced by intravenous adrenaline infusion compared to exercise-induced sweat. The heat-induced sweat in this present study had higher sodium and chloride concentrations than the exercise-induced sweat reported by Carlson and Ocen, and taken together these findings suggest the possibility that the electrolyte composition of equine sweat is significantly dependent on the stimulus to sweating. This subject has been investigated in man by Verde et al (1983), who found no change in sweat sodium, potassium or chloride concentration between heat and exercise-induced sweat, but demonstrated markedly higher calcium and magnesium concentrations in heat-induced sweat. The results of this present study also suggest that there may be some influence of breed on sweat composition, as the ponies showed higher sweat sodium (and possibly also chloride) concentrations than the horses under similar

conditions. These observations suggest that further investigation of the electrolyte composition of equine sweat produced by different stimuli would be valuable.

Sweat magnesium and protein concentrations

Protein concentrations in the initial samples in this study were comparable to those reported by earlier investigators (Leclerc, 1888; Smith, 1890; Jirka and Kotas, 1959). However, in contrast to the findings regarding sweat electrolyte concentrations, these levels were not maintained during prolonged sweating, and after 4.5 hours of sweating sweat protein concentration was generally around 1 g/l. Montgomery et al (1982), who studied skin biopsy samples collected during the heat exposure experiments described here, demonstrated secretory vesicles in the secretory cells in the fundus of the sweat gland which became depleted as sweating progressed. As protein (and glycoprotein) were identified in these vesicles it appears possible that they were the source of the sweat protein. It has been suggested by several authors that sweat protein losses during exercise are high enough to lead to debility or loss of condition in horses in training (Smith, 1890; Jenkinson et al, 1974). However, when the rapid fall in concentration with time seen in this study is taken into consideration, it appears unlikely that total sweat protein loss would be sufficient to lead to a clinical problem.

The very close correlation between sweat magnesium and protein concentrations has not been reported previously, and it suggests that magnesium may be involved in some way in the protein secretory process. The exponential decrease with time is presumably the reason for the wide disparity of previous reports of sweat magnesium, from 7 mmol/l (Meyer et al, 1978) to 2 mmol/l (Carlson and Ocen, 1979), compared to electrolyte concentrations reported in the same publications. The close resemblance of the pattern of decrease of sweat magnesium in this study

to that reported by Strømme et al (1975) for human sweat is quite remarkable, although in man initial concentrations are much lower (under 4 mmol/l) and the lowest concentrations (under 0.4 mmol/l) are reached in less than 60 min. It appears that there has been no investigation as to whether the protein concentration of human sweat also decreases with time.

The identity of the equine sweat protein, its relationship to magnesium and its possible function are discussed further in Section 4.

Sweat glucose

The very low concentration of glucose in the sweat is consistent with the findings in man (Lobitz and Osterberg, 1947).

Conclusions

This study demonstrated that unacclimatised horses generally tolerated an acute period of several hours' exposure to a hot, humid environment very well, but that certain individuals exhibited a poor heat tolerance for unknown reasons. Sweating rates were high but the sweat was largely ineffective and panting also played an important role in thermoregulation.

Changes in PCV and total plasma protein were very poorly related to total fluid losses, and it appears that body fluid shifts during thermal dehydration are much less regular than those associated with muscular exercise. Changes in plasma electrolyte concentrations were not large, and they were always in the direction which would have been expected from the sweat electrolyte concentrations.

Sweat electrolyte concentrations were very much greater than those reported in man, with chloride and potassium always hypertonic and sodium approximately isotonic to plasma, and these concentrations were maintained throughout 4.5 hours of sweating. The production of a hypertonic sweat would normally be considered to be physiologically

disadvantageous because of the resulting high electrolyte losses and the reasons for this finding are so far unknown. Some slight breed differences in sweating responses were apparent between horses and ponies.

Initially a very high concentration of protein was secreted in equine sweat, but the concentration showed an exponential decrease with time. The sweat magnesium concentration was very closely related to the protein concentration and it is suggested that the magnesium may be involved in the protein secretory process. The function of this protein is so far unknown.

Several interesting questions have been opened by this study, particularly the possibility of significant differences in equine sweat electrolyte composition being seen in sweat produced by different stimuli, also the identity of the sweat protein, its relationship to magnesium and its function. These will be discussed further in Section 4.

SECTION FOUR

STUDIES OF BLOOD AND SWEAT COMPOSITION

DURING INTRAVENOUS ADRENALINE INFUSION

Introduction

Pharmacology of the equine sweat gland

The response of the equine sweat gland to a wide variety of pharmacological agents has been extensively studied by Evans and Smith (1956). They recorded that the gland was most sensitive to adrenaline and isoprenaline which both induced copious secretion, but that although noradrenaline caused piloerection it did not induce sweat secretion. Acetylcholine and related compounds were also found to induce sweating but to a much smaller degree, and this effect (but not exercise or adrenaline-induced sweating) was blocked by atropine. On the basis of the failure of direct stimulation of the vagosympathetic trunk to produce sweating, the observation that decentralised skin continued to sweat in response to exercise, and the finding that during exercise venous blood adrenaline concentration was proportional to the degree of sweating (Evans *et al*, 1956), they concluded that sweating in the horse was mediated by humoral adrenaline, not by sympathetic nervous activity. They also observed that peripheral vasoconstriction inhibited adrenaline-induced sweating and evolved a theory whereby neural control of cutaneous circulation was held to explain such phenomena as denervation hypersensitivity and the effect of acetylcholine.

However, Jenkinson and Blackburn (1968) showed that a network of nerves was present around the equine sweat gland, and a subsequent study by Robertshaw and Taylor (1969) elucidated the various roles of humoral and neural mediation of sweating much more precisely. This work demonstrated that heat-induced sweating was mediated entirely by local sympathetic nervous stimulation and was abolished in decentralised skin, that humoral adrenaline alone (either by infusion or stimulated *in vivo* by insulin-induced hypoglycaemia) would cause sweating even in decentra-

lised skin, and that both neural and humoral mechanisms were involved in exercise-induced sweating, which could only be abolished by decentralisation of the skin and adreno-medullary denervation together, not by either procedure alone. It was also demonstrated that while neurally-induced reduction in cutaneous blood flow was important in the response to cold exposure its absence was not sufficient to explain all the effects of denervation hypersensitivity. This study was carried out on donkeys (Equus asinus) rather than horses (Equus caballus), but no evidence has arisen to contest the assumption that the mechanisms in the two closely related species are similar.

A later study (Robertshaw, 1974) demonstrated that equine sweat secretion is mediated by β receptors, being stimulated by isoprenaline and completely blocked by propranolol, while Snow (1977) identified these as β_2 receptors by demonstrating the failure of metoprolol to block secretion.

Robertshaw and Taylor (1969) and Robertshaw (1974) have advanced the hypothesis that the neurotransmitter substance involved in equine sweat secretion is adrenaline itself rather than noradrenaline, based on the failure of noradrenaline to stimulate sweating whether administered systemically or intradermally. However, adrenaline has not been demonstrated to be a neurotransmitter at the peripheral adrenergic nerve endings of any other mammalian system, which would render the horse sweat gland unique were this proven to be the case, and the question still remains an open one.

The induction of sweat secretion by acetylcholine has been held to be irrelevant to normal thermoregulatory sweating in the horse because of the complete failure of atropine to block exercise, heat or adrenaline-induced secretion and the complete blockage of heat-induced secretion by bethanidine (Evans and Smith, 1956; Robertshaw and Taylor, 1969). The presence of cholinesterase in the nerves associated with the

equine sweat gland (Jenkinson and Blackburn, 1968; Bell and Montagna, 1972) may however suggest that cholinergic stimulation of equine sweat glands could have some unidentified physiological function.

The control of thermoregulatory sweating in man is very different from the horse, and has been extensively reviewed by Sato (1977). Briefly, physiological sweating is primarily cholinergic in nature, mediated by intracellular Ca^{2+} and completely blocked by atropine. It does appear, however, that the catecholamines may perform some secondary role. Administration of adrenaline at high dose rates will induce a comparatively low level of sweat secretion and it appears that the human eccrine sweat gland has a dual (cholinergic/adrenergic) innervation (Sato, 1977, 1984). The adrenergic component of human sweating has been reviewed at some length by both Sato (1977) and Robertshaw (1981) without any specific conclusions being reached as to its physiological importance. More recently Sato (1984) has advanced a number of speculations, particularly that the clue to the importance of the adrenergic nervous component may lie in the intracellular accumulation of cAMP and its (so far unknown) role in the regulation of glandular function. He has also reported that the sweat glands of patients suffering from cystic fibrosis, unlike those of normal humans, do not show any sweat secretion in response to isoprenaline despite normal accumulation of cAMP. The significance of this finding appears to be unknown at present.

It is interesting to speculate whether the physiological roles of the cholinergic component of equine sweating and of the adrenergic component of human sweating will prove to be similar in function. This could be the case if the importance of the dual innervation (in man) lies in the marked augmentation of adrenergically-induced accumulation of cAMP which occurs when cholinergic stimulation is also present, as suggested by Sato (1984).

Use of drugs for the induction of sweating

Almost all of the original work on human sweat composition was carried out using heat or exercise-induced sweat. These methods of sweat stimulation are, however, inconvenient for the subjects and may be impossible to use on subjects who are not completely healthy. Schwartz et al (1953) used intradermal injection of mecholyl to stimulate sweating for the purpose of investigating sweat urea concentration and reported that the composition was similar to that of heat-induced sweat. More recently the study of sweat composition of children has become an important diagnostic tool in the investigation of cystic fibrosis and for this purpose the standard method of sweat stimulation is the iontophoresis of pilocarpine (Gibson and Cooke, 1959).

Evans and Smith (1956) used both intradermal injection and iontophoresis of various drugs in horses and reported iontophoresis to be generally disappointing as a method of sweat induction as it led to anhydrosis and induration of the skin. Intradermal injection of adrenaline was also found to cause a transient anhydrosis of up to 10 days' duration and a later study (Evans et al, 1957b) suggested that this was due to degeneration of the sweat glands. Robertshaw and Taylor (1969) suggested that intravenous adrenaline infusion was a more satisfactory method of sweat induction as it did not lead to a state of anhydrosis.

It was therefore decided to use intravenous adrenaline infusion to continue the study of equine sweat composition. The reasons for this were partly economic factors which made further work in the climatic chamber impracticable and (in the case of horse D and pony Y) humanitarian considerations. It was also considered, however, that the study of the composition of adrenaline-induced sweat in comparison with heat and exercise-induced sweat might provide further information about sweating responses in the horse.

Composition of sweat induced by different stimuli

The suggestion that there may be differences in composition between heat- and exercise-induced sweat in man has been made recently by Verde et al (1983) who found double the concentration of calcium and magnesium in heat-induced sweat compared to exercise-induced sweat. It is difficult to relate this finding to differences in the mode of stimulation of sweating as both heat and exercise-induced sweating in man are believed to be induced by the same cholinergic nerves (Sato, 1977). However Verde et al (1983) suggest that there may be some relation to the findings of Nadel et al (1971) who have shown some effect of skin temperature on rate of sweating in man.

Almost all of the reports of equine sweat composition have been concerned only with exercise-induced sweating and only Carlson and Ocen (1979) have also presented results for adrenaline-induced sweat. Examination of these data suggest that there may be a number of differences in composition between exercise and adrenaline-induced sweat. In particular the potassium concentration reported for adrenaline-induced sweat is less than half that reported for exercise-induced sweat. In addition the composition of heat-induced sweat in this present study appeared to be different once again, as discussed in Section 3. It is easier to relate these possible variations in composition to the mode of stimulation of sweating in the horse because of the demonstration of differences in mediation of the sweating response in the three situations.

Purpose of this section

The purpose of this section is first to compare the composition of adrenaline-induced sweat in the horse with that presented for heat-induced sweat in Section 3, and to investigate the changes in plasma parameters consequent on a prolonged period of adrenaline-induced sweating. It was also proposed to use adrenaline-induced sweat to investigate further some of the points noted in Section 3 with particular regard to the protein and magnesium content.

Materials and Methods

A. Sweat collections from unwashed skin

1. Animals

Horses A, B, D and E and ponies V, X, Y and Z were used.

2. Management

The management routine and diet at the time of this study was the same as that described in Section 3 for all animals. Experiments were carried out in winter. The horses received only walking exercise and the ponies were not exercised at all.

3. Procedure

(a) Adrenaline infusion

The clipping and grooming routine was the same as that described in Section 3, except the horses as well as the ponies were trace-clipped.

Adrenaline solutions for infusion were made up by adding adrenaline tartrate (1:1000 solution for injection, Evans Medical Ltd) to a sterile solution of sodium chloride, 150 mmol/l (Viaflex, Travenol Laboratories Ltd). A concentration of 20 µg/ml was used in the horses and 40 µg/ml in the ponies.

The animals were restrained in stocks and the adrenaline solution was infused for a period of three hours via a 16G 12" Teflon cannula (Deseret Pharmaceutical Co) inserted into the left jugular vein. Infusion rates in the horses were adjusted as detailed in Table 35 so as to produce continual free-flowing sweat. In the ponies the dose rate was increased up to a maximum of 1.08 µg/kg/min, but free-flowing sweat was never produced. The total volume of solution infused on no occasion exceeded 800 ml.

Heart rate was monitored by auscultation, rectal temperature by clinical thermometer and respiratory rate by observation. Blood samples were collected immediately before beginning the infusion and then every

TABLE 35

Dose rates of adrenaline used in the horses

Dose rate (µg/kg/min)	Time during which dose rate administered (min)			
	Horse A	Horse B	Horse D	Horse E
0.13	0-60	0-30	0-30	0-60
0.21	60-180	30-180	30-60	60-180
0.31	-	-	60-180	-

30 minutes until one hour after the infusion was terminated. PCV analysis was performed at once, all samples were then immediately centrifuged and the plasma stored at -20°C until analysis. Plasma samples were analysed for total protein, albumin, sodium, potassium, chloride, calcium, magnesium, phosphate, creatinine, urea, glucose and bilirubin by the methods described. Certain samples were also analysed for free glycerol and triglycerides. Urine samples (non-volumetric) were collected from three of the horses during the last 30 minutes of the infusion and these were analysed for sodium, potassium, chloride, creatinine, urea and glucose.

Sweat samples were collected as soon as possible (after about 10 minutes of infusion) and at regular intervals thereafter by the methods described in Section 3. Samples were processed and analysed as described in Section 3, and were also analysed for calcium within 24 hours of sample collection, and for urea.

(b) Control experiments

These were carried out only on horses A and D and ponies V and Y. The protocol followed was exactly as for the adrenaline infusions except that no adrenaline was added to the sodium chloride infusion. No urine (or sweat) was collected.

B. Sweat collection from washed skin

1. Animals

Only horses C and D were used in these experiments.

2. Management

Management, diet and exercise routines were as described in Section 3. Four of the infusions were carried out in early summer and the remaining two in winter.

3. Procedure

Prior to each infusion the horses' necks were clipped as closely as possible with surgical clippers. On two occasions this was done on the morning of the infusion and on the other five occasions it was done one or two days before the infusion. Immediately before each infusion the necks were washed with detergent (Decon 90, Decon Laboratories Ltd), rinsed thoroughly with deionised water and towel-dried. Afterwards the horses were allowed to stand quietly for 30 minutes to ensure complete drying of the skin.

Adrenaline infusions were then carried out as described above except than an initial infusion rate of 0.18 μg/kg/min was used and was found to maintain a suitable sweating rate for the duration of the experiment.

Blood samples were collected before and at 1, 2 and 3 hours of infusion on all but the first occasion. They were processed as above and analysed for PCV, total plasma protein, albumin, sodium, potassium, chloride, calcium, magnesium, phosphate, creatinine, urea, glucose and bilirubin.

No urine samples were collected.

Sweat samples (from the neck only) were collected, processed and analysed as described above. In addition, the samples from the last two infusions were analysed for glucose and pH.

In order to investigate the time taken for the sweat protein to recover after a prolonged period of sweating, five of these infusions were each followed by a short infusion of 10 minutes' duration after an interval of between 3 and 48 hours. Before each of these short infusions the same washing procedure was carried out. The same dose rate of adrenaline (0.18 µg/kg/min) was used and one sweat sample was collected.

4. Statistical analysis

The same statistical techniques were used as described in Section 3. In addition the compositions of heat and adrenaline-induced sweat were compared using the unpaired t test.

5. Investigation of sweat protein and magnesium

In order to determine whether or not the magnesium was bound to the protein, a number of sweat samples were passed through a 13 mm ultrafiltration cell (Millipore Ltd) which allowed passage of molecules with a molecular weight of under 25,000. The samples were then re-analysed.

Electrophoresis of a few representative sweat samples was carried out on agarose gel using an LKB 2117 Multiphor system according to the method described by Miyake et al (1977). Serum harvested from blood collected from horse A and commercially prepared equine serum albumin (Sigma Chemicals Ltd) were run in parallel with the sweat for comparative purposes, and a sweat sample with equine serum albumin added was also run to determine if the alkaline environment of the sweat caused any alteration in the electrophoretic mobility of albumin.

Results

A. Experiments carried out with unwashed skin

These experiments were carried out using a protocol as close as possible to that of Section 3 in order to allow comparison between heat-induced and adrenaline-induced sweat.

Clinical measurements

Respiratory rate and rectal temperatures did not change during adrenaline infusion in any of the animals. Measurement of body weight loss was unfortunately impossible and estimations of sweating rates are subjective.

In all eight animals there was evident polyuria associated with the adrenaline infusion. Three of the horses urinated during the last 30 minutes of infusion in spite of the fact that the muscle tremors made posturing very difficult and they were known to be reluctant to urinate on to a concrete floor. Urine samples were collected from these animals. The other horse and the four ponies urinated copiously as soon as they returned to the stables after the experiment.

In the horses, moderate tremors of the limb muscles began one or two minutes after the start of the experiment and continued for up to three hours after the infusion was stopped. Heart rates increased from 33 ± 2 to 58 ± 6 beats/min during the period of the infusion. The initial dose rates of adrenaline produced free-flowing sweat within five minutes of the start of the experiment. After a variable time the sweating rate was observed to decrease gradually and in order to maintain a flow of sweat sufficient to allow easy sample collection the rate of adrenaline infusion was increased as detailed in Table 35. Visible sweating ceased within two or three minutes of the end of the infusion.

The sweating response of the ponies to adrenaline was much less than that of the horses. Visible dampness was produced, but in spite of increasing the infusion rate to 1.08 µg/kg/min the free-flowing sweating required for sample collection did not occur. At this dose rate muscle tremors were severe and heart rates had increased from 42 ± 3 to 105 ± 12 beats/min and so higher dose rates were not attempted. No results for sweat composition are therefore presented.

During the control experiments there was no sweating, muscle tremor or polyuria, and no change in heart rate, respiratory rate or rectal temperature.

Blood samples

The mean results for the samples taken immediately before, immediately after and one hour after both the adrenaline infusions and the control saline infusions are shown in Table 36. Where the post-adrenaline infusion result is significantly different from the pre-infusion result this is indicated.

The parameters which showed the most marked changes during adrenaline infusion were PCV and total plasma protein, potassium, chloride, phosphate, glucose and glycerol concentrations. These changes are presented graphically in Figure 24.

TABLE 36(a) - Horses

PCV and plasma parameters immediately before, immediately after and one hour after adrenaline infusions and control experiments

Parameter	Adrenaline Infusions (n = 4)			Control Experiments (n = 2)		
	Pre	Post$^{\delta}$	60 min Post	Pre	Post	60 min Post
PCV	0.36 ± 0.01	0.50 ± 0.02**	0.43 ± 0.02	0.40 ± 0.05	0.37 ± 0.04	0.35 ± 0.03
Total protein (g/l)	74 ± 1	80 ± 2*	75 ± 1	66 ± 2	63 ± 2	67 ± 2
Albumin (g/l)	37 ± 2	42 ± 1*	41 ± 1	35 ± 1	33 ± 2	36 ± 2
Na^+ (mmol/l)	134 ± 2	133 ± 2*	135 ± 2	138 ± 1	138 ± 2	136 ± 1
K^+ (mmol/l)	4.2 ± 0.1	3.3 ± 0.1***	2.9 ± 0.1	4.2 ± 0.1	3.6 ± 0.1	3.1 ± 0.1
Cl^- (mmol/l)	100 ± 1	89 ± 1**	94 ± 1	101 ± 0	101 ± 1	101 ± 0
Ca^{2+} (mmol/l)	3.32 ± 0.10	3.20 ± 0.12*	3.27 ± 0.11	2.98 ± 0.01	2.96 ± 0.02	2.97 ± 0.01
Mg^{2+} (mmol/l)	0.76 ± 0.02	0.87 ± 0.08	0.89 ± 0.07	0.77 ± 0.09	0.73 ± 0.08	0.74 ± 0.09
PO_4^{2-} (mmol/l)	1.24 ± 0.08	0.30 ± 0.03***	0.57 ± 0.08	0.92 ± 0.10	0.83 ± 0.11	0.89 ± 0.17
Creatinine (µmol/l)	139 ± 12	170 ± 14**	177 ± 10	150 ± 9	150 ± 26	150 ± 26
Urea (mmol/l)	6.3 ± 0.4	6.1 ± 0.5	6.2 ± 0.3	4.4 ± 0	4.4 ± 0.1	4.5 ± 0.1
Glucose (mmol/l)	5.3 ± 0.3	17.5 ± 1.1**	14.9 ± 1.0	4.9 ± 0.3	5.5 ± 0.7	4.8 ± 0.2
Bilirubin (µmol/l)	41 ± 7	58 ± 11*	46 ± 10	30 ± 1	27 ± 0	30 ± 0
Glycerol (µmol/l)	19 ± 4	2100 ± 644*	1446 ± 703	-	-	-
Triglycerides (µmol/l) (n = 2)	231 ± 128	282 ± 65	146 ± 37	-	-	-

δ Significantly different from pre-infusion values * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

TABLE 36(b) - Ponies

PCV and plasma parameters immediately before, immediately after and one hour after adrenaline infusions and control experiments

Parameter	Adrenaline Infusions (n = 4)			Control Experiments (n = 2)		
	Pre	Post$^{\delta}$	60 min Post	Pre	Post	60 min Post
PCV	0.31 ± 0.01	0.34 ± 0.02	0.31 ± 0.01	0.29 ± 0.01	0.26 ± 0.01	0.27 ± 0.01
Total protein (g/l)	80 ± 4	75 ± 6	78 ± 5	74 ± 3	71 ± 2	73 ± 2
Albumin (g/l)	21 ± 2	21 ± 3	22 ± 3	22 ± 0	21 ± 0	22 ± 1
Na^+ (mmol/l)	135 ± 1	135 ± 1	134 ± 1	135 ± 1	135 ± 1	135 ± 1
K^+ (mmol/l)	4.6 ± 0.1	3.4 ± 0.1**	3.3 ± 0.1	4.4 ± 0.2	3.8 ± 0.2	3.2 ± 0.2
Cl^- (mmol/l)	97 ± 1	87 ± 1**	88 ± 0.2	99 ± 0	99 ± 0	99 ± 1
Ca^{2+} (mmol/l)	2.82 ± 0.08	2.45 ± 0.10**	2.50 ± 0.13	2.80 ± 0.20	2.56 ± 0.21	2.64 ± 0.28
Mg^{2+} (mmol/l)	0.60 ± 0.05	0.66 ± 0.05**	0.64 ± 0.05	0.58 ± 0.09	0.50 ± 0.06	0.53 ± 0.07
PO_4^{2-} (mmol/l)	1.08 ± 0.06	0.69 ± 0.01**	0.79 ± 0.07	1.22 ± 0.02	1.17 ± 0	1.24 ± 0.04
Creatinine (µmol/l)	108 ± 16	130 ± 16	135 ± 18	98 ± 26	101 ± 13	110 ± 13
Urea (mmol/l)	4.4 ± 1.0	4.1 ± 1.0*	4.2 ± 1.0	4.5 ± 0.8	5.0 ± 0.9	5.2 ± 1.0
Glucose (mmol/l)	4.9 ± 0.7	19.4 ± 0.9***	17.5 ± 1.3	5.6 ± 1.7	4.8 ± 0.9	4.8 ± 0.8
Bilirubin (µmol/l)	12 ± 1	16 ± 2	17 ± 1	11 ± 1	11 ± 3	12 ± 3
Glycerol (µmol/l) (n = 2)	43 ± 4	2341 ± 1181	1156 ± 840	-	-	-

$^{\delta}$ Significantly different from pre-infusion values: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

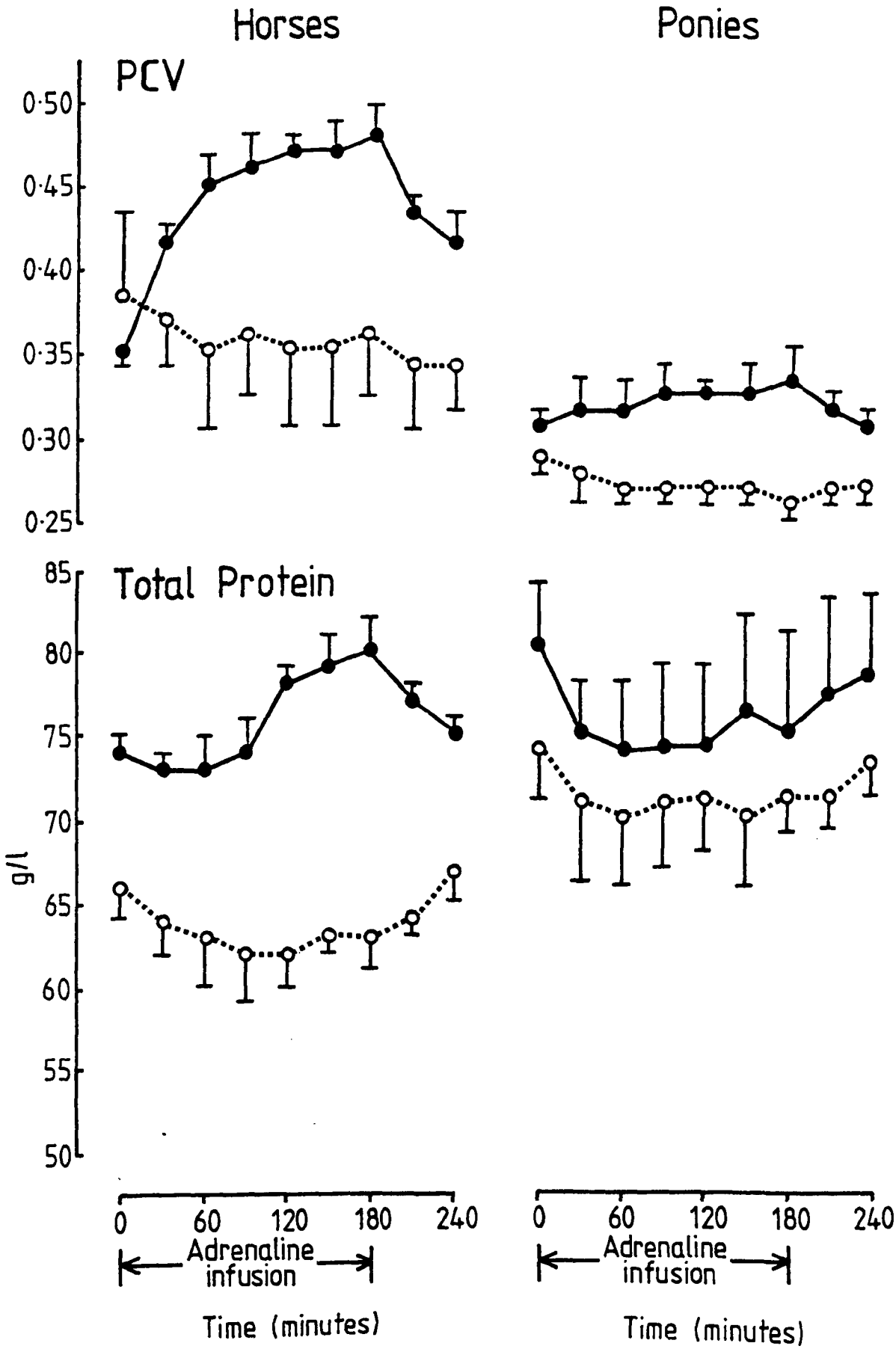


Figure 24(a). Changes in PCV and total plasma protein concentration in horses and ponies during and after intravenous adrenaline infusion (solid line) compared to a control saline infusion (broken line).

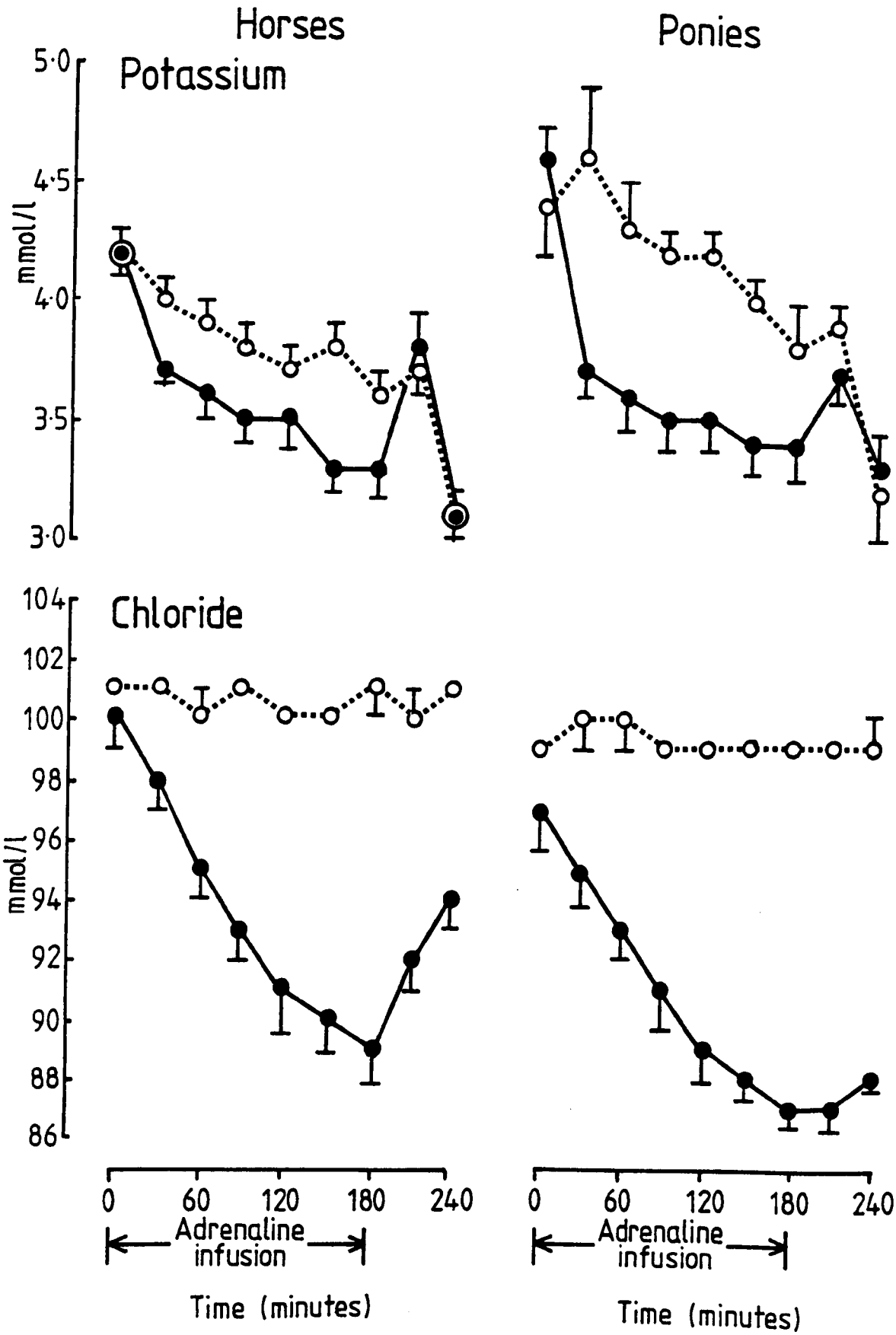


Figure 24(b). Changes in plasma potassium and chloride concentrations in horses and ponies during and after intravenous adrenaline infusion (solid line) compared to a control saline infusion (broken line).

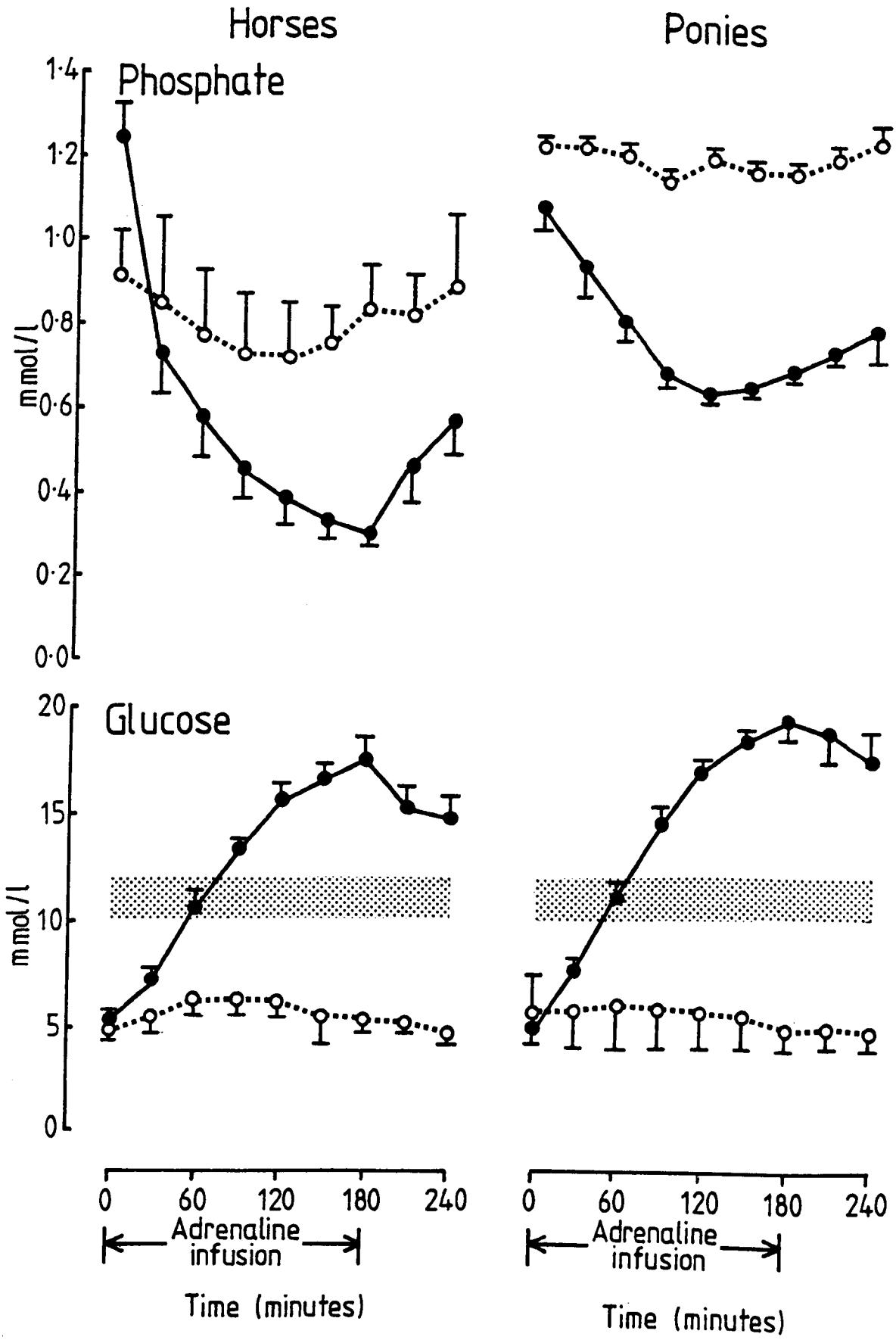


Figure 24(c). Changes in plasma inorganic phosphate and glucose concentrations in horses and ponies during and after intravenous adrenaline infusion (solid line) compared to a control saline infusion (broken line). The shaded area on the glucose graphs indicates the approximate renal glucose threshold.

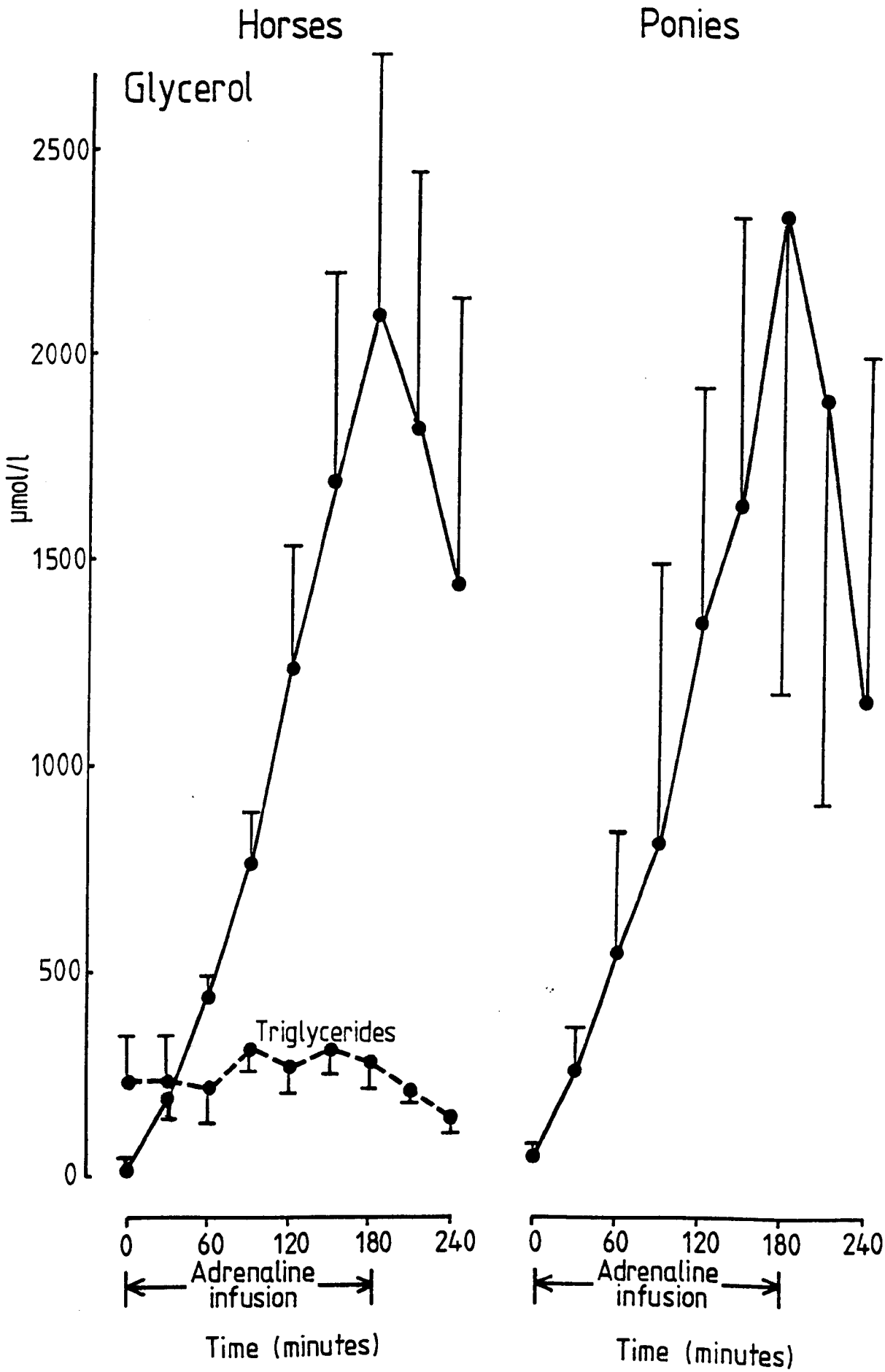


Figure 24(d). Changes in plasma glycerol (horses and ponies, solid line) and triglyceride (horses only, broken line) concentrations during and after intravenous adrenaline infusion.

Urine samples

The results for the urine samples collected during the last 30 minutes of adrenaline infusion from three of the horses are given in Table 37.

TABLE 37

Urine composition towards the end of a 3-hour adrenaline infusion

Parameter	Horse A	Horse D	Horse E
Na^+ (mmol/l)	27	43	30
K^+ (mmol/l)	141	52	126
Cl^- (mmol/l)	60	19	34
Creatinine (mmol/l)	10.9	6.1	8.2
Urea (mmol/l)	121	81	111
Glucose (mmol/l)	124	183	138
pH	7.7	7.7	7.6
Specific gravity	1.031	1.030	1.033

Sweat samples

The appearance of the sweat was identical to that of the heat-induced sweat, initially opaque with a slight yellow colouration. Again the yellow colour disappeared as sweating continued and the white opacity decreased but did not disappear completely.

The changes in sweat concentrations of chloride, sodium and potassium over the period of the experiment are shown in Figure 25, together with the equivalent results from the experiment with washed skin. During the first 40 minutes of infusion there was an irregular decrease in the concentrations of all three electrolytes (in the sweat from the unwashed skin), especially in the sweat from the neck. After this time concentrations remained almost constant. The sweating rate was maintained until the end of the experiment and so the very high values caused by sweat evaporating on the skin towards the end of the

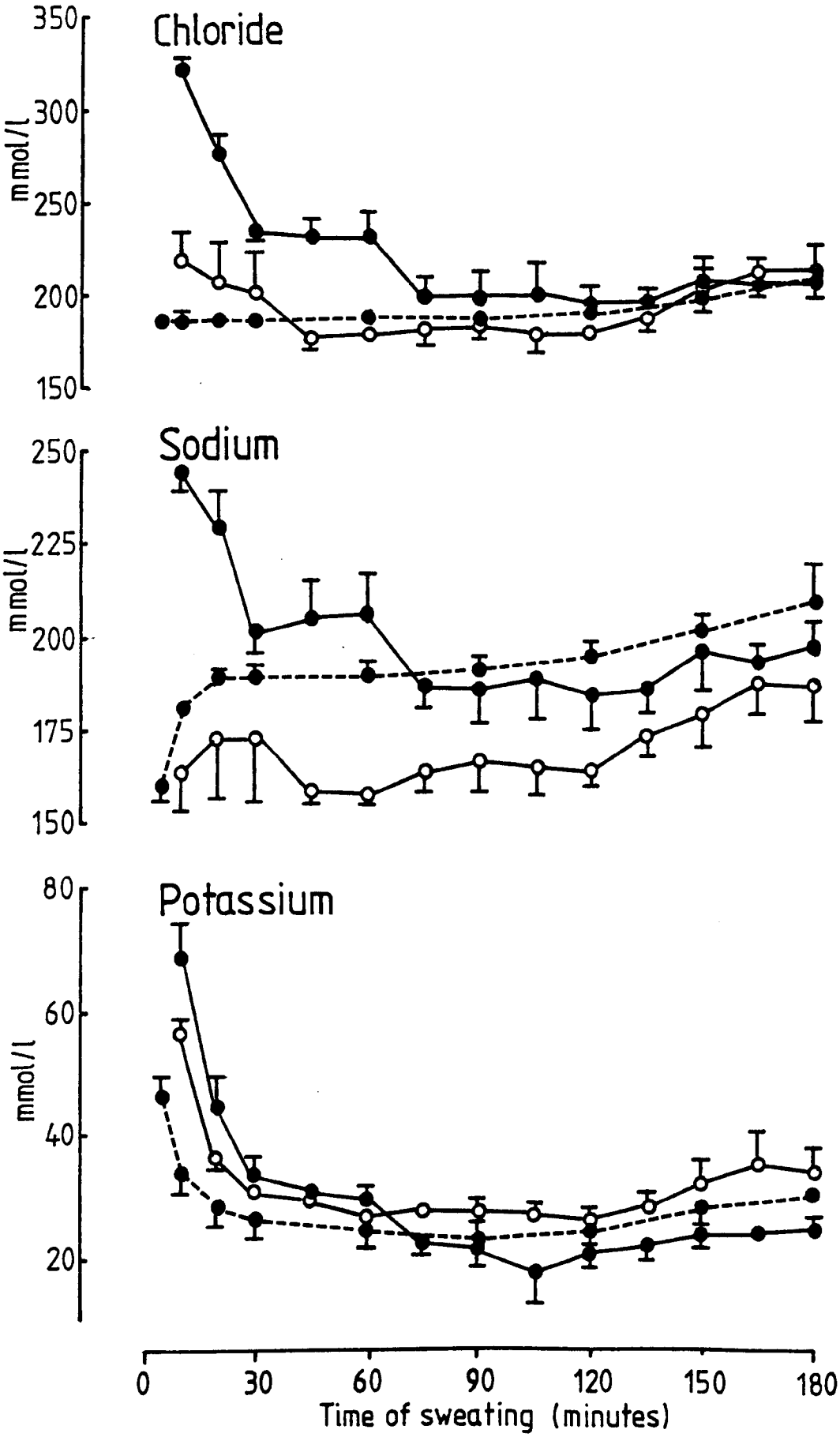


Figure 25. Changes in electrolyte concentrations of adrenaline-induced sweat with time, horses only.

——— unwashed skin
------ washed skin
● sweat from neck
○ sweat from body

heat exposure period were not seen in this experiment. It was considered that the initial decrease was due to contamination of the hair, as with some of the initial samples of heat-induced sweat. Therefore, in order to compare the sweat electrolyte concentrations from this experiment with those of the heat-induced sweat, mean values for each body area of each animal were calculated, omitting those samples collected before 40 minutes of infusion. These figures are presented in Table 38.

TABLE 38

Mean sweat chloride, sodium and potassium concentrations in horses during adrenaline infusion

	Chloride		Sodium		Potassium	
Horse	Neck	Body	Neck	Body	Neck	Body
A	184 ± 4	185 ± 4	177 ± 2	171 ± 4	20 ± 2	23 ± 1
B	215 ± 4	192 ± 8	205 ± 2	180 ± 7	27 ± 1	34 ± 2
D	224 ± 4	191 ± 5	200 ± 3	169 ± 3	26 ± 1	27 ± 1
E	197 ± 10	173 ± 5	186 ± 8	156 ± 3	23 ± 1	31 ± 2
Mean	205 ± 9	185 ± 4	192 ± 6	169 ± 5	24 ± 2	29 ± 2

In contrast to the heat exposure experiment a significant difference in sweat sodium concentration was found between the two body areas sampled ($p < 0.05$). When these figures were compared with the equivalent results for heat-induced sweat from the horses (Table 32) it was found that sodium concentration was significantly higher ($p < 0.001$) and potassium concentration significantly lower ($p < 0.02$) in the adrenaline-induced sweat.

The most striking difference between the sweat produced by the two stimuli was in the patterns of change of sweat sodium:potassium ratio with time (Figure 26). During heat exposure this ratio remained constant at around 3, but during adrenaline infusion it increased from

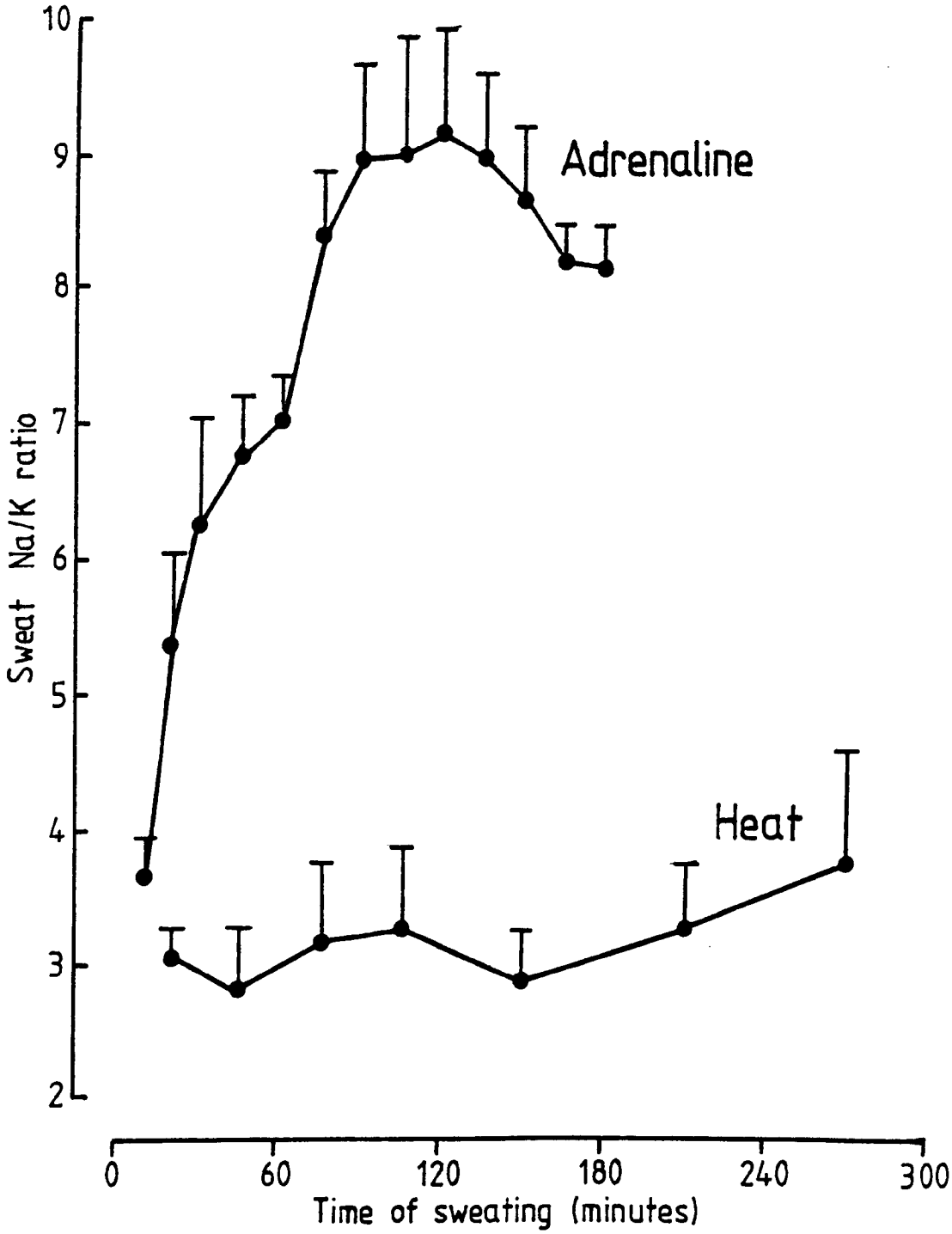


Figure 26. Changes in sweat sodium:potassium ratio with time - adrenaline-induced sweat compared to heat-induced sweat, horses only.

an initial value of 3.64 ± 0.33 to a maximum of 9.15 ± 0.76 after two hours of infusion and thereafter fell slightly. Subjectively, this pattern of change during adrenaline infusion appears to correspond with the observed changes in sweating rate. The steeper increase seen after one hour of sweating coincides with the increase in adrenaline infusion rate in three of the four horses.

The pattern of change in sweat calcium concentration is shown in Figure 27, together with the equivalent results from the sweat collected from washed skin. In the sweat from the neck, calcium concentration decreased throughout the experiment. The concentration in the sweat from the body was slightly lower during most of the experiment, but there was a slight increase in the last 30 minutes.

Sweat protein and magnesium concentrations during the experiment are presented in Figure 28, again with the equivalent results from the experiment with washed skin. As in the heat-induced sweat, there was a close correlation between the two parameters ($r = 0.986$). Both were again initially high in concentration and decreased exponentially, this time until two hours (120 minutes) of sweating, after which they remained constant. Again there was no significant difference between the body areas. As with the heat-induced sweat, exponential regression equations of the form $y = Ae^{Bx}$ were obtained. For magnesium, $A = 17.04$, $B = -0.0202$, and $r = 0.880$. For protein, $A = 15.42$, $B = -0.0194$, and $r = 0.873$.

Sweat urea concentrations are not presented for these experiments as a number of aberrant results were obtained. These extremely low values (< 1.0 mmol/l) appeared to be associated with a fairly marked yellow colouration of the samples and it appeared as if some contaminating substance was interfering with the diacetyl monoxime reaction.

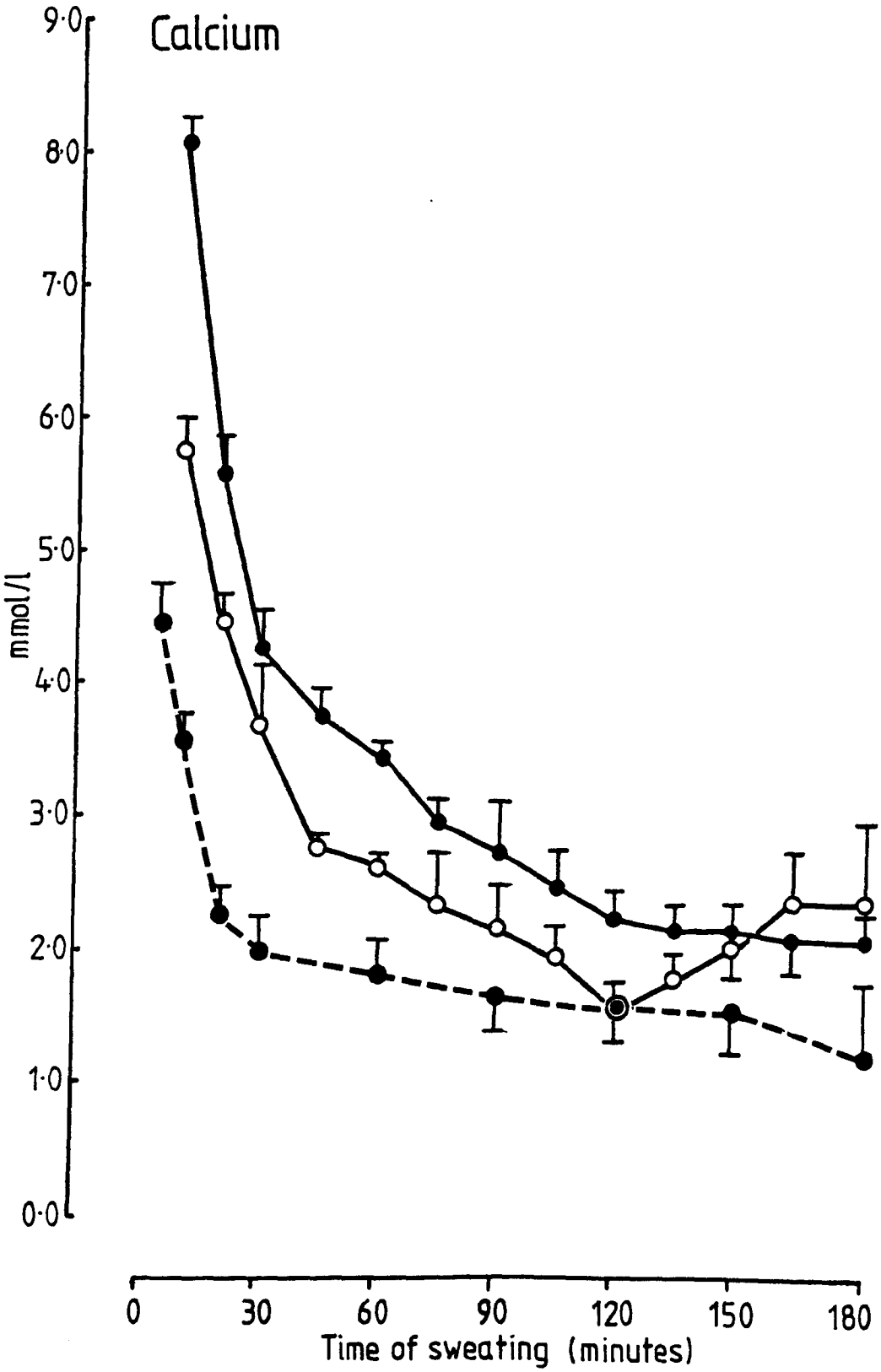


Figure 27. Change in calcium concentration of adrenaline-induced sweat with time, horses only. Symbols as Figure 25.

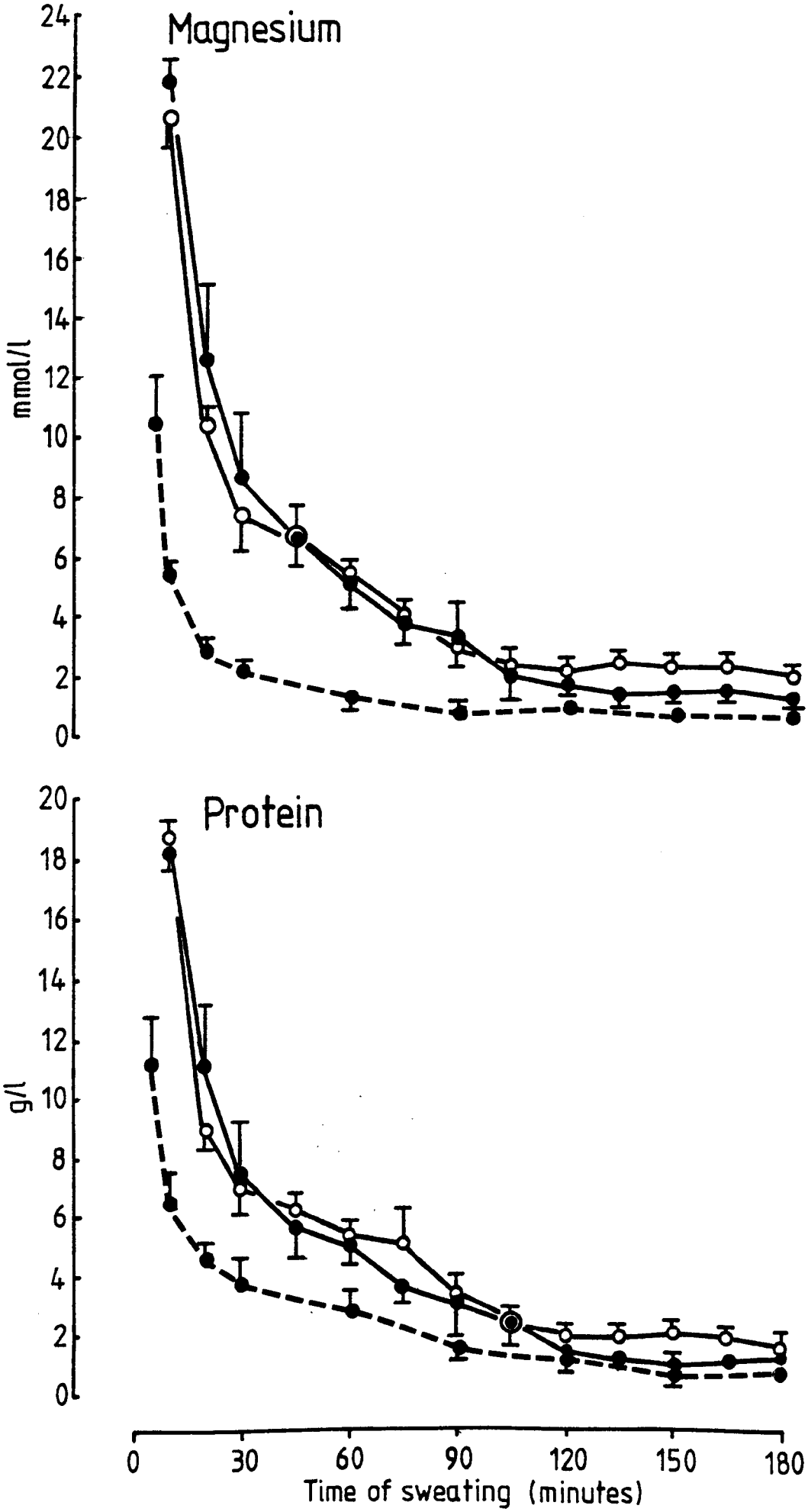


Figure 28. Changes in magnesium and protein concentrations of adrenaline-induced sweat with time, horses only. Symbols as Figure 25.

B. Experiments carried out with washed skin

These experiments were carried out in order to determine the effect of skin and hair contamination on the earlier results and to obtain samples free of such contamination for the further investigation of the sweat protein.

Clinical measurements

The clinical response to adrenaline infusion was exactly as described above. The dose rate of adrenaline used (0.18 µg/kg/min) for these experiments again produced free-flowing sweat within five minutes of the start of the infusion, but although sweating rates were observed to be decreasing towards the end of the three-hour period, sample collection was always possible and the dose rate was not increased.

Blood samples

The results of the blood samples were again virtually identical to those reported above and so only those results which are relevant to the additional sweat analyses (urea and glucose) are presented below.

Sweat samples

The initial sample of sweat from washed skin was completely clear and colourless, while subsequent samples contained variable quantities of a white opaque material which remained in suspension when the sample was centrifuged.

The sweat concentrations of chloride, sodium, potassium, calcium, magnesium and protein are presented in Figures 25, 27 and 28, to allow comparison with the samples from the unwashed skin. The main effect of washing the skin on the chloride, sodium and potassium results was seen during the early part of the infusion. In place of the irregular decrease chloride concentrations were constant while sodium increased significantly ($p < 0.02$) from 159 ± 3 mmol/l at five minutes of infusion to 188 ± 2 mmol/l at 20 minutes and then remained constant. Potassium concentrations still decreased initially ($p < 0.01$) from 46 ± 3 mmol/l

at five minutes to 26 ± 3 mmol/l at 30 minutes, but the initial concentrations were lower than those in the samples from the unwashed skin. Concentrations of all three electrolytes were slightly higher in the last sample compared to the earlier ones, and this was considered to be related to the slight decline in sweating rate having allowed some evaporation of sweat to occur on the skin.

The concentrations of calcium, magnesium and protein in the sweat from the washed skin were lower than those of the samples from the unwashed skin throughout the experiment, but the patterns of change remained the same. Calcium concentration decreased sharply from 4.43 ± 0.33 mmol/l at five minutes to 2.25 ± 0.23 mmol/l at 20 minutes and then continued to decrease more gradually for the remainder of the experiment. The correlation between magnesium and protein was rather less close than in the sweat from the unwashed skin ($r = 0.883$) but was still highly significant. Again both magnesium and protein decreased exponentially with time and regression equations of the form $y = Ae^{Bx}$ were obtained. For magnesium, $A = 5.43$, $B = -0.0191$ and $r = 0.828$. For protein, $A = 7.10$, $B = -0.0182$ and $r = 0.766$.

No problems were encountered with the urea analysis in these experiments. Sweat urea concentrations did not change with time of sweating. There was, however, a marked variation between infusions which appeared to be related to the plasma urea concentration at the time of the infusion, as shown in Figure 29.

Sweat glucose concentration was only measured on two occasions, both from horse D. Figure 30 shows the relationship of sweat glucose concentration to the plasma glucose concentrations on the same occasions. Glucose was not detected in the sweat until the plasma glucose had risen above about 10 - 12 mmol/l.

Sweat pH was also measured on those occasions and mean values were 8.3 ± 0.05 and 8.6 ± 0.03. There was no change with time of sweating.

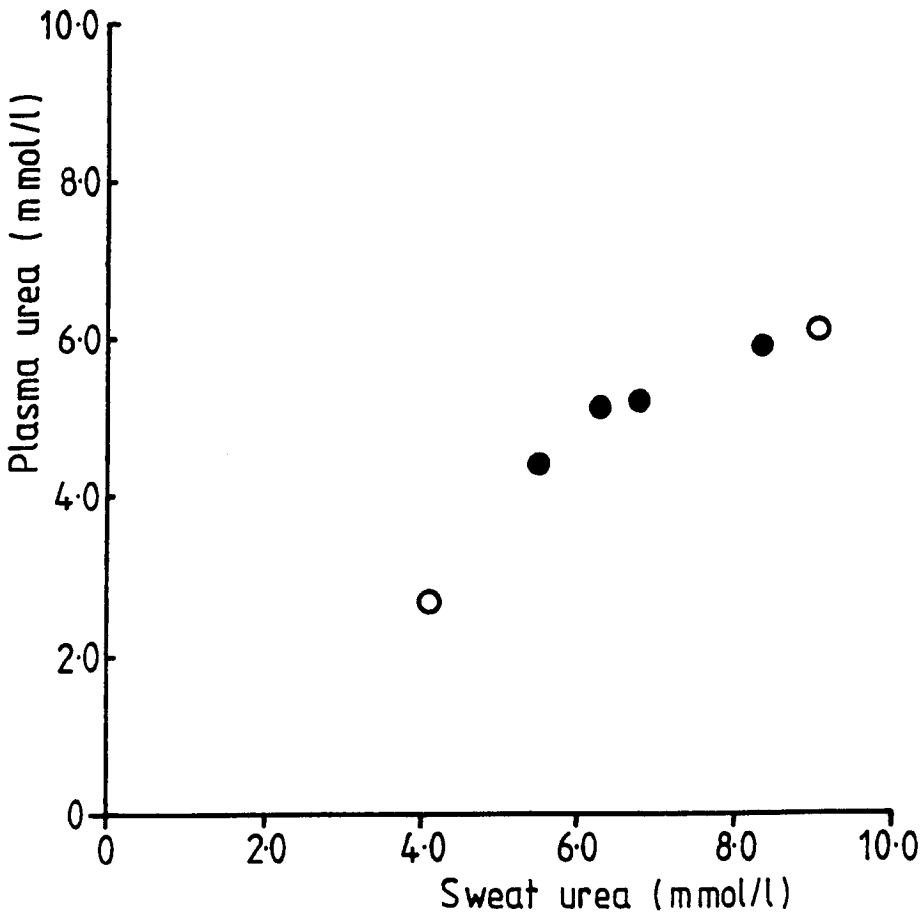


Figure 29. Relationship between simultaneous sweat and plasma urea concentrations in six separate adrenaline infusions, sweat collected from washed skin.

○ Horse C ● Horse D

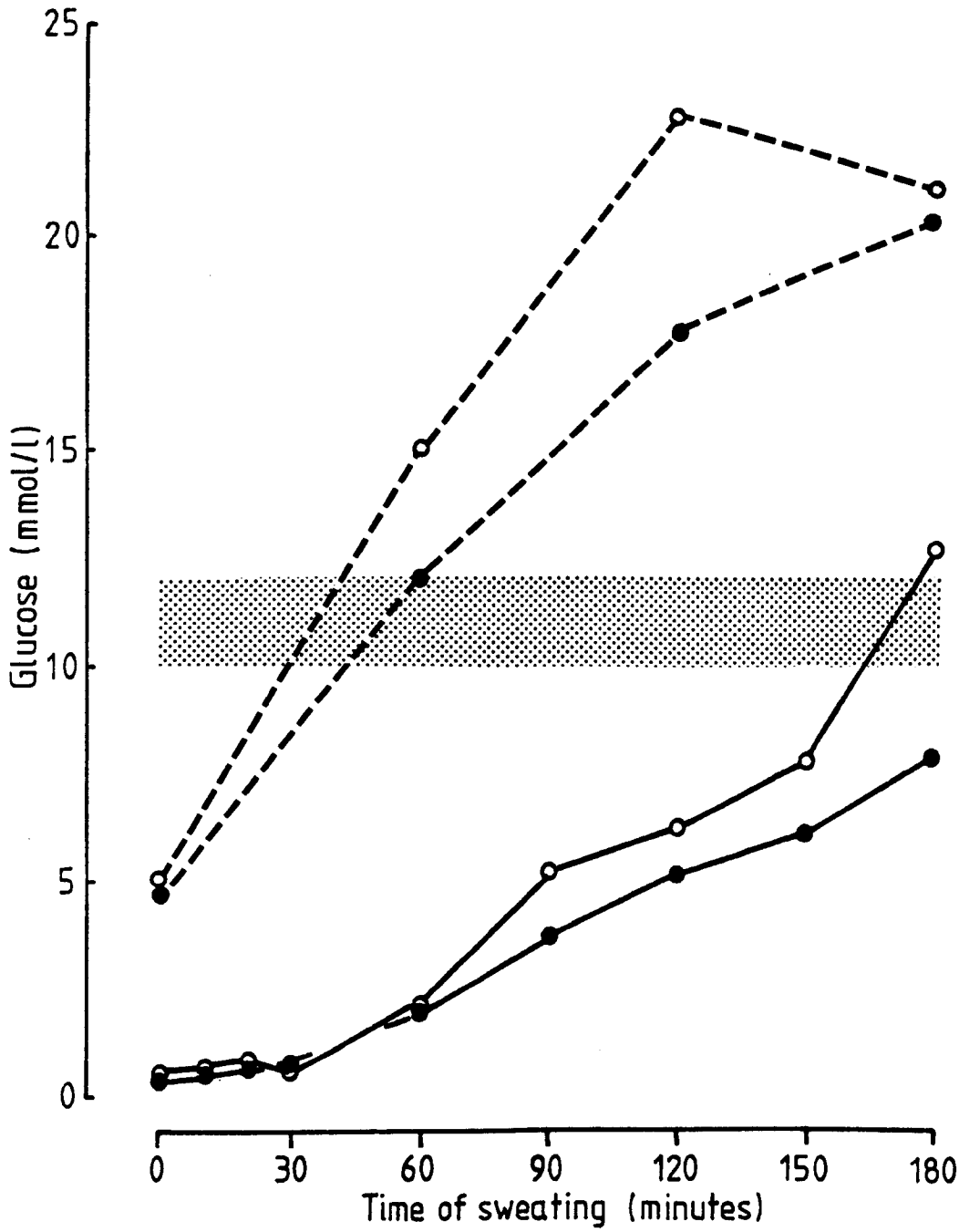


Figure 30. Relationship of sweat glucose concentration (solid line) to plasma glucose concentration (broken line) during intravenous adrenaline infusion in horse D. Sweat and plasma values from the same occasion are shown by the same symbol. The shaded area indicates the approximate renal glucose threshold.

Ultrafiltration experiment

The protein and magnesium concentrations of the eight samples used in this experiment both before and after ultrafiltration are presented in Table 39. Four samples were of heat-induced sweat and four of adrenaline-induced sweat.

Electrophoresis of sweat protein

The electrophoretic pattern of a typical sweat sample with a high protein concentration (16.6 g/l) is shown in Figure 31, together with equine serum and equine serum albumin. There were three protein bands apparent in the sweat sample. One minor fraction had a mobility similar to that of albumin, but the other two fractions were both more mobile than albumin and did not appear to correspond to any serum protein fraction. The mobility of the albumin was unaffected by its being dissolved in the sweat. The electrophoretic patterns of sweat samples of lower protein concentration were similar to that illustrated, but with stain density of all three bands reduced proportionately.

Recovery of sweat protein and magnesium after depletion

The initial concentrations of magnesium and protein in sweat obtained from an adrenaline infusion started a variable time after concentrations had been depleted by a three-hour adrenaline infusion are presented in Table 40.

TABLE 40

Initial sweat protein and magnesium concentrations related to the time elapsed since the end of a three-hour period of adrenaline infusion.

Time elapsed (hours)	Mg^{2+} (mmol/l)	Protein (g/l)
3	2.10	1.14
18	4.80	4.32
24	4.40	4.05
24	7.0	3.20
48	22.0	11.3

TABLE 39

Protein and magnesium concentrations of sweat samples before and after ultrafiltration through a membrane which allowed passage of molecules with a molecular weight of under 25,000

		Protein			Magnesium		
Animal	Stimulus to sweating	Original concentration (g/l)	Filtrate concentration (g/l)	Percentage of original in filtrate	Original concentration (mmol/l)	Filtrate concentration (mmol/l)	Percentage of original in filtrate
A	Adrenaline	17.87	0.78	4.36	21.00	20.80	99.0
B	Adrenaline	15.60	1.68	10.77	19.40	19.60	101.0
C	Heat	10.77	0.54	5.01	10.00	10.13	103.0
W	Heat	9.09	0.74	8.13	10.90	10.77	98.8
Z	Heat	8.66	0.38	4.39	8.90	8.88	99.8
C	Heat	4.20	0.23	5.48	4.20	4.14	98.6
E	Adrenaline	3.20	0.18	5.62	4.40	4.45	101.1
B	Adrenaline	0.82	0.14	17.07	1.12	1.14	101.8
Mean ± SEM				7.73 ± 1.56			100.4 ± 0.56

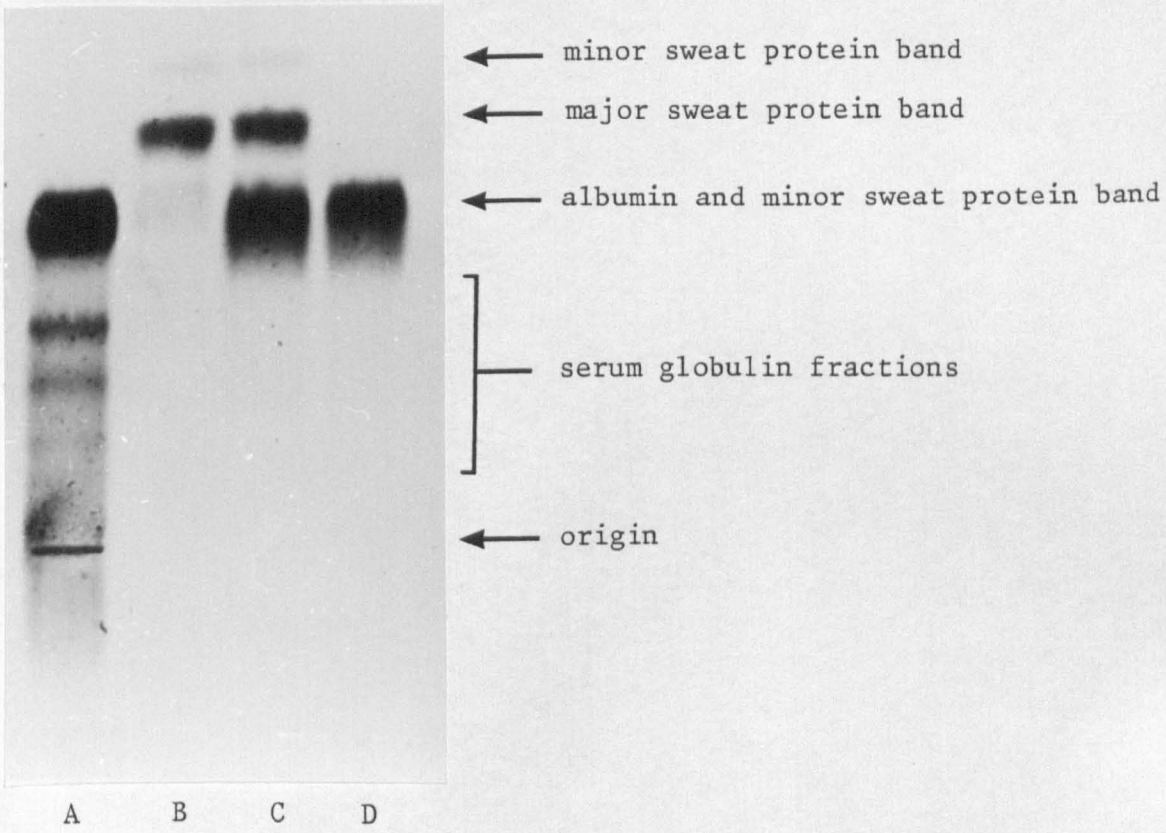


Figure 31. Electrophoretic mobility of sweat proteins compared to those of equine serum and equine serum albumin.

A Equine serum
B Equine sweat
C Equine sweat with equine serum albumin added
D Equine serum albumin.

Discussion

Clinical effects of adrenaline infusion

The most remarkable finding was the very pronounced difference in response to adrenaline seen in the ponies compared to the horses. The increase in heart rate and, subjectively, the severity of the muscular tremors were much greater in the ponies, but this may well have been a result of the higher dose rates administered. In spite of these dose rates, however, the sweating responses of the ponies were minimal compared to those of the horses. This finding is in accordance with that of Langley and Bennett (1923) who reported that the sweat production in response to a subcutaneous adrenaline injection was much less in a "cart horse" compared to other horses of unspecified breeding, presumably hot-blooded horses.

Shetland ponies and draught breeds both belong to the cold-blooded class of horse, as discussed in the General Introduction. It appears from this limited study that there may be a basic difference between cold-blooded and hot-blooded horse types in the response of their sweat glands to circulating adrenaline. This is in marked contrast to the finding in the heat exposure experiment (Section 3) when there was no apparent difference in the amount of sweat secreted between the two groups. Speculation as to the precise pharmacological basis for this phenomenon is outwith the scope of this thesis, however its occurrence did provide a useful basis of distinction, when considering the findings in the blood samples, between those effects directly attributable to the actions of adrenaline (evident in both the horses and the ponies) and those effects attributable to the loss of a considerable volume of sweat (evident only in the horses).

The decline in sweating rates during adrenaline infusion was unexpected. This occurred much earlier in the experiment than the decline seen towards the end of the heat exposure period, and it could be reversed by increasing the dose rate of adrenaline. It therefore appears that this effect was probably not associated with exhaustion of glycogen stores in the sweat gland as was postulated to explain the similar observation in the heat exposure experiment. It may be that the sweat glands were developing an insensitivity to the effects of the adrenaline, as was suggested by Evans et al (1957b) in the more long term case of horses suffering from anhydrosis. In retrospect it might have been interesting to have tested the response of the horses to exogenous adrenaline at the end of the heat exposure period, to ascertain whether or not it was still possible to induce sweating in that situation. It may be that there are two separate factors involved in the progressive decline of sweating rates, one the exhaustion of glycogen, the other a refractory condition of the gland to adrenaline, but it is difficult to speculate on the involvement of either or both of these effects in the development of clinical anhydrosis.

The magnitude of the increase in the horses' heart rates during adrenaline infusion was not very great compared to the rates of over 240 beats/min reported for horses undergoing maximal exercise (Fregin and Thomas, 1983). In contrast the muscular tremors (and sweating) were more pronounced than would be expected in a horse finishing a race, which suggests that sympathetic nervous stimulation may be more important than blood-borne adrenaline in producing the extremely high heart rates measured in galloping horses.

Blood samples

Effects attributable to adrenaline

The effects which have been interpreted as being a direct result of adrenaline action are those which occurred in both the horses and the ponies during adrenaline infusion but which were not seen during the control experiments.

Splenic contraction in the horse is mediated by α adrenoceptors on the splenic capsule (Snow, 1979) and so the increases seen in PCV during adrenaline infusion could well be a direct effect of the adrenaline. However, the mean increase in PCV in the ponies was only 0.03 and the change was not significant. In contrast the PCV in the horses increased by 0.14 ($p < 0.01$) which is inconsistent with the lower dose rate of adrenaline administered to the horses. This apparent anomaly may be partly explained by the fact that total plasma protein and albumin both increased in the horses but not in the ponies which suggests that plasma volume decreased in the horses only, as discussed below. This decrease in plasma volume would tend to increase the PCV's of the horses over and above any increase caused by splenic contraction. The fact that the mean increase in total plasma protein in the horses was only 8.1% compared to a mean PCV increase of 38.9% suggests, however, that about three-quarters of the increase in PCV was due to splenic contraction, i.e. an increase of about 0.1. Considering that the administration of much larger doses of adrenaline to the ponies resulted in a mean PCV increase of only 0.03 it appears very probable that the response of the ponies' spleens to adrenaline was in fact less than that of the horses'.

The effects of adrenaline on carbohydrate and fat metabolism have been extensively documented (Newsholme and Start, 1973). Adrenaline binds to a receptor on the outside of the membrane of the target cell, e.g. muscle cell or adipocyte. These adrenoceptors are classified according to their pharmacological responses and receptor types in the

horse may in some cases be different from the corresponding systems in man (Anderson and Aitken, 1977; Snow, 1979). The hormone/receptor complex is then responsible for the activation of the enzyme adenyl cyclase, and it is considered that the receptor and the adenyl cyclase are probably both asymmetric membrane proteins, the receptor on the outside of the cell and the adenyl cyclase on the inside. The activated adenyl cyclase increases the intracellular concentration of the second messenger, cyclic AMP, which in turn activates the enzyme protein kinase. This protein kinase acts to phosphorylate intracellular enzymes thus changing them from an inactive to an active form. In the muscle cell one of the activated enzymes is itself a kinase, phosphorylase kinase, which in turn acts on glycogen phosphorylase to convert it to its active form (Figure 32). In the adipocyte the activated enzyme is triglyceride lipase (hormone sensitive lipase or HSL). In this way adrenaline stimulates both glycogenolysis and lipolysis in cells containing the appropriate enzyme systems.

The marked hyperglycaemia observed in both the horses and the ponies was presumably mainly a consequence of this increased glycogenolysis and the large increase in plasma glycerol a reflection of the increase in lipolysis (Snow, 1979). There was no change in plasma triglyceride concentration in the two horses in which it was measured.

This hyperglycaemia accounts for the polyuria which was observed towards the end of the adrenaline infusions. It can be seen from Figure 24 that plasma glucose concentrations exceeded the renal glucose threshold of 10 - 12 mmol/l (Stewart and Holman, 1940) by a considerable margin. The consequent glycosuria would naturally lead to an osmotic diuresis and polyuria, and the high glucose concentrations and low creatinine and urea concentrations in the urine samples collected in the later stages of the adrenaline experiments support this hypothesis. The much greater sodium:potassium ratio in these urine samples compared to

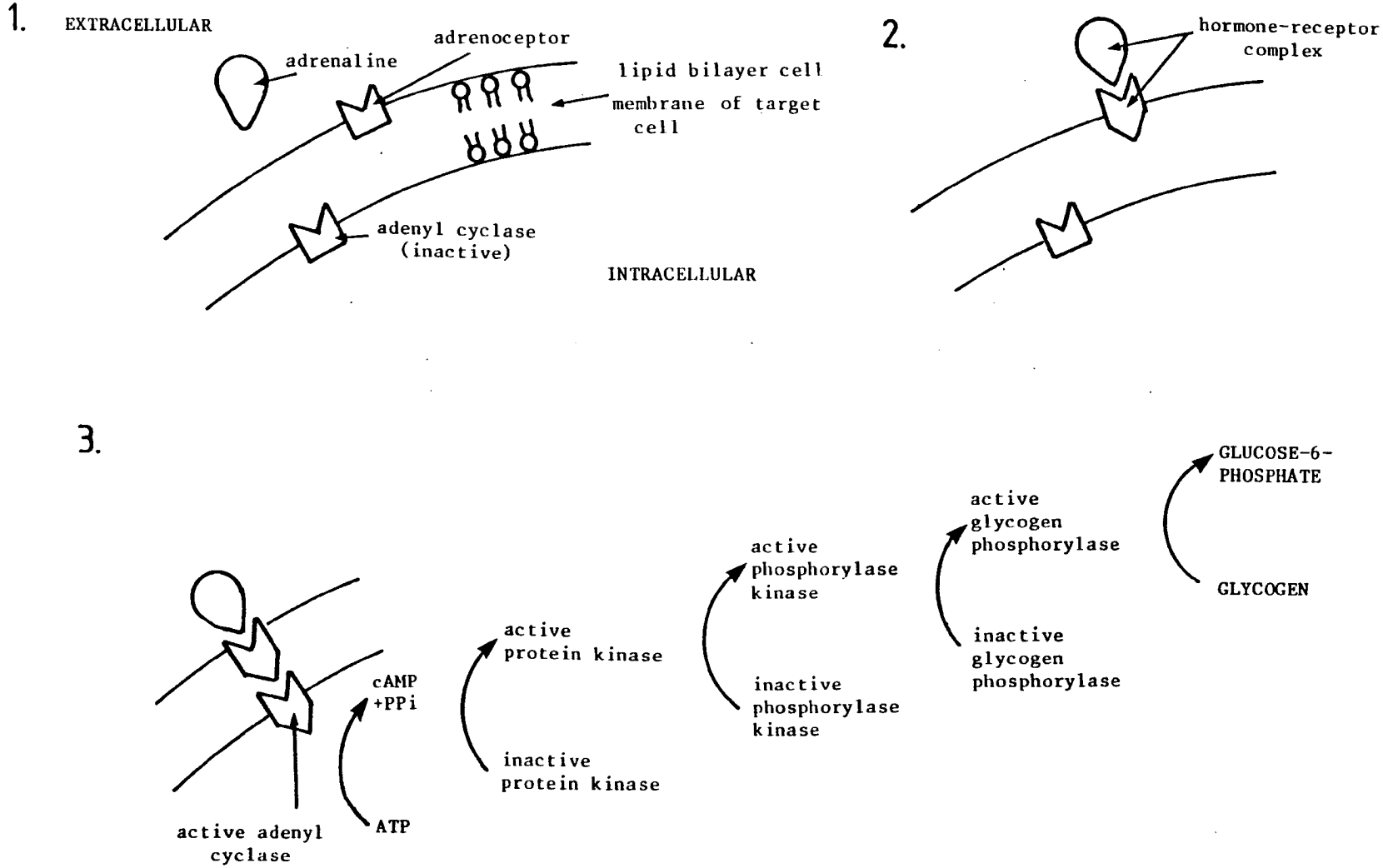


Figure 32. Schematic representation of the mode of action of adrenaline in the stimulation of intramuscular glycolysis.

the samples studied in Section 1 is presumably a result of the higher urine flow rate allowing less time for ionic exchange in the renal tubule. The actual volume of urine excreted during adrenaline infusion was not determined, however the ponies showed no evidence of haemo-concentration, which implies that the volume of urine was small compared to the volume of sweat lost.

It has been shown previously that intravenous infusion of adrenaline causes a decrease in plasma potassium concentration both in man (Massara et al, 1970; Struthers et al, 1983) and in other species which do not sweat in response to adrenaline (D'Silva, 1934; Grassi et al, 1971; Todd and Vick, 1971). This effect is often preceded by a transient increase in plasma potassium concentration which is thought to involve both α and β adrenergic systems, but the sustained decrease appears to be due to an uptake of potassium by a number of cell types, including erythrocytes and muscle cells, which is mediated by a β_2 adrenoceptor linked to Na^+/K^+ ATPase. In this present experiment a gradual decrease in plasma potassium concentration was seen in all animals throughout the adrenaline infusion period, but a similar decrease (which was, however, smaller in magnitude) was also seen during the control saline infusions. These findings were very similar to those observed during the heat exposure experiment in Section 3. Due to the use of only two horses and two ponies for the control experiments statistical interpretation is not possible, but it appears that there is a tendency for plasma potassium concentration to decrease whether or not adrenaline is contained in the infusion and whether or not profuse sweating occurs. The interpretation of this finding appears to be connected with certain findings noted in association with the controlled endurance ride described in Section 5 and will be discussed there.

The reason for the marked decrease in plasma chloride concentration seen during adrenaline infusion is not immediately apparent. It is highly probable that sweat chloride loss contributed to this decrease to some extent in the horses, but considering the very scanty sweating seen in the ponies it is difficult to see how sweat chloride losses could account for the equally large decrease recorded in their plasma samples. Another factor which suggests that some effect other than sweat loss was involved is the fact that decreases in plasma chloride seen during the heat exposure experiment (Section 3) were only about half as great as those occurring during adrenaline infusion. Although the volume of sweat lost during adrenaline infusion was not known, the sweating rates in the horses appeared to be similar to those seen during heat exposure and those in the ponies appeared to be very much less. Considering also the longer duration of the heat exposure experiments it seems unlikely that sweat loss alone was sufficient to account for the magnitude of the decrease in plasma chloride concentration seen during adrenaline infusion in the horses, and even less so in the ponies.

Urine chloride loss also fails to provide a convincing explanation for this finding. Although urine output was observed to increase markedly during the later stages of adrenaline infusion, urine chloride concentration was much lower than sweat chloride concentration, and was in fact hypotonic compared to plasma. It therefore seems impossible that urine chloride loss could have led to any fall in plasma chloride concentration. It also appears unlikely that there was any great increase in any unmeasured plasma anion which might lead to a compensatory decrease in chloride concentration, as discussed below. The reason for the decrease is therefore obscure. The only possible fate of the chloride ions would appear to be a movement into the intracellular fluid, but the possible mechanisms behind any such movement are not clear.

The decrease observed in plasma phosphate concentration was unexpected and in marked contrast to the small regular changes seen at rest (Section 1) and during heat exposure (Section 3) and to the significant increases seen during endurance exercise (Section 2). Beisel et al (1968) noted a decrease in plasma phosphate from 1.06 to 0.74 mmol/l in five men during the first 18 hours of artificially induced hyperthermia. They found sweat phosphate loss to be negligible and urine phosphate excretion to be slightly less than during their control period, and therefore concluded tentatively that this decrease in plasma phosphate was associated with an observed respiratory alkalosis. In this present study no hyperventilation was evident and therefore this explanation would not appear to be feasible; particularly in view of the absence of any decrease in plasma phosphate during heat exposure in the horses when the panting response might have been more likely to lead to hyperventilation.

Sweat phosphate was not routinely measured during this study because preliminary analysis of a number of random samples of both heat and adrenaline-induced sweat indicated that concentrations were so low as to be virtually undetectable. This is in accordance with the findings of Beisel et al (1968). It seems unlikely that adrenaline-induced sweating led to a significant loss of inorganic phosphate, when heat-induced sweating was not accompanied by any decrease in plasma phosphate concentration. It is also unlikely that urinary phosphate losses could be responsible. Urine phosphate concentration in the horse is normally very low (Traver et al, 1977b) and although it was not measured in this present experiment the urine was generally very dilute and a urine phosphate loss sufficient to account for a decrease in plasma phosphate concentration of 0.94 mmol/l (in the horses) or 0.39 mmol/l (in the ponies) does not appear very probable.

A much more likely explanation is provided by the mechanism of action of adrenaline as discussed above. The hormone is responsible for the activation by phosphorylation of phosphorylase kinase and glycogen phosphorylase (in glycogenolysis) and triglyceride lipase (in lipolysis). It is believed that these phosphorylations are only particular cases of a general situation in which most of the actions of adrenaline are brought about via the activation by phosphorylation of specific enzymes contained in the target cells. Other proteins which it has been suggested are phosphorylated by cAMP dependent protein kinase via adrenaline are troponin I, phospholamban (a sarcoplasmic reticular protein) and myosin light chain, all present in muscle. More recently it has been suggested that the second messenger in the adrenergic responses of the human sweat gland is cyclic AMP (Sato, 1977, 1984) and although this pathway has not been elucidated further it again suggests that the phosphorylation of a protein may be an important step. It therefore seems highly probable that the fall in plasma phosphate concentration during adrenaline infusion was a consequence of this multiple protein phosphorylation.

This may also explain the difference in the magnitude of the decrease in plasma phosphate concentration between the horses and the ponies. The ponies showed slightly greater increases in plasma glucose concentration, more severe muscle tremors and markedly greater tachycardia than the horses which is consistent with the higher dose rates of adrenaline they received. However, their sweating responses were much less than those of the horses. It is difficult to believe that this difference alone could account for the much smaller decrease in plasma phosphate concentration seen in the ponies, but if it is symptomatic of a generally lower response of a number of particular adrenergic systems in the ponies as compared to the horses then the difference might be explained. It does not appear as if there is any regular pattern to

those systems which showed a lower response in the ponies, which were sweating (β_2) and splenic contraction (α), compared to heart rate (β), glycogenolysis (uncertain) and lipolysis (β?) which showed a similar or greater response in the ponies. There is, however, some disagreement regarding classification of adrenoceptors in the horse (Anderson and Aitken, 1977; Snow, 1979), which may differ from the equivalent systems in man, and further study of this subject would appear to be warranted.

Although it has been suggested that haemoconcentration may be the cause of a significant increase in plasma bilirubin concentration (Section 1), this effect alone cannot account for the magnitude of the change seen during adrenaline infusion. During the hay feeding experiment the increase in bilirubin concentration of 14.9% was similar to the increase seen in total plasma protein concentration (12.2%). During adrenaline infusion bilirubin concentration increased by 41.5% compared to an increase of only 8.1% in total protein concentration. The explanation for this, as with the similar observation in the endurance horses in Section 2, may be connected to the suggestion that high plasma concentrations of free fatty acids cause a reduction in hepatic uptake of bilirubin by competitive inhibition (Naylor et al, 1980). The very large increase in plasma glycerol during adrenaline infusion strongly indicates that plasma FFA were also very much elevated, and this is confirmed by the results from one horse which are presented in Section 5 as a comparison with endurance exercise. Carlson et al (1965) also found a smaller increase in plasma FFA concentrations in horses during a shorter adrenaline infusion. It is therefore possible that high plasma FFA levels were responsible for much of the increase in bilirubin concentration in this experiment. Although this increase was only statistically significant in the horses, plasma bilirubin also increased in all four ponies (mean increase 33.3%) which also suggests that some effect other than simple haemoconcentration was involved.

Effects attributable to sweat losses

It appears from the results of the control saline infusions that the administration of the volume of saline used in this experiment may have led to a slight haemodilution, as PCV, total plasma protein and albumin fell slightly in all four animals. During adrenaline infusion the ponies, who sweated only slightly, showed no significant change in any of these parameters, while the horses, who sweated copiously, showed significant increases in all three. This indicates that the volume of sweat lost by the horses was sufficient to lead to appreciable haemoconcentration. In spite of this, and of the high sweat electrolyte concentrations, the only change seen in plasma electrolytes which could be attributed to sweat electrolyte losses was a decrease in sodium concentration. This decrease, although statistically significant, was of very small magnitude (1 mmol/l). The changes seen in plasma potassium and chloride concentrations were also seen to an equal extent in the ponies and it appears that additional factors other than sweat losses were involved in these occurrences, as discussed above.

The increases which occurred in plasma creatinine concentrations in the horses during adrenaline infusion may well also have been a result of the haemoconcentration, partly by simple concentration and partly by a consequent reduction in renal perfusion. However, plasma creatinine also increased to a variable extent in all four ponies which tends to suggest that the osmotic diuresis may have itself led to a reduced creatinine clearance. There was no significant change in plasma urea concentration in the horses.

Other changes in plasma parameters

Plasma calcium concentration decreased during adrenaline infusion, however it also decreased during the control experiments, particularly in the ponies. Changes also occurred during adrenaline infusion in plasma magnesium and urea concentrations. These changes were only

statistically significant in the ponies, but they did not occur at all during the control experiments. There is no immediately obvious explanation for these findings.

Sweat composition

Sweat electrolyte concentrations

Concentrations of sodium, potassium and chloride in adrenaline-induced sweat in this experiment were found, as with the heat-induced sweat, to be much greater than those reported for human sweat. Comparison of the sweat electrolyte concentrations seen in this experiment with those of heat-induced sweat indicates, however, that the composition of equine sweat varies according to the stimulus to sweating. It appears to be most valid to make this comparison using the results from unwashed skin for adrenaline-induced sweat as the protocol and sweat collection techniques were designed to be as close as possible to those used in the heat exposure experiment. While it is clear from subsequent results from sweat obtained after thorough washing of the skin that skin and hair contamination must have influenced these findings to some extent there is no reason to believe that this effect was in any way different between the two experiments. In addition, the most striking differences were in the sweat electrolyte ratios, which would not be so seriously influenced by either contamination or evaporation.

When the mean results for each animal throughout the experiment are considered it is clear that sodium concentration was consistently higher and potassium concentration consistently lower in the adrenaline-induced sweat. As a consequence of the higher sodium concentrations the sodium:chloride ratios of the adrenaline-induced sweat were generally higher than those of the heat-induced sweat and in some samples this ratio was greater than one. The most striking illustration of these differences is in the pattern of change of sweat sodium:potassium ratio with time. This ratio did not change with time or with the subjectively

observed changes in sweating rate during the heat exposure experiment. In contrast during adrenaline infusion the ratio almost trebled over the first two hours of the experiment and it appeared that there was a direct relationship between the subjectively observed changes in sweating rate and the sweat sodium:potassium ratio.

It is difficult to explain why circulating adrenaline should produce sweat of a different composition from that induced by the neurotransmitter, whether that be noradrenaline or adrenaline, as both catecholamines presumably act via the same receptor sites. It may be that the blood-borne adrenaline exerts an additional effect on another section of the sweat gland to modify the composition of the secretion. It is perhaps relevant to note that during the final two hours of heat exposure when he was becoming increasingly agitated horse D's sweat sodium:potassium ratio (neck) increased from 3.58 to 5.78 although no such change was seen in any of the other three horses. Speculations as to the modifying role of the adrenergic component of human sweating have not considered any possible effect on sweat composition, and it is not immediately apparent whether any physiological purpose could be served by an adrenaline-induced alteration of the sodium:potassium ratio of equine sweat.

It is clear from the results of the sweat samples collected from the washed skin that skin and hair contamination played a major part in the irregularities noted early in the infusion periods, particularly regarding sodium and chloride concentrations, but that later in the experiments there was very little difference between sweat from washed and unwashed skin. Once skin contamination was eliminated it appears that sweat chloride concentration remained unchanged with time of sweating or with subjectively assessed sweating rate, at about 185 mmol/l. This is in marked contrast to the situation in man, where it has long been observed that sweat chloride concentration varies

directly with rate of sweating (Dill et al, 1938; Johnson et al, 1944). Although sweat sodium concentration remained constant after about 20 minutes of infusion, this time at about 190 mmol/l, there was a significant increase during the first 20 minutes which was not seen in chloride concentration. This meant that sweat sodium:chloride ratio increased from 0.85 to 1.01 during this time. This is again in contrast to human sweat where sodium and chloride always vary in parallel, with a sodium:chloride ratio of about 1.12 (Locke et al, 1951). Although it appears that some of the initial values for potassium concentration in sweat from the unwashed skin were falsely elevated the pattern of a decrease in concentration during the first 40 - 60 minutes of infusion followed by a levelling out at about 20 - 25 mmol/l still remained after washing of the skin.

The fairly close correspondence of electrolyte concentrations in sweat from washed and unwashed skin after the initial 40 - 60 minutes tends to confirm that the conclusions reached concerning these electrolyte concentrations both in the heat exposure and the adrenaline experiments were valid, as comparisons were made using values for sweat collected after this initial period. It is certainly not possible to explain either the hypertonicity of the sweat electrolyte concentrations or the differences between sweat induced by the two stimuli by skin contamination effects. It is interesting to note that the initial sodium and potassium concentrations in the adrenaline-induced sweat were both comparatively close to the concentrations in heat-induced sweat and that it is only in the later samples that the differences between the two stimuli become obvious.

Sweat calcium concentrations

Reports of calcium concentration in horse sweat have ranged from 1.95 mmol/l (Carlson and Ocen, 1979) to 28.5 mmol/l (Soliman and Nadim, 1967). The results of the latter study appear to be improbably high and

may have been affected by sand contamination accumulating during exercise on the exposed skin from which the samples were collected. It certainly appears from this present study that calcium is consistently lower in sweat from washed skin not only in the early stages of sweating but throughout the experiment, and that the calcium concentrations of sweat from unwashed skin should probably be discounted. In the sweat collected from washed skin calcium concentrations decreased during the initial stages of sweating and then levelled off at values similar to those reported by Carlson and Ocen (1979). These later concentrations were below the plasma calcium concentrations and were similar to those reported for human sweat by Costill (1977). Earlier studies of human sweat, however, described even lower calcium concentrations (Talbert et al, 1933; Mitchell and Hamilton, 1949; Robinson and Robinson, 1954), and Mitchell and Hamilton (1949) also described a decrease in concentration during prolonged sweating. More recently Verde et al (1983) have shown that sweat calcium concentration in man depends on the stimulus to sweating, being much higher in heat-induced sweat than in exercise-induced sweat, which may explain some of the discrepancies of previous reports. Unfortunately it was not possible to compare calcium concentration of sweat induced by different stimuli in this present study.

Sweat urea concentration

Schwartz et al (1953) showed that in man sweat urea concentration was consistently higher than plasma urea concentration and postulated that the sweat urea:plasma urea ratio was a constant (1.75) irrespective of plasma urea concentration. It appears, however, that this ratio tends to decrease with increasing sweating rate (Sato, 1977). The more limited results available in this present study suggest that in the case of the horse the sweat urea:plasma urea relationship may not be linear (Figure 29). Although the sweat urea concentration was always higher than the plasma urea concentration, as in man, the ratio varied from

1.24 to 1.52. This direct relationship between plasma and sweat urea may explain the finding of Meyer et al (1978) that the nitrogen content of the sweat was about 50% higher in ponies fed a high protein diet. The deamination of excess dietary protein would be expected to lead to a higher plasma urea concentration and hence to a higher sweat urea (and nitrogen) concentration.

The mechanism whereby the human sweat gland secretes urea in a higher concentration than that of the plasma is still under discussion (Sato, 1977). It would appear either that water must be absorbed from the sweat duct in excess of urea absorption or that urea must be synthesised by the sweat gland itself, but no definitive work has yet been carried out to resolve this question.

Sweat glucose concentration

It appears from the results of this section that glucose can indeed be secreted by the horse sweat gland and that its presence in sweat is directly dependent on the plasma glucose concentration. No glucose was found in the sweat early in the infusion periods, but after about 40 minutes of infusion when plasma glucose had increased to around 10 - 12 mmol/l a gradually increasing concentration of glucose appeared in the sweat. Although these results are somewhat limited, the measurement having been carried out on only two occasions, they suggest the presence of a sweat gland "glucose threshold" very similar to that of the kidney which has been determined as around 10 - 12 mmol/l in the horse (Stewart and Holman, 1940).

This finding is in marked contrast to the situation in man. Lobitz and Osterberg (1947) studied sweat glucose in normal subjects rendered hyperglycaemic (up to 14 mmol/l) by both oral and intravenous glucose administration and reported very low sweat concentrations (under 1.0 mmol/l) which showed no correlation with blood glucose concentration. Similar results were found in patients with long term hyper-

glycaemia due to uncontrolled diabetes mellitus, and the conclusion was reached that the "glucose barrier" in the human sweat gland is highly efficient at both normal and elevated blood glucose concentrations.

This raises an interesting point with regard to the comparison of the sweat gland with the renal tubule which has been discussed by a number of authors (Schwartz et al, 1953). It would certainly appear that, insofar as excretion of glucose is concerned, the equine sweat gland is more closely related to the renal tubule than is the human sweat gland.

Sweat pH

The pH of human sweat is acid and this appears to be due mainly to its high concentration of lactic acid (Thurman and Ottenstein, 1952), although free amino acid content may also be significant (Hier et al, 1946). This high lactic acid concentration is believed to be due to combustion of glycogen by the secretory cells during an active secretory process (Sato, 1977). The limited investigation into sweat pH in this present study confirms the report of Soliman and Nadim (1967) that horse sweat is alkaline, pH about 8.3. This is consistent with the report of Evans et al (1957a) that the concentration of lactic acid in horse sweat is much lower than in human sweat. However, the same authors also report glycogen depletion in horse sweat glands during sweating and the absence of glycogen in severely fatigued glands. More recently Montgomery et al (1982) have also presented evidence that sweat secretion in the horse is an active process at least partially fuelled by glycogen, and the reasons for the high pH and low lactic acid content of horse sweat are not understood. It is however interesting to note that in both the horse and in man the pH of the sweat and the urine in each species are on the same side of neutrality - both acid in man, both alkaline in the horse.

Sweat magnesium and protein concentrations

In the sweat from the unwashed skin the correlation of magnesium to protein and the exponential decrease with time of sweating were almost identical to the findings in the heat-induced sweat (Section 3). However, although the experiments with washed skin also demonstrated a correlation between sweat magnesium and protein concentrations and an exponential decrease with time there were a number of striking differences. The initial concentrations of both parameters were not only lower in the sweat from the washed skin, they also showed a much greater variation between individual infusions. The rate of the exponential decrease was much faster in the sweat from the washed skin, and the correlation between protein and magnesium concentrations was much less close. The steeper exponential decrease may well be a result of the absence of hair allowing the sweat to flow more quickly from the skin, thus reducing carryover in sample collection. However, it appears that a proportion of the protein and magnesium found in the sweat from the unwashed skin, both adrenaline-induced and heat-induced, was almost certainly a result of skin and hair contamination. This further supports the suggestion made in Section 3 that the total amount of protein lost in equine sweat is probably insufficient to lead to any clinical problems. However, in spite of the more variable results and poorer correlation seen in the experiments with washed skin, the protein:magnesium correlation was still significant and the exponential decrease still occurred. Thus the implication remains that protein is being deliberately secreted into the sweat and that magnesium may be involved in the secretory process.

The results of the ultrafiltration experiment indicate that the magnesium is not an integral part of the protein molecule, nor is it bound to it.

The patterns of recovery of sweat protein and magnesium are not clearly defined by the limited number of experiments carried out, but the results generally suggest that around 48 hours are required for protein concentration to approach that found in the initial sweat samples from the resting animal. Sweat magnesium seems to recover more rapidly than protein. In three of the five experiments magnesium concentration was around twice what would have been expected if the usual relationship to protein concentration had been maintained, and the concentration in the sample collected 48 hours after depletion was higher than in any other sample collected from washed skin.

These findings provide evidence that the relationship of sweat protein to magnesium may not be quite so close as earlier data had suggested and the question as to the involvement of magnesium in the protein secretory process still remains an open one. It is however clear that whatever the route of entry of the protein into the sweat some time is required after depletion before the previous initial concentrations are re-established. It may be that this is the time required for synthesis of the protein within the sweat gland.

Characterisation of the sweat protein

Although it is very tempting to assume that the protein is synthesised within the sweat gland and deliberately secreted into the sweat, other possibilities must also be considered. The experiments with washed skin suggest that this is not simply a case of hair or skin surface contamination from exfoliated skin or elsewhere being washed progressively into the sweat, as might at first be suggested by the exponential decrease with time. It seems highly unlikely that such free-flowing sweat could acquire a protein concentration of up to 16.6 g/l in this manner after the meticulous but gentle washing procedure which was used.

The next possibility is connected with the contentious subject of the mode of secretion of the equine sweat gland. The classification of horse sweat glands as apocrine implies that a necrobiotic type of secretion occurs, i.e. that small portions of the surface membrane of the secretory cell are extruded, become detached and are discharged with their contents into the lumen of the gland. This process is also known as decapitation. A microscopic appearance of "pinched-off" areas of apical cytoplasm has been demonstrated on a number of occasions and it has been suggested that this appearance supports the hypothesis of an apocrine mode of secretion (Talukdar et al, 1970). However, Allen and Bligh (1969) have expressed some doubt as to whether such a mode of secretion could possibly produce the profuse sweat secreted by an exercising horse and Jenkinson (1967) has suggested that the "pinched-off" areas of cytoplasm are merely artefacts of fixation. It is conceivable that such a necrobiotic mode of secretion might be partially involved in sweat formation during the early stages of sweating but decrease gradually a sweating continues, thus accounting for the diminishing quantities of protein (and the intracellular ions magnesium and potassium) found in the sweat. This would tend to imply, however, that the proteins present in the sweat would be a mixture of many different types as would occur in the cytoplasm of the secretory cell. In addition, it would imply that a decreasing potassium concentration would always be expected where a decreasing protein concentration was seen, whereas there was no suggestion of such a decrease in potassium concentration during heat-induced sweating.

The earliest report of the protein content of horse sweat (Leclerc, 1888) declared this protein to be albumin and this assumption was generally repeated by later authors (Smith, 1890; Jirka and Kotas, 1959). The results of the electrophoresis presented above

are similar to those presented by Jenkinson et al (1974) who, however, did not run any known proteins other than bovine serum albumin and did not comment on the findings. It can be seen from Figure 31 that the sweat protein was separated into three main fractions, only one of which, and that one not the most abundant, had a mobility corresponding to equine serum albumin. The other two fractions including the most prominent one had a greater mobility than the albumin and did not appear to correspond to any of the proteins present in the horse serum. The mobility of equine serum albumin was unchanged by its being dissolved in the alkaline sweat before electrophoresis. These findings indicate that equine sweat protein is not a particularly heterogeneous mixture and that most of it is neither albumin nor any other protein found in serum. This comparative homogeneity, though not conclusive, tends to suggest that the sweat protein is not merely a by-product of necrobiotic secretion but more probably is intentionally secreted. The electrophoretic pattern also indicates strongly that leakage of serum proteins from the circulation is not involved to any great extent.

Subsequent to the findings presented here, further investigation and characterisation of this protein has been undertaken using a variety of samples of both heat and adrenaline-induced sweat collected during the experiments described in this section and Section 3 of this thesis (Eckersall et al, 1982). These experiments confirmed that serum albumin accounts for less than 1% of the total sweat protein. On SDS gel electrophoresis five major protein components were separated but on gel filtration only two major peaks were found. It appeared that some form of aggregation was taking place on the gel filtration column. The results of that study suggested that the proteins of the two gel filtration peaks might have a similar primary structure but that the higher molecular weight peak also contained a

carbohydrate residue. Further investigation, however, demonstrated that the two peaks differed in their amino acid composition and that both were in fact glycosylated (Eckersall et al, 1984).

Considering all these points together, and the finding by Montgomery et al (1982) of the secretory granules in the fundus cells of the sweat gland (which was discussed in Section 3), it appears much more probable that this protein is deliberately secreted into the sweat for some purpose than that it is a by-product of a partially necrobiotic secretory process. The question still remains, what is this purpose?

Function of the sweat protein

It was initially thought that the protein might be performing some antibacterial role on the surface of the skin. A number of samples of sweat were tested for antibacterial activity but although the sweat samples themselves were found to be sterile no antibacterial activity could be demonstrated. More recently it has been suggested that the protein might function as a detergent-like surfactant to increase the surface area of the sweat and improve evaporation (Eckersall et al, 1984). This suggestion has some merit, as the hair coat of the horse is normally quite difficult to wet externally, even by introducing water to skin level by parting the hair. Carrier (1984) considers that the possession of a hair coat seriously reduces the efficiency of sweating due to pockets of stagnant air between the hairs interfering with evaporation. It is possible that inclusion of the protein as a wetting agent facilitates coating of the hair with sweat, eliminates air pockets between the hairs, reduces run-off of ineffective sweat and so aids heat loss.

Conclusions

This section has demonstrated that although a number of pronounced changes in plasma biochemical parameters occurred during adrenaline infusion most of these could be attributed directly to the effects of the adrenaline and the changes attributable to the effects of the adrenaline-induced sweating were comparatively slight. Some haemoconcentration was evident but there was very little effect of sweating on plasma electrolyte concentrations. It appeared that Thoroughbred horses were much more sensitive than small ponies to a number of the effects of adrenaline, particularly sweating.

Sweat electrolyte concentrations were hypertonic compared to plasma throughout the period of sweating. Chloride concentration was almost identical to that seen in heat-induced sweat but sodium concentration was higher and potassium concentration lower. The most striking difference was seen in the changes in sodium:potassium ratio with time which appeared subjectively to be related to sweating rate in the adrenaline-induced sweat (as with heat-induced sweat). The reasons for these differences in the composition of sweat produced by the two stimuli are unknown.

Sweat urea concentration was found to be related to plasma urea concentration as is the case in man, but in contrast to man it was also found that glucose might be present in horse sweat at certain plasma glucose concentrations. It is suggested that there is a sweat gland glucose threshold in the horse similar to the renal glucose threshold.

It appeared that previous reports of sweat magnesium and protein might have been influenced to some extent by skin and hair contamination, but a correlation between the two constituents and an exponential decrease with time were still evident. The magnesium was not protein-bound. Very little of the protein proved to be serum albumin

and the remainder, which separated into two fractions on electrophoresis, did not correspond to any other serum protein. It was concluded that the protein was more likely to be the result of deliberate secretion into the sweat than to be a by-product of a necrobiotic type of secretory process and a suggestion of a possible function as a wetting agent was discussed.

SECTION FIVE

STUDIES OF HORSES UNDERGOING AN EIGHTY KILOMETRE RIDE UNDER CONTROLLED CONDITIONS

Introduction

Limitations of studies using competing endurance horses

As discussed in Section 2, there are a number of disadvantages inherent in relying on privately-owned horses involved in competitions to investigate the effects of long distance exercise. The most important one is the virtual impossibility of finding a group of even two or three animals which could be considered comparable for statistical purposes due to the large number of variable factors already discussed such as breed, diet, management, training, rider tactics and so on. In addition, as the rider's main concern is usually to win the event and not to further a research project, the amount of help which can reasonably be expected is naturally limited. This factor accounts for most of the missing blood samples and the inadequate number of urine samples obtained in Section 2. It was also apparent that most of the elite horses involved in the Endurance Horse and Pony Society events were not performing to the limits of their capabilities, whether this was because the riders feared veterinary elimination or because they simply underestimated their horses' potential was not clear. Whatever the reason, the result is that studies of competing horses tend to be less informative than studies of human athletes, who are not subject to elimination on medical grounds after having crossed the finishing line and who usually try to push themselves to the limit of their capabilities.

Fluid and electrolyte studies

It is clear from Section 2 that in spite of the decrease in plasma volume which frequently occurs in endurance horses, as shown by the increases in total plasma protein, plasma sodium concentrations change very little. When a change in plasma sodium did occur in the rides in

Section 2 it invariably took the form of an increase. Studies of sweat sodium concentration both in this thesis and elsewhere have however shown that this is isotonic or hypertonic to plasma, which implies that the plasma sodium concentration would be expected to fall, rather than to rise. Although plasma chloride did in fact decrease during the endurance rides the magnitude of the decrease was not usually very great. In order to investigate further the mechanisms by which the horse maintains such constant plasma electrolyte concentrations while losing a remarkably concentrated sweat it will be necessary to obtain both sweat and urine samples as well as blood samples during an endurance ride. This will allow comparison of exercise-induced sweat with the heat- and adrenaline-induced sweat studied in Sections 3 and 4 and provide further information about electrolyte homeostasis during prolonged exercise.

Fuel utilisation

The differences already discussed between privately-owned endurance horses (breed, diet, management, training regime, rider tactics, etc.) mean that body fuel utilisation varies considerably between individuals and it has been shown (Lucke and Hall, 1980c) that patterns of metabolism in horses finishing almost simultaneously may differ considerably. The study of a more uniform group of horses would therefore be expected to yield more precise information regarding body fuel utilisation, particularly regarding the influence of fuel depletion on fatigue.

Plasma enzyme studies

The extreme variability of plasma enzyme activities in endurance horses has been discussed in Section 2, but again due to the multiplicity of variables involved, the necessity of relying on the memories of the owners for background information and the impossibility of following up the animals for any prolonged period after the events, the reasons

for these widely varying enzyme activities remain unclear. Again, the study of a group of horses training and exercising under controlled conditions might provide some illumination of the mechanisms responsible for the elevations in plasma enzyme activities.

Urine composition

Changes in renal function during prolonged exercise in man have been studied by Refsum and Strømme (1975) and Castenfors (1977), in subjects who were allowed free access to drink. Sodium excretion is reported to decrease, and it is believed that this is not related to the action of aldosterone but is due to changes in renal haemodynamics which are incompletely understood. In contrast potassium excretion appears to increase and it is suggested that this may be connected to the haematuria which is a common occurrence in human athletes during heavy exercise. Surprisingly, no decrease in the rate of urinary water excretion has been found, in fact it has been suggested that urinary concentrating ability may be impaired during exercise.

Very little work has been carried out to date concerning changes in renal function or electrolyte excretion during exercise in the horse. Smith (1889) studied 24-hour urine excretion in horses at rest compared to horses which were in regular work, but the findings are hard to interpret due to the limited analytical facilities available and the lack of any statistical treatment of the results. However, the suggestion is made that daily sodium, potassium and chloride excretion were all less during work than when the horses were rested. In almost a century following this very painstaking piece of work practically no attempt has been made to follow it up using modern analytical methods.

Carlson (1980) has stated that urine output appears to decrease during an endurance ride, that urine sodium and chloride concentrations decrease markedly and that urine potassium concentration also decreases although to a lesser extent. However, no numerical data at all are

given to support these statements. In a later publication (Carlson, 1983) urine specific gravity, pH and concentrations of sodium, potassium and chloride were compared between 11 horses at the 48 km stage and 8 horses at the finish of a 160 km competitive endurance ride. Significant decreases were reported in pH and in sodium and potassium concentrations between 48 and 160 km, while specific gravity remained unchanged and a very substantial decrease in chloride concentration was not statistically significant. No details were given of the diets of the horses, of their food and water intake during the ride or of their urine composition while at rest, although as the horses were privately owned these were probably variable. These results may indeed give some reflection of the responses of the kidney to prolonged exercise, as they are all consistent with the sort of effects which would be expected considering the extent of the losses of all of these electrolytes in sweat. However, urine electrolyte concentrations, as discussed in Section 1, are entirely at the mercy of the level of diuresis occurring at or just prior to sample collection. In horses at rest points on an endurance ride, when water is routinely offered, this would be expected to vary considerably depending on the time elapsed between drinking and urinating in each individual. In addition it has been shown in Section 1 (and it was suggested by Sir Frederick Smith as early as 1889) that both concentrations and excretion rates of most urinary constituents vary widely over a 24-hour period. It is therefore impossible to place any reliance on statistically significant changes occurring in the composition of the urine of horses over a period of about 12 hours - such changes are equally easy to demonstrate in the resting animal.

Previous controlled studies of long distance exercise in the horse

Studies of horses undergoing endurance exercise under controlled conditions are very few in number compared to publications reporting the results of blood samples taken from horses involved in competition.

Both Murakami and Takagi (1974) and Snow and Mackenzie (1977b) have studied submaximal exercise over a distance of 22 km, but if the distance of the shortest competitive ride organised by the EHPS, 40 km, is taken as a definition of "endurance" exercise, then it appears that only three controlled studies of this nature have been conducted.

Lindholm et al (1974a) compared muscle glycogen depletion patterns in three horses which trotted (without riders) for 72 km at 18 km/hr to a shorter period of faster exercise (30 km at 30 km/hr). This study indicated that although the slow twitch fibres were not completely depleted of glycogen when the horses began to show signs of fatigue these fibres were very heavily utilised during endurance exercise. It was suggested that it may be changes in these fibres in particular which are important in the impairment of exercise capacity in fatigued horses. Blood lactate, the only blood parameter measured, increased very little in the endurance trial compared to the faster exercise.

The effect of dietary fat levels on endurance performance has been investigated by both Hintz et al (1978) and Hambleton et al (1980). The former study compared horses fed a "high fat" diet with those fed a normal diet, using a 60 km trail ride as the exercise test. The results appear to have been somewhat inconclusive, showing no difference between the groups in terms of muscle glycogen stores or plasma FFA concentrations and indicating no actual benefit of the high fat diet in terms of performance. However, it was considered that some sparing of glycogen was evident and that over a longer distance some improvement in endurance performance might have been seen. As the horses in this study only travelled at 9.6 km/hr and it was considered that they were "not worked hard" it would appear that the results do not represent horses performing to the limits of their endurance capacity.

The same is probably true of the latter study (Hambleton et al, 1980), where the speed was 11.5 km/hr and the distance about 65 km, but the horses were again not carrying riders, the ground was perfectly level and the environmental temperature only 0°C. Four different levels of dietary fat were used and the exercise test was carried out at four stages of training. A number of effects of diet and training were noted, but many were not statistically significant, perhaps because only four horses were used. In particular, training led to increases in resting muscle glycogen and plasma inorganic phosphate concentrations, a greater exercise-induced decrease in plasma calcium concentration and smaller exercise-induced increases in plasma CK and AST activities. Increasing dietary fat levels resulted in larger exercise-induced increases in plasma glucose and cholesterol concentrations. Findings in relation to plasma free fatty acids were, however, somewhat bizarre, with linoleic acid (C18:2) the most abundant at 60% of the total, and very little change (sometimes a decrease) in total plasma concentrations occurring with exercise. These results have been contradicted by later studies which will be discussed below (Luther et al, 1981; Rose and Sampson, 1982). Both Hintz et al (1978) and Hambleton et al (1980) have reported that the feeding of increased levels of dietary fat had no adverse effects and recommend it as a useful method of providing concentrated energy to working horses.

Purpose of this section

The work reported in this section was therefore carried out in order to study the composition of exercise-induced sweat in the horse, the mechanisms of electrolyte homeostasis during prolonged exercise-induced sweating, the patterns of utilisation of body fuels by horses undergoing endurance exercise, the relationship between fuel depletion and fatigue and the correlation between plasma enzyme activities and any clinical signs of muscular injury.

Materials and Methods

1. Animals

Horses A, D, E and F were used in this Section.

2. Management

The stable routine at the time of this study was as described in Section 1, except that the bedding used was wood shavings.

3. Diet

The diet consisted of 8 kg of the commercial cube diet (Spillers' Complete Cubes) and 11 kg hay daily during training and until after the first endurance ride. Before the second endurance ride (six days before in horses A and E, 10 days before in horses D and F) an increased starch content was added to the ration in the form of 1.4 kg maize, 0.5 kg oats and 0.5 kg bran. On the days of the endurance rides the horses were given half their feed two hours before the ride. On returning to the stable after the ride hay was offered immediately and the remainder of the concentrate feed was given two hours later.

4. Training

Exercise was increased progressively over a period of two months until the horses were being worked at the trot and canter for a period of 2 - 3 hours/day, five days per week.

5. Procedure

The endurance rides were carried out with the horses in pairs, A and E, D and F. They were saddled in the stable and then ridden at a walk to the location of the exercise area, a large undulating field 1.6 km away. They were then ridden at a steady canter with short periods of active trotting around a figure-of-eight course (total length 1.6 km) within the field either for 80 km or until withdrawal due to fatigue. Fatigue in this context was assessed by the horses' marked unwillingness to maintain the steady cantering pace. The horses were

stopped for sampling at 16 km (5 - 10 minutes), 40 km (20 minutes, as this included riders' lunch break) and 64 km (5 - 10 minutes). On the occasions when one horse of a pair did not complete the course he was held in the field until the other horse had finished, but sample timing was calculated from the time of cessation of exercise. After the ride the horses were ridden at a walk back to the stables. This procedure was carried out twice with each pair of horses. There was a two-week interval between rides for horses A and E and a four-week interval for horses D and F.

Weather conditions during the first ride (both pairs) were fine to overcast with a moderate wind, and the ground was heavy. During the second ride (horses A and E) the weather was wet and very windy with very heavy ground while for the second pair (horses D and F) there was occasional light rain and the ground was fair to heavy.

The degree of fatigue shown by the horses at the end of the rides was subjectively graded according to the following criteria:

1. Withdrawn early due to lameness, not unduly fatigued.
2. Completed 80 km and appeared able to continue at the same pace (in rider's opinion).
3. Completed 80 km but appeared unable to continue further at the same pace.
4. Withdrawn early due to fatigue as shown by extreme unwillingness to maintain a cantering pace.

6. Sampling

(a) **Blood samples**

These were collected in the stable before saddling, in the field before beginning the ride, at 16, 40, 64 and 80 km (or point of withdrawal) and then 30 minutes, and 1, 2, 4 and 18 hours after the ride. Samples were placed in ice immediately after collection and were centrifuged no more than one hour later. Plasma was stored at -20°C

until analysis. Samples were analysed for total protein, albumin, sodium, potassium, chloride, calcium, magnesium, phosphate, creatinine, urea, uric acid, glucose, cortisol, glycerol and bilirubin. Certain samples were also analysed for CK, AST, ALP and free fatty acids (both total and individual). Additional samples were collected for enzyme analysis only at 1, 2 and 3 days after each ride then at intervals of 3 - 7 days for 2 - 6 months following the experiment. All methods were as described in the General Materials and Methods section and the reaction rate method was used for the ALP analysis.

In order to study further the changes seen in plasma free fatty acid composition seen, individual free fatty acids were also quantified in two plasma samples from one of the adrenaline infusions described in Section 4 (horse D), and in the extracted triglyceride fractions of two samples of adipose tissue obtained post mortem from a horse of unknown identity. Methods of fatty acid extraction and analysis are also described in the General Materials and Methods section.

(b) **Urine samples**

The time of the last urine passed before the rides was noted for each horse. No urine was passed during the rides. After the horses had returned to the stable urine bags were fitted so that the entire volume of the first urination after the rides could be collected. The time of this urination was also noted in order to calculate the rate of urine production during the period of exercise. The samples were processed and analysed for specific gravity, pH, urea, creatinine, sodium, potassium and chloride by the methods described.

(c) **Sweat samples**

The horses' backs were washed on the day before each ride but the hair was not clipped. Each horse was fitted underneath the saddle with a cotton saddle-cloth partially lined with plastic. Two pads of absorbent material (CLD2, Southalls Ltd) with a narrow adhesive strip on

one side were placed under this cloth so that the adhesive fixed the pads to the plastic lining (one on either side of the back). After 16, 40 and 64 km the sweat-soaked pads were removed and placed in sealed plastic bags in ice, and fresh pads placed in position. After 80 km the final pads were removed. Within one hour of retrieval sweat was squeezed from the pads into a plastic sample tube and thereafter processed as described in Section 3. Samples were analysed for sodium, potassium, chloride, magnesium, total protein and urea by the methods described.

In order to assess the comparability of this sweat collection method with that used in Sections 3 and 4, a solution of similar electrolyte composition to equine sweat was prepared and four pads were soaked in the solution for two hours. The composition of the solution before and after soaking in the pads was compared (Table 41). In addition, sweat was collected by this method during two of the adrenaline infusions described in Section 4, using horse D. Collection pads were changed every hour on one occasion and every 30 minutes on the other, and the composition of the sweat compared to the average composition of the sweat collected from the washed area of the neck during the same period (Table 42).

(d) **Other samples**

Blood samples were also collected for haematological estimations and muscle samples were collected by percutaneous needle biopsy from the middle gluteal muscle for fibre typing, glycogen analysis and ultra-structural studies. These results are not included in this thesis but have been reported elsewhere (Nimmo and Snow, 1982; Snow et al, 1982).

7. Statistical analysis

The results of both rides have been considered together, i.e. n = 8 for all parameters (except the 80 km samples where n = 6 due to the failure of two horses to proceed beyond the 64 km point). All comparisons between different distances and times were made using the paired t test.

TABLE 41

Comparison of electrolyte solution before and after soaking in absorbent pads for two hours

		Fresh Solution	After Soaking (n = 4)
Chloride	(mmol/l)	195	198 ± 2
Sodium	(mmol/l)	155	157 ± 3
Potassium	(mmol/l)	32	31 ± 1
Calcium	(mmol/l)	1.36	1.3 ± 0.1
Magnesium	(mmol/l)	4.9	4.7 ± 0.1

TABLE 42

Comparison between sweat collected directly from the neck and sweat soaked into pads under a saddle during two adrenaline infusions in horse D

Time of Infusion (minutes)	Cl^- (mmol/l)		Na^+ (mmol/l)		K^+ (mmol/l)		Ca^{2+} (mmol/l)		Mg^{2+} (mmol/l)		Protein (g/l)		Urea (mmol/l)		pH	
	Neck	Saddle	Neck	Saddle	Neck	Saddle	Neck	Saddle	Neck	Saddle	Neck	Saddle	Neck	Saddle	Neck	Saddle
1st infusion																
0-60	187	182	193	186	23	22	2.5	2.6	3.6	7.2	4.9	8.5	5.3	5.4	8.4	7.4
60-120	189	186	205	185	18	18	2.3	2.2	1.7	5.2	2.4	3.3	5.5	4.9	8.3	7.4
120-180	191	178	210	190	22	19	2.0	1.9	1.0	4.0	1.0	1.6	5.9	4.7	8.3	7.5
2nd infusion																
0-30	181	188	182	179	22	24	3.2	3.2	7.2	11.7	7.7	13.4	6.4	6.6	8.7	7.9
30-60	187	186	193	178	20	20	2.7	2.7	2.6	5.6	3.9	5.4	6.4	6.4	8.6	7.7
60-90	185	180	194	168	18	19	2.5	2.6	1.7	3.7	2.9	3.7	6.4	6.5	8.6	7.8
90-120	193	200	205	191	18	20	2.4	2.4	1.2	3.2	1.9	3.4	6.9	6.5	8.6	6.6
120-150	202	180	213	172	21	18	2.4	2.0	1.0	2.1	1.2	1.8	7.1	6.6	8.5	6.9
150-180	206	182	217	178	23	18	2.3	1.4	0.9	1.4	0.8	1.5	7.5	6.5	8.5	6.8
Mean	191	185	201	181	21	20	2.5	2.3	-	-	-	-	6.4	6.0	8.5	7.3
± SEM	±3	±2	±4	±3	±1	±1	±0.1	±0.2					±0.2	±0.3	±0.1	±0.2

Results

A. Performance and clinical observations

Horse F did not complete the 80 km distance on either occasion due to fatigue, while Horse A had to be withdrawn during the second ride due to lameness. Otherwise the horses completed the 80 km. All four horses began to sweat visibly within the first 1 km and, subjectively, sweating rates then appeared to increase gradually over the next 2 - 3 km until their coats were saturated. No noticeable decrease in sweating rates was observed before the end of the rides. The distances completed by each horse, the times taken, and the subjective grades of fatigue manifested after the rides are shown in Table 43.

No medical problems were encountered in any of the horses, except that muscle tremors which persisted for up to four hours were seen in horse E after both rides and in horse A after the first ride. On returning to the stables the horses drank copiously and readily ate the hay and concentrates when offered. On the day following the rides all horses showed some signs of stiffness and tiredness and after the second ride horses A and E had slight oedema of the lower limbs, probably due to the particularly heavy ground conditions. Otherwise the horses appeared well.

TABLE 43

Performance characteristics of the horses

Horse	Ride	Distance Covered (km)	Time Taken (minutes)	Average Speed (km/hr)	Degree of Fatigue
A	1	80	288	16.7	2
	2	58∂	195	17.8	1
D	1	80	288	16.7	3
	2	80	265	18.1	2
E	1	80	266	18.0	3
	2	80	260	18.5	3
F	1	61∂	207	17.7	4
	2	72*	235	18.4	4

*Finishing blood and sweat results included with 80 km group.

∂Finishing blood and sweat results included with 64 km group.

B. Blood samples

(a) Parameters indicative of plasma volume changes

Changes in packed cell volume and total plasma protein and albumin concentrations during and after the rides are shown in Figure 33 and certain mean values are also included in Table 44. All three parameters were already showing significant increases by the 16 km stage (compared to the values at the start) and by the end of the 80 km increases were substantial: PCV 40.9%, total protein 24.6% and albumin 29.7%. In addition a significant increase ($p < 0.001$) in PCV was seen between the samples collected in the stable (0.38 ± 0.01) and those collected at the start (0.45 ± 0.01). Immediately after the finish all three parameters began to decrease.

(b) Plasma electrolyte concentrations

Changes in plasma sodium, potassium and chloride concentrations during and after the rides are shown in Figure 34 and selected mean values are also included in Table 44. Plasma sodium concentration increased gradually throughout the rides and began to decrease again immediately after the finish with the sharpest decrease occurring between 30 and 60 minutes after the finish. Plasma chloride concentration decreased during the first half of the rides but after 40 km a constant concentration was maintained which had not shown any significant return towards the pre-ride value even by 18 hours after the finish. Plasma potassium concentration increased by a substantial amount (1.4 mmol/l) between the samples collected in the stable and at the start ($p < 0.001$). There was then no significant change during the rides, but immediately after the finish concentrations decreased very sharply to their lowest value of 2.8 ± 0.1 mmol/l two hours after the ride ($p < 0.001$). This was followed by a gradual rise between two and

TABLE 44

Packed cell volume and plasma concentrations of total protein, albumin, electrolytes and minerals at certain stages before, during and after the controlled 80 km rides (n = 8 except for 80 km figures when n = 6)

	Stable	Start	40 km	80 km	2 hours after ride
PCV	0.38 ± 0.01***	0.45 ± 0.01	0.54 ± 0.005***	0.62 ± 0.01***	0.52 ± 0.02***
Total protein (g/l)	68 ± 1	69 ± 1	77 ± 1***	86 ± 2***	82 ± 3***
Albumin (g/l)	34 ± 1	37 ± 1	42 ± 1***	48 ± 2***	45 ± 1***
Sodium (mmol/l)	140 ± 0.8	139 ± 0.9	141 ± 0.9	148 ± 1.5***	141 ± 1.0
Potassium (mmol/l)	3.2 ± 0.2***	4.6 ± 0.1	4.6 ± 0.2	4.4 ± 0.2	2.8 ± 0.1***
Chloride (mmol/l)	99.0 ± 0.6	98.6 ± 0.6	95.2 ± 0.6***	95.5 ± 0.8*	94.6 ± 1.0**
Calcium (mmol/l)	2.97 ± 0.06	3.04 ± 0.07	3.10 ± 0.07	3.20 ± 0.15	3.07 ± 0.06
Magnesium (mmol/l)	0.71 ± 0.01	0.73 ± 0.01	0.72 ± 0.02	0.85 ± 0.06*	0.82 ± 0.04
Phosphate (mmol/l)	0.94 ± 0.06*	0.88 ± 0.05	1.04 ± 0.03**	1.03 ± 0.04*	0.84 ± 0.05

Significantly different from start values: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

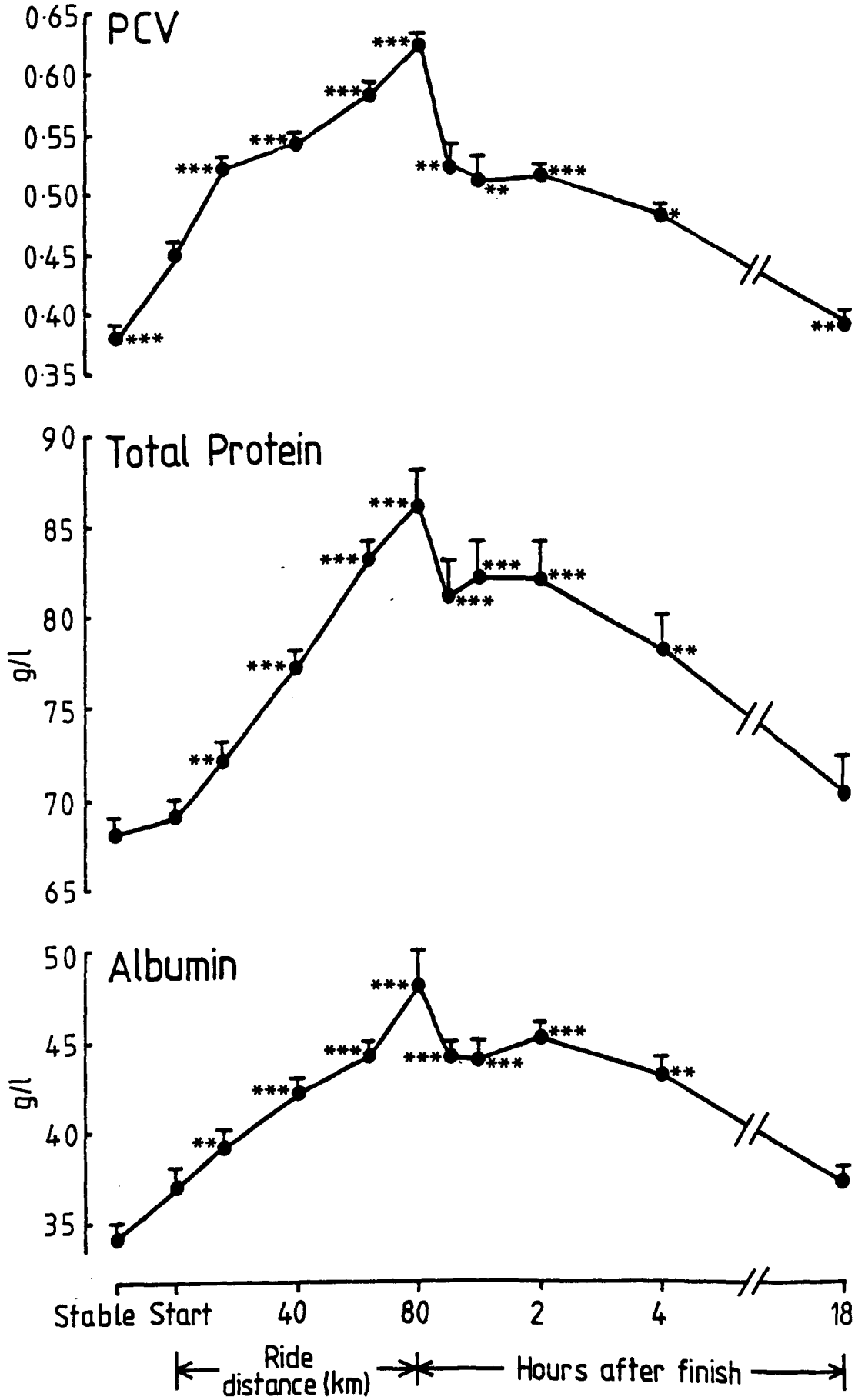


Figure 33. Changes in PCV and plasma protein concentrations before, during and after the controlled 80 km rides. Significantly different from start value: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

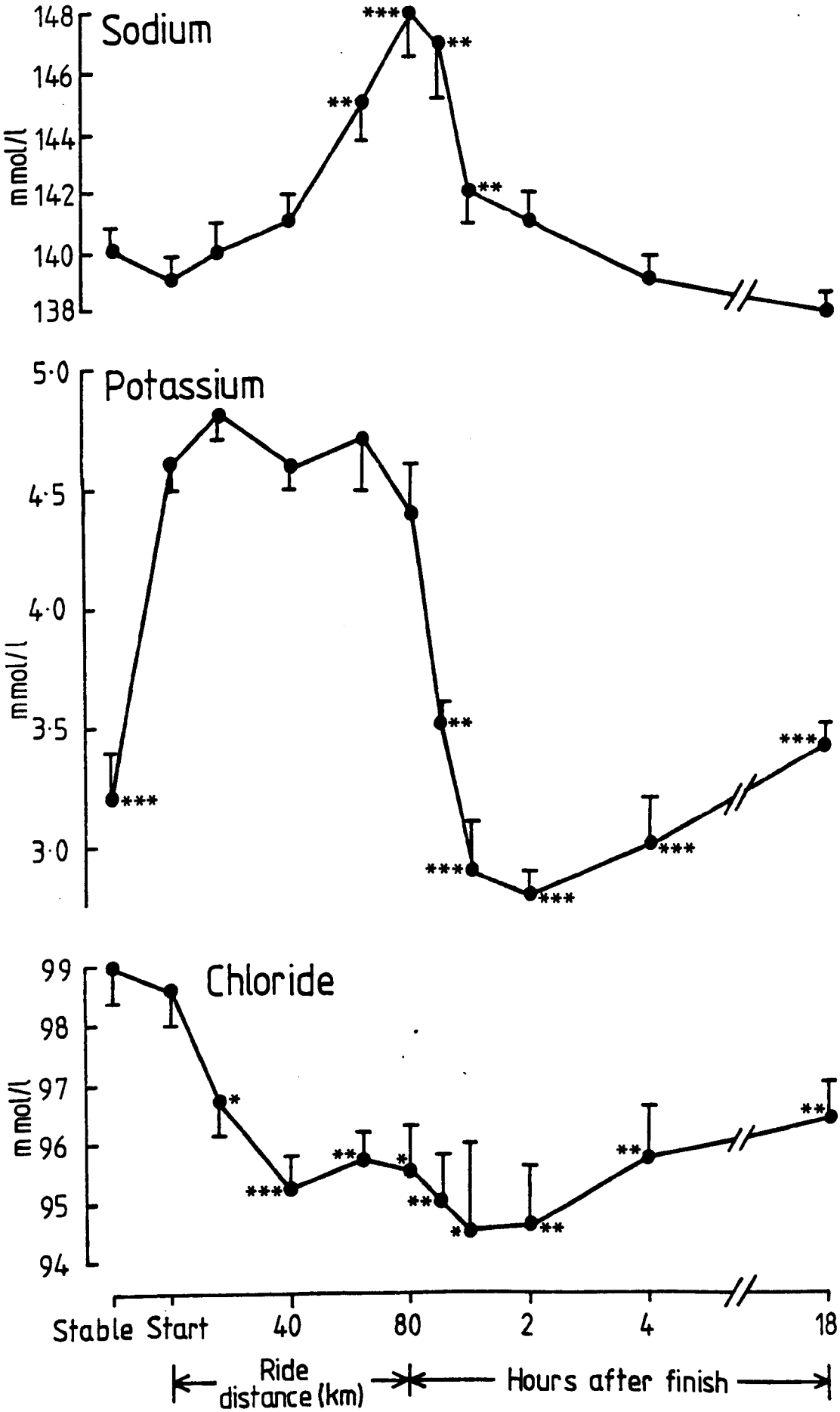


Figure 34. Changes in plasma electrolyte concentrations before, during and after the controlled 80 km rides. Statistics as Figure 33.

18 hours after the ride ($p < 0.01$), however none of the samples collected after the finish was significantly different from that collected in the stable before the start.

(c) Plasma mineral concentrations

Plasma calcium, magnesium and phosphate concentrations during and after the rides are shown in Figure 35 and selected mean values are also included in Table 44. All three parameters increased slightly during the rides and decreased after the finish.

(d) Fuel utilisation - plasma parameters indicative of metabolic status

Changes in plasma concentrations of glucose, cortisol, glycerol, bilirubin, creatinine, urea and uric acid are shown in Figure 36 and selected mean values are presented in Table 45. Plasma glucose concentration showed a complex pattern of change. Initially a decrease was noted between the samples collected in the stable and at the start, this was followed first by an increase during the first half of the rides and then by a decrease during the second half. After the finish, concentrations increased steadily, reaching their highest level of all four hours later. The other parameters in this group all increased steadily during the rides and all but urea began to decrease again immediately after the finish. Urea concentration continued to increase for at least four hours afterwards.

The composition of the plasma free fatty acid pool during the rides is presented in Table 46. Although the total plasma FFA concentration increased considerably during the rides, the concentrations of some of the less abundant fatty acids, such as C12:1 and C14:2, did not change. A significant change in the percentage composition was noted during the rides, particularly a 47% decrease in stearic acid (C18:0) content and a 47% increase in oleic acid (C18:1) content. Figure 37 presents some examples of gas liquid chromatography traces: free fatty acid composition before and after the second ride in horse D is compared to that

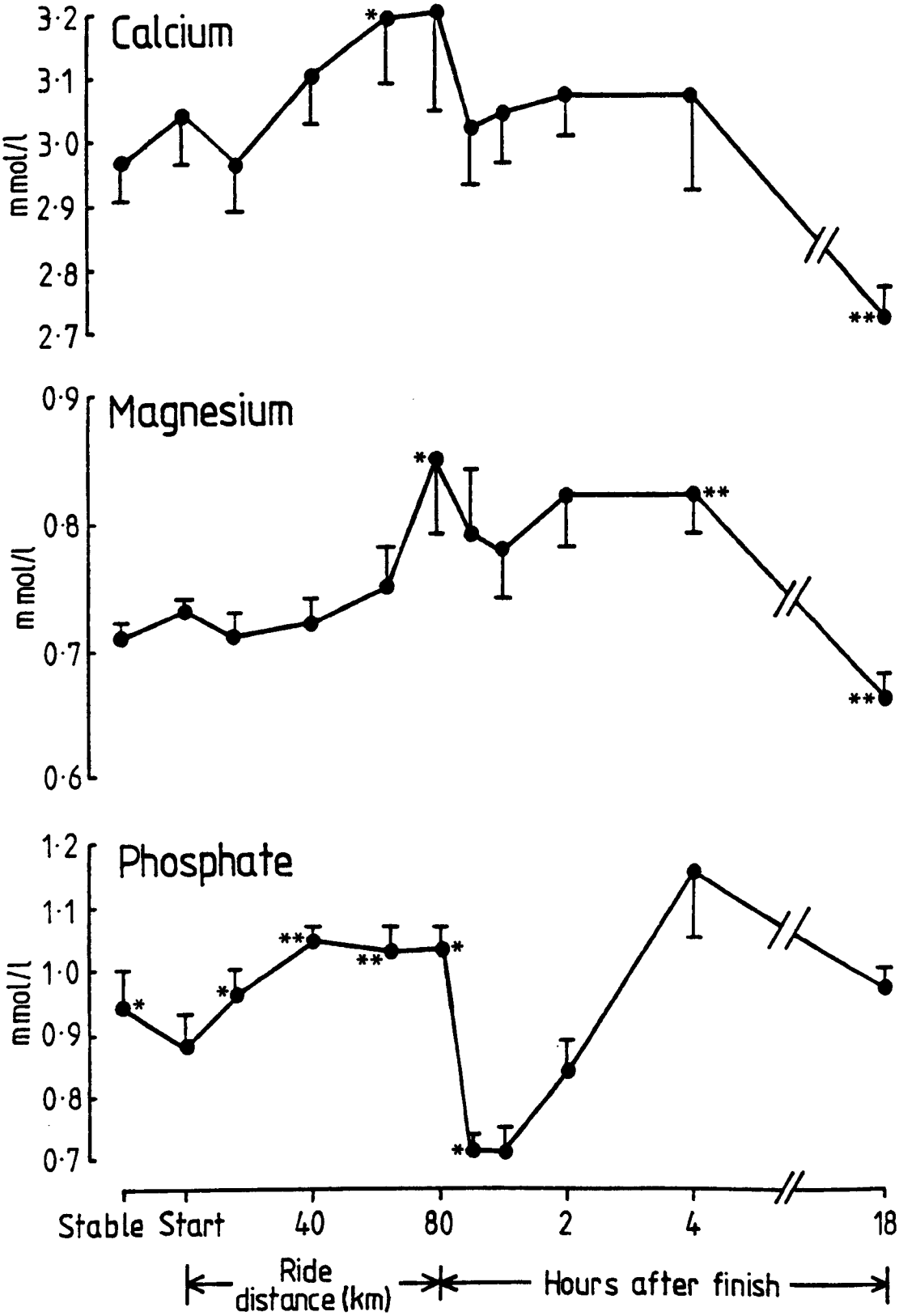


Figure 35. Changes in plasma mineral concentrations before, during and after the controlled 80 km rides. Statistics as Figure 33.

TABLE 45

Plasma concentrations of glucose, cortisol, glycerol, bilirubin, creatinine, urea and uric acid at certain stages before, during and after the controlled 80 km rides. (n = 8 except for 80 km figures where n = 6).

	Stable	Start	40 km	80 km	2 hrs after ride
Glucose (mmol/l)	5.0 ± 0.1***	4.3 ± 0.1	5.9 ± 0.2***	1.9 ± 0.4***	5.8 ± 0.3**
Cortisol (nmol/l)	162 ± 16	203 ± 22	438 ± 58**	757 ± 33***	356 ± 58
Glycerol (µmol/l)	30 ± 6	38 ± 6	314 ± 18***	995 ± 83***	179 ± 31**
Bilirubin (µmol/l)	27 ± 4	28 ± 4	40 ± 5***	64 ± 9***	63 ± 8***
Creatinine (µmol/l)	129 ± 4	124 ± 4	170 ± 4***	205 ± 9***	171 ± 9***
Urea (mmol/l)	4.7 ± 0.3	4.9 ± 0.3	5.6 ± 0.3***	7.3 ± 0.5***	8.1 ± 0.4***
Uric acid (µmol/l)	14 ± 2	-	36 ± 7**	140 ± 23**	64 ± 12**

Significantly different from start values: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$
(Stable value for uric acid)

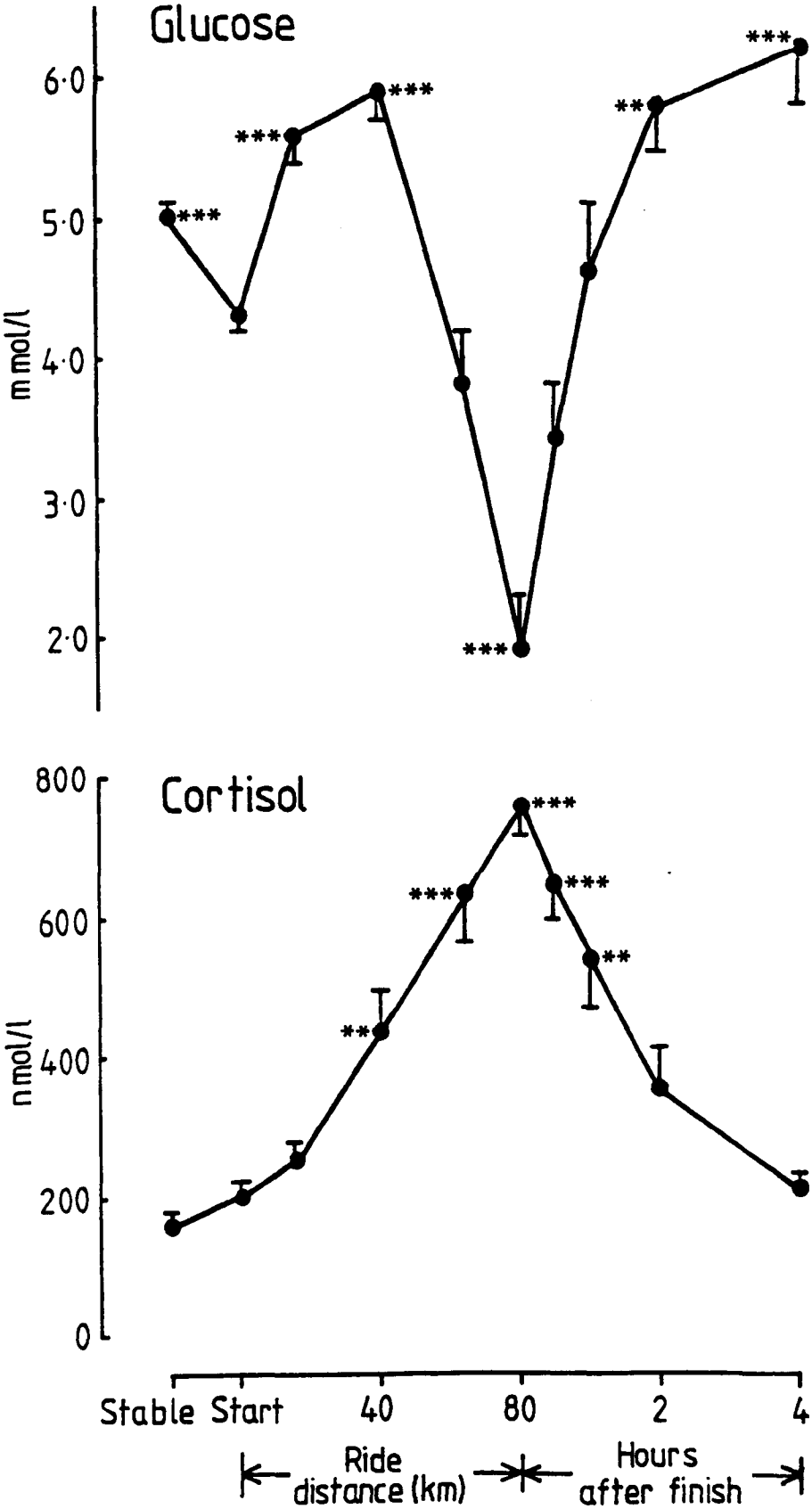


Figure 36(a). Changes in plasma glucose and cortisol concentrations before, during and after the controlled 80 km rides. Statistics as Figure 33.

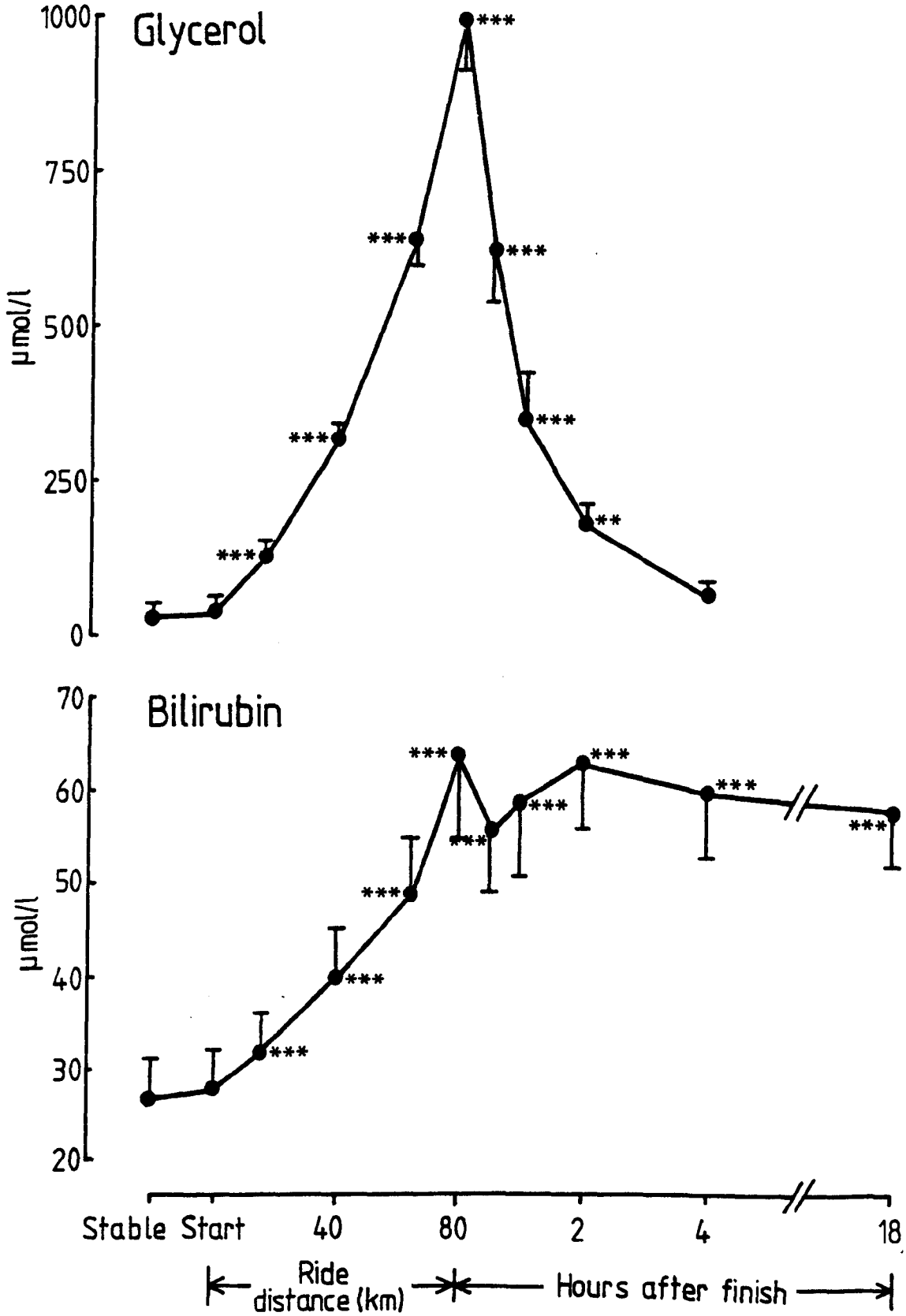


Figure 36(b). Changes in plasma glycerol and bilirubin concentrations before, during and after the controlled 80 km rides. Statistics as Figure 33.

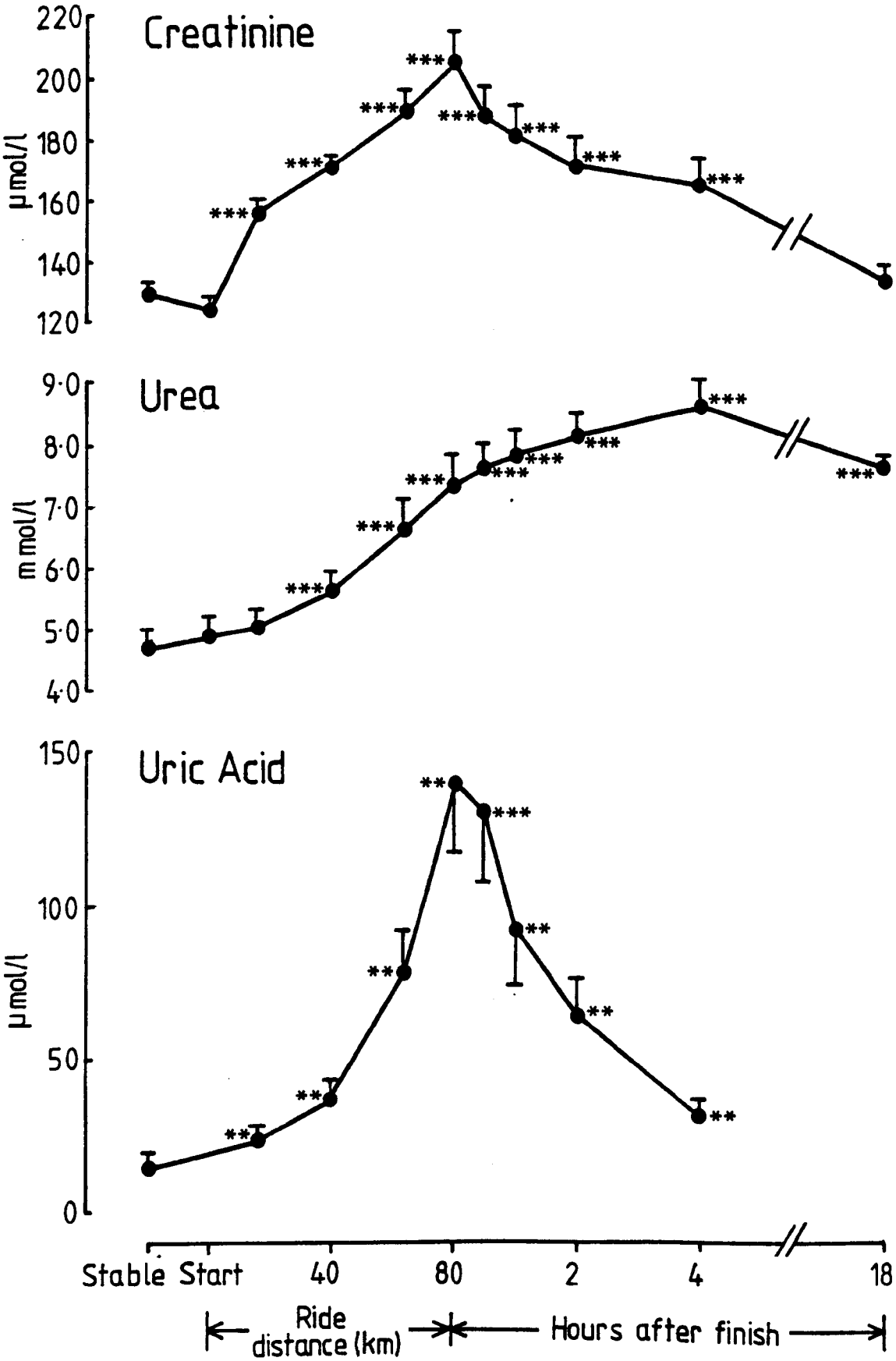


Figure 36(c). Changes in plasma creatinine, urea and uric acid concentrations before, during and after the controlled 80 km rides. Statistics as Figure 33.

TABLE 46

Change in composition of the plasma free fatty acid pool during the controlled 80 km rides (Stable, n = 8; 40 km and 80 km, n = 6).

Fatty Acid	Absolute values (µmol/l)			Percentage of total		
	Stable	40 km	80 km	Stable	40 km	80 km
C 12:0 (Lauric)	1.67 ± 0.43	2.94 ± 0.48	3.19 ± 0.53	0.65 ± 0.27	0.32 ± 0.04	0.27 ± 0.06
C 12:1	0.47 ± 0.10	1.01 ± 0.41	0.66 ± 0.17	0.14 ± 0.03	0.10 ± 0.04	0.05 ± 0.01
C 14:0 (Myristic)	11.4 ± 2.2	31.9 ± 3.5	36.5 ± 3.2	4.09 ± 1.31	3.51 ± 0.19	2.78 ± 0.21
C 14:1 (Myristoleic)	5.25 ± 0.86	18.3 ± 4.5	18.1 ± 3.4	1.84 ± 0.52	1.92 ± 0.27	1.33 ± 0.22
C 14:2	3.19 ± 1.59	7.06 ± 3.58	3.95 ± 2.44	0.86 ± 0.40	0.83 ± 0.40	0.33 ± 0.20
C 16:0 (Palmitic)	89.0 ± 13.1	257 ± 25.6	399 ± 67.2	26.6 ± 2.1	28.3 ± 0.53	28.7 ± 1.3
C 18:0 (Stearic)	63.2 ± 6.4	103 ± 6.6	139 ± 24.2	19.2 ± 1.6	11.5 ± 0.6	10.2 ± 1.1**
C 18:1 (Oleic)	80.2 ± 15.5	271 ± 25.8	468 ± 75.0	23.0 ± 2.0	30.0 ± 1.3	33.8 ± 1.0**
C 18:2 (Linoleic)	59.1 ± 10.7	150 ± 18.6	217 ± 21.7	17.1 ± 2.6	16.4 ± 0.5	16.3 ± 0.9
C 18:3 (Linolenic)	22.4 ± 5.9	54.8 ± 8.6	85.5 ± 12.0	6.44 ± 1.57	5.94 ± 0.47	6.2 ± 0.2
TOTAL ± SEM	336 ± 36	897 ± 88***	1370 ± 192***			

Significantly different from stable values: ** $p < 0.01$; *** $p < 0.001$.

(Only total FFA concentration and changes in percentage of individual FFA's tested.)

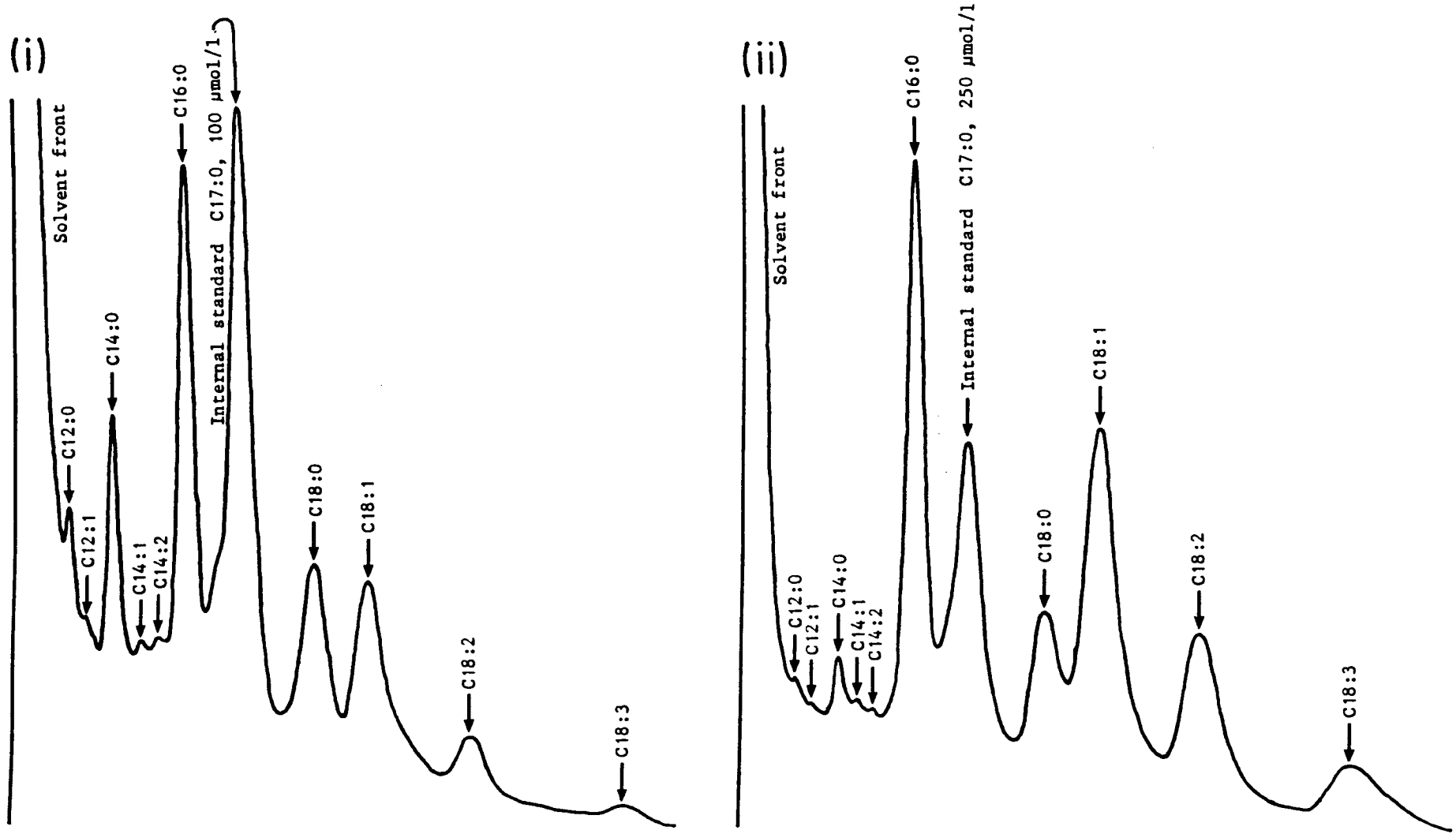


Figure 37(a). Gas liquid chromatography traces of plasma free fatty acids in horse D, (i) immediately before and (ii) at the finish of the first of the controlled 80 km rides.

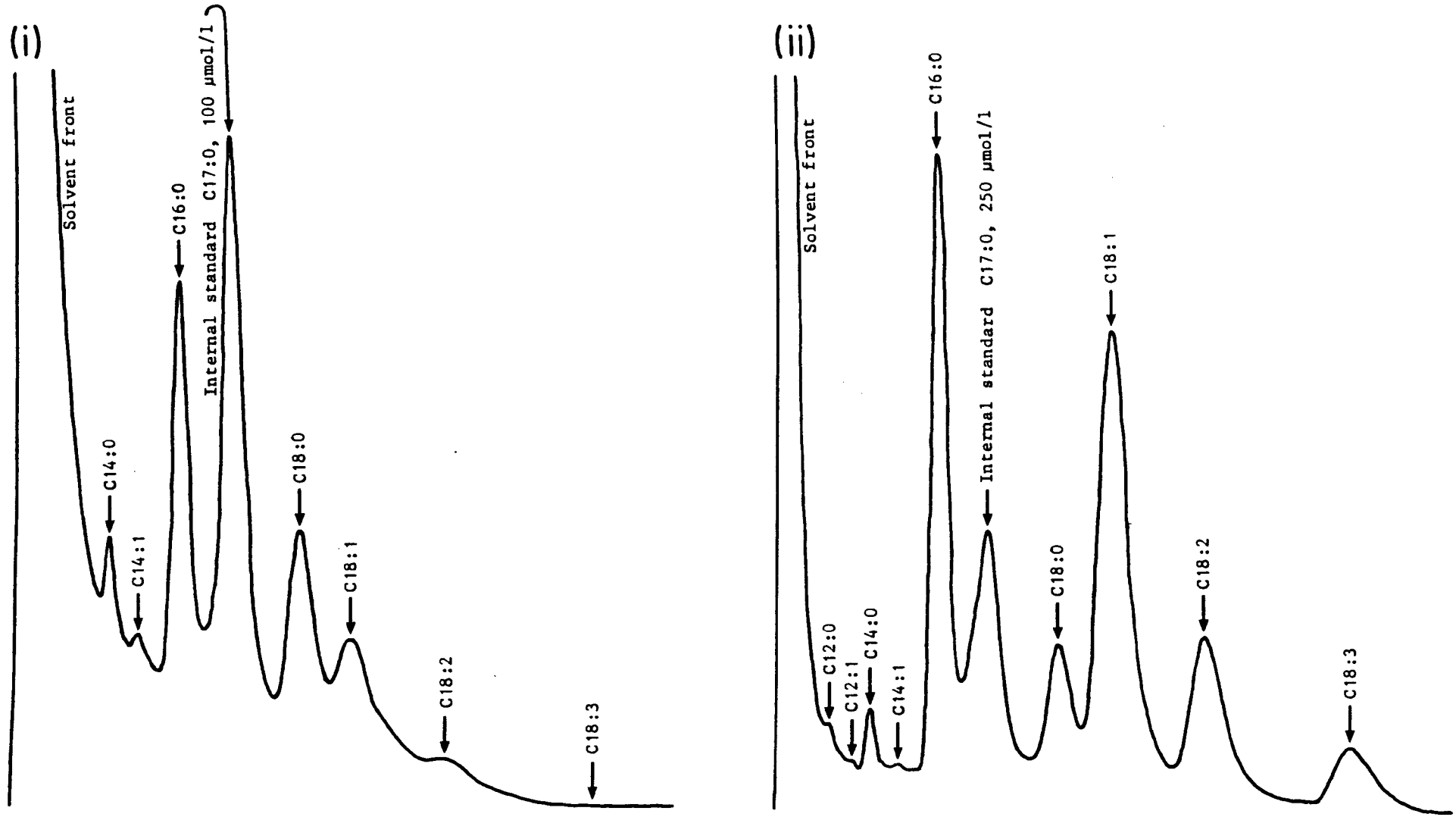


Figure 37(b). Gas liquid chromatography traces of plasma free fatty acids in horse D, (i) immediately before and (ii) after 3 hours of intravenous adrenaline infusion at a rate of 0.18µg/kg/min.

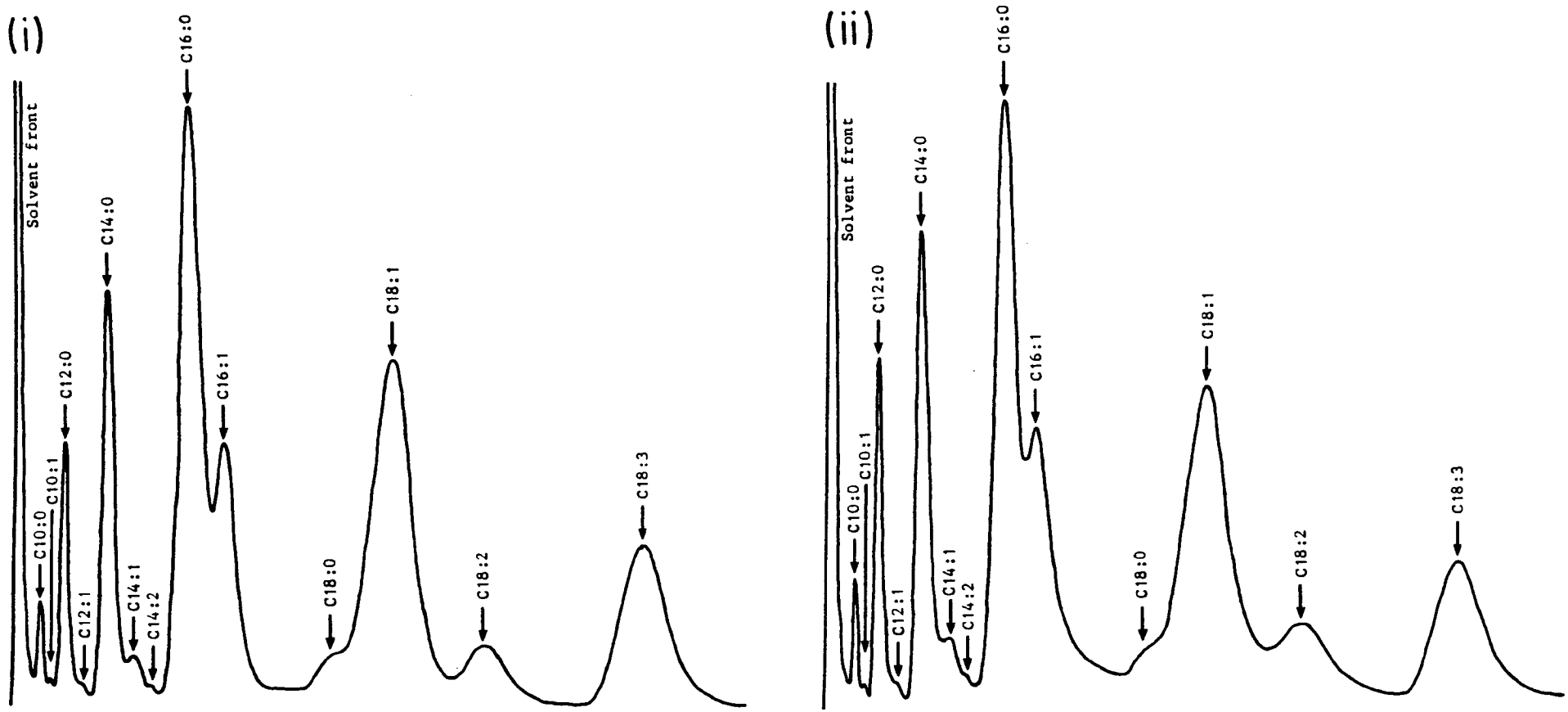


Figure 37(c). Gas liquid chromatography traces of the fatty acids of the triglyceride fractions of (i) subcutaneous (ii) mesenteric fat from an unknown horse.

before and after one of the three-hour adrenaline infusions described in Section 4 in the same horse and to that of the extracted triglyceride fractions from two samples of adipose tissue obtained post mortem from another horse.

(e) Plasma enzyme activities

Changes in mean plasma activities of CK, AST and ALP during and after the rides are shown in Table 47 and Figure 38. Plasma CK activity showed the greatest changes with increases in AST and ALP being relatively small in magnitude. Although CK activity decreased very sharply between 4 and 18 hours after the rides it was still significantly above pre-ride values 24 hours after the finish ($p < 0.01$). AST and ALP activities were still significantly above pre-ride values 48 hours after the finish ($p < 0.01$ and $p < 0.05$ respectively).

Individual variations in plasma enzyme activities were very marked with 80 km values for CK ranging from 679 iu/l (horse E, second ride) to 2121 iu/l (horse D, first ride). In addition horse D's pre-ride value for AST at the second ride was surprisingly high, 912 iu/l, which represented an increase of 550 iu/l over the value recorded in this horse three weeks earlier, six days after the first ride. The comparisons between individual increases in CK and AST activity during the rides and subjectively assessed degree of fatigue are presented in Figure 39, together with comparison of end of ride plasma cortisol concentration with fatigue.

Figure 40 shows the fluctuations which occurred in plasma CK and AST activities in horses A and D in the six months following the rides. In horse D plasma CK returned to normal about two months after the second ride and remained there and plasma AST followed about a month later. In horse A plasma CK remained slightly elevated and plasma AST

TABLE 47

Plasma enzyme activities at certain stages before, during and after the controlled 80 km rides.
(n = 8 except for 80 km figures where n = 6.)

	Stable	40 km	80 km	4 hrs after ride	24 hrs after ride
Creatine kinase (iu/l)	154 ± 9	502 ± 85**	1209 ± 216**	1176 ± 226**	293 ± 35**
Aspartate aminotransferase (iu/l)	381 ± 82	493 ± 94**	589 ± 130**	519 ± 94**	495 ± 69*
Alkaline phosphatase (iu/l)	562 ± 59	679 ± 67***	808 ± 77***	739 ± 71**	654 ± 55**

Significantly different from stable value: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

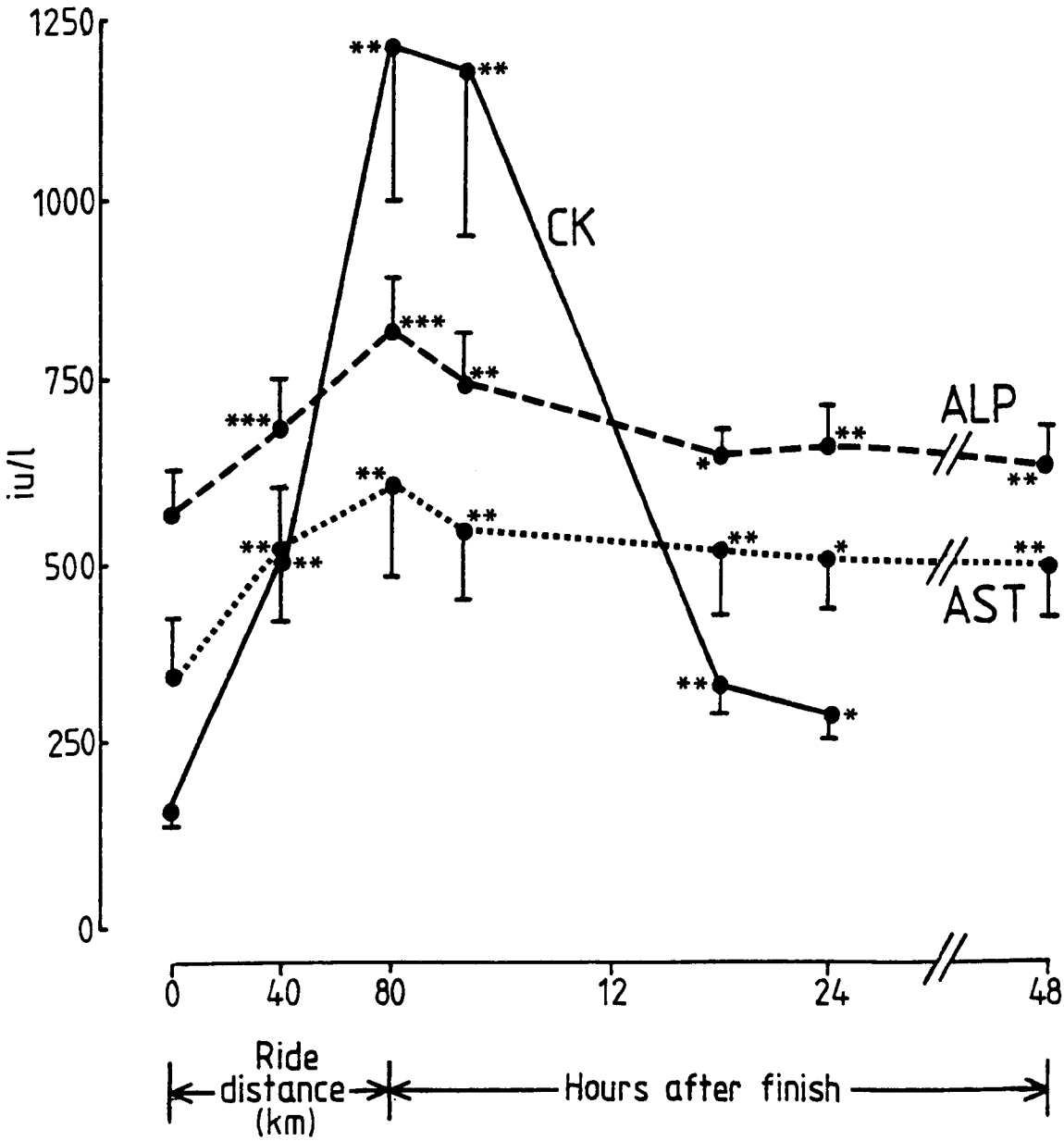


Figure 38. Changes in plasma enzyme activities during and after the controlled 80 km rides. Significantly different from pre-ride value: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

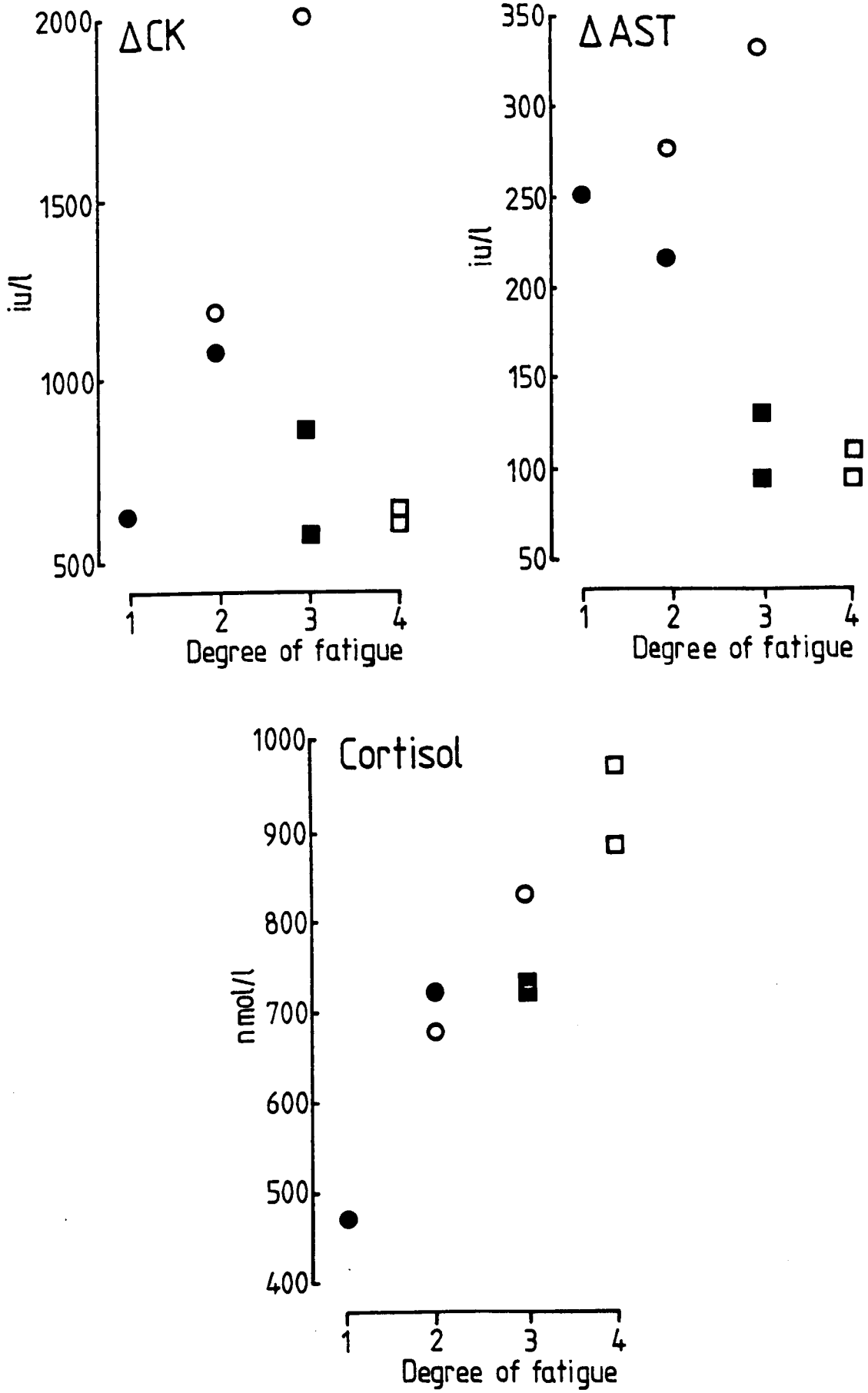


Figure 39. Increases in plasma CK and AST activities during the controlled 80 km rides and end-of-ride plasma cortisol concentration compared to the subjectively assessed degree of fatigue in each horse.

● Horse A ○ Horse D ■ Horse E □ Horse F

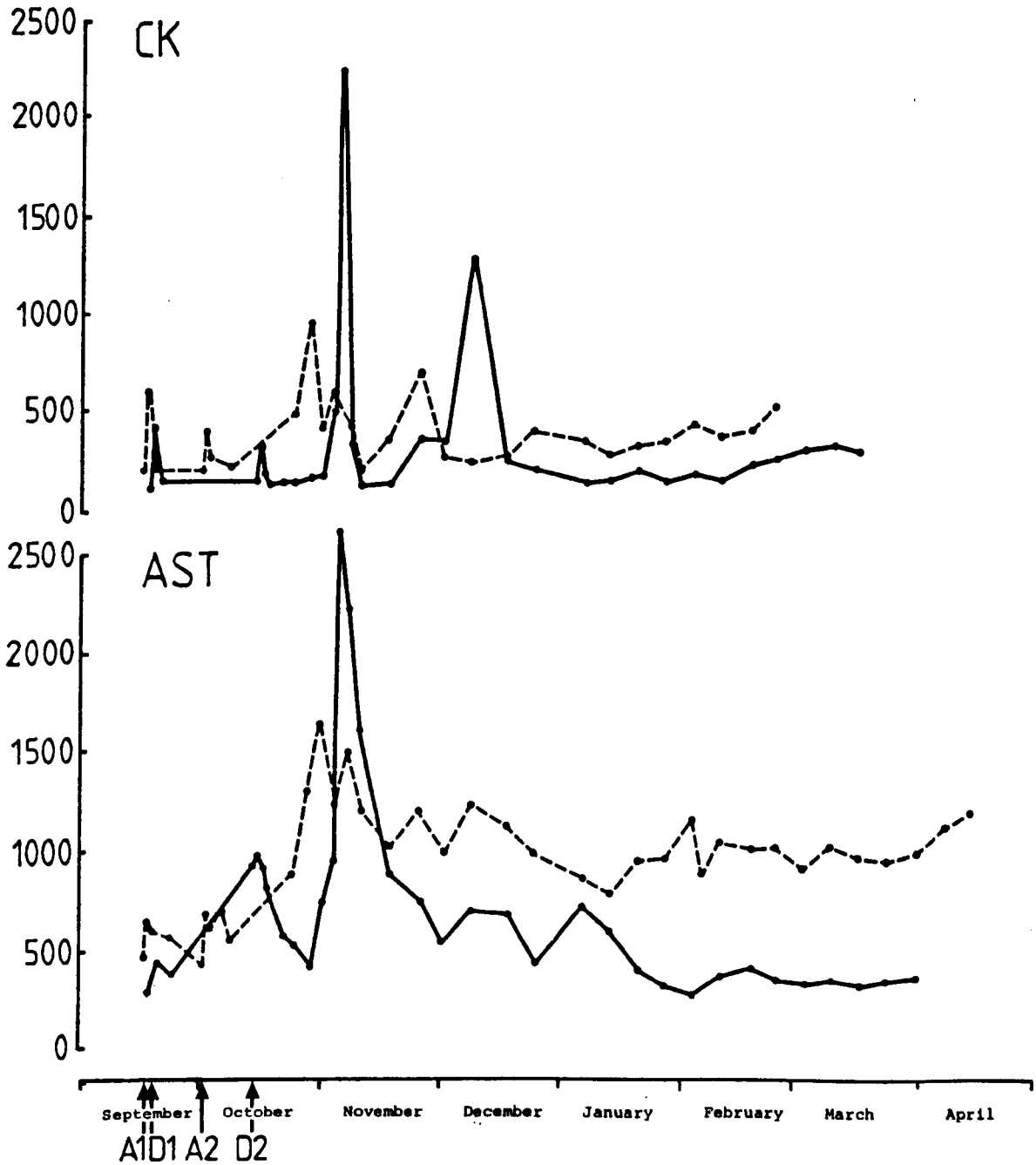


Figure 40. Fluctuations in plasma enzyme activities in horse A (broken line) and horse D (solid line) during the time of the controlled 80 km rides and for six months afterwards.

A1	First ride, horse A	A2	Second ride, horse A
D1	First ride, horse D	D2	Second ride, horse D

quite markedly elevated as long as sampling was continued. In horses E and F plasma CK remained less than 200 iu/l and plasma AST less than 300 iu/l for the two months following the rides.

C. Urine samples

All horses urinated between 8.00 hrs and 9.30 hrs on the morning of the rides. No urine was passed during the rides and so the urine collected after the rides, in all cases within one hour of the finish, largely represented urine which was formed during the ride. In fact 60% of the time covered by the urine sampling was spent in endurance exercise while a proportion of the remainder was spent walking to and from the ride location. The composition of this urine is compared to the mean values for the same four animals during the same period of the day (approximately 8.30 hrs to 16.30 hrs) in the urine collected during the experiment described in Section 1, when the animals were at rest (Table 48). The urine excreted on the days of the endurance rides was generally more dilute, as indicated by the lower specific gravity. Rate of creatinine excretion was slightly less than during the resting days, but the most marked differences were in pH, which was much more alkaline in the endurance samples, and in rate of chloride excretion, which was only 0.35 of the rate recorded at rest.

TABLE 48

Comparison of daytime urine composition between horses at rest and undergoing endurance exercise (n = 8).

	At rest	Exercising
Time during which urine secreted (minutes)	427 ± 17	424 ± 19
Urine flow rate (ml/min)	4.52 ± 0.30	6.30 ± 0.76
pH	7.4 ± 0.06	8.4 ± 0.07***
Specific gravity	1.038 ± 0.001	1.030 ± 0.002**
Creatinine concentration (mmol/l)	24.1 ± 1.5	15.7 ± 1.6
Creatinine excretion rate (μmol/min)	106.7 ± 4.9	91.4 ± 3.0*
Urea concentration (mmol/l)	312 ± 20	216 ± 26
Urea excretion rate (mmol/min)	1.38 ± 0.08	1.27 ± 0.13
Sodium concentration (mmol/l)	38.2 ± 15.6	40.6 ± 17.3
Sodium excretion rate (mmol/min)	0.191 ± 0.081	0.335 ± 0.168
Potassium concentration (mmol/l)	383 ± 18	283 ± 22
Potassium excretion rate (mmol/min)	1.72 ± 0.12	1.69 ± 0.12
Chloride concentration (mmol/l)	315 ± 15	77 ± 11
Chloride excretion rate (mmol/min)	1.43 ± 0.13	0.51 ± 0.12***

Significantly different from resting values: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
(Concentrations not tested)

D. Sweat samples

Changes in sweat chloride, sodium and potassium concentrations during the rides are shown in Figure 41. All the electrolytes decreased significantly between the first and second samples (Cl^- and Na^+, $p < 0.02$; K^+, $p < 0.05$). Thereafter chloride concentration remained constant, sodium concentration increased significantly ($p < 0.05$ between the second and third samples) and potassium concentration continued to decrease ($p < 0.01$). The higher electrolyte concentrations, particularly the chloride concentration, in the first samples strongly suggested that these had been affected by hair and skin contamination, in spite of the precautions taken. These samples were therefore excluded when mean values of sweat electrolyte concentrations were calculated for each horse for comparison with heat and adrenaline-induced sweat. Table 49 shows the overall mean electrolyte concentrations of the exercise-induced sweat compared to the equivalent values for heat-induced sweat (from Table 32) and adrenaline-induced sweat (from Table 38). Significant differences were found in all three electrolytes.

TABLE 49

Comparison of sweat electrolyte concentrations in exercise, heat and adrenaline induced sweat

	Chloride (mmol/l)	Sodium (mmol/l)	Potassium (mmol/l)
Exercise	166 ± 2	160 ± 2	30 ± 1
Heat	198 ± 10	146 ± 2	53 ± 8
Adrenaline	195 ± 6	180 ± 5	26 ± 2

Significant differences between groups:
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

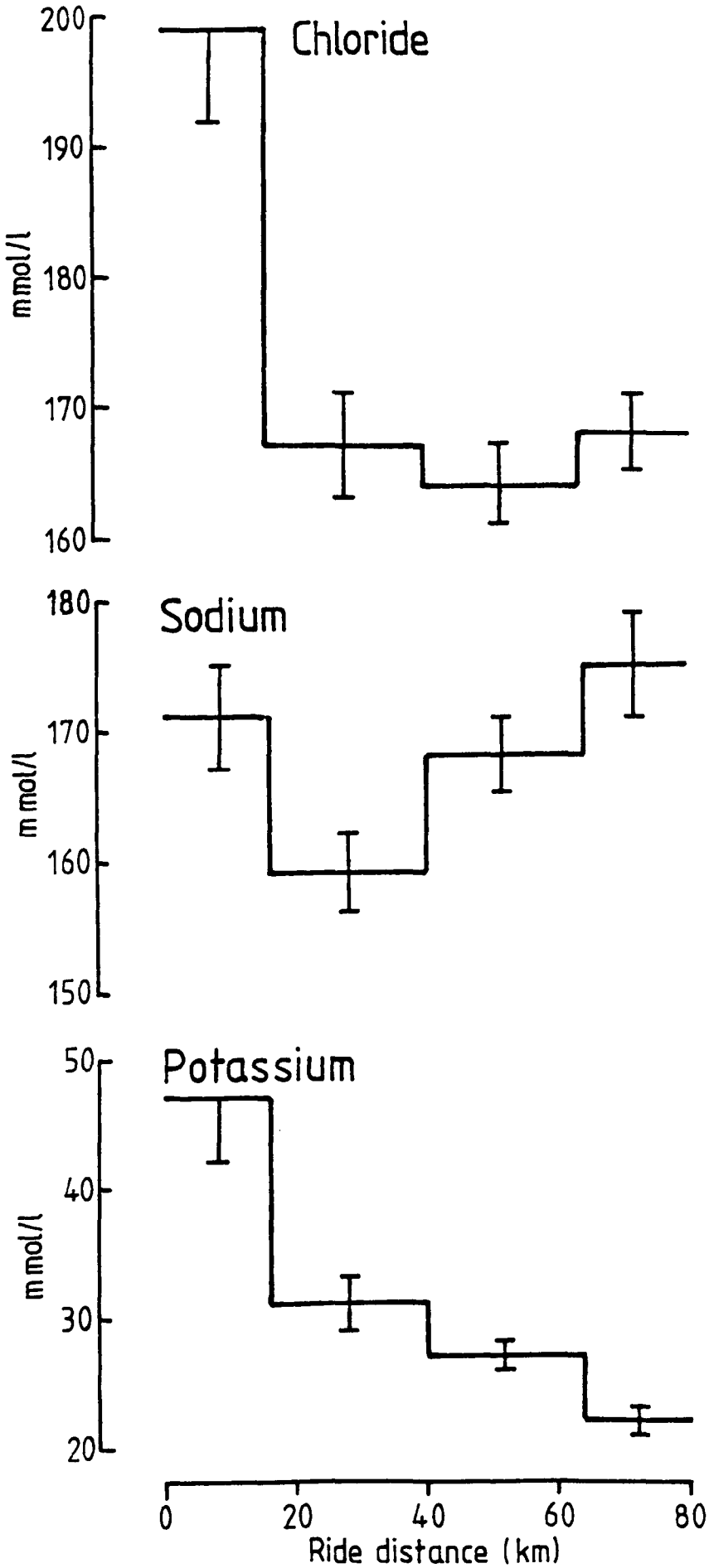


Figure 41. Changes in electrolyte concentrations of exercise-induced sweat with time.

The most marked difference in sweat composition between sweat induced by the three stimuli was again seen in the pattern of change of sodium:potassium ratio with time, which is presented in Figure 42. For comparative purposes a distance of 16 km is considered approximately equivalent to one hour's sweating.

Changes in sweat magnesium and protein concentrations during the rides are shown in Figure 43. It appears that the pattern of change in both constituents was similar to that seen during heat and adrenaline-induced sweating and the correlation between them was again significant ($r = 0.890$). However, protein concentrations were higher compared to magnesium concentrations than was the case in the heat or the adrenaline-induced sweat.

Sweat urea concentration increased significantly during the rides ($p < 0.01$), apparently in parallel with the increasing plasma urea concentration (Figure 44). (Sweat urea results for horse F in ride 1 have been omitted from this figure as they appeared to have been affected by the same problem as was described in relation to the samples of adrenaline-induced sweat collected from unwashed skin in Section 4.)

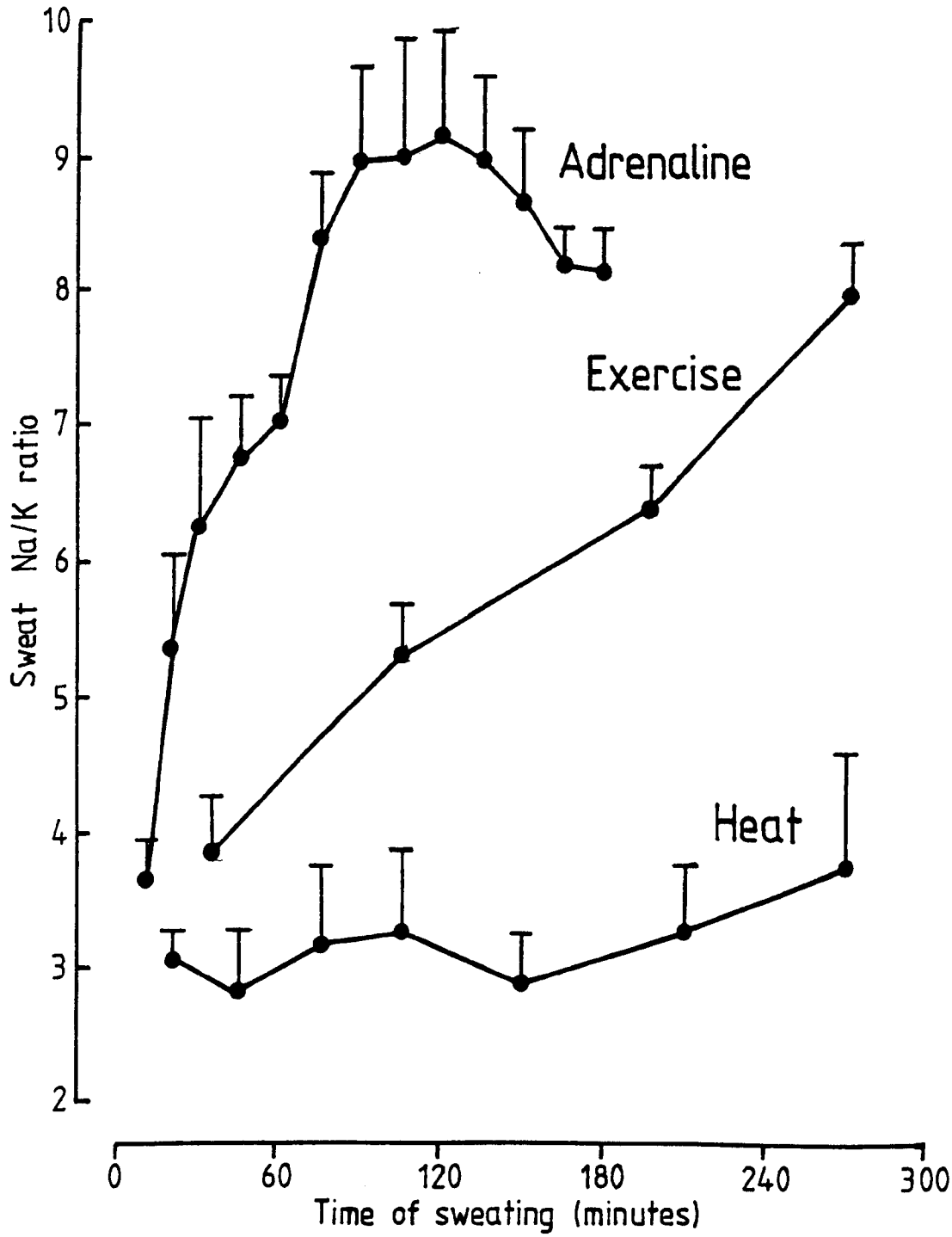


Figure 42. Changes in sweat sodium:potassium ratio with time - comparison of heat, exercise and adrenaline-induced sweat. For comparative purposes 16 km is considered to be equal to 60 minutes.

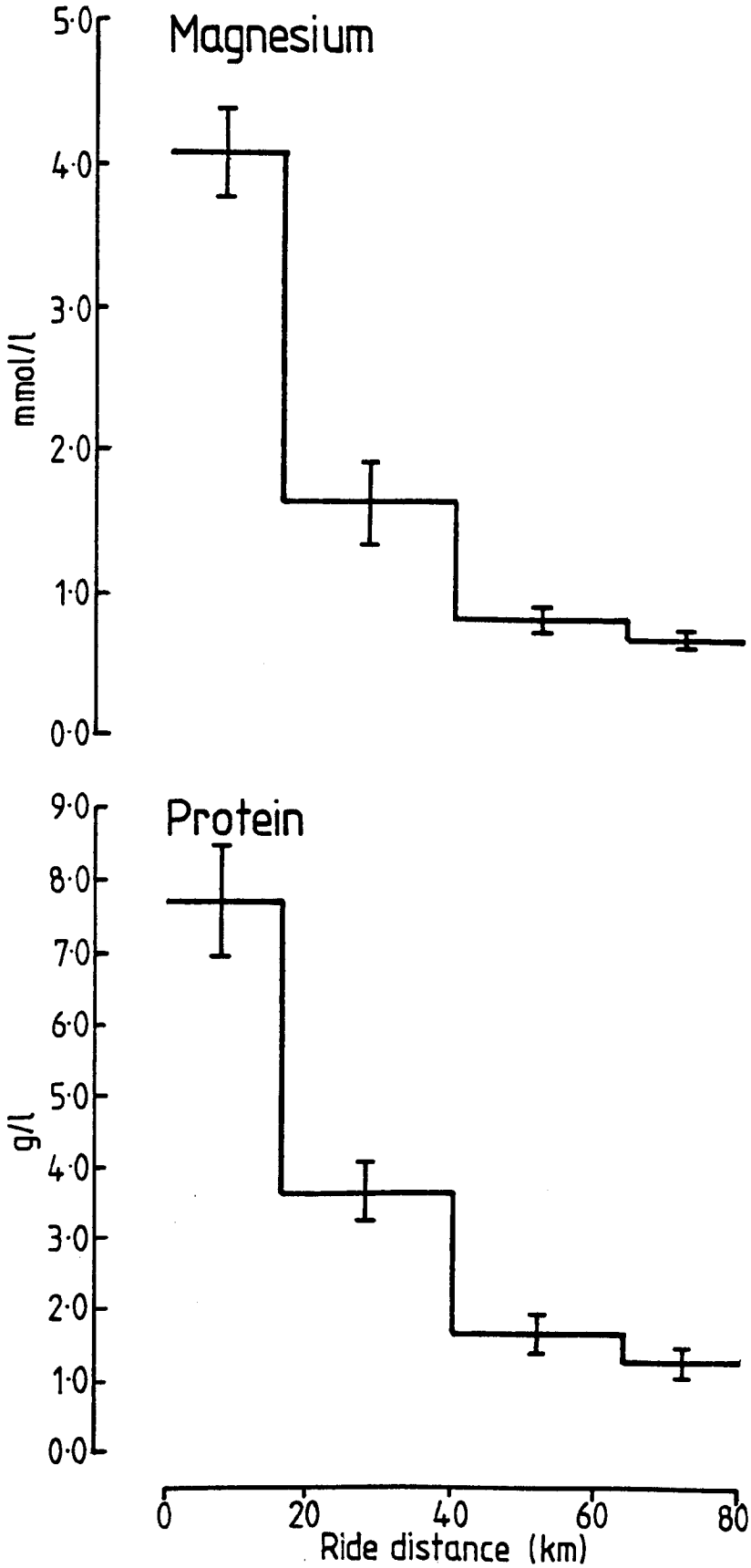


Figure 43. Changes in magnesium and protein concentrations of exercise-induced sweat with time.

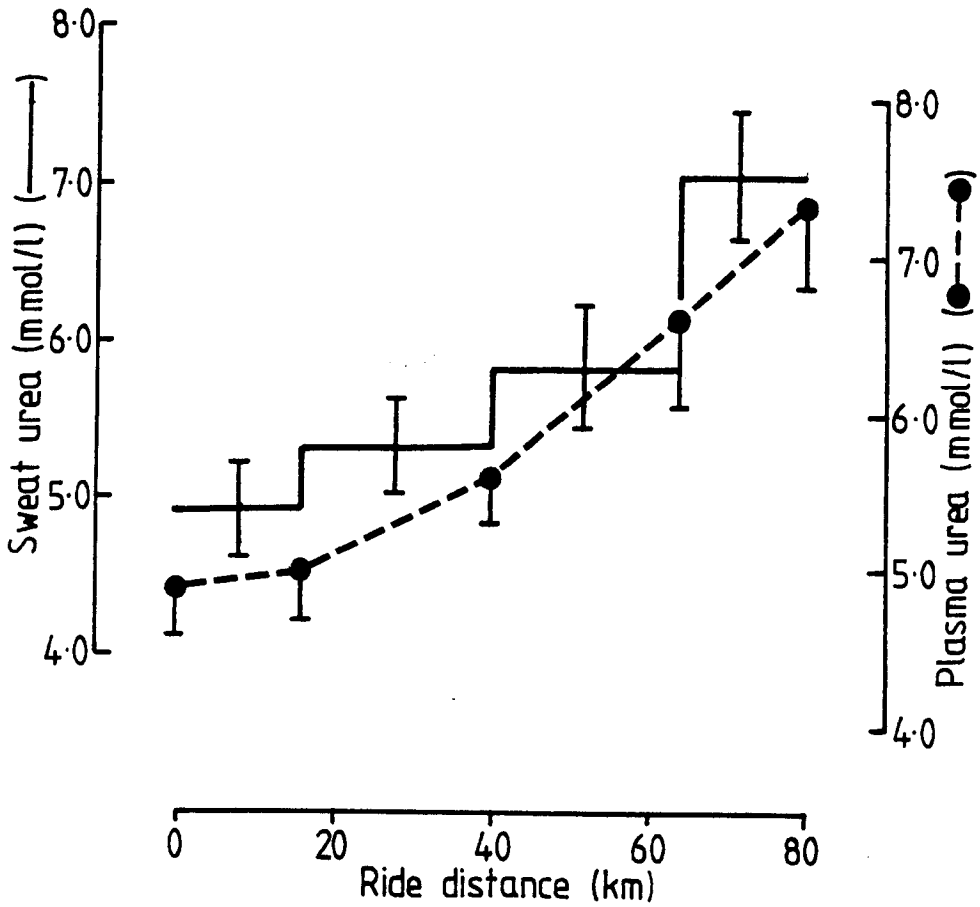


Figure 44. Relationship of sweat urea concentration (solid line) to plasma urea concentration (broken line) during the controlled 80 km rides.

E. Calculations of fluid and electrolyte losses

The decreases in the body weight of the horses during the rides together with the approximate volumes of water drunk are given in Table 50. The sum of these two figures was considered to be equal to the total fluid loss (sweat plus respiratory water loss) occurring during the rides. The proportion of this value which could be attributed to respiratory water loss was calculated by the method detailed in Section 3. (In order to compensate for the supposed contamination error in the first sweat samples discussed above, it was assumed that sweat chloride concentration had in fact remained constant, as observed in Section 4 after washing of the skin, at a value of 165 mmol/l. The sodium concentration of each of the initial samples was therefore reduced by a factor of $(165/[Cl^-])$ before mean values for each animal were calculated for this present purpose.) These calculated volumes of sweat and respiratory water loss are shown in Table 50. It appears that about 22% of the water lost during the rides was from the respiratory tract.

Mean sweat concentrations of chloride and potassium during the rides were also calculated for each horse, again reducing the values in the initial samples according to the assumption made above. These values were used to calculate the absolute losses of electrolytes in the sweat and these figures are presented in Table 51, together with the theoretical concentration of each electrolyte in the total body fluid loss. It can be seen that while chloride loss overall was hypertonic to plasma chloride concentration, sodium loss was hypotonic.

TABLE 50

Body weight losses, volumes of water drunk, and calculated volumes of sweat and respiratory water loss during the 80 km rides

Horse	Ride	Body Weight Lost (kg)	Water Drunk (l)	Total Fluid Loss (l)	Volume of Sweat (l)	Volume of Resp. Water (l)
A	1	32	0	32	24.5	7.5
	2	41	0	41	35.5	5.5
D	1	46	4	50	38.7	11.3
	2	29	6	35	27.6	7.4
E	1	29	0	29	20.4	8.6
	2	39	0	39	27.1	11.9
F	1	21	8	29	23.0	6.0
	2	34	8	42	34.0	8.0
Mean ± SEM				37 ± 3	29 ± 2	8.3 ± 0.8

TABLE 51

Electrolyte losses during 80 km rides - mean sweat concentrations, absolute losses and theoretical "concentrations" in total body fluid losses

		Sweat electrolyte concentrations (mmol/l)			Total electrolyte losses (moles)			Electrolyte "concentration" in total body fluid loss (mmol/l)		
Horse	Ride	Chloride	Sodium	Potassium	Chloride	Sodium	Potassium	Chloride	Sodium	Potassium
A	1	162	152	37	3.97	3.73	0.91	124	117	28
	2	177	162	35	6.28	5.75	1.24	153	140	30
D	1	169	170	28	6.54	6.58	1.08	131	131	22
	2	171	158	27	4.72	4.36	0.75	135	125	21
E	1	160	159	32	3.26	3.25	0.65	112	112	22
	2	163	167	27	4.42	4.52	0.73	113	116	19
F	1	162	159	29	3.73	3.66	0.67	129	126	23
	2	163	156	26	5.54	5.30	0.88	132	126	21
Mean ± SEM		166±2	160±2	30±1	4.81±0.43	4.64±0.41	0.86±0.07	129±5	124±3	23±1

Discussion

Physical performance of the horses

There is no doubt that the amount of time spent preparing these four horses for this study was much less than that spent by the riders of top-class endurance horses before an event of the same length and it is very probable that as a consequence they were not so fit. In addition these horses were Thoroughbreds, which are generally considered to be less suitable for distance riding than Arabs. With these considerations in mind the generally good performance and the lack of any medical problems were gratifying. It is true that the terrain was much less severe than that of most endurance rides, but the horses maintained speeds which are almost never seen in events in Britain and which were comparable to the riding speeds in events in Australia and the USA.

It is difficult to say whether or not these horses would have passed a veterinary inspection as "fit to continue" after the standard 30 minutes' rest, as the veterinary examinations laid down by the EHPS were not carried out. It is perhaps unfortunate that heart rates at least were not measured, but considering the level of activity involved in the collection of blood, urine and muscle samples, and weighing the horses, it is very doubtful if it would have been possible to obtain genuinely basal resting heart rates at that time. From the subjective reports of the riders it was however clear that the horses graded as 3 and 4 in Table 43 had reached the limits of their endurance capabilities and those graded as 2 were probably close to their limits. It is therefore probable that samples collected at the end of the rides (with the exception of horse A in ride 2, who was eliminated lame) represent horses virtually maximally exerted in terms of endurance performance. It is interesting to note that the muscle tremors seen in horses A and E

after the rides appeared identical to those seen during and after adrenaline infusion, and although attempts to measure plasma adrenaline concentrations in this study were not successful, it has been shown that these concentrations do increase quite markedly in the exercising horse (Evans et al, 1956, 1957b; Snow and Rose, 1981).

Horse A's lameness highlights the fact that very often orthopaedic rather than metabolic problems are the limiting factor to endurance performance and can render controlled investigations of this type difficult to carry out (Hintz et al, 1978). In fact five horses began training for this present study but horse C had to be dropped at an early stage, also due to lameness. It is possible that the fear of this type of injury is one of the factors which deters riders from attempting higher ride speeds in British events, and the fact that lameness of one kind or another resulted in the elimination of four of the ten starters in the 160 km ride reported in Section 2 suggests that such a fear may be well grounded. However, the situation is not entirely clear-cut, as it seems likely that excessive fatigue and consequent incoordination may be the cause of a number of cases of lameness, and this factor is very difficult to quantify.

Blood samples

Many of the findings in this Section were similar to those presented in Section 2. However, the controlled nature of this study led to results which were much more uniform and clear-cut, and so much more reliable interpretation was possible.

Plasma volume changes

In the horse it can be very difficult to distinguish the relative polycythaemia caused by loss of water from the plasma from that caused by splenic contraction, as discussed in Section 2. The increase in PCV from 0.38 ± 0.01 in the stable to 0.44 ± 0.01 at the start of the experiment was almost certainly a result of the horses becoming excited

in anticipation of the coming exercise. The further increase to 0.62 ± 0.01 during the actual rides was undoubtedly due in part to a loss of plasma water, but it is difficult to assess to what extent splenic contraction was also a contributing factor. The mean percentage increase in PCV over the 80 km (39.0 ± 3.9%) was certainly somewhat larger than the mean percentage increases in total plasma protein (25.6 ± 2.6%) and albumin (32.7 ± 3.6%). This difference, however, could be due either to augmentation of the PCV by splenic erythrocytes or to escape of protein molecules (but not erythrocytes) into the interstitial fluid, or to a combination of both factors.

As in ride F in Section 2, plasma albumin concentration was found to increase by a larger percentage than total plasma protein concentration, in other words the plasma albumin:globulin ratio increased. As already discussed, this is the opposite of what might be expected to happen as a consequence of increased leakage of protein from capillary beds. It may be that synthesis of albumin increased during the rides or that globulin synthesis decreased, but the information available is not really sufficient for any firm conclusion to be reached. It is difficult, therefore, to derive a numerical estimate of the decrease in plasma volume during the rides from increases in PCV or plasma proteins, due to the number of other factors affecting these parameters. However, it was considered that the inclusion of dye or isotope dilution techniques in this experiment would not be practicable. That being so, the changes in plasma protein concentrations appear to be the best available means of assessing the extent of plasma water loss. It appears from these figures that plasma water decreased by about 25 - 30% during the rides, which represents a loss of about 6.2 - 7.5 litres in these horses.

The total fluid loss in these horses (Table 50) represents 33.5 ± 2.7% of the total ECF volume (assuming ECF volume = 0.22 bodyweight), but only 12.7 ± 0.8% of the total body water content (assuming total body water = 0.6 bodyweight). It is probable that the decrease in plasma volume is approximately representative of the decrease in volume of the ECF as a whole, i.e. about 25 - 30%. Any discrepancy is likely to be in the direction of a greater decrease in interstitial fluid volume than in plasma volume, as the increased protein concentration in the plasma would tend to retain water (Costill, 1977). These figures therefore suggest that most of the observed fluid loss in the horses was probably borne by the extracellular fluid with reductions in intracellular fluid being comparatively small in magnitude. This is borne out by the finding of Snow et al (1982) that there was no change in the water content of the muscle biopsy samples collected from the horses during the rides. This is in accordance with the findings of Saltin (1964), Kozlowski and Saltin (1964) and Costill (1977), who agree that most of the water loss during exercise occurs from the extracellular fluid, in particular the interstitial fluid. Dill and Costill (1974) have pointed out that this is largely an effect of the much higher protein concentration of the intracellular environment.

It is well known that in the early stages of exercise in man there is a substantial movement of water into the intracellular space from the plasma (van Beaumont et al, 1973; Costill, 1977). However, Costill and Fink (1974) suggest that this movement is transient and is followed by a gradual redistribution of body water during prolonged exercise, and there were no findings in this present study which could be directly related to this phenomenon.

Plasma sodium and chloride concentrations

During the period of exercise plasma sodium concentration increased while plasma chloride concentration decreased. At first sight this would seem to be paradoxical when the hypertonic concentrations of both these electrolytes in the sweat are considered. However, it must be appreciated that sweat is not the only source of body fluid loss and that the other major route of loss, expired air (which may account for as much as 22% of the total volume lost), is essentially electrolyte-free. When this factor is included in the calculations it becomes evident that the effective concentration of sodium in the total fluid lost is in fact hypotonic compared to plasma while chloride remains hypertonic (Table 51).

This explains both the directions of change in the plasma concentrations of these two electrolytes and the comparatively small magnitude of these changes in comparison with the substantial absolute losses of the electrolytes in the sweat. Sodium and chloride are approximately equimolar in equine sweat, but in plasma sodium is about 40 mmol/l higher than chloride. When the hypertonic sweat is combined with respiratory water loss, the effective electrolyte concentrations in the total fluid loss are almost midway between the plasma sodium and chloride concentrations - about 20 mmol/l hypotonic for sodium and 20 mmol/l hypertonic for chloride. Given the equimolar sweat, this is as close as it is possible to come to an isotonic fluid loss. The effect of this is that however great the volume of fluid lost the plasma electrolyte concentrations will tend to alter very little. This is probably the reason for the discrepancies between different reports of changes in sodium concentration during endurance rides (Table 6). When respiratory water losses are relatively high plasma sodium concentration will tend to rise slightly, and when they are relatively low it will tend to fall slightly.

It seems likely that this minimal disruption of plasma electrolyte concentrations is the source of the physiological explanation for the hypertonicity of equine sweat. The use of sweating as a major thermoregulatory mechanism inevitably leads to the loss of a large volume of water from the body. The horse appears to be prepared to allow a considerable amount of sodium and chloride to accompany this water in order to maintain the tonicity of the remaining extracellular fluid, and the sweat electrolyte concentrations seem to be maintained at a level which will accomplish this when sweating and respiratory water loss occur together. This is in marked contrast to the situation in man. Even in the unacclimatised individual human sweat is hypotonic to plasma for sodium and chloride. During acclimatisation to heat or exercise the occurrence of a negative sodium balance increases the secretion of aldosterone which acts on the sweat glands to reduce sweat sodium and chloride concentrations by a considerable amount (Streeten et al, 1960). It therefore appears that in man sodium conservation takes priority over the maintenance of constant plasma electrolyte concentrations and that any resultant changes in these concentrations are dealt with by altering renal electrolyte excretion (Itoh, 1952).

The fact that plasma chloride concentrations were still significantly below pre-ride values 18 hours after the end of the rides is probably an indication of the severity of the chloride depletion which occurred in the horses. However, the magnitude of the hypochloraemia was not great (only about 4 mmol/l below pre-ride levels) and no hyponatraemia was seen at any time during or after the rides.

Considering that the horses drank large amounts of tap water on returning to the stables it is surprising that no haemodilution was observed. Plasma sodium concentration did decrease at this stage, but only to return to pre-ride levels. It is possible that the water drunk was not all absorbed immediately, which is supported by the fact that

total plasma protein decreased only gradually during the four hours following the ride. It is also possible that the large caecum of the horse, which normally contains semi-digested fermenting vegetable matter, may contain some sodium and chloride which acts as a reservoir to prevent any serious fall in plasma concentrations during the recovery period, and this hypothesis is considered further in the General Discussion. Grosskopf and van Rensburg (1983) have reported the case of one horse which finished an 80 km ride in a severely dehydrated condition (total plasma protein concentration 102 g/l) and died one hour later after drinking a large volume of water. A diagnosis of osmotic shock was made on the basis of the finding of "vastly overfilled stomach and intestines" at post mortem, however this was not confirmed biochemically and it seems equally possible that the overfilled condition of the gastro-intestinal tract *per se* was a major contributing factor to the horse's death.

Plasma potassium concentrations

The patterns of change in plasma potassium concentrations were markedly different from those of the other two electrolytes. No significant change was seen during the actual exercise period while very marked alterations occurred immediately before the start and after the finish. It has been known for some time that potassium tends to move out of muscle cells and into the extracellular fluid when the muscle becomes active (Sreter, 1963; Laurell and Pernow, 1966; Korge and Viru, 1971). It appears that the muscular activity involved in the walk from the stable to the start of this ride was sufficient to cause a very marked increase in plasma potassium concentrations. It is possible that the sharp decrease observed after the ride was simply a reversal of this process. The drinking of water after the finish would also tend to cause potassium concentration to fall as would the fact that the horses were given their hay feeds at this point. However, it can be seen from

Table 51 that potassium loss in the sweat was very considerably hypertonic to plasma even when respiratory water loss is taken into account. It follows therefore that potassium must have been moving from the intracellular to the extracellular compartment throughout the rides for there to have been no decrease in the plasma concentration during the exercise period, and the decrease to slightly below the pre-ride (stable) concentration by one hour after the finish probably represents the return of only a small proportion of the intracellular potassium deficit. In retrospect it is questionable whether it was entirely prudent to allow the horses access to hay at this point, when such a sharp fall in plasma potassium concentration was liable to occur, as the consumption of the hay itself was liable to lead to a further decrease (Section 1). However, clinically significant hypokalaemia was not seen and there were no untoward effects.

It is probable that this tendency for a marked increase in plasma potassium concentration to occur when the horse moves from complete stabled rest to mild walking exercise is the source of the explanation for the remarkable tendency of this parameter to show a gradual steady decrease during both the experimental and the control periods of the investigations described in Sections 3 and 4. Immediately prior to both of these studies the horses were taken from their stables and walked short distances to the location of the environmental chamber or the stocks, usually via a weighbridge. It seems likely that a release of potassium from the muscle cells occurred at this point, similar to that seen in this present study. Following this, the horses in Sections 3 and 4 were restrained in stocks for the duration of the investigations, and particularly during the control periods they generally stood very quietly. The gradual decrease in plasma potassium concentration was

therefore probably a result of the return to the intracellular fluid of the potassium which had left the muscle cells just prior to the start of the experiments.

It is difficult to interpret changes in plasma potassium concentrations with respect to sweat losses against this background of apparently hair-trigger shifts of potassium between fluid compartments. It seems likely that this factor is the reason for the marked discrepancies between different reports of plasma potassium changes during endurance rides as discussed in Section 2. The finding of a significant increase, no change, or a significant decrease will depend entirely on whether the pre-ride sample was collected with the horse at rest or after the "trotting-up" included in the veterinary examination, and whether the post-ride sample was collected immediately or delayed somewhat. It is certainly noticeable that the pre-ride potassium concentrations recorded for the 160 km ride in Section 2, when the sample was collected from (mostly) stabled horses on the afternoon before the event, were generally lower than those recorded for the other rides when this sample was taken from "warmed-up" horses immediately before they crossed the starting line.

Plasma mineral concentrations

Plasma calcium, magnesium and phosphate concentrations each showed some statistically significant variation from the start value at some point during the experiment, but in general changes were small in magnitude and rather irregular. These findings were in marked contrast to those observed in connection with the competitive rides discussed in Section 2.

Plasma calcium and magnesium concentrations tended to increase during the rides in this Section and the maximum mean concentrations of both parameters were seen at the 80 km point. However, the percentage increases were considerably less than the percentage increases in the

plasma protein concentrations, which implies that the total plasma content of these minerals actually decreased. Considered in this way, it is possible to reconcile these findings with the significant decreases in concentrations observed in most of the competitive rides studied, and with the controlled study of Hambleton et al (1980), in which significant decreases in plasma calcium were seen (in spite of "minimal" sweating). If sweat calcium and magnesium losses were in fact the major factor involved in these changes, it would be expected that plasma concentrations would continue to decrease as dehydration became more pronounced. However,this does not appear to be the case. On the contrary, those rides in which the greatest degree of haemoconcentration was seen, that is the experimental rides discussed in this Section and ride C in Section 2, actually showed slight non-significant increases in the mean plasma calcium concentrations. This suggests that there may be an exercise-induced decrease in total plasma calcium content which is independent of the volume of sweat lost. The factors influencing this occurrence may be very complex, as already discussed in Section 2, and further discussion of this point is outwith the scope of this thesis.

Changes in plasma magnesium concentrations observed during this study have also been difficult to explain. In addition to the contradiction between the regular decreases in the competitive rides in Section 2 and the slight increase in the controlled rides in this Section, significant decreases were seen during the control experiments in Sections 3 and 4 and a significant increase during the morning in the resting stabled horses in Section 1. Plasma magnesium concentrations in the horse and the factors which influence them have not been widely studied, perhaps due to the fact that horses do not generally suffer from hypomagnesaemia in the same way as do cattle and sheep. The variety of changes observed during this present study and the lack of

any obvious explanation for them suggest that these factors, as with plasma calcium, may be very complex, and certainly require further investigation.

A significant increase in plasma phosphate concentration was an almost universal finding in the horses studied in Section 2, and this was believed to be a reflection of the breakdown of intramuscular stores of phosphocreatine. In this present experiment the increase was much smaller in magnitude, and significant decreases were seen before the start and after the finish. It is possible to speculate that these four rather excitable Thoroughbred horses had a higher level of adrenergic activity during exercise than the comparatively phlegmatic endurance horses and that the tendency of adrenergic activity to decrease plasma phosphate concentration (Section 4) combined with the tendency of endurance exercise to increase it to produce these rather irregular fluctuations. There is, however, no firm evidence to support this speculation.

Carbohydrate utilisation

On the mornings of the rides included in this section the horses were fed earlier than on the days of the 24-hour experiments in Section 1. It is therefore most probable that the decrease in plasma glucose concentration seen between the samples collected in the stable and at the start of the rides was due to the horses being in the descending section of the plasma glucose peak which occurs after the morning feed (Figure 4). During the rides themselves plasma glucose alterations appeared to occur in two distinct phases. In the first 40 km an increase was seen, probably due to adrenergically-induced mobilisation of liver glycogen stores and gluconeogenesis in excess of glucose utilisation. After the half-way point these stores appear to have been progressively exhausted and although plasma glycerol was high, as were cortisol and, presumably, adrenaline, gluconeogenesis was

obviously not sufficient to maintain plasma glucose concentrations. This is similar to the pattern seen in the competitive rides except that the hypoglycaemia at the finish was more marked, possibly because the higher exercising speeds in this present experiment required a greater proportion of carbohydrate to be used relative to fat. Lucke and Hall (1980c) have theorised that the distance after which hypoglycaemia would be expected to develop in the exercising horse is about 60 km, and the results of this present study tend to support this suggestion.

It is tempting to try to relate the hypoglycaemia seen at the end of the rides to the degree of fatigue, however horse F, who demonstrated the most marked fatigue of all the horses, showed only moderate hypoglycaemia (2.3 and 2.4 mmol/l) at the points where he refused to continue. In contrast horse A, who was apparently capable of continuing further when he finished the first ride, had a plasma glucose concentration then of only 1.9 mmol/l. The association of depletion of muscle glycogen stores with fatigue, both in endurance horses and in long distance runners, has already been discussed (Section 2), and this association also appeared to hold good for the horses in this present study. Finishing plasma glucose and muscle glycogen concentrations are compared to the degree of fatigue for each horse in Table 52. All five performances which were graded for fatigue as 3 or 4 (i.e. unable to continue) had muscle glycogen concentrations of under 5 µmol glucose units/g dry weight (i.e. undetectable) while the three performances graded as 1 or 2 (i.e. able to continue, apart from injury) had muscle glycogen concentrations of 76 - 156 µmol glucose units/g dry weight. These findings offer further confirmation that in the horse, as in man, it is muscle glycogen stores which determine an individual's ability to continue exercising rather than plasma glucose.

TABLE 52

Plasma glucose and muscle glycogen concentrations at the cessation of exercise compared to the subjectively observed degree of fatigue.

Horse	Ride	Degree of Fatigue	Plasma glucose (mmol/l)	Muscle glycogen (μmol/g dry wt)*
A	2	1	4.1	116
D	2	2	3.5	156
A	1	2	1.9	76
D	1	3	0.7	< 5
E	1	3	1.2	< 5
E	2	3	1.5	< 5
F	1	4	2.3	< 5
F	2	4	2.4	< 5

* Taken from Snow et al, 1982.

The increase in plasma glucose concentration after the rides was very rapid and had begun before the horses were fed. This indicates that once the demands of exercise had ceased gluconeogenesis was sufficient to replenish plasma glucose. This is in accordance with the findings of Terblanche et al (1981) who showed that liver glycogen increased very rapidly after exercise in rats fed on glycerol compared to fasting rats and suggested that glycerol was an excellent substrate for post-exercise gluconeogenesis. In the horses the increase in plasma glucose in the four hours following the rides was accompanied by a decrease in plasma glycerol from extremely elevated concentrations (995 ± 83 μmol/l) to a level approaching the pre-ride value.

Fat utilisation

The substantial increase in free fatty acid mobilisation and uptake which occurs in horses during both adrenaline infusion and exercise was first demonstrated by Carlson (1965), and the importance of fat utilisation during submaximal exercise is now well established (Goodman et al, 1973; Rose and Sampson, 1982). The hydrolysis of one triglyceride molecule yields one molecule of glycerol and three of fatty acid. Therefore if the products of lipolysis were not metabolised further the increase in plasma free fatty acid concentration would be three times as great as the increase in plasma glycerol concentration. In all three endurance competitions studied by Lucke and Hall (1978, 1980a,b) the observed FFA increase was proportionately very slightly greater than this, but in this present study increases in the two parameters were almost equimolar. This could be due to a greater uptake of the available FFA in this present experiment, or to a lesser utilisation of the glycerol for gluconeogenesis, and when it is considered that the actual end of ride plasma glycerol concentrations in this study were much greater than those of the competing horses, while the end of ride plasma FFA concentrations were very similar, the latter explanation appears to be the more probable. This is supported by the fact that the hypoglycaemia seen at the end of the rides in this present study was more profound than any reported by Lucke and Hall and much more profound than in any of the competitions studied in Section 2 of this thesis. If gluconeogenesis was indeed less efficient in the experimental horses the reasons are not immediately clear as plasma cortisol concentrations were actually higher at the end of the experimental rides. The apparent efficiency of post-ride gluconeogenesis has been discussed above.

Armstrong et al (1961) have postulated that uptake of FFA by the exercising muscle is directly related to plasma FFA concentration on a simple mass-action basis. Friedberg et al (1963) demonstrated that the

increase in plasma FFA concentration during exercise is preceded by an initial transient decrease, and considered that this was due to an active uptake by the muscle occurring prior to mobilisation of fat depots. However, extensive investigations have since been carried out into the uptake and utilisation of FFA by muscle during exercise (summarised by Gollnick, 1977), and no active carrier system for the movement of FFA into muscle has been identified. It is now assumed that this occurs by simple diffusion through the cell membrane, and that the initial decrease in plasma FFA concentration is caused by the increase in muscle blood flow which occurs at the start of exercise. It is still not possible, however, to draw any firm conclusions about FFA utilisation from plasma FFA concentrations alone, as although oxidation of the FFA taken up by the muscle is complete at low work intensities this decreases to about 60% at higher work intensities, with the capacity of the adenyl transferase enzyme considered to be the limiting factor (Hagenfeldt and Wahren, 1971). It also appears that individual fitness may influence this proportion, both in man (Poldene and Parizkova, 1975; Hultman and Sjöholm, 1983) and the horse (Goodman et al, 1973).

As in Section 2, the increase in plasma bilirubin concentration (over 100% on this occasion) could not be attributed to haemoconcentration alone, and it seems likely that once again this was partly a consequence of the high plasma free fatty acid concentrations leading to a reduction in hepatic uptake of bilirubin (Naylor et al, 1980). It was noticeable that the highest end of ride plasma FFA concentration (2172 µmol/l) and bilirubin concentration (94 µmol/l) occurred in the same individual (Horse E, ride 1). The metabolism of haemoglobin released from erythrocytes damaged during the ride is almost certainly another contributory factor towards the high bilirubin concentration, as suggested by Rose et al (1977), and this may well be the reason for the very slow return towards normal levels seen after the finish.

The two previous reports of individual plasma free fatty acid proportions in the horse have exhibited a number of differences (Luther et al, 1981; Rose and Sampson, 1982), and the results of this present study, considering the resting (pre-ride) samples, are slightly different yet again. In particular Luther et al reported 12.9% of C14:0 while only 4.1% was found in this study and only 0.8% by Rose and Sampson, the 19.2% of C18:0 found in this study was similar to the 21.7% reported by Luther et al while Rose and Sampson reported only 2.6%, and Rose and Sampson reported 29.9% of C18:2 while only 17.1% was found in this study and only 2.8% by Luther et al. The extent of these variations is surprising. It appears that a training effect is unlikely to be involved as the horses studied by Rose and Sampson (1982) were also about to set out on an 80 km (competitive) ride, and it is possible that the differences may be caused by some effect of diet. However, although the horses used by Luther et al (1981) were not fed hay, it seems unlikely that the diets of the privately owned horses sampled by Rose and Sampson (1982) would have been significantly different from those fed in this present study, and so this question must remain an open one.

It has been suggested by Robb et al (1972) that while the composition of the fatty acid content of adipose tissue is fairly constant between different fat depots in the same individual it can vary quite widely between different animals, and a dietary effect is again suggested. Because of this, it may not be entirely valid to compare the changes in the plasma fatty acids in the exercising horses with the fat depot composition of an entirely different individual, and it might have been more valid to try to obtain fat biopsies from the experimental horses themselves. However, this was not done, and using the available information it does appear that during exercise the composition of the plasma FFA pool became closer to that of adipose tissue. In particular

the proportion of C18:1, the most abundant fatty acid in the depot fat, increased by nearly 50% while that of C18:0, which was almost absent in the depot fat, was nearly halved. It can also be seen from Figure 37 that the change in plasma free fatty acid composition following intravenous adrenaline infusion was almost identical to that following exercise. These findings are in contrast to those of Rose and Sampson (1982), who reported that proportions of individual FFAs remained "much the same" in horses finishing an 80 km endurance ride, but are similar to those reported in man. Rothlin et al (1962) found increasing percentages of C18:1 and decreasing percentages of C16:0 and C18:0 with increasing total plasma FFA concentration in human subjects given intravenous noradrenaline infusion while Liesen et al (1975) reported an almost exactly similar effect in marathon runners. In both cases these findings are interpreted as indicating that the increase in plasma free fatty acids is due almost entirely to lipolysis with very little hepatic synthesis being involved.

Protein as a body fuel

While carbohydrates and fats are accepted as normal body fuel substances, protein is traditionally seen only as a last resort in an emergency, akin to Phineas Fogg burning the structure of the wooden paddle steamer in her own boiler when she ran out of fuel in "Around the World in Eighty Days". However, studies of both human and equine endurance athletes have invariably reported that plasma urea concentration increases steadily throughout exercise and Riley et al (1975) consider that the magnitude of the increase is too great to be accounted for by decreased urinary excretion and suggest that increased protein turnover is probably involved.

Available data in this present study tend to support this hypothesis. The urinary excretion rate of urea was not significantly different from that seen at rest (although, as discussed below, it is possible

that the comparison is not strictly valid) and when the extra output of urea in the sweat (about 160 mmoles, equivalent to 0.38 mmol/min over the whole period of the urine collections) is taken into consideration it appears that total urea excretion may actually have been higher during exercise. In spite of this, plasma urea concentration increased considerably over the period of exercise and was still increasing four hours after the finish. This suggests strongly that urea production was increased, i.e. that amino acid deamination (and hence protein breakdown) was increased.

Both Cerny (1975) and Haralambie and Berg (1976) have demonstrated that in man the increase in plasma urea does not begin until one hour after the start of exercise. A similar pattern was seen in this present study, with plasma urea concentration at 16 km (just under one hour from the start) being unchanged from the start value, and subsequently increasing almost linearly with time. Lemon and Nagle (1981) have suggested that the fuel supply situation after one hour of exercise is similar to that seen in short term starvation, in that amino acids are released from muscle to provide a substrate for increased gluconeogenesis in order to protect the supply of glucose to the CNS. However, in this present study it was clear that plasma urea concentration began to increase while plasma glucose concentration was still increasing and, presumably, hepatic glycogen stores were still available. In addition, the suitability of glycerol produced from triglyceride hydrolysis as a substrate for gluconeogenesis has been discussed above, and glycerol availability continued to increase throughout exercise. It is therefore difficult to believe that protein catabolism increases after only one hour of exercise because of a pressing need to protect the CNS from hypoglycaemia.

The concept of the existence of "labile protein stores" in the body which are available both to replace a protein deficit and as a source of energy has been discussed at length by Munro (1964). He considers that the liver, not the muscle, is the site of storage of the majority of this protein and that the nucleolus of the hepatocyte contains RNA which has the specific function of laying down protein stores. He also considers that some breakdown of these protein stores during the post-absorptive period is a normal occurrence to maintain blood amino acid levels between meals, and the increase in plasma urea concentration during the overnight fast in the horses reported in Section 1 may support this hypothesis. More recently Decombaz et al (1979) interpreted changes in serum free amino acids and urea in human competitors in a 100 km run as indicating "a stimulation of gluconeogenesis at the expense of the amino acid pool without induction of muscle catabolism". It seems, therefore, that a degree of protein catabolism may be a normal component of fuel metabolism during medium to long term exercise.

It has been shown that in the horse, unlike man, plasma alanine concentration decreases during prolonged exercise (Lucke and Hall, 1980a). This indicates that the muscle-liver alanine-pyruvate cycle which is believed to be an important route of protein catabolism during exercise in man is probably not of great importance in the horse. This may be taken as further evidence for the major site of protein catabolism being elsewhere than in the muscle, however, it is also possible that gluconeogenic amino acids other than alanine are released from equine muscle during exercise. This possibility has not yet been investigated.

The increase in plasma uric acid concentration seen in this study was similar to that reported by Lucke and Hall (1980a) in endurance horses, but of much greater magnitude than that reported by Riley et al (1975) in marathon runners. It is possible that this finding is another

reflection of increased protein catabolism in a similar way to the increase in plasma urea concentration, as suggested by Munro (1964). However, it is equally possible that it is a consequence of increased ATP breakdown, which may result in some purine nucleotides being completely metabolised to uric acid.

Plasma enzyme activities

The patterns of increase in plasma enzyme activities seen during the rides, as with the competing horses in Section 2, were strongly indicative of an alteration in the integrity of the muscle cell membrane. However, it is again extremely difficult to show that the magnitude of the rise in the activity of any of the enzymes in the plasma was in any way correlated with the severity of the muscle damage occurring. It can be seen from Figure 39 that there was no relationship between increases in either CK or AST activity and the subjectively assessed degree of fatigue, and that in fact end-of-ride plasma cortisol concentration appeared to show a much better agreement, probably due to the association of this hormone with general stress which has already been discussed. However, as discussed above, the observed onset of fatigue seemed to coincide with the exhaustion of the muscle glycogen stores. If the limiting factor in exercise was therefore primarily a fuel supply matter, rather than being connected to loss of muscle cell integrity, then the lack of correlation between fatigue and increases in plasma enzyme activities proves nothing about the relationship of these changes to the severity of muscle damage. Studies which indicate that the magnitude of plasma enzyme increases over a standard exercise test is less in trained individuals, both human (Fowler et al, 1962) and equine (Hambleton et al, 1980), suggest that such a relationship may well exist, but without some other reliable index of the degree of damage to muscle cells this is difficult to confirm. As in Section 2, it was found that changes in plasma CK activity immediately after the

rides (considering the four-hour post ride period in this case) were almost evenly divided between increases and decreases, and that this did not appear to be related to the horses' performances. Plasma AST and ALP decreased in the four-hour post ride period in almost all cases.

The fluctuations in plasma CK and AST activities seen in horses A and D in the months following their rides, when all four horses were receiving only minimal walking exercise, were quite remarkable. None of the horses exhibited any evidence of a continuing or intermittent muscle problem, and yet while plasma enzyme activities of horses E and F returned to their baseline values soon after the second endurance ride and remained there, those of horses A and D continued to fluctuate, often rising much higher than on the days of the actual exercise. The patterns of these fluctuations, especially in horse D in early November, again strongly suggest release of enzymes from damaged muscle cells. In that case the sudden increase in CK activity followed by an immediate fall coincided with a sudden increase in AST activity which fell more gradually over several weeks, which is again similar to the pattern seen in cases of rhabdomyolysis, but of rather smaller magnitude. Another similar episode seems to have occurred about a month later, after which CK activity returned to baseline and stayed there, to be followed about a month after that by AST. In contrast horse A did not demonstrate such dramatic peaks in enzyme activities, although one is tempted to speculate about the timing of the two highest CK activities, which occurred almost exactly four and eight weeks after the second ride in this horse, just as they did in horse D. The continuing elevation of plasma AST activity at about 1000 iu/l combined with a slight continuing elevation of CK activity at about 350 iu/l tend to suggest some chronic but low grade interference with muscle cell integrity in this horse. This was still evident as late as the following August, when the hay feeding experiment was carried out (see Appendix I).

When these observations are compared to the frequent finding of "elevated" pre-ride plasma AST (and sometimes also CK) activities in the competing endurance horses in Section 2, the suggestion must be made that this type of occurrence is fairly common among horses in training (or just following training) for endurance work. It seems almost certain that the plasma enzyme changes must be reflecting some disruption of muscle cells, however there is no evidence to suggest that this is at all pathological or even liable to interfere with performance. Horses A and D, and the affected competing horses, were all generally good performers, and the observations recorded concerning horse 11 in the 160 km ride in Section 2 (Figure 20) indicate that it is possible for a horse to perform well in competition even while an episode of this kind is actually in progress. There is no real indication either as to why certain horses are affected and others apparently not. Horses A and D were a few years older than horses E and F, but there did not seem to be any consistent effect of age among the privately-owned horses. It appears, therefore, in the absence of any evidence that these unusually high plasma enzyme activities are connected to any pathological condition, that such elevated enzyme activities in endurance horses should not be considered a cause for concern unless accompanied by clinical evidence of a muscular problem.

Urine composition

Limitations of this present study

The findings in this present study with regard to urine composition have been criticised by Carlson (1983) on the grounds that the time over which the urine samples were secreted was substantially longer than the actual time spent in exercise. To a certain extent this is a valid point, but principally so far as it refers to a time lag after the rides when the horses were fed and were drinking large amounts of water. In fact most of the post-ride urine samples were collected almost immedi-

ately the horses returned to their stables (after walking back from the ride location, weighing, etc.) before much (or any) water was drunk and certainly before they were fed. The major portion of the time discrepancy occurred in the morning before the rides, and as there is no reason to believe that renal metabolism at that time would be any different from any other morning it follows that significant differences between the exercise day and the resting day are overwhelmingly likely to be effects of the exercise. Similarly, as at least 60% of the time covered by the urine collections was spent in endurance exercise the absence of any difference between the two days would be a strong indication that the urine being produced during exercise was not significantly different from that produced at rest. Unless the horses' bladders had been catheterised (a procedure impossible without the use of tranquillisers) the protocol adopted was probably the most satisfactory which could be devised, as results are considered as excretion rates, not as concentrations, and so the effects of the level of diuresis are minimised.

A criticism of the present study which is probably more valid concerns the use of results from Section 1 as the control data. Although these results are from the same horses at the same time of day as the exercise study, it was not originally appreciated that their diets were significantly different on that occasion. This discrepancy affects some constituents more than others, but it is to be regretted that the control experiment was not repeated for this purpose when the horses were receiving the same diet.

Urine flow rate, specific gravity and excretion of nitrogenous constituents

Although the difference between the rates of urine production on the control days and on the exercising days was not statistically significant the mean urine flow rate was substantially greater on the exercising days. This is extremely surprising considering the amount of

fluid loss which was occurring from other sources at the time and especially considering that the data for the control day included one of the periods of diuresis thought to be associated with periprandial drinking as discussed in Section 1. It is not clear how this finding could be due to any error introduced by the differences in diet between the control and exercising days, and it can only be concluded that, at least in this present instance, long distance exercise in the horses was not associated with any reduction in the rate of urine production. Although unexpected, this finding is similar to previous reports of urine flow rate in human athletes (Refsum and Strømme, 1975), which suggest a decrease in urinary concentrating ability during heavy exercise. The lower specific gravity of the urine produced during exercise reflects the generally lower concentration of most of the urinary solutes.

Urinary creatinine excretion was significantly lower on the exercise days compared to the control days, and it would not be expected that this parameter would be particularly affected by any differences in diet. This lower creatinine excretion rate was accompanied by an increase in plasma creatinine concentration which implies that creatinine clearance rate was lower on the exercising days. In a steady state situation this would imply a lower glomerular filtration rate (Knudsen, 1959; Gelså, 1979), however it is entirely possible that increased breakdown of intramuscular stores of phosphocreatine leads to an increased rate of creatinine production during long term exercise. It is therefore difficult to draw any firm conclusions regarding renal function in endurance horses from changes in creatinine clearance alone.

Urinary urea excretion, unlike creatinine, is liable to be affected by the diet, particularly by its protein content, and so it is possible that conclusions drawn by comparing the exercising days with a time when the horses were fed differently may not be entirely valid. It appears,

as discussed above, that urinary urea excretion during exercise was not significantly different from that seen in the resting animal, and that when sweat urea losses are taken into account total urea output may actually have been higher on the exercising days. This has been used to argue that the steady increase in plasma urea concentration seen during exercise could not have been due to a reduction in excretion and must therefore have been a reflection of an increase in protein catabolism. However, it may be that the higher protein content of the diet during the exercising period had led to a higher daily urea output than that recorded in Section 1. In that case the urea excretion rate seen during exercise might actually represent a reduction compared to the resting excretion rate on the same diet. Thus some uncertainty must still remain when considering the reason for the increasing plasma urea concentrations.

Urine pH and electrolyte excretion

The significantly higher urine pH seen during exercise is in marked contrast to the findings of Carlson (1983) who reported a change in the opposite direction between 48 km and the finish of a 160 km ride. It is possible that the alkaline urine observed after 80 km in the present experiment and after 48 km in the Tevis Cup ride (Carlson, 1983), pH 8.4 in both cases, are actually comparable and that in more prolonged exercise of over 80 km the urine pH, in common with several plasma parameters discussed in Section 2, tends to return towards the resting level.

It is often assumed that a metabolic alkalosis occurs in horses involved in endurance rides due to the hypochloraemia, caused by sweat chloride losses, being balanced by an increase in plasma bicarbonate (Carlson, 1975, 1980; Fowler, 1980a). However Rose et al (1979) have demonstrated that arterial and venous pH and bicarbonate concentration did not change over a 100 km endurance ride, and indeed reported that

the leading horses, which completed the ride at mean speeds of over 18 km/hr and were therefore probably comparable to the horses in this present study, all showed a metabolic acidosis due, probably, to a degree of anaerobic metabolism. Other studies have confirmed the lack of change in plasma bicarbonate concentration (Rose et al, 1977; Lucke and Hall, 1980b) and there does not appear to be any other factor, such as an increase in urine potassium excretion, which might explain the alkalinity of the urine. This therefore remains an anomalous observation, and it must be questioned whether, in spite of the efforts made to process samples promptly, the pH measurement of the urine samples on the exercising days might have been delayed long enough to lead to erroneous results, as was suggested in Section 2 when considering very similar findings.

The electrolyte excretion rates, as with urea, might be expected to be affected by the discrepancy in the diets between the exercising and the control days. However, due to the heterogeneous nature of the diet fed, it is difficult to assess the extent of this problem and the results are considered as they stand.

The only electrolyte in which a lower excretion rate could be demonstrated on the exercising days was chloride. This was hardly surprising considering the loss of almost half the total chloride content of the extracellular fluid in the sweat, and the fact that as the fluid lost was hypertonic for chloride a significant hypochloraemia developed. It is noteworthy however, that although the chloride concentrations in the urine samples collected after the rides were generally much lower than those seen at any time of day in the resting horses (Section 1), the overall chloride excretion rates on the exercising days were not very much lower than those calculated for urine samples collected first thing in the morning or during the night in Section 1. This underlines the fact that comparisons of urinary

parameters measured before and after exercise can be very misleading and comparisons must be made between samples representing the same times of day.

No significant reductions in urinary excretion of either sodium or potassium could be demonstrated in association with endurance exercise. The findings with regard to sodium excretion were mainly remarkable, as in Section 1, for their extreme variability between individuals and in the same individual on different occasions. The lack of any obvious tendency towards renal sodium conservation is consistent with the fact that the hypotonic nature of the overall sodium loss led, in fact, to a slight increase in plasma sodium concentration. In any case, the resting level of urinary sodium excretion in the horse is so low that very little benefit would seem likely to result from a further reduction, and this subject is considered further in the General Discussion.

The situation regarding urinary potassium excretion is similar in that as plasma concentrations at no time fell significantly below the levels seen in the horses at rest a reduction in renal output would not immediately be expected. However it is highly probable, as discussed above, that there was a considerable potassium deficit in the intracellular fluid by the end of the rides, and it is perhaps surprising that no effect of this deficit on renal excretion was observed. It may be that such an effect would have been seen to occur if urine sampling had been continued for several hours after the ride when plasma potassium concentration was lower than during exercise. Comparison of the total sweat electrolyte losses (Table 51) with the amounts excreted in the urine over 24 hours in the resting animals (Table 2) suggests, however, that the potassium deficit is of comparatively minor importance

compared to the chloride deficit, and that its gradual replacement under the normal conditions of a high dietary intake may not require a very great reduction in renal excretion.

Sweat collection technique

The various sweat collection techniques used in exercising human subjects have been described in Section 3 and their advantages and disadvantages considered. The body washdown method is, as already discussed, quite impractical for use in the horse, and it appears that the collection of exercise-induced sweat directly from an uncovered skin surface was a major source of error in most of the early studies of equine sweat electrolyte concentrations. It therefore appears that collection from under an impermeable covering is the only feasible method of obtaining exercise-induced sweat from horses. The most convenient means of accomplishing this is to collect sweat from under the saddle by means of an absorbent pad, as described by Carlson and Ocen (1979) and Rose *et al* (1980). It has been suggested that the use of absorbent pads may overcome the problems of altered sweat composition associated with the enclosure of the skin with impermeable material, as such pads appear to behave as though permeable until they have become saturated with sweat (Verde *et al*, 1983). However, there is no doubt that the pads used in this present experiment were actually saturated in many cases, particularly after the 24 km stages. The omission of the plastic lining on the saddle cloths might have improved things in this respect, but it was considered that such a layer of plastic was essential. This was because all saddle cloths/blankets worn by horses sweating heavily on long distance rides were observed to become so saturated with sweat that contamination from the leather saddle and the soap used in cleaning it tended eventually to soak back through the blanket. The layer of plastic eliminated this problem but was observed

to have caused some erythema and even slight skin abrasions in one or two horses, and so it may be that some interference with the sweat composition did occur.

The analysis of the known electrolyte solution after soaking in the absorbent pads indicated that although some alteration in electrolyte concentrations may have occurred the changes were small in magnitude and probably negligible in comparison with other sources of error such as hair contamination.

The comparison between sweat collected from the neck and from under a saddle by the method under discussion during adrenaline infusion was not exact, as it is impossible to compare a number of spot samples (neck) with one continuous mixed sample (saddle), particularly when considering constituents which demonstrate exponentially decreasing concentrations. This is probably the reason for the discrepancies between the two methods in the magnesium and protein concentrations, but the fact that the sweat collected from under the saddle showed patterns of exponential decrease in the concentrations of these two parameters very similar to those of the neck samples indicates that the saddle collection method is probably valid.

Comparison of chloride results from the two methods of collection indicates that the major discrepancy occurred towards the end of the adrenaline infusions, when chloride concentration in the sweat from the neck was noticeably higher. At this stage in the experiments sweating rate was observed to be decreasing and it was considered that some evaporation of sweat was occurring on the skin. This would not have affected the samples collected from under the saddle and the constant (or even slightly decreasing) chloride concentration in these was probably the more accurate. A similar effect is evident in the potassium, calcium and urea concentrations.

Sodium concentrations also exhibit this effect, but it appears that the sodium concentration of the sweat from under the saddle was consistently lower than that of the neck samples throughout the experiments. This was obviously not due to any retention of sodium in the absorbent pads, as was demonstrated above. However, a significant difference in sodium concentration between sweat collected from the neck and from the body during adrenaline infusion has already been demonstrated in Section 4 (see Table 38) and the same finding involving the saddle collection technique seems to provide further evidence for this effect of body area on sweat composition.

The only major discrepancy between the two sweat collection methods was the markedly lower pH of the samples collected from under the saddle. For this reason, pH measurements were omitted from the analysis of exercise-induced sweat. In general, the close correspondence of the results from the two methods was encouraging, as it not only suggested that the saddle method was probably valid for use on exercising horses, but provided confirmation that, except possibly to some extent towards the end of the experiments, evaporation of sweat from the exposed skin did not give rise to gross errors in the electrolyte concentrations reported in Sections 3 and 4.

There was no obvious suggestion that contamination from the unclipped hair on the back led to erroneously high electrolyte concentrations in the samples collected from under the saddle in the early stages of the adrenaline infusions, as appeared to have been the case during exercise. It may be that the washing procedure was carried out more thoroughly before the adrenaline infusions, but it is more likely that the weight of the rider in the saddle and the powerful movements of the horses' back muscles during exercise tended to transfer more extraneous material from the coat into the pads than would occur when the horse was stationary.

Sweat composition

Sweat electrolyte concentrations

The apparent effects of skin and/or hair contamination on the first sweat samples collected (i.e. 0 - 16 km) have been discussed above. However, after these effects have been discounted it appears that exercise-induced sweat, in common with that induced by other stimuli, is hypertonic compared to plasma for sodium, potassium and chloride.

Chloride concentration of the exercise-induced sweat was significantly lower than that of either heat or adrenaline-induced sweat. It is possible that this is a basic effect of the mode of stimulation of sweating, but it is also possible that it was a result of the endurance training undergone by the horses prior to this experiment. During acclimatisation to heat exposure and exercise in man increased aldosterone secretion leads to decreases in sweat electrolyte concentrations (Streeten et al, 1960), but this has not been shown to occur in the horse. The reduction in sweat chloride concentration seen in this experiment (only 20 - 25 mmol/l less than the heat- and adrenaline-induced sweat in the untrained horses) was much less than the reduction seen in acclimatising human subjects, and the actual concentration, hypertonic to plasma, was very much higher than that of even unacclimatised man. It therefore does not appear as if any physiologically significant electrolyte conservation was occurring in the endurance-trained horses, and indeed if the hypothesis advanced above is correct that the horse prefers to lose an isotonic thermoregulatory fluid in order to prevent disturbances of extracellular fluid balance, a marked reduction in sweat electrolyte concentrations during acclimatisation to heat or exercise would not be expected. In retrospect it might have been informative to carry out adrenaline infusions on the horses while

they were in a state of endurance training to determine conclusively whether this difference in sweat chloride concentration was due to the training regime or to the mode of stimulation of sweating.

Mean sodium and potassium concentrations of exercise-induced sweat were, in contrast, intermediate between those of heat and adrenaline-induced sweat. This again tends to discount an electrolyte-conserving effect of training. The patterns of change in these electrolytes with time were in fact more similar to those of adrenaline than of heat-induced sweat. After allowance was made for the apparent contamination error in the first samples the chloride concentration, so far as could be ascertained, remained unchanged throughout the period of exercise. In contrast sodium concentration tended to increase and potassium concentration tended to decrease, changes which were also seen during adrenaline infusion but not during heat exposure. However, the most striking difference in sweat composition between the three stimuli to sweating was again the change in sodium:potassium ratio with time (Figure 42), with the exercise-induced sweat following a line intermediate between those of heat and adrenaline-induced sweat. This difference between heat and adrenaline-induced sweat has been discussed earlier (Section 4) and it was suggested that it was an effect of the mode of induction of sweating in that heat-induced sweat is entirely stimulated by sympathetic nervous activity with no increase in adrenomedullary adrenaline secretion, while no sympathetic nervous activity was involved in the adrenaline infusions. As both sympathetic nervous activity and increased circulating adrenaline are involved in the stimulation of sweating during exercise (Robertshaw and Taylor, 1969), an intermediate pattern of change in sweat sodium: potassium ratio is not surprising. This tends to confirm that the effect is likely to be a function of

circulating adrenaline levels (whether in association with sweating rate or not), but the mechanism behind the changing electrolyte ratio is still not entirely clear.

Sweat urea concentration

In contrast to the adrenaline infusion experiments, where no change in urea concentration with time was observed, sweat urea increased steadily during the period of exercise with the mean concentration of the last samples being over 2 mmol/l greater than that of the first samples. This increase appeared to parallel the increasing plasma urea concentration, which rose by a mean of 2.4 mmol/l over the same period. This is in accordance with the suggestion discussed in Section 4 that in the horse, as in man, sweat urea concentration is dependent on plasma urea concentration. It is difficult, as already discussed, to compare spot samples (plasma) with a mixed sample collected continuously over a period of time (sweat). However, it appears from Figure 44 that the sweat urea:plasma urea ratio was somewhat lower during exercise than the 1.24 to 1.52 seen during adrenaline infusion. The contribution of sweat urea to the total body urea excretion was not negligible when set alongside urine urea excretion, and this point has been discussed above.

Sweat magnesium and protein concentrations

Even if one accepts that the concentrations in the first samples may have been somewhat overestimated due to skin and/or hair contamination, as appeared to be the case with the electrolyte concentrations, it is evident that sweat magnesium and protein concentrations showed an exponential-type pattern of decrease with time during exercise very similar to that seen during heat exposure and adrenaline infusion. Although the correlation between them was still significant, due partly to their similar patterns of change with time, the protein concentration was consistently much higher in comparison with the corresponding magnesium concentration than was seen to occur in any of the other

sections of this thesis. It may be that this was an effect of the slight erythema and abrasion of the skin caused by the plastic lining of the saddle cloths which was mentioned above. Slight inflammation may possibly have caused an addition of non-sweat proteins to the samples collected, although as no further analyses were carried out this is impossible to confirm. However, the relatively high protein concentration is in fact most evident in the earlier samples, while any effect of abrasion of the skin as described would have been expected to be absent in the early samples and most obvious in the later ones. It therefore seems possible that the magnesium:protein ratio in exercise-induced sweat may indeed be different from that in heat and adrenaline-induced sweat, which adds another variable to those discussed in Section 4. It appears that the relationship between magnesium and protein in equine sweat is not nearly so clear-cut as the early results in this study seemed to suggest, and it may be that further investigation of the mechanism of secretion of the sweat protein is required before the connection can be established.

Conclusions

The most interesting findings in this section concern the degree of fluid and electrolyte loss in the sweat of the exercising horses and the response of the horses to these losses, which seems to be very different from that of man. Sweat electrolyte composition differed slightly from that of sweat secreted in response to other stimuli, but was still hypertonic to plasma. However, when the respiratory water loss was included in the calculations it became clear that sodium and chloride "concentrations" in the total body water loss were in fact almost isotonic. Thus although the horses lost about a third of their extracellular fluid volumes during the rides plasma electrolyte concentrations changed very little - a slight increase in sodium and a

slight decrease in chloride. Fluid replacement after the rides appeared to be regulated in order to avoid haemodilution. Changes in plasma potassium concentration seemed to be connected to shifts of potassium out of the intracellular fluid immediately at the start of exercise and back in after exercise had ended. The only renal response which could be related to fluid/electrolyte homeostasis was a marked decrease in chloride excretion.

Other constituents of sweat yielded findings similar to those in earlier sections of the thesis: urea concentration increased with plasma urea concentration while magnesium and protein concentrations showed an exponential type pattern of decrease with time. However, the magnesium: protein ratio was much lower than that of heat or adrenaline-induced sweat. There was no evidence of any disturbance of plasma mineral concentrations during exercise, either due to sweat losses or to other causes.

Fuel metabolism appeared to be very similar to the findings in human athletes. During the second half of the rides the progressive development of hypoglycaemia suggested that liver glycogen stores were becoming exhausted, however onset of fatigue was related to exhaustion of muscle glycogen stores rather than to the severity of the hypoglycaemia. A huge increase in plasma free fatty acid concentration and a shift in composition of this plasma FFA pool closer to that of depot fat indicated that mobilisation of fat stores was an important aspect of endurance fuel supply. The simultaneous increase in plasma glycerol concentration provided a substrate for gluconeogenesis which in this study appeared to be most important in the immediate post-exercise period. There was some evidence that combustion of protein stores was occurring from fairly early in the exercise periods, and it was suggested that the liver rather than the muscle might be the source of this labile protein.

Plasma activities of enzymes known to be in high concentration in the muscle cells increased during the rides by varying degrees in the different animals. Due to the absence of any other means of quantifying the extent of damage to muscle cells occurring during exercise in each animal it was not possible to determine whether the magnitude of these increases in plasma enzyme activities was related to the degree of severity of muscle damage in the horses. In the months following the rides a pattern of fluctuations in plasma enzyme activities was seen in two of the four horses which strongly suggested that chronic or recurrent intermittent disruption of muscle cells was occurring. However, there was no evidence that this occurrence, which it was suggested was also seen in a number of the privately-owned endurance horses, was connected to any pathological condition.

GENERAL DISCUSSION

THERMOREGULATION AND FLUID/ELECTROLYTE HOMEOSTASIS IN THE EXERCISING HORSE

THERMOREGULATION AND FLUID/ELECTROLYTE HOMEOSTASIS IN THE EXERCISING HORSE

The findings in this study relating to subjects such as body fuel utilisation and plasma enzyme changes have been largely self-explanatory and in most cases have been very much in line with the findings in human athletes. In contrast, the findings relating to fluid and electrolyte balance and in particular to sweat composition have been very different from those in man, and suggest that the two species may have evolved different mechanisms for dealing with the problems of fluid and electrolyte losses which arise from the use of evaporative thermoregulatory mechanisms. Investigation of this area of equine endurance exercise has formed the major part of this study and different aspects have been dealt with in each section. It is therefore appropriate to discuss this particular subject in greater detail in order to integrate the findings of the different sections and to try to arrive at a unified picture of fluid/electrolyte homeostasis in the exercising horse.

Sweating as a thermoregulatory mechanism

Compared to devices such as the internal combustion engine, the biological combustion of fuels is an extremely efficient process. Nevertheless, a certain amount of metabolic heat is always produced, and during exercise this heat production increases considerably. It has been suggested that when a cheetah, for example, is running at 100 km/hr, its rate of metabolic heat production is over 60 times as great as at rest (Taylor and Rowntree, 1973). Obviously, this degree of heat production cannot be sustained for very long before the animal's body temperature rises towards a lethal level, which has been shown to

be only about 42 - 44°C in small to medium sized mammals (Adolph, 1947). The larger the animal, and hence the smaller the surface area:volume ratio, the greater the problem of dissipating this heat load, and a number of different solutions to this problem are utilised by various species according to their different lifestyles and exercise requirements.

Species which have a requirement to run for only short distances may indeed allow the heat load to accumulate. The body then cools down when the animal is at rest - deferred heat dissipation. It appears that the cheetah is an example of this type of thermoregulation, and it is suggested that the cheetah's observed habit of abandoning a pursuit if it has not caught its prey within about 1 km may be a result not of fatigue but of the animal's body temperature reaching a critical level of 40.5°C (Taylor and Rowntree, 1973). It seems likely that the horse, and indeed all large mammals, use deferred heat dissipation to a certain extent while sprinting. The obvious disadvantage of this system is that it severely curtails the duration of exercise. If an animal wishes to run for an extended period of time it must devise some means of dissipating the increased metabolic heat load while exercise is continuing, and this must be efficient enough to stabilise its body temperature at a tolerable level.

The major methods of heat transport are conduction, convection, radiation and evaporation. While the first three methods are involved to some extent in thermoregulation and increase with increasing cutaneous blood flow, most large mammals rely primarily on evaporative cooling to stabilise body temperature during prolonged exercise. This is accomplished by two separate mechanisms - panting, which involves forced evaporation of saliva and nasal secretions from the oral or nasal mucous membranes by increasing the respiratory rate, and sweating, which involves the secretion of a watery fluid from specialised glands on the

body surface. "Sweat" glands are found in a wide variety of species producing secretions of different composition for a number of different purposes, from improving grip to scent production (Jenkinson, 1973). However, it appears that only a very few species use sweat secretion as their major thermoregulatory mechanism - man (and other Old World Anthropoids), the horse and the camel. Virtually all other large mammals rely mainly on panting.

The relative advantages and disadvantages of sweating versus panting have been discussed at length by Carrier (1984), who makes a persuasive case for the combination of bipedal running and efficient thermoregulation by sweating as the main reason for man's evolutionary success as a hunter.

The advantages of panting are said to be threefold. Firstly, the panting animal does not lose electrolytes. Secondly, panting involves actively forced evaporation which may be more efficient than relying on a wind. Thirdly, the temperature gradient across the skin surface of a panting animal is more favourable - non-evaporative heat loss will be more efficient when environmental temperature is lower than body temperature and heat gain from the environment will be less when environmental temperature is higher. One could also add that there is no such thing as ineffective panting - all fluid lost while panting is actually evaporated and hence effective in cooling the animal, while under certain circumstances sweat may run from the body while still liquid.

Two advantages of sweating are discussed. Firstly, the evaporative surface area provided by the skin of a sweating animal is much higher than the oral or nasal mucous membranes can provide. Secondly, sweating is independent of the respiratory cycle and so any conflict between the need for oxygen supply and the need for heat loss will be avoided. In

addition, one may add that sweating does not require the muscular effort needed for panting, and so avoids the generation of even more metabolic heat.

Considering these points it appears that panting may be a more suitable mechanism for the dissipation of non-exercise heat loads, in common with techniques such as saliva spreading and mud wallowing. The problem of accommodating oxygen demand does not arise and the advantage of forced evaporation may be very important. However, a running animal requires an adequate oxygen supply as a major priority, while it creates a good deal of air movement over the body as it runs and so ineffective sweating is much less likely to occur. This suggests that sweating is a more suitable thermoregulatory mechanism for use during exercise and it is therefore very surprising to find its use restricted to so few species.

It is interesting to note, in this context, that while man appears to be exclusively committed to sweating, the horse makes active use of both systems. In the heat exposure experiments in Section 3, when no exercise was involved and the conditions of high humidity and lack of air movement were unfavourable for efficient sweating, the horses began to pant immediately. It appeared that they were not in fact able to prevent the loss of large volumes of sweat in spite of the impossibility of its being evaporated, but the efficiency of the panting response was demonstrated by pony W, who did not begin to sweat for one-and-a-half hours and maintained a stable rectal temperature during that time by panting alone. In contrast, man appears to be incapable of this type of respiration. The people who were in the environmental chamber with the horses reported no impulse to alter their respiratory patterns and indeed were incapable of matching the horses' respiration rates voluntarily for more than a few seconds.

At the gaits of canter and gallop, however, the horse's respiratory rate is strictly tied to the stride frequency, due to the involvement of the muscles of the upper forelimb in both respiration and locomotion (Attenburrow, 1983). This means that the horse has in fact no freedom to alter its respiratory rate even to accommodate oxygen demand (even swallowing requires careful timing), and respiratory evaporation, while it is clearly far from negligible, as shown in Section 5, is totally inflexible. Under these circumstances sweating becomes the major controllable means of thermoregulation.

It is possible, then, to argue that the thermoregulatory mechanisms of the horse are particularly well developed, with panting and sweating both available for use in appropriate circumstances, and the question arises as to why man lacks the ability to pant. Man is a bipedal runner and so there is no obligatory coupling of respiration to locomotion, which suggests that it should be possible for him to pant even while running, if necessary. It may be, however, that the particularly short, flat face of the human species (possibly a result of the necessity of balancing a large-brained head above an upright body) and the consequent small area of oral and nasal mucous membrane in comparison to body size, has rendered panting so inefficient that it was abandoned. Carrier (1984) points to the hairless condition of man as being particularly important in improving the efficiency of cutaneous evaporative heat loss, and advances the hypothesis that this is one evolutionary reason for the loss of the hair coat, as it improved man's performance as a persistence hunter. Considering this from the viewpoint of man as a non-panting animal, however, it may be that the improved efficiency of sweating provided by the absence of a hair coat is a necessary substitute for panting in man as a means of thermoregulation while <u>at</u>

rest. Indeed, while the horses were beginning to pant as they entered the environmental chamber, the humans were removing all the outer clothing that decency permitted!

The presence of protein in the sweat of the horse may be an attempt to improve sweating efficiency to some degree while retaining the hair coat, by ensuring efficient wetting action, reducing run-off of ineffective sweat and eliminating pockets of air trapped between the hairs.

Hypertonic versus hypotonic sweat

The horse and man are the only two species of athletes involved in endurance competitions in temperate or tropical climates, and, apart from the camel (which has been studied very little), they are the only two species which use sweating as their major means of thermoregulation during exercise. It is therefore extremely interesting to note that the physiology of sweating in the two species is almost completely dissimilar. Indeed, it appears that the production of a watery fluid for the purpose of cutaneous evaporative heat loss is almost the only similarity between the sweat glands of the two species. The differences in embryological origin, morphology and physiological control discussed in Sections 3 and 4 seem to demonstrate almost conclusively that these glands have evolved quite separately towards the same purpose - an example of parallel evolution. It is, however, the differences in composition of the sweat which are the most interesting, as they seem to demonstrate two quite different approaches to the problem of reconciling the maintenance of fluid/electrolyte balance with evaporative thermoregulation.

The absolute importance of salt conservation as an overriding consideration in hot environments or during prolonged exercise is seldom questioned. If one considers the vast majority of mammalian species

which do not sweat to any great extent, presumably because the cost of producing this specialised thermoregulatory fluid is not balanced by the advantages to the animal of improved heat loss, it would appear that the loss of electrolytes is the main disincentive to the production of large volumes of sweat. It therefore comes as no surprise to find that human sweat has a low electrolyte concentration compared to plasma and that in cases where a negative sodium balance develops sweat sodium is further reduced to extremely low levels. However, there are two sides to this argument, and it must be questioned whether the retention of electrolytes during a period of water loss, with the obvious consequence of developing hypertonicity of body fluids, is preferable to the maintenance of normal extracellular fluid electrolyte concentrations at the expense of net loss of electrolytes from the body.

The human approach to this problem, and presumably the approach of all the species which rely mainly on panting, is to give electrolyte conservation priority by reducing the electrolyte content of the thermoregulatory fluid as much as possible. However the consequence of this, as discussed by Itoh (1952) and Kuno (1956), is that plasma electrolyte concentrations will tend to rise. This leads to increased renal electrolyte excretion in order to prevent hypertonicity of the extracellular fluid. It therefore appears that in this case urinary electrolyte loss has merely been substituted for sweat electrolyte loss, and the advantages become less apparent. However, compared to the horse, man is very poorly adapted for carrying large amounts of water in the gastro-intestinal tract and must therefore drink more frequently (Newman, 1970). In the natural situation, unlike organised marathon races, the fluid drunk will probably be almost salt-free. This means that drinking will tend to lower plasma electrolyte concentrations and under these conditions urinary electrolyte excretion decreases (Itoh, 1952). Thus it appears that the hypotonic sweat of the human runner is

designed to favour electrolyte homeostasis in circumstances where fairly frequent drinking occurs, and that the kidney is of major importance in adjusting any imbalance between fluid/ electrolyte secretion and ingestion.

The horse, on the other hand, appears to give priority to the production of an isotonic thermoregulatory fluid, considering sweat and respiratory water loss together, thus maintaining extracellular electrolyte concentrations fairly constant with little need for adjustment of urine composition. This may allow more prolonged exercise without the necessity for drinking, but at the expense of fairly heavy electrolyte losses which are obligatory and cannot be reversed even if the animal does stop to drink water. It seems surprising that the horse, which has a normal diet very low in sodium compared to that of man, is prepared to allow such a high level of sodium loss.

The probable reasons for the adoption of this approach are concerned with the differences between man and the horse with regard to urinary electrolyte excretion, which were discussed in Section 1. This study was an extension of that of Tasker (1967a), showing that the main urinary cation in the horse is potassium with urinary sodium excretion even at rest varying from low to non-existent. Tasker (1967a) considered that this was an effect of the diet of the horses, which was low in sodium and high in potassium. However, it can be seen from the figures in his paper that only about 2% of the daily sodium intake actually appeared in the urine, and similarly in this present study urinary sodium excretion was only about 180 mmol/day while about 540 mmol/day was being ingested in the concentrate feed alone. It seems clear that in the horse the main route of sodium excretion is elsewhere than in the urine. Michell (1985) has made a convincing case for the role of the gut as the main regulator of sodium balance in herbivores, particularly ruminants, and there is strong evidence that the situation

in the horse is similar. This means that while it may indeed be possible to increase renal sodium excretion if the plasma becomes hypertonic the opposite adjustment is not possible. It therefore appears that reliance on the kidney to maintain an isotonic extracellular fluid in circumstances where swings in concentration in either direction are liable to occur is not an option which is available to the horse.

On the other hand, if one considers the gastro-intestinal tract, particularly the large intestine, as being the main organ of sodium homeostasis in the horse, it is clear that this species may possess a biological "reservoir" of sodium which is not available to man. The combined capacity of the caecum and colon of the horse is about 80 - 90 litres (Sisson and Grossman, 1953). Lindner et al (1983) have reported that the sodium concentration in the semi-digested contents of these organs, in ponies fed an "adequate" sodium diet, was around 120 mmol/l. This implies a total sodium content of about 10 moles, which appears to be available to the animal if required, as the sodium concentration of the large intestinal contents in ponies rendered severely sodium deficient was almost zero. It seems therefore that this hypothetical "reservoir" may well be capable of dealing with the 4 - 5 mole sodium deficits seen in Section 5 of this study.

The strategy of the horse in dealing with the problem of fluid/electrolyte balance raised by the use of evaporative cooling is therefore, according to this hypothesis, to maintain the tonicity of its extracellular fluid at the expense of heavy electrolyte losses, then to use the large capacity of its intestinal tract and the sodium contained in it to allow controlled replacement of these fluid and electrolyte losses after exercise. This would explain why the horses were able to drink copiously after the endurance rides in Section 5 without showing any signs of haemodilution.

A problem would appear to arise, however, when repeated days of heavy sweating persistently give rise to sodium losses which are greater than daily sodium intake. It has been shown in man that individuals who maintain high sweat sodium concentrations over a period of heavy sweating (due to lack of response of their sweat glands to aldosterone) are unable to acclimatise to heat but continue to suffer symptoms of heat exhaustion (Streeten et al, 1960). It is therefore suggested that the responsiveness of the sweat glands to the sodium-conserving actions of aldosterone is a critical factor in man's ability to acclimatise to heat. The lowest sweat sodium concentrations which have ever been reported in horses (132 mmol/l, Carlson and Ocen, 1979) are still double those seen in non-acclimatising human subjects (about 65 mmol/l, Streeten et al, 1960), and considering the low sodium content of the normal diet of the horse it is difficult to see how these high sweat losses can be sustained for any length of time. It was in fact evident that some of the endurance horses studied in Section 2 had very low (90 mmol/l or less) resting (pre-ride) plasma chloride concentrations, although their plasma sodium concentrations were almost always normal. It may be that this was a consequence of repeated heavy sweat losses during training and competition, but there was no evidence that these horses' performances were adversely affected.

Under natural conditions some of the electrolytes lost in the sweat may actually be recovered later in the course of grooming and coat licking, and this behaviour has been observed to be markedly increased in sodium-depleted ponies (Lindner et al, 1983). However, endurance horses are usually washed down after a competition to remove dried sweat from the coat which eliminates this opportunity for electrolyte recovery. Under these circumstances the provision of salt licks in the stable would appear to be particularly important.

It may be that this problem of cumulative sodium losses is a factor in the development of anhydrosis in horses exported to tropical climates. These horses are usually reported to sweat particularly profusely when first exported, followed by a gradual diminution of the sweating response, until they become completely dry and are unable to maintain normal thermoregulation in spite of very marked panting (Evans et al, 1957b). The effect of a reduction in work and/or removal to a temperate environment in reversing this process is well established (Gilyard, 1944; Arnold, 1950; Marsh, 1961) and several authors recommend treatment with sodium chloride (Gilyard, 1944; Arnold, 1950; Stewart, 1961; Correa and Calderin, 1966). However, the original report by Gilyard (1944) of an extremely low blood (plasma?) chloride concentration in one case of the disease (equivalent to 44 mmol/l chloride, normal values quoted as 72-82 mmol/l) has not been pursued except by Correa and Calderin (1966), who merely state that sodium and chloride concentrations are "below normal" in 90% of cases without giving any figures. It appears that a sodium deficiency may be associated with a reduced ability to sweat, as Lindner et al (1983) have reported a 55% reduction in sweat output in ponies rendered hyponatraemic by an extremely low sodium diet. It would appear that further investigation of this subject may be warranted, in particular to establish why only a proportion of susceptible animals (15%, Gilyard, 1944) develop the condition, and whether there is any association with adrenocortical insufficiency as originally suggested by Wallace (1938).

The question as to whether the horse or man has developed the more effective means of electrolyte homeostasis during sweating is almost certainly a meaningless one. Comparison of the incidence of clinical fluid/electrolyte problems at long distance athletic events is completely unhelpful, because of the enormous differences in athlete motivation between the two species and the much stricter medical

supervision of the equine events. Each species has probably developed the methods most appropriate to its basic physiology and exercising requirements. However, if the hypotheses advanced above are valid it would appear that the horse may have the advantage in medium-term sustained exercise of several hours' duration while man may be more capable of continuing this type of exercise for a number of days in succession.

Hormonal control of sweat composition

The effect of aldosterone in reducing the sodium and chloride concentration of human sweat has been discussed above. This effect is in line with the general strategy of human evaporative thermoregulation, which appears to be to reduce electrolyte losses as much as possible. The possible disadvantages to the horse of allowing electrolyte losses to continue at a high level during prolonged sweating have also been discussed. However, it would be inconsistent to expect aldosterone to reduce equine sweat electrolyte concentrations to the extent seen in man, if the proposition that the horse gives priority to the maintenance of the tonicity of the extracellular fluid during sweating is in fact valid.

There are a number of findings which support this point of view. Firstly, Carlson and Ocen (1979) report sweat sodium and chloride concentrations of 132 and 174 mmol/l respectively in trained endurance horses in California. It might be expected that these animals would be fully heat-acclimatised, yet although the sweat sodium concentration is lower than any recorded in this present study it does not approach that of even the unacclimatised human subject. Secondly, Lindner et al (1983) have reported sweat electrolyte concentrations in ponies subjected to extreme sodium deprivation compared to those fed a normal diet. Although the low-sodium group became significantly hyponatraemic

their sweat sodium concentrations were only about 20 mmol/l below those of the control group (130 mmol/l compared to 150 mmol/l). Thirdly, although the training of the horses for Section 5 of this present study took place during an unusually cool Scottish summer, it might have been expected that some "acclimatisation" effect would have been seen as a result of the repeated days of moderately heavy sweating involved. However, sweat sodium and chloride concentrations were again only about 20 mmol/l below those recorded in the untrained horses (in response to different stimuli), and were still hypertonic compared to plasma. In addition, preliminary experiments indicate that the administration of exogenous aldosterone to horses at a dose rate of 0.1 µg/kg/day for five days has no effect at all on the electrolyte concentration of either adrenaline or exercise induced sweat (Kerr, unpublished data). It therefore appears most likely that, in contrast to man, the horse does not reduce its sweat electrolyte concentration to any great extent in response to a negative sodium balance.

There is, however, some suggestion arising from this present study that equine sweat electrolyte concentration can be hormonally modified, not by aldosterone but by adrenaline itself. During heat exposure, when there is no increase in adrenomedullary adrenaline secretion (Robertshaw and Taylor, 1969), sweat sodium concentration was only around 150 mmol/l. In contrast, during intravenous adrenaline infusion sweat sodium concentration rose to over 180 mmol/l. It was therefore suggested (Section 4) that there might be an adrenoceptor present somewhere on the duct of the sweat gland which is activated by humoral adrenaline while being out of range of neurotransmitter released by the sympathetic nerve ending, and that this adrenoceptor governs the sodium:potassium ratio of the sweat. This suggestion is highly speculative, as no other evidence for the existence of such an adrenoceptor has been shown. However, the catecholamines have been

shown to increase the influx of potassium into a number of different types of cells such as rat muscle (Clausen and Flatman, 1980), human erythrocytes (Bodemann et al, 1982) and avian erythrocytes (Schmidt and McManus, 1977), and this effect is generally considered to be mediated via a β_2 adrenoceptor linked to Na^+/K^+ ATPase (Clausen and Flatman, 1980; Struthers et al, 1983). It is therefore not inconceivable that a similar adrenoceptor might be present in the equine sweat gland, perhaps mediating potassium uptake from the secretion during its passage down the sweat duct. It is possible, also, that such an effect might have a useful physiological function.

During heat exposure the proportion of total body water loss which was accounted for by respiratory evaporation was only about 10%. Under these circumstances a sweat sodium concentration which was only slightly greater than plasma sodium concentration would be sufficient to balance the respiratory water loss and maintain plasma sodium concentration constant. This in fact appeared to be the case (Section 3). However, during endurance exercise the proportion of water loss attributable to respiratory evaporation increased to over 20%. In this situation a higher sweat sodium concentration would obviously be necessary to balance the respiratory water loss - indeed, the balance during exercise was not exact and plasma sodium concentration did increase slightly in spite of a sweat sodium concentration about 15 mmol/l greater than that of heat-induced sweat. This may have been connected to the need to prevent plasma chloride concentration from decreasing by too great an amount, as discussed in Section 5. If the proportions of total water loss which were found to be due to respiratory evaporation during heat exposure and exercise in this study are typical, it appears that it would be to the horse's advantage to secrete sweat with a higher sodium concentration during exercise.

During heat exposure, there is no increase in adrenomedullary adrenaline secretion in the horse (Robertshaw and Taylor, 1969), while during exercise plasma adrenaline concentration increases considerably (Evans et al, 1956) and may contribute significantly towards the stimulation of sweat secretion. It may be that the horse makes use of this difference in plasma adrenaline concentration between the two situations to ensure that sweat sodium concentration is higher during exercise than when at rest. Re-examination of the data presented in Section 4 tends to support the suggestion that sweat sodium:potassium ratio may be directly related to the dose rate of adrenaline being administered to the animal, although it seems that the response was not immediate and that the ratio took some time to alter after the dose rate of adrenaline was changed.

However, two additional possibilities must be considered. The apparent association of the changing sweat sodium:potassium ratio with sweating rate during adrenaline infusion was discussed in Section 4, and as it is difficult to separate an effect of sweating rate from an effect of the dose rate of adrenaline this connection must still remain under consideration. In addition, it has been suggested that sweat sodium: potassium ratio may be affected by the dietary sodium:potassium ratio (Meyer et al, 1978). It therefore appears that the entire subject of the control of sweat electrolyte concentrations in the horse merits further investigation.

Conclusions

It appears that the strategy adopted by the horse in dealing with the problems of fluid and electrolyte homeostasis which arise due to the use of evaporative means of thermoregulation is very different from that adopted by man. The maintenance of sweat sodium and chloride concentrations at a level which, when added to the necessary respiratory water loss, will result in the total thermoregulatory fluid being almost isotonic with the extracellular fluid, may give the horse an advantage in medium-term sustained exercise, as ECF electrolyte concentrations can be kept fairly constant without the horse being obliged to stop to drink. It also appears that the horse has evolved a mechanism to prevent dangerous haemodilution occurring when the fluid deficit is finally replaced by drinking water, probably involving sodium "stored" in the large intestine. However, the electrolyte losses involved in this strategy seem to be very high in comparison to the normal dietary intake, and this may be disadvantageous when heavy sweating occurs repeatedly for a number of days or weeks.

Although this study has clarified a considerable number of points in connection with fluid/electrolyte homeostasis during endurance exercise in the horse, many questions still remain to be answered. In particular, the effect on the horse of repeated days of heavy sweating and the factors involved in the control of sweat electrolyte concentrations are subjects whose further investigation would seem likely to be particularly rewarding.

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APPENDICES

For reasons of space the units have been omitted from the column headings in the appendices and the parameter names have been abbreviated. The abbreviations and units are as follows:

Time	(24 hour experiment)	Time of day, British Summer Time
	(all other experiments)	Time from start of experiment, minutes.
Bwt	Body weight, kg	
HR	Heart rate, beats/min	
RR	Respiratory rate, respirations/min	
RT	Rectal temperature, °C	
PCV	Packed cell volume	
Prot	Total protein, g/l	
Alb	Albumin, g/l	
Na^+	Sodium, mmol/l	
K^+	Potassium, mmol/l	
Cl^-	Chloride, mmol/l	
Ca^{2+}	Calcium, mmol/l	
Mg^{2+}	Magnesium, mmol/l	
PO_4^{2-}	Inorganic phosphate, mmol/l	
Creat	Creatinine, µmol/l (plasma) mmol/l (urine)	
Urea	Urea, mmol/l	
Ur.A	Uric acid, µmol/l	
Gluc	Glucose, mmol/l	
Cort	Cortisol, nmol/l	
Ins	Insulin, mU/l	
Glyc	Free glycerol, µmol/l	
Trig	Triglycerides, µmol/l	
FFA	Free fatty acids, µmol/l	
B/rub	Bilirubin, µmol/l	
ALP	Alkaline phosphatase, iu/l	
AST	Aspartate aminotransferase, iu/l	
CK	Creatine kinase, iu/l	
Vol	Volume (urine), ml	
SG	Specific gravity	
pH	pH	

In appendices II and V the abbreviations denoting sampling time or distance travelled are the same as those used in Sections 2 and 5.

APPENDIX I

Individual Data from Section 1

24-hour experiment

ADAM

First occasion - urine samples only

Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
11.50	630	1.044	7.5	634	31.21	105	158	118
13.00	680	1.030	7.6	362	14.02	82	209	192
14.40	830	1.030	7.6	332	12.36	85	264	220
18.30	2210	1.038	7.2	365	12.33	27	268	292
21.00	1150	1.040	7.2	328	11.35	3.0	254	324
9.00	2010	1.043	7.9	516	26.61	0.5	328	150

Second occasion - blood samples

Time	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	B/rub	ALP	AST
9.00	0.39	71	138	3.5	97	3.46	0.70	0.91	159	4.9	4.7	32	148	487
12.00	0.36	71	130	4.2	103	3.11	0.69	0.78	159	5.1	5.8	34	145	516
15.00	0.34	72	132	3.7	98	2.66	0.64	0.85	132	5.1	5.7	33	142	479
18.00	0.36	74	132	3.1	101	2.79	0.71	0.95	123	4.8	4.5	34	144	516
21.00	0.39	75	134	3.8	94	2.15	0.67	1.10	123	4.7	4.3	36	152	516
24.00	0.40	73	139	3.2	94	2.81	0.64	0.99	132	4.8	4.7	33	151	516
3.00	0.40	73	138	3.5	96	2.52	0.64	1.16	159	5.1	4.4	35	153	516
6.00	0.38	74	131	3.4	96	2.75	0.66	1.19	185	5.4	4.5	38	152	457
9.00	0.35	71	132	3.1	95	2.58	0.64	1.12	176	5.5	4.7	40	141	501

Second occasion - urine samples

Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
10.30	590	1.045	7.7	345	35.85	0.2	474	288
12.30	340	1.045	7.2	413	33.63	0.3	364	366
14.00	530	1.040	7.2	361	22.28	0.2	386	380
16.30	690	1.038	7.4	339	20.22	0.2	450	354
18.00	460	1.040	8.0	329	23.43	0.5	553	266
22.30	840	1.043	7.9	352	29.27	0.7	551	276
4.30	630	1.047	7.8	445	42.65	0.3	484	216
9.00	650	1.045	7.8	466	40.23	0.8	453	260

Third occasion - blood samples

Time	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	B/rub	ALP	AST
9.00	0.34	67	131	3.3	99	2.82	0.69	1.05	167	4.6	4.9	37	146	457
12.00	0.34	64	132	3.6	98	2.34	0.76	0.77	221	4.8	6.7	37	156	457
15.00	0.38	67	138	3.5	97	2.50	0.77	0.81	194	4.7	5.7	37	154	523
18.00	0.42	74	132	3.1	94	2.66	0.80	0.91	194	4.7	4.1	44	178	560
21.00	0.45	71	139	3.2	99	2.52	0.81	0.81	212	4.7	4.0	38	169	516
24.00	0.43	69	140	4.1	97	3.15	0.78	0.92	194	4.6	4.5	36	170	538
3.00	0.42	72	140	3.9	96	2.96	0.76	1.08	203	4.8	4.5	39	169	560
6.00	0.40	66	140	3.8	98	2.87	0.78	1.04	229	5.0	4.5	37	163	605
9.00	0.43	69	140	3.7	98	2.67	0.76	1.05	238	5.1	4.5	38	166	575

Third occasion - urine samples

Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
10.00	530	1.041	7.4	340	31.73	79	429	304
14.00	840	1.038	7.3	277	22.91	71	532	328
15.00	790	1.033	7.1	285	15.42	100	286	330
18.00	620	1.035	7.5	287	17.65	113	351	326
19.00	410	1.037	7.3	292	19.02	39	447	358
22.00	1090	1.037	7.1	274	16.88	5.9	451	380
4.00	1430	1.041	7.1	332	24.55	0.5	451	332
8.30	730	1.046	7.3	402	32.06	0.0	417	372

ASTRYL

First occasion - urine samples only

Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
11.45	750	1.034	7.2	399	17.74	26	214	228
14.40	1250	1.038	7.1	429	15.48	6	293	342
18.30	830	1.040	7.0	495	17.00	0.9	251	350
20.00	610	1.044	7.1	490	15.46	0.6	274	318
20.50	450	1.040	7.2	446	14.90	1.4	296	252
22.50	540	1.037	7.7	396	14.43	1.0	374	144
9.00	1190	1.043	7.3	499	19.42	1.3	316	250

Second occasion - blood samples

Time	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	B/rub	ALP	AST
9.00	0.30	63	132	4.2	95	3.05	0.71	0.82	88	5.6	4.8	19	138	306
12.00	0.29	60	134	3.3	96	3.56	0.86	0.93	115	5.9	6.0	21	141	280
15.00	0.30	62	135	4.9	96	2.60	0.67	0.83	106	5.1	5.0	29	101	324
18.00	0.37	75	135	4.0	101	3.13	0.78	0.88	124	5.2	4.4	30	125	365
21.00	0.33	67	131	4.7	98	3.44	0.75	1.00	115	4.9	4.9	26	119	328
24.00	0.34	67	134	4.2	95	3.61	0.74	1.04	124	5.1	4.7	30	131	321
3.00	0.30	65	134	3.2	99	3.33	0.67	1.01	124	5.4	4.0	25	124	332
6.00	0.32	67	135	3.8	96	3.50	0.65	1.05	124	5.5	4.6	26	125	339
9.00	0.31	65	139	4.2	97	2.90	0.63	1.02	115	5.7	4.6	26	125	343

24-hour experiment, Astryl, cont'd.

Second occasion - urine samples

Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
10.30	990	1.043	8.2	91	5.48	43	76	57
12.30	660	1.032	7.1	228	17.70	26	265	258
14.00	400	1.031	7.4	250	14.72	12	350	278
15.00	390	1.031	7.4	256	14.53	8.1	354	294
16.00	440	1.031	7.5	261	14.12	16	279	276
18.30	820	1.035	7.6	263	17.89	15	412	242
22.30	1180	1.035	7.6	240	17.78	5.9	459	296
6.00	950	1.043	7.4	318	35.70	0.4	424	216

Third occasion - blood samples

Time	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	B/rub	ALP	AST
9.00	0.30	63	137	3.7	98	3.39	0.73	0.96	106	4.6	4.5	18	137	332
12.00	0.30	64	133	4.0	99	2.12	0.70	0.81	115	4.7	5.0	18	131	357
15.00	0.32	63	132	4.0	98	2.40	0.72	0.68	124	4.7	5.2	18	131	332
18.00	0.37	71	132	4.5	100	3.05	0.78	0.84	115	4.7	4.2	20	152	361
21.00	0.35	66	133	4.7	98	3.86	0.71	0.85	124	4.6	4.7	18	150	350
24.00	0.35	68	130	3.9	98	3.11	0.76	0.82	115	4.7	4.3	20.	150	346
3.00	0.32	65	133	4.3	101	3.35	0.75	0.88	106	5.0	4.8	19 ·	143	335
6.00	0.30	63	129	4.1	99	3.90	0.72	0.90	106	5.2	5.0	17	139	339
9.00	0.33	63	134	4.0	100	3.66	0.78	0.89	115	5.1	4.3	16	139	332

Third occasion - urine samples

Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
10.00	1420	1.010	7.3	64	3.61	31	58	57
13.00	1040	1.030	7.2	169	14.49	43	262	266
15.00	890	1.032	7.0	246	14.06	36	268	334
18.00	1020	1.034	7.1	241	15.17	39	331	312
20.00	950	1.032	7.3	200	11.68	27	412	290
24.00	990	1.036	7.1	220	17.26	5.4	370	304
7.30	1730	1.046	7.1	300	21.39	0.5	323	364

BEN (Blood samples only)

Second occasion

Time	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	B/rub	ALP	AST
9.00	0.26	72	138	3.4	94	2.96	0.62	0.98	168	6.0	4.9	31	159	191
12.00	0.27	73	147	4.1	96	2.74	0.66	0.83	150	6.2	5.5	32	157	195
15.00	0.24	74	142	4.2	93	2.27	0.68	0.78	141	6.4	5.5	33	159	180
18.00	0.34	83	138	4.0	106	3.00	0.69	0.82	141	6.4	4.6	40	180	195
21.00	0.33	80	136	4.4	100	2.98	0.68	0.89	141	6.1	4.5	39	172	199
24.00	0.31	82	141	4.0	93	2.63	0.61	0.99	150	6.3	4.7	37	174	195
3.00	0.30	81	145	4.4	91	3.10	0.62	1.01	168	6.5	4.6	40	177	191
6.00	0.28	77	140	4.1	91	2.84	0.59	1.04	150	6.7	4.5	39	175	191
9.00	0.29	78	146	4.4	94	2.51	0.59	1.01	150	6.8	4.6	43	174	199

Third occasion

Time	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	B/rub	ALP	AST
9.00	0.30	78	131	3.8	92	2.55	0.61	0.98	141	4.9	4.6	42	177	195
12.00	0.30	80	130	3.9	97	2.96	0.67	0.77	150	5.0	6.1	39	166	206
15.00	0.29	72	137	3.5	99	3.00	0.78	0.77	150	5.2	5.2	37	166	184
18.00	0.36	88	147	3.8	96	3.63	0.77	0.75	150	5.1	4.1	43	187	221
21.00	0.37	85	142	4.5	100	2.80	0.76	0.71	150	4.9	4.6	38	178	206
24.00	0.33	77	135	4.1	98	3.20	0.76	0.83	141	4.7	4.8	33	185	199
3.00	0.33	83	149	4.2	95	2.92	0.75	0.90	141	4.7	4.7	34	194	191
6.00	0.31	84	140	4.0	94	2.69	0.65	0.83	133	4.8	4.6	37	192	180
9.00	0.29	77	145	4.0	91	2.88	0.73	0.96	168	4.9	4.6	36	180	195

DIRK

First occasion - urine samples only

Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
10.50	710	1.024	8.5	146	6.19	145	96	95
13.00	360	1.040	6.9	562	23.05	9.1	122	240
14.40	950	1.037	7.1	507	18.69	4.2	313	307
16.10	420	1.035	7.0	511	14.43	2.3	233	285
18.30	660	1.039	7.1	434	15.53	1.8	286	307
20.30	850	1.037	7.0	376	13.16	0.9	276	328
22.30	680	1.037	7.1	358	13.16	1.0	270	288
9.00	2920	1.039	7.5	495	20.04	0.2	257	128

Second occasion - blood samples

Time	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	B/rub	ALP	AST
9.00	0.38	61	138	3.9	94	2.62	0.64	1.05	159	4.9	4.8	30	67	273
12.00	0.37	63	134	4.2	98	2.33	0.71	0.91	133	5.2	5.4	24	78	335
15.00	0.38	62	138	4.1	98	2.21	0.71	0.92	167	5.2	5.2	35	95	332
18.00	0.46	69	141	3.2	100	2.97	0.73	1.04	150	5.0	3.8	38	113	369
21.00	0.41	68	138	4.0	99	2.85	0.71	1.13	150	4.6	3.8	37	126	365
24.00	0.43	68	133	4.0	97	2.86	0.68	1.15	159	4.5	4.1	38	118	372
3.00	0.38	65	136	3.7	96	2.83	0.66	1.21	150	4.6	4.2	37	114	332
6.00	0.41	66	138	3.9	98	3.04	0.64	1.28	159	4.8	4.4	39	117	350
9.00	0.42	65	136	3.9	94	3.01	0.67	1.31	176	4.9	4.5	40	121	361

24-hour experiment, Dirk, cont'd.

Second occasion - urine samples

Time	Vol	SG	Urea	Creat	Na^+	K^+	Cl^-
12.00	500	1.040	320	33.99	9.2	392	164
13.30	450	1.037	330	23.02	2.8	355	288
15.00	440	1.036	323	19.32	2.5	398	298
16.00	380	1.035	312	17.35	3.6	355	306
17.30	600	1.036	297	17.71	19	409	282
18.30	400	1.036	300	19.05	59	429	294
21.00	630	1.039	306	24.08	7.7	500	296
24.00	340	1.038	290	24.62	0.8	495	304
3.00	670	1.043	344	35.88	0.1	484	270
7.30	410	1.047	415	41.75	0.0	505	168

Third occasion - blood samples

Time	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	B/rub	ALP	AST
9.00	0.41	63	135	4.0	96	2.90	0.67	1.24	167	4.6	4.4	32	124	324
12.00	0.38	61	135	4.0	100	2.53	0.78	1.01	176	4.9	5.0	28	117	265
15.00	0.40	63	132	4.2	102	2.93	0.81	1.05	176	5.1	5.1	31	123	317
18.00	0.45	67	141	3.8	102	2.85	0.79	1.08	185	4.9	3.7	33	132	383
21.00	0.46	66	136	4.3	101	2.53	0.79	1.04	185	4.7	4.1	31	142	339
24.00	0.42	66	145	3.9	100	2.79	0.89	1.14	203	4.5	4.0	29	132	335
3.00	0.44	66	138	4.5	98	3.40	0.83	1.12	203	4.3	4.1	29	129	339
6.00	0.44	64	138	4.3	98	2.95	0.85	1.23	212	4.4	4.3	28	124	357
9.00	0.40	65	138	4.0	96	3.03	0.85	1.20	212	4.5	4.4	26	121	324

Third occasion - urine samples

Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
13.00	1320	1.038	7.3	314	23.46	8.2	368	318
15.00	790	1.036	7.2	302	18.43	5.2	325	366
18.00	1020	1.036	7.1	290	17.16	5.5	330	410
20.00	870	1.036	7.1	267	15.78	1.6	389	406
21.00	490	1.034	7.1	225	13.91	2.2	337	376
24.00	1000	1.038	6.9	268	18.80	0.2	359	396
3.00	840	1.040	6.9	279	19.33	0.2	354	380
7.30	870	1.041	7.2	303	25.19	0.6	431	310

JOHNNY WALKER

First occasion - urine samples only

Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
10.50	740	1.032	8.4	352	18.04	311	109	108
11.30	300	1.032	7.9	445	17.34	228	137	116
13.00	800	1.027	7.7	324	11.91	176	154	194
14.40	450	1.030	7.5	344	12.72	91	216	230
16.10	350	1.035	7.3	395	13.06	37	242	286
18.30	870	1.040	7.4	466	17.51	6.2	295	264
20.00	580	1.040	7.2	480	16.73	2.4	282	302
22.30	700	1.041	7.2	480	16.88	1.1	287	288
9.00	2400	1.040	7.4	497	19.38	0.8	308	188

Second occasion - blood samples

Time	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	B/rub	ALP	AST
9.00	0.46	69	145	4.2	90	3.14	0.70	1.18	167	6.6	4.4	61	157	228
12.00	0.43	64	130	3.2	93	2.10	0.77	1.03	124	7.6	7.4	63	137	162
15.00	0.42	63	139	4.1	99	3.54	0.67	0.83	124	6.1	5.2	56	137	206
18.00	0.50	72	142	3.5	99	2.50	0.76	0.90	124	6.5	3.9	61	149	184
21.00	0.50	70	140	4.4	95	3.35	0.75	1.01	150	5.4	4.6	64	167	177
24.00	0.47	66	140	4.4	96	2.86	0.70	1.13	159	5.6	4.3	56	161	225
3.00	0.51	62	136	4.2	97	3.05	0.64	1.21	141	6.0	4.3	56	157	225
6.00	0.44	64	136	4.0	96	3.00	0.67	1.19	141	7.1	4.4	61	155	228
9.00	0.45	63	132	3.8	95	3.03	0.64	1.21	167	6.5	4.4	67	156	206

Second occasion - urine samples

Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
10.30	350	1.043	8.2	380	36.67	5.4	470	178
12.00	230	1.040	7.7	426	30.75	5.5	365	190
15.00	500	1.040	7.4	407	26.99	4.0	416	268
17.00	550	1.039	7.3	369	21.95	4.3	432	328
20.00	600	1.039	7.7	341	21.27	6.4	453	270
21.00	390	1.039	7.7	333	25.94	3.0	488	290
24.00	510	1.040	7.6	313	30.45	1.0	462	284
6.00	930	1.045	7.7	425	41.31	0.4	432	238

Third occasion - blood samples

Time	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	B/rub	ALP	AST
9.00	0.42	61	139	3.9	95	3.37	0.66	1.08	150	5.5	4.3	51	156	214
12.00	0.41	60	133	3.9	97	2.26	0.82	0.84	150	5.9	5.5	49	161	180
15.00	0.45	63	133	4.0	95	2.51	0.86	0.99	150	5.8	4.7	41	138	206
18.00	0.51	67	141	3.4	100	2.66	0.79	0.85	115	5.2	3.5	49	165	232
21.00	0.50	66	141	3.4	101	3.17	0.80	0.71	159	5.5	3.9	42	170	232
24.00	0.50	66	140	3.9	99	2.74	0.82	0.91	133	5.0	4.5	45	171	228
3.00	0.47	65	141	4.2	96	3.03	0.83	1.01	124	5.2	4.2	43	178	236
6.00	0.50	64	139	4.0	96	3.05	0.81	1.10	124	4.9	4.4	43	177	228
9.00	0.50	65	139	4.1	96	3.30	0.83	1.04	124	5.6	4.2	44	173	236

24-hour experiment, Johnny Walker, cont'd.

Third occasion - urine samples

Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
12.00	650	1.038	7.4	297	25.15	110	346	272
13.00	360	1.035	7.0	294	18.46	78	288	382
15.00	910	1.035	7.0	279	16.56	93	317	352
18.00	830	1.035	7.1	275	17.60	125	320	344
20.00	590	1.035	7.3	247	16.11	67	390	344
21.00	580	1.035	7.0	234	15.13	25	447	380
24.00	1090	1.035	7.0	240	15.53	7.6	440	388
3.00	920	1.038	7.1	242	18.77	1.0	424	368
6.00	590	1.038	7.0	188	22.23	0.8	453	358
8.30	630	1.040	7.0	216	24.41	0.7	431	366

SMOKEY (Urine samples only)

First occasion

Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
11.30	930	1.030	8.5	345	18.61	231	91	57
13.00	730	1.031	7.7	372	16.24	98	201	136
14.20	580	1.033	7.8	347	15.31	61	274	144
15.50	850	1.031	7.3	328	12.23	47	230	198
18.30	1000	1.035	7.4	359	13.87	24	259	244
19.50	930	1.036	7.4	338	12.64	12	275	270
20.50	820	1.035	7.3	332	12.47	6.7	316	266
9.00	3540	1.035	7.6	349	15.70	6.1	227	168

Second occasion

Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
12.30	760	1.043	7.8	340	36.67	25	475	228
14.00	390	1.040	7.1	335	28.98	6.2	402	360
15,30	400	1.039	7.2	332	22.86	9.7	429	374
18.00	820	1.035	7.4	274	16.34	18	446	354
23.30	1040	1.038	8.2	281	24.70	60	496	240
3.00	590	1.040	8.3	309	31.63	27	529	184
9.00	1020	1.043	8.2	331	37.32	7.3	557	206

Third occasion

Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
13.00	830	1.034	7.5	191	21.76	123	353	340
14.00	530	1.035	7.0	203	19.49	78	366	396
15.00	570	1.032	7.2	203	15.81	66	353	360
18.00	860	1.035	7.1	195	17.81	33	394	384
20.00	690	1.037	7.0	227	18.75	15	411	390
24.00	920	1.034	7.1	198	17.24	13	448	386
1.30	750	1.037	6.9	230	21.62	5.1	393	406
6.00	700	1.038	7.0	243	23.63	5.9	415	418
7.30	510	1.038	7.1	267	25.99	12	428	372

Hay-feeding experiment

Time	PCV	Prot	Alb	Na^+	K^+	Cl^-	B/rub	ALP	AST
ADAM									
Test day									
0	0.30	69	36	140	3.6	98	29	568	1290
60	0.36	77	40	142	3.3	99	32	706	1582
120	0.37	77	40	140	3.4	98	33	-	-
180	0.37	74	39	138	3.7	98	40	-	-
Control day									
0	0.32	67	36	138	3.9	99	26	587	1393
60	0.32	67	37	141	4.0	100	27	541	1307
120	0.37	70	38	141	4.3	101	30	-	-
180	0.38	68	37	141	4.3	101	31	-	-
BEN									
Test day									
0	0.32	82	31	135	4.3	97	31	660	173
60	0.40	94	36	139	3.7	98	35	792	232
120	0.38	91	35	139	4.1	97	35	-	-
180	0.35	84	32	136	4.0	98	29	-	-
Control day									
0	0.35	79	32	136	4.0	98	28	680	166
60	0.34	80	32	135	4.2	97	27	693	184
120	0.32	79	31	136	4.3	97	27	-	-
180	0.32	79	30	135	3.6	97	27	-	-

Hay feeding experiment, cont'd.

Time	PCV	Prot	Alb	Na^+	K^+	Cl^-	B/rub	ALP	AST
DIRK									
Test day									
0	0.39	74	33	138	3.9	98	21	548	173
60	0.42	83	35	141	3.7	101	22	634	258
120	0.42	81	35	143	3.5	101	22	-	-
180	0.44	80	34	139	4.0	98	21	-	-
Control day									
0	0.37	75	33	137	4.0	100	24	561	184
60	0.37	75	32	137	3.8	99	23	548	196
120	0.37	74	32	137	3.7	101	24	-	-
180	0.34	74	32	137	4.5	100	24	-	-
JOHNNY WALKER									
Test day									
0	0.35	73	33	137	3.6	96	36	726	184
60	0.42	85	38	142	3.2	98	42	884	210
120	0.41	82	37	141	3.2	99	41	-	-
180	0.39	75	33	137	4.4	98	33	-	-
Control day									
0	0.34	73	32	138	4.1	98	32	693	166
60	0.35	73	32	139	3.9	98	32	700	207
120	0.34	74	33	138	3.8	99	34	-	-
180	0.32	73	32	140	3.1	98	38	-	-
SMOKEY									
Test day									
0	0.35	77	33	137	3.8	95	17	1135	225
60	0.40	88	36	141	3.0	96	20	1228	295
120	0.39	82	35	138	3.3	96	18	-	-
180	0.38	81	35	136	4.0	95	18	-	-
Control day									
0	0.34	76	32	137	3.7	95	28	858	292
60	0.36	76	32	137	3.8	95	30	944	236
120	0.33	76	32	137	3.8	95	32	-	-
180	0.36	80	34	137	3.7	96	37	-	-
BEWITCHED									
Test day									
0	0.33	72	36	140	3.8	101	22	469	435
60	0.36	80	41	139	2.8	101	26	469	472
120	0.36	78	40	139	3.6	99	22	-	-
180	0.36	77	39	138	3.2	101	21	-	-
Control day									
0	0.35	74	37	138	3.6	100	27	488	524
60	0.34	73	37	138	3.8	99	27	475	517
120	0.35	76	39	138	3.6	99	28	-	-
180	0.34	75	38	138	3.6	99	30	-	-
PEARL									
Test day									
0	0.37	75	36	138	3.8	101	21	898	328
60	0.42	84	40	137	3,0	101	26	950	384
120	0.41	83	39	137	2.9	101	23	-	-
180	0.41	80	39	136	3.8	99	22	-	-
Control day									
0	0.34	74	36	138	3.7	101	25	845	295
60	0.34	76	37	138	3.4	101	27	818	292
120	0.35	77	37	136	3.6	101	29	-	-
180	0.36	78	38	136	3.8	101	32	-	-
SUNNY									
Test day									
0	0.33	64	35	139	3.8	102	33	244	295
60	0.39	71	39	138	2.8	102	38	277	328
120	0.39	71	39	138	2.9	101	37	-	-
180	0.38	69	38	136	3.3	103	34	-	-
Control day									
0	0.37	66	37	137	4.1	101	35	251	328
60	0.38	66	37	137	4.1	99	40	251	310
120	0.36	68	38	136	4.2	99	46	-	-
180	0.35	68	38	136	4.0	100	48	-	-

APPENDIX II

Individual Data from Section 2

Breamore 1978 (Ride B)

	PCV	Prot	Na^+	K^+	Cl^-	Ca^{++}	Mg^{++}	PO_4^{--}	Creat	Urea	Gluc	Ins	Cort	Glyc	Trig	B/rub	ALP	AST	CK
BRIG O'DOON																			
S	0.35	71	137	3.9	99	3.00	0.72	1.08	124	5.9	6.4	50.4	124	0	219	14	559	398	141
Jway	0.46	75	136	4.0	98	2.72	0.69	1.32	150	6.9	4.8	3.9	483	81	290	21	629	428	272
F	0.49	72	143	4.8	99	2.78	0.68	1.70	141	8.4	4.2	7.6	551	263	99	29	637	568	439
F+30	0.43	75	150	3.7	98	3.07	0.67	1.62	141	9.5	5.9	1.5	436	74	162	30	455	420	400
NIZETTE																			
S	0.36	65	139	4.2	109	2.96	0.72	0.96	141	6.2	5.0	13.0	396	3	132	22	208	405	219
Jway	0.44	68	140	4.6	112	3.11	0.71	1.15	133	7.1	5.5	4.0	280	25	110	29	212	442	340
F	0.39	69	145	4.5	104	2.71	0.58	1.27	159	8.4	3.1	0.2	459	63	80	42	237	472	467
SHAABAN																			
S	0.39	69	140	4.9	96	2.91	0.60	1.04	141	6.3	5.0	8.9	351	0	244	14	131	1549	233
Jway	0.43	73	139	4.4	104	3.20	0.63	1.02	159	7.9	4.2	9.8	415	0	67	18	143	1672	499
F	0.40	72	141	4.3	89	2.91	0.57	1.57	177	8.4	4.0	3.1	466	179	91	30	165	1771	647
F+30	0.36	72	135	3.5	88	3.07	0.59	1.35	168	8.6	3.2	1.7	381	14	62	31	167	1771	364
NIZZOLAN																			
S	0.42	72	139	4.9	98	3.20	0.72	0.80	133	6.1	5.0	15.6	176	0	295	15	336	328	230
Jway	0.40	73	136	3.6	100	3.36	0.67	1.27	141	8.6	5.1	13.5	409	4	181	27	252	350	219
F	0.46	75	130	3.5	99	3.05	0.52	1.47	150	9.1	1.8	0.1	594	221	91	34	281	442	276
F+30	0.41	71	142	3.9	95	3.00	0.50	1.42	141	9.9	2.7	0.1	472	77	66	37	281	383	279
TARQUIN																			
S	0.37	78	138	5.5	105	2.86	0.74	1.14	150	8.6	-	16.0	224	0	126	10	241	413	792
F	0.40	71	145	4.0	95	2.97	0.70	1.38	194	13.0	3.1	1.6	372	7	86	35	192	405	209
F+30	0.39	73	135	3.2	109	3.02	0.74	1.36	194	12.9	3.9	1.1	375	32	52	32	195	369	230
JASON																			
S	0.35	70	138	4.9	100	2.96	0.62	1.39	-	6.3	5.8	23.4	337	17	126	10	379	568	233
Jway	0.40	72	140	4.9	96	2.86	0.65	1.98	106	7.4	6.3	16.7	537	46	148	21	411	730	598
F	0.41	74	140	4.9	100	2.86	0.69	1.98	106	9.2	2.8	2.8	450	11	200	30	456	830	990
F+30	0.39	74	139	4.1	95	3.00	0.71	1.83	106	9.6	5.1	7.6	369	18	75	34	464	863	990
GAY WILLIAM																			
S	0.43	74	141	4.8	97	3.00	0.67	1.07	115	7.0	5.4	10.8	290	21	223	22	243	380	173
Jway	0.42	77	143	3.9	101	2.96	0.62	1.40	115	7.7	5.2	8.9	422	126	42	35	265	479	435
F	0.45	76	142	4.3	97	2.89	0.62	1.46	133	9.6	5.3	4.1	432	130	106	45	294	361	605
F+30	0.41	76	142	4.6	86	3.32	0.52	1.33	150	9.8	6.9	7.6	299	32	111	49	266	450	630
THE STORK																			
S	0.40	72	137	4.6	84	3.04	0.57	0.97	159	5.2	7.7	23.9	569	0	17	32	246	446	152
F	0.53	72	142	6.4	100	3.18	0.48	1.18	-	7.2	4.6	2.1	848	224	71	57	224	664	842
F+30	0.49	77	147	4.2	84	3.36	0.51	1.47	-	7.7	4.4	2.2	778	116	78	55	314	649	864
ZODIAC																			
S	0.32	82	140	4.5	83	3.29	0.86	1.28	106	7.6	5.5	14.4	347	31	247	20	190	627	258
Jway	0.42	85	148	4.4	81	3.25	0.77	1.38	141	9.4	5.4	7.1	467	175	52	26	216	686	439
F	0.48	89	147	4.2	76	3.25	1.08	2.38	159	11.8	2.8	0.4	943	528	45	43	250	701	736
F+30	0.48	91	147	4.3	75	3.29	1.22	2.41	150	12.3	5.6	4.3	773	231	72	47	265	774	806
MYEBON																			
S	0.37	75	144	4.4	99	2.94	0.68	0.63	141	5.8	9.5	56.2	232	0	152	23	475	479	350
Jway	0.30	76	151	4.6	86	3.36	0.60	0.83	124	6.7	4.1	3.1	316	7	97	31	298	461	821
F	0.40	72	146	4.4	89	3.00	0.51	0.88	124	7.8	3.3	3.7	406	84	152	42	314	464	594

Gloucester 1979 (Rides G and K)

	PCV	Prot	Na^+	K^+	Cl^-	Ca^{++}	Mg^{++}	PO_4^{--}	Creat	Urea	Gluc	Cort	B/rub	ALP	AST	CK
KAZMAHAL																
Jway	0.52	72	134	4.8	97	2.53	0.43	1.14	141	6.3	4.9	353	45	168	516	438
F	0.53	80	136	4.1	97	2.85	0.41	1.86	133	7.7	2.4	422	47	202	405	452
F+30	0.54	80	133	3.9	96	2.93	0.45	1.71	133	8.0	3.9	287	50	216	420	516
CAIRO																
Jway	0.50	73	136	3.7	97	2.55	-	1.35	124	6.2	4.5	408	53	167	664	456
F	0.48	73	134	3.6	97	2.82	0.43	1.35	124	6.2	3.2	655	77	170	664	693
F+30	0.50	77	136	3.4	101	3.01	0.47	1.43	115	7.2	3.4	406	90	181	1107	792
DARREN'S PAL																
S	0.42	64	133	4.1	98	2.58	0.78	0.95	80	7.4	5.8	154	27	379	188	168
Jway	0.53	70	136	3.9	97	2.59	0.65	0.88	159	8.9	5.0	420	44	477	236	325
F	0.50	71	137	3.8	96	2.54	0.62	1.08	150	10.1	3.6	585	58	529	269	686
F+30	0.50	69	136	3.3	96	2.83	0.60	1.22	141	10.3	4.6	388	59	530	269	530
HOLLY																
S	0.35	69	133	4.7	99	2.99	0.67	1.01	62	5.6	5.3	188	26	241	350	131
Jway	0.46	75	133	4.5	94	2.92	0.62	0.83	80	7.0	5.1	401	40	287	302	258
F	0.47	69	133	4.4	94	2.75	0.55	1.15	106	8.1	3.7	463	47	324	303	424
F+30	0.43	69	133	3.5	93	2.57	0.53	1.09	106	8.2	3.7	343	45	261	343	530
SHAABAN																
S	0.38	65	135	3.9	98	2.87	0.73	0.98	106	6.5	5.4	127	28	136	3321	658
Jway	0.48	66	136	4.0	97	2.82	0.53	0.61	141	7.3	4.7	281	44	157	2856	836
F	0.42	65	137	3.7	97	3.12	0.48	1.25	150	8.1	3.7	269	49	168	2922	1011
F+30	0.44	69	136	3.8	98	3.13	0.54	1.11	141	8.2	4.6	261	57	171	3321	1039
TARQUIN																
S	0.37	66	135	4.2	102	2.77	0.69	0.91	97	8.6	5.8	322	23	174	295	255
Jway	0.46	68	134	4.7	99	2.67	0.56	0.73	150	10.0	5.0	357	35	197	295	283
F	0.45	69	137	4.2	99	2.69	0.53	1.49	186	11.7	3.3	538	45	239	302	1075
F+30	0.43	68	134	3.2	98	2.80	0.56	1.21	177	12.1	4.2	421	43	252	265	467
BRIG O'DOON																
S	0.42	67	134	4.2	100	2.79	0.69	1.05	97	7.0	6.4	298	32	338	324	189
Jway	0.45	68	136	3.5	100	2.75	0.60	0.85	124	7.2	5.5	325	39	353	343	287
F	0.47	68	138	4.0	99	2.95	0.58	0.97	133	8.2	4.7	441	-	-	413	283
F+30	0.42	68	137	3.5	97	2.92	0.62	1.00	115	8.7	4.9	285	50	371	302	300
SANDPIPER																
S	0.35	64	135	3.9	102	2.81	0.64	1.28	80	6.7	6.0	244	24	321	250	109
Jway	0.42	66	140	3.3	102	2.67	0.50	0.86	115	7.8	5.2	433	33	255	232	170
F	0.41	67	142	3.6	99	2.79	0.49	1.23	106	8.7	3.2	674	39	415	324	219
F+30	0.42	71	142	2.9	101	2.96	0.50	0.80	159	9.0	4.2	639	41	438	243	262

Gloucester 1979, cont'd.

	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	Cort	B/rub	ALP	AST	CK
UNKNOWN HORSE		(50)														
S	0.44	66	137	3.8	101	3.02	0.62	0.80	150	4.4	6.4	248	33	146	243	113
F	0.55	75	145	5.2	102	3.20	0.62	0.93	159	5.4	5.7	542	51	189	346	255
F+30	0.46	71	138	3.0	99	3.16	0.48	1.23	150	5.5	5.5	316	53	177	291	276

Breamore 1979 (Rides C and H)

	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	Cort	B/rub	ALP	AST	CK
CAIRO																
S	0.38	66	146	4.6	93	3.08	0.64	1.06	141	5.2	4.3	150	30	135	369	124
½way	0.51	87	135	3.8	92	3.41	0.69	2.16	159	5.9	6.2	535	43	186	479	200
F	0.60	83	143	3.8	91	3.28	0.67	1.91	194	7.4	3.0	578	77	242	516	460
F+30	0.50	79	138	3.0	87	3.65	0.59	1.01	194	7.4	4.7	555	71	217	575	445
KAZMAHAL																
S	0.37	66	138	4.0	90	3.28	0.66	0.90	150	6.0	7.4	386	19	171	575	208
½way	0.45	78	139	4.4	89	3.51	0.67	1.74	176	7.0	3.4	551	25	209	738	288
F	0.54	84	140	4.2	94	3.39	0.63	2.23	212	8.2	3.4	659	38	294	738	560
F+30	0.48	81	139	3.6	83	3.44	0.58	2.08	212	8.3	4.5	506	38	263	738	601
TARQUIN																
S	0.36	74	138	4.5	95	3.24	0.75	1.77	168	9.4	5.4	369	14	202	324	360
½way	0.42	77	139	4.4	92	3.23	0.65	1.58	212	11.4	6.9	491	27	238	324	209
F	0.48	79	144	4.1	89	3.40	0.63	1.96	256	13.4	4.4	633	45	243	398	474
F+30	0.40	76	140	2.4	91	3.30	0.65	1.89	265	14.0	6.3	451	46	231	354	431
SHAABAN																
S	0.36	75	129	3.4	88	3.68	0.62	1.58	194	5.4	4.8	306	33	208	1892	2014
½way	0.43	81	141	4.0	94	4.24	0.73	2.29	194	6.8	5.2	548	50	158	2332	3394
F	0.50	80	143	3.8	91	3.51	0.64	2.94	221	7.5	3.3	419	63	194	2446	3712
F+30	0.40	75	148	3.4	94	3.59	0.60	2.75	230	7.7	4.9	397	59	178	2132	3606
BRIG O'DOON																
S	0.36	68	139	3.7	91	3.32	0.73	1.53	124	6.5	6.3	218	18	386	339	170
½way	0.50	76	144	4.0	92	3.13	0.61	1.69	141	7.3	6.1	411	23	444	383	230
F	0.46	76	144	3.8	94	3.18	0.62	1.95	150	8.3	5.3	397	28	456	376	304
F+30	0.45	76	150	4.0	90	3.40	0.63	1.91	150	8.2	-	356	31	475	354	339
DOROCINA KISS-KISS																
S	0.43	69	136	4.4	93	3.29	0.63	1.83	168	5.3	4.9	376	27	173	546	197
F	0.48	73	138	4.8	92	3.26	0.67	2.01	203	6.6	5.7	442	40	201	582	311
F+30	0.50	74	139	4.0	95	3.56	0.76	1.88	212	7.1	4.7	224	38	211	664	293
LEEWOOD ISOLDE																
S	0.37	77	138	4.6	92	3.45	0.76	1.87	168	7.4	6.1	505	18	344	1255	346
F	0.45	85	144	4.4	94	3.58	0.77	2.22	176	9.1	5.9	854	25	431	1462	792
F+30	0.43	87	147	4.2	98	3.70	0.79	2.32	203	9.5	4.9	695	25	428	1682	403
WOOTON JASPER																
S	0.35	82	142	4.6	89	3.45	0.79	2.21	168	6.7	5.0	252	13	236	272	209
½way	0.42	87	140	4.6	93	3.52	0.89	2.06	186	7.5	4.9	343	17	257	236	227
F	0.42	83	142	4.2	91	3.30	0.79	2.16	186	7.8	5.3	156	19	268	265	251
F+30	0.42	83	144	2.7	92	3.47	0.86	2.18	194	8.2	4.8	141	20	264	280	230
LINNET																
S	0.40	78	139	4.2	92	3.54	0.77	2.16	141	6.8	-	380	12	294	472	283
F	0.44	80	142	3.4	93	3.50	0.83	2.32	168	8.6	5.4	259	19	336	575	353
F+30	0.46	83	144	3.7	94	3.68	0.84	2.34	159	8.8	4.9	120	18	362	546	375
PAL-O-MINE																
S	0.39	77	142	4.0	94	3.57	0.82	2.08	150	6.1	5.7	279	29	326	335	212
½way	0.49	83	142	4.2	94	3.59	0.86	2.20	186	6.6	5.8	599	37	375	391	223
F	0.46	80	138	4.2	92	3.69	0.83	2.47	176	7.3	5.1	378	40	365	369	258
F+30	0.41	79	140	3.6	94	4.00	0.79	2.36	186	7.3	5.5	278	37	357	346	251
MINNIE																
S	0.39	90	139	5.4	92	3.40	0.79	2.32	124	7.7	4.8	294	10	532	457	276
F	0.43	91	138	5.0	89	3.38	0.77	2.86	141	9.0	4.7	283	14	550	509	389
F+30	0.41	95	142	3.7	92	3.57	0.81	2.83	159	9.5	5.6	164	13	589	496	389

Red Dragon 1979 (Rides E and J)

	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	Cort	B/rub	ALP	AST	CK
SILVER SABRE																
	0.36	67	136	4.0	90	2.97	0.77	0.91	106	6.2	4.6	343	16	211	287	347
	0.43	73	138	6.4	90	2.74	0.68	1.22	123	7.1	4.7	538	22	236	343	403
	0.41	75	135	4.8	89	2.60	0.73	1.46	123	9.1	2.7	624	36	255	383	990
CAIRO																
S	0.40	70	131	3.8	91	3.06	0.61	0.80	123	5.3	5.0	218	30	101	317	136
½way	0.51	79	136	3.9	90	3.26	0.62	1.26	141	6.2	5.7	478	38	110	361	223
F	0.55	77	136	3.4	87	3.11	0.58	1.67	171	7.5	2.5	878	60	138	391	439
F+30	0.48	77	135	2.5	83	3.14	0.54	1.14	167	7.9	3.2	769	61	138	405	601
TARQUIN																
S	0.41	76	143	4.5	93	3.16	0.74	0.84	132	8.6	6.1	319	22	142	335	195
½way	0.46	77	140	4.1	91	3.00	0.60	0.86	176	10.3	5.5	539	27	148	335	233
F	0.46	84	135	2.8	84	2.60	0.56	1.00	238	13.4	3.6	953	47	188	428	432
F+30	0.43	78	134	3.6	84	2.75	0.59	0.91	238	13.6	4.1	859	46	178	435	531
HARVEY																
S	0.36	78	144	3.9	98	3.18	0.67	0.68	141	7.0	5.9	344	23	156	472	187
½way	0.47	83	145	4.2	90	3.05	0.71	0.76	203	8.8	6.3	720	32	179	523	318
F	0.66	86	144	2.7	87	2.80	0.64	1.08	230	11.9	2.2	989	47	224	590	707
F+30	0.45	84	140	2.5	84	2.88	0.65	1.13	221	12.4	3.1	923	49	218	583	736
LOUCHI																
S	0.32	76	159	4.6	102	3.07	0.73	1.39	106	7.6	5.7	296	15	193	398	347
½way	0.41	75	150	4.8	93	3.00	0.64	1.48	141	8.2	6.9	531	19	167	413	637
F	0.44	73	145	3.3	89	2.61	0.65	1.67	150	10.2	3.5	654	25	123	494	1655
F+30	0.36	72	139	2.4	87	2.71	0.73	1.36	141	10.8	3.3	531	34	166	509	1677
DANNY																
S	0.36	80	138	3.6	91	2.96	0.70	1.32	106	8.6	4.4	468	15	176	457	523
½way	0.45	83	141	5.9	89	2.63	0.68	1.26	176	11.0	8.0	840	21	195	472	538
F	0.36	81	146	3.8	81	2.40	0.68	1.24	160	14.3	5.0	515	35	208	575	1132
PHILLE																
S	0.42	76	135	4.2	90	3.26	0.74	1.02	123	7.5	6.4	518	19	323	369	219
½way	0.43	76	141	3.9	92	3.30	0.76	1.18	123	8.3	-	475	19	332	472	1613
F	0.52	78	145	3.5	87	2.70	0.67	1.63	150	9.6	5.2	648	31	402	575	2165
F+30	0.47	79	146	3.0	90	2.88	0.80	1.43	141	9.9	5.5	815	39	376	619	2207

Red Dragon 1979, cont'd.

	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	Cort	B/rub	ALP	AST	CK
BRACKEN																
S	0.38	70	138	3.8	97	2.65	0.71	1.10	87	5.9	5.4	326	10	503	546	877
F	-	79	145	4.0	89	2.38	0.59	1.21	132	8.5	3.7	648	25	531	634	976
F+30	0.41	79	148	3.0	91	2.40	0.63	0.85	123	8.8	3.3	449	26	549	597	1019
PIXIE																
S	0.31	71	145	3.9	96	3.10	0.67	0.94	88	7.5	4.6	244	10	366	258	474
F	0.40	73	149	3.4	97	3.00	0.65	0.98	123	9.5	3.6	571	14	391	261	623
F+30	0.34	77	152	3.0	98	2.86	0.70	0.86	132	9.8	3.4	447	15	367	236	693
KAZMAHAL																
S	0.39	64	139	4.2	96	2.70	0.64	0.82	141	6.7	10.1	304	22	114	324	219
½way	0.43	76	150	4.6	90	3.13	0.67	1.02	177	7.4	4.5	334	21	229	306	361
F	0.43	71	147	3.9	92	2.96	0.57	1.27	185	7.6	5.1	474	24	288	361	403
F+30	0.43	72	132	-	91	2.87	0.60	1.08	177	7.2	4.7	436	30	240	302	545
RUSTY																
S	0.42	82	141	4.0	92	2.93	0.81	0.91	132	6.3	6.2	287	17	301	280	187
F	0.49	69	144	3.0	85	2.88	0.71	1.28	194	7.4	7.5	603	16	407	295	354
F+30	0.41	75	145	2.4	92	2.93	0.73	0.97	185	7.5	6.0	428	18	343	335	410
ROYAL LORD																
S	0.44	68	137	3.9	92	2.99	0.63	0.63	123	5.9	7.8	282	22	346	442	143
F	0.47	71	145	4.0	89	2.95	0.55	1.04	141	7.1	5.3	410	33	402	372	202
F+30	-	68	143	3.4	94	2.99	0.58	0.85	141	7.4	5.6	317	33	462	354	223
GELERT																
S	0.38	70	149	3.9	91	2.93	0.67	1.06	132	7.7	7.1	197	13	425	376	150
F	-	77	148	3.7	84	2.79	0.65	1.09	203	8.9	6.6	450	21	500	413	304
F+30	0.44	80	145	2.7	92	2.94	0.69	0.99	194	9.5	4.7	448	20	562	501	403
KUSHTI BOK																
S	0.36	76	146	3.9	90	2.93	0.86	1.38	132	9.3	5.2	413	12	489	1100	396
F	0.36	71	151	3.0	89	2.74	0.73	1.67	159	10.2	4.4	746	19	504	1118	410
F+30	0.34	83	158	2.6	92	2.96	0.87	1.70	159	10.9	4.6	552	22	529	1066	396
POLLY																
S	0.44	75	140	3.9	91	2.98	0.81	0.86	150	8.5	7.5	306	21	524	567	297
F	0.43	77	139	3.1	85	2.82	0.66	1.13	194	9.8	5.9	550	29	589	584	375
F+30	0.41	93	138	2.9	92	3.01	0.75	1.12	194	10.8	5.6	583	37	664	602	382
ZAYIR																
S	0.38	67	145	4.1	92	3.20	0.68	1.12	159	5.4	4.8	245	23	300	254	134
F	0.43	94	140	3.5	98	3.10	0.72	1.22	203	7.8	5.4	339	40	348	269	237
F+30	0.44	78	129	2.4	93	3.20	0.67	1.14	185	7.1	4.8	400	31	314	314	269
ROXENA																269
S	0.34	73	140	3.4	91	3.10	0.79	1.05	123	7.3	6.4	262	37	423	184	403
F	0.35	73	145	2.8	92	2.93	0.62	1.43	159	7.9	5.2	584	63	437	276	396
F+30	0.33	75	145	2.5	94	2.95	0.62	1.24	159	8.1	4.7	452	72	449	265	
BETHAN																
S	0.33	79	149	3.9	93	2.96	0.64	1.21	132	9.7	5.2	224	10	393	420	495
½way	0.34	80	150	3.8	96	2.82	0.67	1.45	150	11.2	5.1	249	14	400	391	545
WYERE LAD																
S	0.38	85	147	4.0	93	3.32	0.76	1.23	141	7.6	7.8	490	14	361	376	181
F	0.44	80	130	3.1	91	2.93	0.64	1.20	167	8.0	6.0	594	15	395	413	226
F+30	0.38	84	147	3.0	97	3.01	0.65	1.04	159	8.2	5.0	552	19	398	442	255

Breamore 1980 (Rides D and I)

	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	Cort	B/rub	ALP	AST	CK
WASHINGTON PROUD SIR																	
S	0.37	-	-	-	-	-	-	-	1.15	88	6.5	-	224	10	363	383	269
MAGNUS																	
S	0.43	75	33	137	4.7	102	3.32	0.75	0.93	160	7.2	4.6	101	26	396	328	108
½way	0.47	79	37	140	4.0	102	3.15	0.58	1.15	194	7.3	5.4	257	30	442	413	1486
F	0.53	82	38	145	3.4	101	3.21	0.46	1.47	212	8.6	3.7	610	52	521	546	2462
F+30	0.51	82	39	143	3.6	102	3.20	0.47	1.34	212	8.7	4.4	412	55	527	568	2419
TARQUIN																	
S	0.38	68	33	136	4.8	100	3.09	0.59	1.07	115	10.3	5.5	229	12	323	295	106
½way	0.48	75	36	138	5.2	97	3.00	0.52	1.13	159	11.7	5.3	462	18	356	332	219
F	0.50	78	38	142	3.8	95	3.09	0.51	1.54	194	14.2	2.9	684	32	396	391	340
F+30	0.46	77	37	140	3.3	94	3.31	0.53	1.54	186	14.7	3.9	435	34	415	376	347
MAELOR																	
S	0.43	71	33	138	4.3	100	3.27	0.56	1.20	71	6.0	4.7	190	16	627	1410	-
F	0.48	75	35	140	4.0	101	3.24	0.54	1.18	115	6.7	3.8	468	29	726	1651	-
F+30	0.48	77	37	141	2.6	102	3.19	0.58	1.08	88	6.9	4.3	337	31	739	1616	-

Summer Solstice 1980 (Ride A)

	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	Cort	Glyc	Trig	B/rub	ALP	AST	CK
ROXENA																			
Pre	0.45	79	33	139	3.9	100	3.19	0.70	0.69	88	5.8	5.9	121	43	80	16	481	309	120
40km	0.47	78	33	141	4.1	99	3.05	0.73	1.04	106	6.9	6.0	485	156	91	23	508	284	2373
80km	0.45	74	33	142	3.5	96	2.93	0.63	1.28	114	9.0	4.5	396	169	130	37	521	328	2576
120km	0.45	75	34	139	3.2	97	2.76	0.64	1.36	123	11.4	4.5	393	338	46	55	613	365	2099
160km	0.41	75	35	140	3.0	96	2.89	0.64	1.23	141	12.5	3.8	252	143	65	70	574	398	1806
F+30	0.40	73	33	141	3.4	98	3.02	0.66	1.13	132	12.6	4.5	245	117	111	73	580	424	1755
F+12h	0.40	75	33	141	3.7	103	2.88	0.72	1.14	114	10.4	5.9	113	32	102	48	574	501	585
DRESDEN																			
Pre	0.40	66	35	141	3.7	101	3.00	0.72	0.88	123	5.6	5.2	172	27	128	23	280	5865	1873
40km	0.43	72	39	144	4.4	99	3.00	0.61	1.20	150	6.2	5.4	579	468	137	32	283	5865	20856
80km	0.42	67	37	143	4.1	97	2.83	0.61	1.47	159	7.3	5.9	396	638	78	41	310	5676	35486
120km	0.44	70	37	140	3.7	97	2.91	0.63	1.39	150	8.5	4.4	411	599	26	55	336	8136	28017
160km	0.41	72	38	143	3.4	98	2.63	0.60	1.19	159	9.4	5.7	338	429	26	76	382	8892	23342
F+30	0.40	66	39	140	3.8	101	-	-	-	-	9.6	-	303	260	78	-	-	8668	22176
F+12h	0.39	69	36	139	3.4	102	3.06	0.73	0.98	114	6.3	4.5	118	54	128	58	402	8136	3871
F+12d	-	69	33	137	3.6	98	3.00	0.76	1.12	115	5.8	-	-	-	-	24	435	1992	201

Summer Solstice 1980, cont'd.

	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	Cort	Glyc	Trig	B/rub	ALP	AST	CK
BADGER																			
Pre	0.44	80	38	139	3.8	100	3.18	0.78	0.87	114	8.7	4.9	153	0	107	18	587	509	138
40km	0.43	76	37	143	5.2	100	3.15	0.72	0.65	141	9.4	5.7	391	78	104	15	594	501	181
80km	0.42	77	37	141	5.3	101	3.00	0.78	0.89	141	11.0	4.4	346	221	156	21	594	546	550
110km	0.41	77	39	143	4.5	104	3.00	0.76	0.85	123	11.2	4.8	428	52	78	31	699	602	211
F+12h	0.40	76	37	137	3.6	101	3.03	0.67	0.82	114	8.4	5.5	294	-	-	17	660	509	138
MAELOR																			
Pre	0.47	73	34	138	3.9	102	3.10	0.78	0.84	97	5.6	5.5	157	-	-	20	607	756	185
40km	0.51	74	36	144	4.3	103	2.90	0.70	1.02	123	6.0	4.2	498	-	-	29	640	774	202
STEFANO																			
Pre	0.38	72	36	142	3.2	107	3.12	0.72	0.77	150	7.8	4.3	30	21	70	18	409	1634	314
40km	0.39	80	38	142	4.6	107	3.00	0.75	1.11	176	8.4	5.0	375	78	130	24	448	1703	374
80km	0.43	76	38	141	4.3	104	2.99	0.68	1.26	176	9.7	4.1	361	377	104	35	475	1686	456
110km	0.38	78	37	139	3.5	104	3.03	0.69	0.96	167	12.5	5.2	146	102	59	49	534	1789	516
F+12h	0.36	70	35	140	3.2	103	2.85	0.85	1.13	132	12.1	4.6	91	-	-	42	541	-	178
TARQUIN																			
Pre	0.41	75	37	140	3.3	101	3.19	0.74	1.11	141	7.6	5.9	119	0	123	22	333	335	138
40km	0.49	77	39	137	5.6	101	2.91	0.63	1.02	176	9.3	4.6	525	260	104	26	336	324	185
80km	0.50	77	40	137	5.9	100	3.03	0.64	1.23	176	10.7	2.9	634	468	13	36	382	350	271
120km	0.39	73	39	138	4.3	105	2.89	0.66	1.07	176	12.5	4.0	234	273	104	49	389	380	654
160km	0.42	72	38	140	3.5	103	2.84	0.61	0.94	176	14.3	4.3	234	208	33	61	409	479	1531
F+30	0.40	73	39	137	3.7	104	2.86	0.66	0.88	167	14.5	5.2	258	85	84	62	429	509	1445
F+12h	0.41	75	39	140	3.7	104	3.04	0.65	0.97	132	10.2	5.8	94	32	54	26	462	501	499
MAGNUS																			
Pre	0.47	76	38	141	3.5	101	3.15	0.72	1.10	132	6.0	5.3	38	24	254	20	356	328	138
40km	0.47	78	39	145	4.9	103	3.14	0.75	1.27	194	6.7	5.2	290	182	26	33	415	376	305
80km	0.52	76	39	141	4.5	103	3.12	0.63	1.39	194	7.1	3.0	204	364	140	42	462	420	305
120km	0.53	78	40	142	5.5	104	2.97	0.63	1.35	185	8.1	3.1	299	364	85	64	541	553	1411
160km	0.48	76	39	141	4.5	105	2.90	0.58	1.20	194	9.3	4.1	348	429	78	88	561	686	2408
F+30	0.47	80	40	141	4.4	107	3.10	0.63	0.95	185	9.6	4.5	216	124	130	92	567	715	2580
NIZAR																			
Pre	0.40	69	34	138	3.9	102	3.00	0.65	0.89	114	5.2	9.8	153	19	77	27	398	398	176
40km	0.50	73	37	142	4.1	99	2.92	0.64	0.92	132	6.2	5.2	544	143	91	35	448	442	568
80km	0.50	73	37	142	4.1	100	2.90	0.61	1.04	141	7.9	3.7	691	377	78	37	488	487	705
120km	0.51	75	38	-	-	-	2.93	0.61	0.98	141	9.3	3.0	496	390	104	43	561	501	723
160km	0.47	70	36	140	3.9	101	2.80	0.53	0.91	132	10.2	3.1	479	299	0	61	547	567	705
F+30	0.45	65	36	140	3.4	101	3.11	0.73	0.79	123	10.4	4.9	387	117	52	62	567	590	705
F+12h	0.40	67	33	140	3.6	101	2.96	0.79	0.93	106	8.0	6.0	187	21	65	34	587	664	211

Red Dragon 1980 (Ride F)

	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	Cort	Glyc	B/rub	ALP	AST	CK
ROXENA																		
S	0.36	73	30	143	3.6	101	2.88	0.72	1.24	141	8.3	4.7	252	36	18	514	346	304
½way	0.42	74	31	145	3.1	100	3.01	0.70	1.38	150	9.3	6.8	483	221	23	528	372	361
F	0.44	75	34	144	3.1	97	2.84	0.67	1.61	194	9.8	2.0	671	377	32	600	405	646
F+30	0.41	75	32	140	2.2	99	2.86	0.62	1.49	150	10.2	4.2	605	141	38	587	313	821
DRESDEN																		
S	0.31	68	34	145	3.5	101	3.02	0.73	0.65	115	6.3	5.5	188	62	19	290	322	686
½way	0.44	72	36	142	3.3	97	2.85	0.61	1.12	168	7.6	5.7	420	455	28	347	636	3181
F	0.41	72	36	144	3.5	93	2.58	0.54	1.65	133	9.4	5.3	524	494	37	376	928	8589
F+30	0.37	71	36	142	2.4	96	2.80	0.58	1.37	177	9.7	4.7	355	99	46	369	997	8691
DIDO																		
S	0.31	70	27	142	3.9	102	2.90	0.73	0.80	97	6.3	5.0	356	10	10	640	287	304
½way	0.36	77	29	145	3.6	98	2.97	0.75	0.65	124	8.3	6.7	488	208	13	686	276	389
End	0.40	71	27	142	3.2	95	2.81	0.64	0.69	106	8.9	-	507	83	17	726	309	778
BOSTON BAY																		
S	0.41	70	34	141	3.7	98	2.99	0.80	1.03	141	4.9	5.4	301	52	32	343	232	115
½way	0.54	81	40	144	3.4	96	2.81	0.68	1.06	194	6.5	7.4	615	364	54	481	295	311
F	0.52	81	42	146	3.3	95	2.60	0.61	1.46	230	10.1	3.3	793	853	95	567	343	856
F+30	0.45	76	39	141	2.8	93	2.56	0.59	1.47	221	10.4	5.3	705	302	97	495	339	891
WYERE LAD																		
S	0.37	71	34	141	3.8	98	3.02	0.78	0.86	133	5.5	5.1	360	31	15	376	313	173
½way	0.46	84	38	144	3.3	96	2.88	0.69	1.05	159	7.0	6.1	606	377	23	462	383	276
F	0.52	90	42	148	2.9	95	3.02	0.79	1.36	248	9.8	5.1	840	260	28	547	413	552
F+30	0.46	85	39	141	2.9	95	3.13	0.74	1.31	194	10.2	5.7	674	219	38	508	420	594
TARQUIN																		
S	0.34	77	35	140	3.3	102	2.87	0.73	0.63	133	8.8	5.5	288	47	19	501	273	170
½way	0.47	82	38	140	3.2	95	2.58	0.54	0.68	194	11.6	6.3	543	768	30	521	332	219
F	0.48	85	41	138	2.8	89	2.87	0.58	1.17	265	15.1	2.1	961	910	50	580	391	778
F+30	0.46	85	41	138	2.6	90	3.03	0.65	1.18	274	15.7	3.5	740	193	58	600	391	962
GLAS-MYNYDD HONEY																		
S	0.32	71	29	140	3.3	98	2.81	0.68	1.06	203	5.0	5.1	158	62	14	481	334	199
½way	0.44	79	32	140	3.6	97	2.81	0.79	1.20	212	7.4	5.7	479	234	23	600	405	361
F	0.41	75	32	142	3.2	92	2.48	0.84	1.74	248	10.4	2.2	569	494	31	633	468	891
F+30	0.38	74	32	141	2.4	93	2.43	0.80	1.58	194	10.9	3.8	570	214	32	613	487	877
GLOSS																		
S	0.36	69	32	143	3.9	103	2.63	0.69	1.58	167	8.9	5.6	159	99	22	778	273	260
½way	0.51	77	37	140	3.7	94	2.63	0.63	1.38	203	10.2	4.1	476	520	36	910	313	332
F	0.44	72	35	141	3.0	87	2.24	0.79	1.86	212	12.1	2.2	487	390	54	897	383	1203
F+30	0.41	72	35	141	2.8	91	2.39	0.81	1.95	203	12.3	2.6	496	255	57	897	398	1330
LITTLE SOULA II																		
S	0.30	73	30	144	3.9	103	2.69	0.69	1.43	177	7.3	5.3	186	21	18	660	261	152
½way	0.50	93	39	142	3.8	90	2.49	0.83	1.35	345	9.7	7.1	824	651	32	990	361	552
+30	0.46	93	39	139	3.6	90	2.56	0.81	1.22	256	10.4	5.9	731	224	34	1003	324	594

APPENDIX III

Individual Data from Section 3

ASTRYL

Control experiments - clinical data and blood samples

Time	Bwt	HR	RR	RT	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Cort
0	499	40	Q	37.5	0.43	68	145	4.6	104	3.98	0.73	0.95	80	4.8	150
60	-	35	Q	37.5	0.34	66	143	4.1	104	3.55	0.68	0.77	80	4.4	255
120	-	37	Q	37.6	0.35	66	141	3.9	103	3.23	0.68	0.85	97	4.4	162
180	-	33	Q	37.4	0.36	64	148	4.0	106	3.29	0.65	1.08	106	4.0	157
240	-	35	Q	37.5	0.37	64	143	3.6	102	3.23	0.66	1.22	97	4.0	205
300	497	37	Q	37.5	0.39	65	144	3.4	101	3.20	0.60	1.25	71	4.1	240

Heat exposure - clinical data and blood samples

Time	Bwt	HR	RR	RT	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Cort
0	496	37	Q	37.4	0.35	65	141	4.0	102	3.22	0.72	0.98	80	4.8	147
60	-	40	110	37.5	0.35	64	148	3.9	106	3.20	0.65	0.96	62	5.0	181
120	-	45	140	38.0	0.35	65	141	3.4	104	3.47	0.66	1.02	80	4.9	200
180	-	40	140	38.3	0.35	68	140	3.1	102	3.33	0.63	0.99	88	4.9	238
240	-	40	130	38.6	0.35	70	142	3.1	102	3.26	0.61	1.04	97	5.0	192
300	483	37	130	38.3	0.36	71	141	2.9	101	3.25	0.63	1.08	115	5.3	252

Sweat samples - neck

Time	Cl	Na^+	K^+	Mg^{2+}	Prot
20	205	146	62	20.64	18.87
40	198	137	57	9.25	8.44
70	220	145	64	6.50	4.81
100	210	146	61	3.84	3.33
150	195	142	56	2.16	1.77
210	220	161	63	1.36	1.02
270	230	167	65	1.00	0.55

Sweat samples - body

Cl^-	Na^+	K^+	Mg^{2+}	Prot
-	-	-	-	-
235	165	84	16.42	15.38
260	160	83	12.10	10.00
230	152	70	8.60	7.30
225	151	67	5.70	4.90
220	155	60	3.04	2.21
235	165	65	2.28	1.40

BEN

Control experiments - clinical data and blood samples

Time	Bwt	HR	RR	RT	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Cort
0	528	45	Q	37.5	0.38	68	140	4.3	102	3.57	0.72	0.69	142	7.4	201
60	-	40	Q	37.2	0.38	69	142	4.0	103	3.80	0.63	0.69	186	7.4	210
120	-	43	Q	37.6	0.39	72	141	3.9	102	3.98	0.59	0.75	142	7.4	144
180	-	40	Q	37.5	0.37	74	139	3.7	101	3.42	0.57	0.99	168	7.4	165
240	-	45	Q	37.7	0.36	74	140	3.8	102	3.44	0.54	1.06	171	7.4	143
300	527	40	Q	37.6	0.35	76	141	3.8	101	3.93	0.54	1.12	177	7.4	269

Heat exposure - clinical data and blood samples

Time	Bwt	HR	RR	RT	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Cort
0	530	40	Q	37.4	0.38	67	138	4.3	103	3.51	0.66	0.71	115	7.6	122
60	-	48	20	37.7	0.37	67	139	4.4	103	3.50	0.57	0.55	97	7.6	191
120	-	55	50	38.2	0.38	69	142	4.2	104	3.90	0.62	0.63	142	7.6	139
180	-	50	130	38.3	0.39	72	148	3.8	104	3.60	0.60	0.72	124	7.5	232
240	-	48	140	38.4	0.40	75	144	3.6	104	3.75	0.51	0.74	168	7.8	237
300	516	45	140	38.3	0.40	77	141	3.3	99	3.60	0.58	0.91	168	8.1	359

Sweat samples - neck

Cl^-	Na^+	K^+	Mg^{2+}	Prot
177	132	45	18.78	20.46
215	128	75	8.66	9.90
195	135	65	4.70	6.55
190	135	69	3.56	3.72
220	147	78	3.39	4.11
172	128	54	1.08	1.51
217	158	70	1.12	1.22

Cl^-	Na^+	K^+	Mg^{2+}	Prot
-	-	-	-	-
210	140	61	10.00	10.77
207	145	58	9.63	8.95
200	143	56	9.02	8.00
215	146	66	8.60	7.12
234	158	79	6.20	6.35
240	146	81	4.20	4.20

DIRK

Control experiment - clinical data and blood samples

Time	Bwt	HR	RR	RT	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Cort
0	502	40	Q	37.6	0.46	59	149	4.0	102	3.56	0.84	2.11	133	6.9	221
60	-	38	Q	37.5	0.44	64	149	3.9	104	2.40	0.53	2.26	133	6.1	325
120	-	35	Q	37.8	0.44	62	149	3.8	102	2.98	0.62	2.16	150	6.6	285
180	-	38	Q	37.6	0.43	64	147	3.7	104	2.64	0.54	1.99	133	6.6	250
240	-	35	Q	37.6	0.46	64	150	3.8	104	2.72	0.55	2.43	141	6.2	148
300	500	35	Q	37.7	0.47	65	150	3.7	104	2.55	0.53	2.43	133	6.3	231

Dirk, cont'd.

Heat exposure - clinical data and blood samples

Time	Bwt	HR	RR	RT	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Cort
0	510	40	Q	37.6	0.43	62	145	4.4	101	2.55	1.00	1.00	133	5.7	102
60	-	45	140	38.0	0.43	65	147	4.1	104	2.93	0.67	1.14	133	5.6	263
120	-	50	200	38.2	0.42	66	148	4.0	104	3.06	0.68	1.45	133	5.9	200
180	-	60	210	38.7	0.47	75	145	3.4	99	2.38	0.65	1.24	159	6.2	432
240	-	90	210	39.2	0.54	77	147	3.3	99	2.53	0.45	1.18	186	6.5	710
300	485	105	210	39.5	0.53	89	144	3.3	92	2.80	0.41	0.72	168	7.5	1361

Sweat samples - neck

Time	Cl^-	Na^+	K^+	Mg^{2+}	Prot
20	195	133	38	15.11	16.94
40	185	132	37	6.91	7.02
70	190	149	38	4.40	5.10
100	190	146	40	1.53	3.50
150	180	136	38	1.08	1.32
210	180	151	34	0.71	0.66
270	170	156	27	0.53	0.42

Sweat samples - body

Cl^-	Na^+	K^+	Mg^{2+}	Prot
-	-	-	-	-
212	149	53	10.34	10.54
205	142	51	8.40	8.80
195	140	48	5.20	6.10
170	129	43	1.55	3.28
180	143	37	1.22	1.28
175	153	32	0.71	0.52

JOHNNY WALKER

Control experiment - clinical data and blood samples

Time	Bwt	HR	RR	RT	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Cort
0	474	45	Q	37.5	0.44	66	148	3.9	103	3.06	0.76	1.00	115	4.9	204
60	-	43	Q	37.4	0.44	60	151	4.4	103	2.61	0.71	1.26	124	5.1	257
120	-	45	Q	37.7	0.44	63	150	4.0	102	2.94	0.67	1.33	141	5.0	222
180	-	40	Q	37.7	0.41	63	149	3.9	103	2.90	0.65	1.33	133	5.3	259
240	-	40	Q	37.6	0.44	66	147	3.8	101	2.96	0.63	1.37	141	5.3	159
300	472	43	Q	37.5	0.41	64	148	3.6	102	3.09	0.61	1.40	141	5.4	401

Heat exposure - clinical data and blood samples

Time	Bwt	HR	RR	RT	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Cort
0	470	40	Q	37.8	0.45	67	142	4.6	102	2.92	0.70	1.29	97	5.3	276
60	-	50	95	38.1	0.40	67	142	4.0	103	3.04	0.71	1.21	124	5.0	266
120	-	50	120	38.2	0.43	72	144	3.4	101	3.20	0.70	1.26	141	4.9	372
180	-	53	140	37.8	0.50	76	150	3.5	101	3.14	0.70	1.38	159	5.1	260
240	-	55	140	37.9	0.48	78	148	3.1	97	3.19	0.69	1.54	168	5.3	296
300	446	50	140	38.1	0.47	83	152	2.9	96	3.19	0.65	1.63	186	5.7	485

Sweat samples - neck

Time	Cl^-	Na^+	K^+	Mg^{2+}	Prot
20	170	126	38	23.00	20.80
40	175	128	36	9.20	7.43
70	174	155	35	3.92	3.15
100	165	161	33	1.37	1.33
150	200	171	49	0.94	0.66
210	174	150	41	0.85	0.54
270	196	164	42	0.75	0.46

Sweat samples - body

Cl^-	Na^+	K^+	Mg^{2+}	Prot
-	-	-	-	-
218	142	55	7.63	8.52
166	137	34	3.80	4.05
160	140	35	1.59	2.47
168	143	39	0.96	0.58
160	140	39	0.78	0.40
163	147	39	0.74	0.42

BRIGADIER

Control experiment - clinical data and blood samples

Time	Bwt	HR	RR	RT	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Cort
0	247	48	Q	38.0	0.32	74	134	4.4	100	2.07	0.77	1.62	97	7.9	252
60	-	45	Q	38.0	0.30	77	133	4.1	99	1.41	0.69	1.18	88	7.5	203
120	-	45	Q	38.0	0.30	73	137	4.0	99	1.55	0.64	1.14	97	7.6	317
180	-	48	Q	37.9	0.30	75	136	4.4	100	1.43	0.59	1.24	97	7.2	182
240	-	50	Q	37.8	0.30	75	136	3.7	98	1.55	0.58	1.28	97	7.6	258
300	245	45	Q	37.8	0.28	79	135	3.7	98	2.18	0.52	1.21	97	7.6	252

Heat exposure - clinical data and blood samples

Time	Bwt	HR	RR	RT	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Cort
0	246	45	Q	38.0	0.29	75	139	4.7	104	2.50	0.53	1.23	97	6.4	303
60	-	65	80	38.2	0.30	76	137	4.3	104	2.11	0.49	1.20	106	6.4	283
120	-	90	140	38.7	0.32	82	140	3.9	103	1.63	0.49	1.08	115	6.8	305
180	-	80	120	38.5	0.33	82	137	3.7	100	2.33	0.49	1.25	115	7.0	411
240	-	80	140	38.4	0.32	84	136	3.5	98	2.50	0.47	1.21	124	7.0	229
300	239	70	120	38.4	0.32	82	138	3.5	98	2.55	0.47	1.26	124	7.4	213

Sweat samples - neck

Time	Cl^-	Na^+	K^+	Mg^{2+}	Prot
50	365	234	100	10.90	9.09
80	292	198	61	4.44	4.09
110	310	200	76	4.84	4.35
180	302	200	72	1.66	1.38
240	275	200	84	1.30	0.93

Sweat samples - body

Cl^-	Na^+	K^+	Mg^{2+}	Prot
330	220	78	13.30	12.29
260	177	61	7.90	7.42
260	170	65	6.00	5.89
240	175	71	4.00	3.73
276	171	74	3.24	3.28

BIANCA

Control experiment - clinical data and blood samples

Time	Bwt	HR	RR	RT	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Cort
0	158	55	Q	37.6	0.27	86	136	4.9	99	1.63	0.67	0.83	88	4.9	169
60	-	50	Q	37.9	0.26	85	137	4.3	100	1.18	0.61	0.65	80	4.6	130
120	-	53	Q	38.0	0.26	86	136	5.2	99	1.17	0.53	0.61	71	4.5	142
180	-	53	Q	38.1	0.27	87	139	4.2	98	1.29	0.45	0.66	71	4.6	229
240	-	50	Q	37.8	0.28	90	139	3.9	99	1.21	0.44	0.68	80	4.7	112
300	157	50	Q	37.9	0.26	87	140	4.2	99	1.35	0.44	0.73	71	4.7	163

Heat exposure - clinical data and blood samples

Time	Bwt	HR	RR	RT	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Cort
0	156	50	Q	37.9	0.27	84	140	4.9	101	1.29	0.51	0.66	80	4.1	178
60	-	80	110	38.3	0.27	85	138	5.1	100	1.61	0.46	0.82	80	4.3	203
120	-	60	200	39.2	0.27	88	141	4.3	101	1.38	0.38	0.63	80	4.2	340
180	-	70	170	39.2	0.27	89	138	3.5	97	1.24	0.36	0.49	97	4.3	371
240	-	60	170	39.2	0.27	88	138	3.5	96	1.43	0.34	0.55	106	4.5	389
300	153	55	150	39.1	0.28	87	139	3.6	95	1.70	0.39	0.58	106	4.6	269

Sweat samples - neck

Time	Cl	Na^+	K^+	Mg^{2+}	Prot
50	215	178	37	4.48	4.42
80	245	193	48	2.70	2.20
110	255	186	57	1.83	1.83
180	260	191	69	1.41	1.95
240	250	188	60	1.35	1.29

Sweat samples - body

Cl^-	Na^+	K^+	Mg^{2+}	Prot
250	200	49	14.50	12.94
270	197	58	7.25	6.23
235	168	54	5.91	4.96
250	175	56	3.16	3.07
240	172	49	2.22	2.11

MEENIE

Control experiment - clinical data and blood samples

Time	Bwt	HR	RR	RT	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Cort
0	136	50	Q	37.9	0.31	75	137	4.2	100	2.11	0.59	0.90	62	3.1	428
60	-	48	Q	38.0	0.31	75	137	3.9	99	1.53	0.54	0.83	62	3.0	326
120	-	48	Q	38.3	0.31	75	140	3.4	99	1.41	0.52	0.77	62	3.4	270
180	-	45	Q	38.2	0.32	73	137	3.7	99	1.27	0.59	0.72	62	3.3	275
240	-	48	Q	38.1	0.31	71	139	4.9	100	1.25	0.58	0.80	62	3.4	463
300	135	48	Q	37.9	0.31	71	137	5.1	101	1.30	0.56	0.61	53	3.3	292

Heat exposure - clinical data and blood samples

Time	Bwt	HR	RR	RT	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Cort
0	143	50	Q	37.4	0.29	79	138	4.2	100	3.07	0.68	1.33	141	2.7	177
60	-	60	140	37.8	0.27	77	140	4.3	100	2.94	0.62	1.35	176	2.7	383
120	-	80	180	38.6	0.28	79	137	4.1	98	2.89	0.60	1.23	185	2.8	416
180	-	90	180	38.8	0.29	80	136	3.7	98	2.86	0.55	1.07	194	3.0	463
240	-	100	220	39.0	0.29	82	130	3.1	94	2.73	0.54	1.01	176	3.2	504
300	130	130	230	39.2	0.31	81	137	3.1	92	2.54	0.49	0.85	203	3.4	698

Sweat samples - neck

Time	Cl	Na^+	K^+	Mg^{2+}	Prot
50	334	226	96	22.8	16.40
80	325	220	97	14.7	10.25
110	310	214	93	12.2	8.00
180	275	208	77	4.28	2.91
240	244	195	63	2.28	1.53

Sweat samples - body

Cl^-	Na^+	K^+	Mg^{2+}	Prot
258	173	95	17.4	12.47
235	151	75	8.9	6.48
230	152	77	6.24	5.48
215	151	65	3.98	3.24
197	150	48	2.42	1.79

MO

Control experiment - clinical data and blood samples

Time	Bwt	HR	RR	RT	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Cort
0	132	45	Q	37.9	0.27	71	130	4.2	95	1.74	0.57	1.56	80	4.3	947
60	-	45	Q	38.1	0.30	72	128	4.0	95	1.78	0.60	1.30	80	3.9	822
120	-	48	Q	38.2	0.30	79	137	4.1	99	2.14	0.66	1.36	88	4.1	718
180	-	50	Q	38.0	0.30	71	134	3.9	97	2.28	0.60	1.34	80	3.9	648
240	-	45	Q	37.6	0.28	71	133	4.0	97	2.02	0.55	1.35	80	4.1	751
300	131	48	Q	37.6	0.29	73	133	5.2	98	1.99	0.59	1.49	80	4.1	560

Heat exposure - clinical data and blood samples

Time	Bwt	HR	RR	RT	PCV	Prot	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Cort
0	138	50	Q	37.3	0.28	85	137	4.5	97	3.08	0.67	1.48	167	3.6	443
60	-	70	140	37.8	0.27	86	137	4.1	96	3.10	0.65	1.49	150	3.6	483
120	-	70	180	38.4	0.28	93	138	4.0	94	3.11	0.69	1.44	150	3.8	620
180	-	70	170	38.8	0.27	95	137	3.4	93	2.82	0.62	1.16	150	4.2	640
240	-	70	200	38.5	0.28	95	134	3.4	92	3.19	0.64	0.93	176	4.5	928
300	134	70	190	38.7	0.28	92	135	3.2	91	2.70	0.63	1.17	176	4.0	569

Sweat samples - neck

Time	Cl	Na^+	K^+	Mg^{2+}	Prot
50	230	192	42	8.90	8.66
80	173	154	31	4.25	2.58
110	184	163	34	2.52	1.74
180	191	171	36	1.16	0.80
240	206	182	40	1.18	0.82

Sweat samples - body

Cl^-	Na^+	K^+	Mg^{2+}	Prot
284	222	66	12.4	9.00
193	164	37	4.32	3.52
194	165	38	3.44	2.89
192	166	37	2.64	1.64
209	178	43	2.08	1.48

APPENDIX IV

Individual Data from Section 4

Experiments without washing the skin

ADAM

Control saline infusion - heart rate and blood samples

Time	HR	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	B/rub
0	35	0.35	68	34	137	4.2	101	2.97	0.68	1.02	141	4.4	5.2	32
30	-	0.35	66	34	135	4.0	101	2.95	0.67	1.05	124	4.5	6.3	31
60	37	0.31	66	34	136	3.9	100	2.90	0.65	0.93	115	4.5	7.3	30
90	-	0.33	65	33	136	3.8	101	2.94	0.67	0.87	115	4.5	7.1	29
120	30	0.31	64	30	135	3.6	100	2.95	0.68	0.85	133	4.6	6.9	29
150	-	0.31	65	31	136	3.7	100	2.94	0.65	0.84	124	4.7	7.1	28
180	32	0.32	65	30	136	3.6	101	2.93	0.65	0.94	124	4.6	6.2	27
210	-	0.30	65	32	137	3.5	101	2.94	0.63	0.93	115	4.6	5.8	28
240	35	0.32	69	34	137	3.0	101	2.97	0.65	1.07	124	4.6	4.5	30

Adrenaline infusion - heart rate and blood samples

Time	HR	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	B/rub	Glyc
0	31	0.34	75	41	140	4.3	100	3.54	0.71	1.05	159	5.2	5.4	24	8
30	-	0.44	73	40	143	3.6	98	3.49	0.71	0.48	203	5.2	7.8	21	256
60	45	0.46	70	41	140	3.6	96	3.63	0.78	0.32	186	5.2	12.3	24	588
90	-	0.51	68	42	143	3.6	91	3.50	0.86	0.24	194	5.3	14.7	26	1132
120	58	0.49	78	44	140	3.7	88	3.62	0.92	0.22	203	5.4	17.6	29	2180
150	-	0.50	81	43	141	3.6	87	3.49	1.04	0.20	203	5.9	18.2	31	3186
180	60	0.49	80	44	139	3.6	86	3.52	1.07	0.22	194	5.6	19.8	33	4025
210	-	0.46	78	43	138	4.2	89	3.59	1.06	0.33	194	5.7	17.8	35	3702
240	42	0.46	75	45	140	2.9	92	3.49	1.10	0.37	194	5.7	17.2	32	3528

Sweat samples - neck

Time	Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}	Prot
10	316	238	84	8.12	21.0	17.87
20	268	234	55	4.82	9.0	9.60
35	222	191	39	3.80	5.20	5.32
45	206	179	31	3.46	4.50	4.53
60	200	180	27	3.25	3.92	4.80
75	180	174	18	2.90	2.88	4.56
90	172	170	16	2.37	1.88	2.18
105	174	171	15	2.09	1.48	1.59
120	170	170	15	1.89	1.10	1.05
135	174	172	16	1.78	1.04	0.94
150	176	173	17	1.68	1.00	0.73
165	192	188	21	1.71	1.07	1.01
180	196	189	23	1.79	1.12	0.82

Sweat samples - body

Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}	Prot
218	160	34	5.12	21.20	18.60
188	163	33	4.04	9.10	8.87
178	164	24	2.96	4.80	5.32
182	160	28	2.86	4.50	5.34
180	158	27	2.78	4.40	6.04
202	179	27	3.51	5.12	7.92
176	169	21	2.50	2.56	3.68
176	164	20	2.08	1.64	2.21
176	167	20	1.88	1.48	1.78
180	171	20	2.05	1.58	1.80
178	167	21	1.80	1.40	1.61
208	194	20	3.11	1.47	1.69
188	184	23	1.68	1.60	1.75

ASTRYL

Adrenaline infusion - heart rate and blood samples

Time	HR	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	B/rub	Glyc	Trig
0	33	0.34	74	33	136	3.9	100	3.32	0.73	1.23	115	6.4	5.9	43	16	103
30	-	0.38	72	33	135	3.6	98	2.92	0.69	0.81	124	5.7	7.2	40	111	127
69	48	0.46	77	36	135	3.3	96	2.88	0.71	0.64	124	5.4	9.9	45	320	126
90	-	0.48	79	37	136	3.3	94	2.92	0.72	0.50	132	5.2	12.9	49	590	241
120	62	0.49	80	38	134	3.1	92	3.17	0.72	0.38	141	5.2	15.2	51	836	205
150	-	0.50	82	39	134	3.1	91	3.20	0.76	0.38	150	5.5	16.7	57	936	254
180	75	0.52	84	40	135	3.0	91	3.12	0.71	0.35	150	4.8	17.9	60	1371	217
210	-	0.44	77	37	135	3.6	94	3.23	0.80	0.68	141	5.7	15.7	52	1103	206
240	40	0.40	76	38	137	3.3	95	3.42	0.81	0.76	150	5.6	15.6	50	501	183

Sweat samples - neck

Time	Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}	Prot
10	318	244	69	8.07	22.3	17.9
20	295	236	50	5.90	19.4	15.6
35	242	203	36	4.48	12.8	11.06
45	228	208	30	3.08	8.1	6.62
60	242	214	34	3.31	7.5	6.35
75	208	198	26	2.72	4.36	3.69
90	200	200	22	2.68	3.18	2.69
105	202	202	24	2.40	2.48	2.35
120	216	204	26	2.40	1.90	1.68
135	206	202	25	2.12	1.40	1.18
150	222	212	27	1.98	1.66	1.34
165	210	204	26	2.08	1.46	1.26
180	220	210	27	2.03	1.63	1.32

Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}	Prot
200	149	56	5.85	20.30	18.95
186	152	39	4.16	11.40	8.58
180	155	29	3.65	7.21	6.99
164	152	30	2.32	6.30	6.19
180	160	29	2.42	4.48	4.45
176	160	29	1.65	2.72	3.16
210	183	37	2.71	4.84	4.24
188	174	31	1.97	3.40	2.76
185	172	28	1.61	3.15	2.51
200	186	33	2.05	3.12	2.46
232	202	41	2.61	3.32	2.64
224	200	37	2.61	3.16	2.38
250	216	44	3.21	1.80	1.28

Experiments without washing the skin, cont'd.

DIRK

Control saline infusion - heart rate and blood samples

Time	HR	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{++}	Mg^{++}	PO_4^{--}	Creat	Urea	Gluc	B/rub
0	33	0.45	64	36	139	4.1	101	3.00	0.86	0.82	159	4.4	4.3	29
30	-	0.40	62	35	139	4.0	101	3.03	0.83	0.65	159	4.3	4.7	29
60	30	0.41	60	34	138	4.0	100	2.92	0.82	0.61	159	4.4	5.4	27
90	-	0.41	59	34	139	3.9	100	2.95	0.82	0.59	168	4.2	5.4	25
120	35	0.41	60	35	140	3.8	100	2.93	0.82	0.59	168	4.4	5.3	25
150	-	0.41	62	36	141	3.9	99	2.92	0.76	0.65	168	4.4	4.0	26
180	30	0.41	61	35	141	3.7	100	2.98	0.81	0.71	177	4.3	4.8	27
210	-	0.39	63	37	141	3.8	99	2.96	0.80	0.72	177	4.4	4.7	27
240	33	0.38	65	38	139	3.2	101	2.96	0.83	0.72	177	4.4	5.0	30

Adrenaline infusion - heart rate and blood samples

Time	HR	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{++}	Mg^{++}	PO_4^{--}	Creat	Urea	Gluc	B/rub	Glyc
0	31	0.38	73	37	132	4.1	103	3.07	0.82	1.44	159	6.9	4.6	39	23
30	-	0.46	72	38	131	3.7	100	3.00	0.79	0.68	168	6.7	7.0	61	212
60	38	0.52	69	38	131	3.6	97	3.09	0.80	0.55	177	6.7	11.4	69	408
90	-	0.51	74	40	130	3.5	95	2.97	0.82	0.47	177	6.6	13.1	73	654
120	48	0.51	77	40	129	3.6	94	2.94	0.85	0.41	186	6.8	15.3	77	1020
150	-	0.53	76	42	130	3.3	92	2.96	0.91	0.39	186	6.8	16.4	81	1263
180	52	0.55	77	42	130	3.2	91	2.93	0.94	0.34	194	6.9	17.7	85	1398
210	-	0.49	75	40	131	3.9	93	3.01	0.91	0.45	186	6.7	15.2	79	1203
240	38	0.47	72	41	132	3.1	96	3.04	0.89	0.63	186	6.5	14.5	72	720

Sweat samples - neck

Time	Cl^-	Na^+	K^+	Ca^{++}	Mg^{++}	Prot
10	312	234	62	7.60	20.4	18.8
20	246	200	40	5.66	13.4	12.8
35	234	196	34	4.86	11.6	9.9
45	238	205	32	4.28	8.9	7.92
60	228	197	27	3.49	5.80	6.40
75	220	194	25	3.42	4.95	4.22
90	235	200	28	3.85	6.62	5.64
105	246	214	29	3.38	4.20	4.56
120	208	194	22	2.72	2.86	2.36
135	206	191	23	2.65	2.04	1.69
150	226	210	24	2.75	1.96	1.16
165	212	193	24	2.69	2.01	1.12
180	222	207	25	2.74	1.98	1.20

Sweat samples - body

Cl^-	Na^+	K^+	Ca^{++}	Mg^{++}	Prot
194	147	53	4.97	21.53	19.10
182	151	32	4.39	11.30	10.42
173	146	28	3.01	10.00	9.85
176	153	27	3.03	9.00	7.85
178	157	22	2.63	6.10	6.10
188	164	27	2.51	4.88	5.41
190	169	27	2.36	2.76	3.36
190	173	25	2.47	2.88	3.06
178	164	25	1.75	2.06	1.19
182	166	26	1.81	2.23	1.35
209	184	29	2.53	2.54	1.94
204	180	30	2.36	2.59	2.06
220	182	31	3.55	2.67	2.38

JOHNNY WALKER

Adrenaline infusion - heart rate and blood samples

Time	HR	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{++}	Mg^{++}	PO_4^{--}	Creat	Urea	Gluc	B/rub	Glyc	Trig
0	37	0.37	73	39	130	4.3	98	3.36	0.77	1.24	124	6.8	5.5	40	27	160
30	-	0.43	75	40	130	3.8	95	3.05	0.70	0.93	133	6.6	6.9	49	201	134
60	40	0.44	76	40	129	3.8	93	3.06	0.68	0.81	150	7.1	8.9	51	439	301
90	-	0.44	75	39	129	3.7	91	3.30	0.69	0.59	141	7.2	12.8	67	692	179
120	52	0.46	77	40	130	3.5	88	3.19	0.71	0.50	124	7.0	15.1	53	936	335
150	-	0.45	77	41	129	3.4	89	3.24	0.75	0.34	159	7.1	15.6	51	1423	362
180	45	0.45	78	42	129	3.3	90	3.24	0.77	0.28	141	7.0	14.5	53	1605	347
210	-	0.43	77	41	131	3.6	91	3.40	0.80	0.39	150	6.8	12.7	41	1298	211
240	35	0.38	76	41	132	3.0	93	3.14	0.76	0.52	177	6.8	12.3	31	1036	109

Sweat samples - neck

Time	Cl	Na^+	K^+	Ca^{++}	Mg^{++}	Prot
10	336	257	58	8.43	23.7	17.68
20	290	246	33	6.00	8.2	6.25
35	234	214	25	3.96	4.8	3.54
45	250	228	29	4.02	4.40	3.20
60	256	232	30	3.66	3.36	2.79
75	182	178	21	2.85	2.44	2.03
90	180	169	19	2.23	1.12	1.01
105	170	166	19	2.10	1.03	0.76
120	172	163	19	2.05	0.84	0.65
135	184	176	20	2.07	0.86	0.62
150	198	185	24	2.10	0.89	0.47
165	190	182	23	1.86	0.76	0.62
180	184	179	24	1.64	0.65	1.05

Sweat samples - body

Cl	Na^+	K^+	Ca^{++}	Mg^{++}	Prot
262	197	62	6.31	19.61	7.71
268	220	38	5.04	9.4	7.65
270	224	38	5.04	7.2	5.90
184	166	30	2.77	6.24	5.03
172	152	26	2.60	5.77	4.32
154	150	25	1.58	2.32	3.50
162	143	28	1.22	1.62	1.85
150	143	28	1.29	1.25	1.33
168	154	29	1.10	1.60	1.56
182	164	30	1.23	1.62	1.70
180	160	33	1.25	1.61	1.23
206	170	50	1.23	1.60	1.04
176	160	35	1.01	0.98	0.60

BRIGADIER (Blood samples only)

Control saline infusion

Time	HR	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{++}	Mg^{++}	PO_4^{--}	Creat	Urea	Gluc	B/rub
0	40	0.30	78	22	134	4.6	99	2.60	0.49	1.20	124	5.3	3.9	13
30	-	0.30	77	22	137	4.9	99	2.65	0.46	1.20	115	5.5	4.0	14
60	35	0.28	75	21	135	4.5	99	2.50	0.46	1.17	115	5.7	3.8	13
90	-	0.28	75	21	135	4.3	99	2.61	0.48	1.09	115	5.8	3.9	13
120	38	0.27	74	21	135	4.3	99	2.58	0.49	1.19	115	5.8	3.8	14
150	-	0.27	73	21	134	4.2	99	2.42	0.48	1.16	115	5.9	3.9	13
180	35	0.27	73	21	136	4.1	99	2.33	0.44	1.17	115	6.0	3.8	15
210	-	0.28	74	21	134	4.0	99	2.37	0.43	1.23	115	5.9	3.9	15
240	40	0.27	75	21	134	3.5	98	2.36	0.46	1.28	124	6.3	4.0	15

Experiments without washing the skin, Brigadier, cont'd.

Adrenaline infusion - heart rate and blood samples

Time	HR	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	B/rub
0	35	0.31	80	27	136	4.9	95	2.94	0.64	0.95	150	7.4	4.5	12
30	-	0.30	72	27	136	3.9	93	2.79	0.61	1.00	141	7.3	7.6	12
60	58	0.31	78	27	135	3.8	92	2.72	0.54	0.83	150	7.1	11.8	13
90	-	0.35	75	28	134	3.6	89	2.70	0.53	0.75	168	6.9	14.5	15
120	74	0.36	78	28	135	3.6	87	2.74	0.56	0.68	141	7.4	16.5	16
150	-	0.37	81	29	137	3.5	87	2.90	0.55	0.70	150	7.0	17.7	18
180	82	0.37	82	29	137	3.6	85	2.53	0.72	0.69	159	6.9	18.3	21
210	-	0.34	81	29	135	3.6	85	2.64	0.75	0.65	159	6.7	17.3	20
240	75	0.32	82	30	134	3.5	88	2.78	0.67	0.59	177	6.8	16.6	19

CHANCE (Blood samples only)

Adrenaline infusion

Time	HR	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc	B/rub
0	40	0.29	91	17	133	4.6	94	2.97	0.72	1.15	71	3.3	5.9	8
30	-	0.28	85	16	134	3.9	93	2.75	0.66	0.95	80	3.2	9.1	9
60	62	0.28	83	16	133	3.8	92	2.77	0.71	0.81	80	3.3	12.4	9
90	-	0.28	88	16	132	3.9	90	2.71	0.72	0.66	88	3.4	16.4	10
120	80	0.29	86	17	131	3.8	88	2.76	0.76	0.63	88	3.4	17.7	12
150	-	0.29	91	17	131	3.8	87	2.71	0.77	0.65	88	3.4	18.1	12
180	88	0.29	88	17	132	3.7	87	2.70	0.77	0.70	88	3.1	18.0	13
210	-	0.28	91	17	131	3.8	88	2.66	0.77	0.76	88	3.5	16.5	14
240	72	0.27	91	17	132	3.6	89	2.66	0.75	0.79	97	3.6	15.7	13

MEENIE (Blood samples only)

Control saline infusion

Time	HR	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc
0	42	0.27	71	22	136	4.2	99	3.00	0.67	1.25	71	3.7	7.3
30	-	0.26	66	20	135	4.2	100	2.88	0.62	1.24	80	3.8	7.8
60	40	0.25	66	20	134	4.1	100	2.83	0.61	1.23	88	3.9	8.2
90	-	0.26	67	22	134	4.0	99	2.83	0.59	1.18	88	4.0	7.9
120	45	0.26	68	22	134	4.1	99	2.90	0.61	1.20	88	4.0	7.5
150	-	0.26	67	21	135	3.9	99	2.85	0.59	1.18	97	3.8	7.3
180	42	0.25	68	21	135	3.6	99	2.76	0.56	1.17	88	4.1	5.7
210	-	0.25	69	21	136	3.8	99	2.93	0.57	1.18	88	4.1	5.7
240	40	0.26	71	24	135	3.0	100	2.93	0.61	1.20	97	4.2	5.6

Adrenaline infusion

Time	HR	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc
0	45	0.32	70	20	136	4.3	99	2.63	0.52	1.03	106	4.0	4.8
30	-	0.37	69	19	138	3.6	98	2.53	0.52	0.73	115	3.6	6.7
60	88	0.35	66	18	139	3.2	96	2.47	0.54	0.69	133	3.7	8.9
90	-	0.34	63	18	138	3.3	95	2.47	0.58	0.59	141	3.7	13.2
120	118	0.33	61	18	139	3.2	92	2.49	0.64	0.61	150	3.8	16.0
150	-	0.32	62	18	136	3.2	90	2.47	0.64	0.64	150	4.0	19.6
180	121	0.33	61	18	136	3.1	88	2.25	0.61	0.72	150	3.8	21.8
210	-	0.33	64	19	135	3.5	87	2.42	0.64	0.80	150	3.7	22.7
240	90	0.34	67	20	136	2.9	88	2.27	0.62	0.83	150	3.7	21.4

MO (Blood samples only)

Adrenaline infusion

Time	HR	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Gluc
0	48	0.33	78	22	137	4.8	99	2.76	0.50	1.20	106	3.0	4.4
30	-	0.33	74	21	140	3.6	96	2.45	0.50	1.07	115	2.7	7.3
60	96	0.35	71	21	139	3.6	94	2.41	0.51	0.93	115	2.7	11.0
90	-	0.36	71	21	139	3.4	91	2.45	0.54	0.77	115	2.7	14.7
120	122	0.35	73	22	138	3.5	90	2.40	0.53	0.64	124	2.7	17.9
150	-	0.35	72	22	139	3.3	88	2.34	0.55	0.61	124	2.7	18.1
180	130	0.36	71	21	137	3.2	87	2.31	0.56	0.65	124	2.7	19.3
210	-	0.34	72	22	136	4.1	88	2.36	0.55	0.77	124	2.8	18.8
240	98	0.31	73	22	135	3.2	88	2.30	0.53	0.94	115	2.7	16.4

Experiments with washed skin (sweat samples only)

BEN

First occasion

Time	Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}	Prot	Urea
5	176	161	45	3.00	6.40	6.65	4.3
10	180	182	36	2.86	4.98	4.05	4.1
20	182	185	33	1.16	1.36	3.60	4.2
30	173	180	27	1.08	0.76	1.45	3.9
60	178	183	23	0.88	0.62	1.21	3.8
90	184	187	26	1.02	0.49	0.69	4.1
120	181	186	24	1.00	0.40	0.42	4.1
150	200	194	33	0.96	0.44	0.51	4.6
180	203	197	28	0.82	0.39	0.35	4.2

Second occasion

Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}	Prot	Urea
188	157	35	3.44	6.56	14.0	8.9
192	177	42	3.48	5.06	11.2	8.9
195	187	40	1.74	3.08	7.32	9.2
190	184	40	1.45	2.67	7.50	9.1
190	182	41	1.14	1.97	5.89	9.2
186	183	36	0.83	1.23	3.18	8,7
189	188	34	0.75	0.91	2.34	8.6
196	187	42	0.93	0.86	1.97	9.0
220	210	46	0.76	0.62	1.20	10.2

DIRK

First occasion

Time	Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}	Prot	Urea
5	186	164	37	5.10	9.00	6.60	4.7
10	184	184	29	4.04	5.58	3.87	4.5
20	186	188	22	2.82	1.94	2.24	4.6
30	182	185	18	2.44	1.34	1.92	4.3
60	194	192	19	2.50	0.76	1.06	4.6
90	188	187	18	2.37	0.76	0.63	4.7
120	188	186	20	2.37	0.82	0.54	4.8
150	209	205	20	2.23	0.53	0.30	5.4
180	212	208	22	1.24	0.44	0.25	5.0

Second occasion

Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}	Prot	Urea
190	166	45	5.04	11.50	14.20	6.9
192	186	33	4.14	6.40	9.22	6.6
194	198	28	2.22	3.80	6.10	6.8
186	198	24	1.90	2.50	5.00	6.4
182	191	18	1.99	1.78	3.33	5.8
176	181	18	1.61	1.02	2.21	5.6
176	189	17	1.56	0.76	1.27	5.7
178	195	19	1.08	0.48	0.61	6.1
208	215	24	1.30	0.55	0.44	7.0

Third occasion

Time	Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}	Prot	Urea
5	196	154	56	5.27	9.26	7.20	9.1
10	198	174	42	4.10	5.74	4.52	9.1
20	188	180	34	2.37	3.35	2.50	8.7
30	190	177	31	1.93	2.21	1.89	8.2
60	186	178	26	1.37	1.05	0.78	7.9
90	188	187	23	0.95	0.69	0.38	8.0
120	196	189	29	0.72	0.59	0.44	8.3
150	190	187	28	0.55	0.49	0.33	8.0
180	206	200	33	0.69	0.49	0.27	8.7

Fourth occasion

Time	Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}	Prot	Urea	Gluc	pH
5	186	168	41	4.40	11.20	12.25	5.3	0.4	8.4
10	184	182	35	3.26	6.16	6.32	5.4	0.5	8.3
20	180	186	21	2.64	2.40	4.90	5.2	0.7	8.4
30	184	192	20	2.28	1.96	3.55	5.2	0.6	8.3
60	192	205	19	2.18	1.68	3.08	5.4	2.0	8.5
90	186	204	18	2.35	1.40	2.70	5.5	5.2	8.1
120	188	205	17	2.30	0.94	1.43	5.6	6.2	8.2
150	190	218	23	2.34	1.15	1.04	6.0	7.7	8.2
180	195	206	27	1.32	0.84	0.99	6.2	12.5	8.4

Fifth occasion

Time	Cl^-	Na^+	K^+	Ca^{2+}	Mg^{2+}	Prot	Urea	Gluc	pH
5	180	146	40	4.78	18.60	16.60	6.7	0.3	8.7
10	168	178	17	2.84	3.60	5.13	5.9	0.5	8.7
20	179	190	19	2.82	3.42	4.82	6.4	0.4	8.7
30	190	197	20	2.85	3.20	4.37	6.6	0.7	8.7
60	184	189	19	2.65	1.94	3.49	6.3	1.9	8.6
90	186	199	17	2.43	1.40	2.40	6.6	3.6	8.6
120	200	211	20	2.44	1.09	1.45	7.2	5.1	8.6
150	204	215	22	2.46	0.99	0.95	7.4	5.9	8.5
180	209	219	25	2.23	0.86	0.61	7.6	7.7	8.6

APPENDIX V

Individual Data from Section 5

ADAM

First ride - blood samples

	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Ur.A	Gluc	Cort	Glyc	FFA	B/rub	ALP	AST	CK
Stabl	0.36	68	32	140	3.6	96	3.18	0.76	0.83	141	3.7	20	5.1	149	28	172	19	660	450	177
Start	0.43	70	34	139	4.6	96	3.01	0.74	0.77	133	4.1	-	4.4	151	34	-	21	-	-	-
16km	0.50	71	37	140	4.9	97	2.98	0.73	0.87	159	4.1	23	5.5	218	112	-	24	-	-	-
40km	0.53	79	39	141	4.9	95	3.21	0.71	1.00	177	4.5	30	5.8	320	252	695	31	792	560	509
64km	0.54	82	42	144	4.9	94	2.98	0.71	1.07	186	5.4	50	4.2	555	526	-	38	-	-	-
80km	0.56	83	44	149	4.4	95	2.96	0.74	1.09	203	5.9	112	1.9	718	1198	743	48	1036	664	1244
+½hr	0.48	84	43	149	3.3	93	2.81	9.68	0.73	194	6.7	130	3.5	671	694	-	47	-	-	-
+1hr	0.49	86	44	144	2.8	91	2.89	0.70	0.64	194	6.7	67	5.1	351	403	-	50	-	-	-
+2hr	0.50	87	46	140	2.8	92	3.13	0.79	0.97	194	6.9	50	6.7	157	213	-	52	-	-	-
+4hr	0.47	81	45	140	2.0	93	3.47	0.85	1.54	168	6.9	26	6.2	199	73	-	48	976	671	1548
+18hr	0.38	85	41	140	2.6	95	2.89	0.70	0.95	141	7.3	-	-	-	-	-	46	759	642	566

First ride - sweat samples

	Cl^-	Na^+	K^+	Mg^{2+}	Prot	Urea
0-16km	212	128	66	4.48	10.72	3.9
16-40km	160	147	40	1.72	5.46	4.1
40-64km	158	160	31	0.88	2.55	4.5
64-80km	168	172	27	0.72	1.85	6.2

First ride - post-ride urine

Elapsed Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
450	2300	1.030	8.4	176	17.1	7.81	348	55

Second ride - blood samples

	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Ur.A	Gluc	Cort	Glyc	FFA	B/rub	ALP	AST	CK
Stabl	0.33	73	35	144	2.8	99	2.89	0.69	0.89	124	4.7	10	5.5	207	64	299	20	858	413	188
Start	0.44	69	33	145	4.8	99	3.11	0.75	0.79	106	4.7	-	4.5	266	42	-	24	-	-	-
16km	0.46	71	36	145	4.7	97	2.98	0.71	0.94	150	4.9	17	5.5	265	112	-	25	-	-	-
40km	0.53	78	39	144	4.2	94	3.17	0.77	1.10	159	5.3	25	5.1	367	274	-	32	990	656	502
58km	0.52	81	41	148	4.0	96	3.26	0.80	1.00	168	6.3	61	4.1	469	683	1186	38	1069	664	792
+½hr	0.43	80	40	147	2.8	93	3.03	0.74	0.78	159	6.7	48	4.3	389	269	-	36	-	-	-
+1hr	0.45	83	40	147	3.7	98	3.18	0.72	0.79	159	6.9	42	4.7	334	157	-	41	-	-	-
+2hr	0.49	81	42	144	3.1	96	2.98	0.70	0.86	161	7.2	31	4.8	207	101	-	44	-	-	-
+4hr	0.46	85	42	144	2.0	97	2.97	0.75	1.17	150	7.5	20	5.3	119	78	-	47	1095	583	1160
+18hr	0.33	69	34	140	3.7	97	2.62	0.62	0.94	124	7.2	-	-	-	-	-	43	844	627	361

Second ride - sweat samples

	Cl^-	Na^+	K^+	Mg^{2+}	Prot	Urea
0-16km	214	184	47	3.40	8.60	5.5
16-40km	184	167	37	1.10	4.11	5.5
40-58km	178	173	31	0.61	2.10	6.4

Second ride - post-ride urine

Elapsed Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
455	2200	1.035	8.4	250	20.8	6.49	355	66

DIRK

First ride - blood samples

	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Ur.A	Gluc	Cort	Glyc	FFA	B/rub	ALP	AST	CK
Stabl	0.36	67	30	140	3.8	100	3.13	0.71	0.64	150	5.5	5	5.0	119	17	257	16	349	280	171
Start	0.44	70	36	140	4.6	99	2.84	0.71	0.65	141	5.6	-	4.2	91	73	-	21	-	-	-
16km	0.54	76	39	140	5.1	98	3.11	0.71	0.83	186	6.0	20	6.0	207	188	-	25	-	-	-
40km	0.56	76	42	142	4.9	96	3.05	0.71	0.90	194	6.9	28	6.1	287	347	713	30	514	376	1004
64km	0.61	85	45	146	4.8	95	3.13	0.71	0.90	230	8.1	70	4.4	718	706	-	41	-	-	-
80km	0.65	94	54	147	5.0	95	3.29	0.89	0.91	248	9.1	159	0.7	828	997	1181	58	679	567	2121
+½hr	0.60	89	46	149	3.6	94	2.83	0.87	0.75	239	9.7	150	1.6	828	974	-	55	-	-	-
+1hr	0.58	86	48	146	2.7	94	3.03	0.85	0.74	230	10.0	99	2.7	828	728	-	57	-	-	-
+2hr	0.55	90	50	141	2.8	92	3.00	0.86	0.97	212	10.1	60	5.4	593	319	-	66	-	-	-
+4hr	0.52	86	46	138	3.2	93	3.14	0.90	1.14	203	10.5	31	5.8	254	163	-	72	653	501	2544
+18hr	0.39	72	38	136	3.6	96	2.64	0.65	0.92	150	8.9	-	-	-	-	-	70	580	490	431

First ride - sweat samples

	Cl^-	Na^+	K^+	Mg^{2+}	Prot	Urea
0-16km	198	176	48	4.15	7.72	4.8
16-40km	170	172	26	1.24	3.40	6.5
40-64km	168	175	26	0.88	1.28	7.2
64-80km	176	184	22	0.72	0.77	8.9

First ride - post-ride urine

Elapsed Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
480	2320	1.035	8.0	333	19.3	1.41	200	70

Second ride - blood samples

	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Ur.A	Gluc	Cort	Glyc	FFA	B/rub	ALP	AST	CK
Stabl	0.40	62	33	137	3.3	102	2.98	0.73	0.64	124	3.5	15	5.0	149	22	306	21	409	312	159
Start	0.40	65	33	138	4.2	102	2.74	0.71	0.65	124	3.6	-	4.6	174	48	-	21	-	-	-
16km	0.56	70	38	137	4.3	99	2.68	0.69	0.83	150	3.8	17	5.1	229	98	-	25	-	-	-
40km	0.56	71	39	140	4.4	97	2.68	0.64	0.90	177	4.5	20	6.0	351	286	865	29	508	1060	630
64km	0.59	75	40	141	4.3	96	2.64	0.62	0.90	194	5.6	26	5.5	414	386	-	35	-	-	-
80km	0.61	78	43	144	3.9	94	2.64	0.64	0.91	203	6.4	47	3.5	676	616	1215	42	607	1187	1443
+½hr	0.51	74	39	142	3.1	96	2.62	0.62	0.75	194	6.9	36	4.1	598	398	-	43	-	-	-
+1hr	0.49	79	39	139	2.4	96	2.64	0.62	0.74	186	7.3	27	4.5	555	198	-	46	-	-	-
+2hr	0.51	78	41	138	2.8	97	2.87	0.74	0.97	177	7.9	19	5.8	317	112	-	49	-	-	-
+4hr	0.48	73	38	137	3.9	98	2.75	0.85	1.14	177	8.7	16	5.3	199	48	-	48	521	1084	1231
+18hr	0.37	66	33	134	3.5	98	2.72	0.61	0.92	133	7.0	-	-	-	-	-	62	495	963	332

Second ride - sweat samples

	Cl^-	Na^+	K^+	Mg^{2+}	Prot	Urea
0-16km	190	178	31	3.20	5.45	5.3
16-40km	184	162	30	3.44	2.32	5.3
40-64km	170	158	28	1.04	1.75	5.7
64-80km	158	155	22	0.69	0.63	6.1

Second ride - post-ride urine

Elapsed Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
438	2660	1.027	8.2	127	14.1	90.1	211	140

JOHNNY WALKER

First ride - blood samples

	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Ur.A	Gluc	Cort	Glyc	FFA	B/rub	ALP	AST	CK
Stabl	0.41	69	34	141	2.7	99	3.01	0.73	1.13	133	5.4	15	5.0	187	17	370	49	429	217	177
Start	0.49	73	41	137	5.0	98	3.38	0.76	0.93	124	5.3	-	4.2	199	28	-	49	-	-	-
16km	0.55	72	41	138	5.0	98	3.19	0.73	0.92	150	5.5	20	5.0	240	106	-	52	-	-	-
40km	0.55	77	44	139	5.3	97	3.27	0.80	0.96	168	6.0	29	5.9	370	274	992	61	455	309	332
64km	0.60	85	47	144	5.7	96	3.50	0.77	1.13	186	6.6	61	3.7	497	638	-	75	-	-	-
80km	0.64	90	52	147	4.7	93	3.42	0.98	1.15	194	7.2	213	1.2	718	963	2172	94	640	369	976
+½hr	0.57	84	47	149	3.8	94	3.19	1.01	0.65	194	7.6	233	1.8	718	795	-	88	-	-	-
+1hr	0.60	90	51	142	3.0	89	2.91	0.97	0.65	194	7.8	192	2.5	747	560	-	96	-	-	-
+2hr	0.59	92	52	141	2.4	93	3.27	1.06	0.64	177	8.7	127	5.9	593	241	-	108	-	-	-
+4hr	0.54	81	45	136	3.4	95	3.65	0.93	0.70	168	8.9	57	6.8	287	45	-	93	547	317	877
+18hr	0.47	79	41	139	3.2	98	3.00	0.79	0.93	141	7.9	-	-	-	-	-	88	547	350	269

First ride - sweat samples

	Cl^-	Na^+	K^+	Mg^{2+}	Prot	Urea
0-16km	194	160	54	4.46	10.25	3.9
16-40km	152	151	32	1.54	4.37	5.8
40-64km	164	172	29	1.16	1.94	6.4
64-80km	162	174	22	0.73	1.23	7.3

First ride - post-ride urine

Elapsed Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
485	2950	1.030	8.5	215	13.3	19.7	306	62

Second ride - blood samples

	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Ur.A	Gluc	Cort	Glyc	FFA	B/rub	ALP	AST	CK
Stabl	0.38	68	37	143	2.5	100	2.72	0.75	1.11	124	5.7	8	5.1	157	45	513	36	534	236	118
Start	0.49	66	36	140	4.2	99	3.26	0.78	1.06	124	5.7	-	4.3	179	34	-	40	-	-	-
16km	0.51	70	39	143	4.8	95	3.11	0.77	1.06	150	5.9	22	6.0	188	162	-	48	-	-	-
40km	0.54	77	43	146	4.0	95	3.25	0.79	1.05	159	6.4	36	5.2	414	370	1291	58	600	273	233
64km	0.61	84	46	153	4.6	97	3.43	0.90	1.15	186	7.5	92	2.7	635	706	-	73	-	-	-
80km	0.65	87	48	154	4.6	98	3.70	1.01	1.10	194	7.9	145	1.5	721	1064	1445	85	910	332	679
+½hr	0.59	80	47	157	4.1	97	3.39	0.96	0.73	186	8.4	135	3.0	593	806	-	84	-	-	-
+1hr	0.58	81	45	142	2.1	94	3.31	0.83	0.63	177	8.6	97	5.4	334	308	-	90	-	-	-
+2hr	0.55	79	44	146	2.9	93	3.36	0.81	0.62	177	8.7	76	5.9	240	252	-	91	-	-	-
+4hr	0.50	72	41	138	3.5	96	3.31	0.80	0.89	159	9.0	31	8.2	177	48	-	80	640	332	587
+18hr	0.43	64	36	138	3.5	96	2.64	0.69	0.83	141	8.0	-	-	-	-	-	69	594	273	198

Second ride - sweat samples

	Cl^-	Na^+	K^+	Mg^{2+}	Prot	Urea
0-16km	164	156	32	2.90	7.80	4.7
16-40km	158	157	30	0.76	4.32	4.6
40-64km	162	172	24	0.56	1.61	4.5
64-80km	172	184	20	0.42	1.76	6.4

Second ride - post-ride urine

Elapsed Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
368	1600	1.035	8.6	287	20.4	4.09	351	41

SMOKEY

First ride - blood samples

	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Ur.A	Gluc	Cort	Glyc	FFA	B/rub	ALP	AST	CK
Stabl	0.40	68	33	139	4.0	98	3.11	0.67	1.07	115	4.5	20	4.4	229	31	412	23	587	232	166
Start	0.46	74	42	139	4.9	97	3.03	0.68	1.07	115	4.8	-	3.6	118	25	-	24	-	-	-
16km	0.51	76	42	140	5,1	94	2.66	0.62	1.12	150	4.8	36	6.3	367	162	-	28	-	-	-
40km	0.53	81	46	140	4.7	92	3.20	0.65	1.14	168	5.4	82	6.5	718	198	-	35	739	309	452
61km	0.56	87	48	144	4.5	94	3.25	0.71	1.09	177	6.5	149	2.1	986	706	1167	45	805	328	792
+½hr	0.46	77	43	140	3.9	94	3.02	0.64	0.72	159	7.0	147	4.6	593	482	-	46	-	-	-
+1hr	0.45	77	44	138	3.0	93	3.14	0.73	0.85	150	7.5	101	6.6	467	123	-	46	-	-	-
+2hr	0.47	77	45	139	2.7	94	3.06	0.69	0.93	141	7.7	84	5.0	351	95	-	47	-	-	-
+4hr	0.44	78	44	141	3.6	94	2.93	0.72	1.35	141	7.7	37	-	265	67	-	49	726	317	792
+18hr	0.38	80	38	138	3.6	93	2.57	0.62	0.98	115	7.2	-	-	-	-	-	41	613	302	187

First ride - sweat samples

	Cl^-	Na^+	K^+	Mg^{2+}	Prot	Urea
0-16km	232	188	62	3.00	6.41	-
16-40km	160	161	25	0.74	2.72	-
40-61km	163	175	23	0.60	1.74	-

First ride - post-ride urine

Elapsed Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
420	3660	1.027	8.3	220	12.3	62.9	271	71

Second ride - blood samples

	PCV	Prot	Alb	Na^+	K^+	Cl^-	Ca^{2+}	Mg^{2+}	PO_4^{2-}	Creat	Urea	Ur.A	Gluc	Cort	Glyc	FFA	B/rub	ALP	AST	CK
Stabl	0.41	70	39	139	3.0	98	2.77	0.67	0.87	124	4.9	15	4.6	97	17	357	28	673	306	188
Start	0.42	67	38	137	4.1	99	2.93	0.67	0.88	124	5.0	-	4.2	279	22	-	27	-	-	-
16km	0.52	74	41	137	4.6	96	2.95	0.72	1.06	141	4.9	30	5.0	334	76	-	32	-	-	-
40km	0.56	80	46	138	4.4	96	3.00	0.70	1.10	159	5.4	41	6.2	676	314	898	41	832	399	332
64km	0.61	84	47	143	4.7	98	3.30	0.78	1.08	186	6.4	119	3.4	828	773	-	50	-	-	-
71km	0.59	84	48	144	3.9	98	3.16	0.83	1.02	186	7.0	166	2.4	883	1131	1466	54	977	413	792
+½hr	0.51	78	45	143	3.6	99	3.24	0.79	0.76	168	7.5	163	4.4	778	571	-	51	-	-	-
+1hr	0.48	74	43	140	3.6	103	3.18	0.79	0.83	159	7.7	110	5.5	718	308	-	50	-	-	-
+2hr	0.47	71	42	138	2.7	100	2.94	0.91	0.91	159	7.8	63	5.1	389	101	-	47	-	-	-
+4hr	0.43	69	41	138	2.8	100	2.34	0.79	1.06	150	8.2	33	6.7	207	56	-	44	752	351	665
+18hr	0.39	67	39	137	3.3	98	2.71	0.59	1.14	124	6.9	-	-	-	-	-	43	673	389	278

Second ride - sweat samples

	Cl^-	Na^+	K^+	Mg^{2+}	Prot	Urea
0-16km	192	162	36	5.00	4.90	6.3
16-40km	168	158	27	2.40	3.18	5.3
40-64km	153	157	22	0.80	0.86	6.0
64-71km	173	180	21	0.88	1.21	7.0

Second ride - post-ride urine

Elapsed Time	Vol	SG	pH	Urea	Creat	Na^+	K^+	Cl^-
317	3290	1.022	8.5	117	8.5	152	172	109

Individual Free Fatty Acids (as percentage of total)

	ADAM			DIRK		JOHNNY WALKER			SMOKEY	
First ride	Stable	40km	80km	Stable	40km	80km	Stable	40km	80km	Stable	61km
C12:0	2.45	0.31	0.47	1.04	0.21	0.22	0.10	0.35	0.07	0.33	0.27
C12:1	-	-	-	0.21	-	0.07	0.07	0.20	0.03	0.13	0.05
C14:0	11.86	3.23	3.16	7.70	3.35	2.56	2.78	3.70	1.90	1.81	2.37
C14:1	5.20	1.41	1.19	2.02	2.27	0.56	1.56	1.71	1.02	1.41	1.72
C14:2	-	1.88	0.05	2.74	-	0.92	1.09	2.13	-	-	-
C16:0	36.92	26.62	27.99	25.37	28.75	23.37	29.73	27.02	31.63	23.98	28.02
C18:0	15.06	13.28	10.79	20.19	13.51	14.73	22.76	11.49	11.10	18.09	9.68
C18:1	17.71	30.79	33.65	26.07	31.42	33.36	23.08	24.80	36.00	20.61	33.91
C18:2	6.99	15.54	16.42	10.78	15.29	18.04	15.80	14.92	12.20	23.28	16.37
C18:3	1.47	6.91	6.12	3.95	5.18	6.20	2.99	6.56	5.99	12.38	7.96

Second ride	Stable	58km	Stable	40km	80km	Stable	40km	80km	Stable	40km	71km
C12:0	0.38	0.04	0.35	0.47	0.34	0.29	0.33	0.16	0.10	0.24	0.35
C12:1	0.15	0.28	0.31	0.27	0.10	0.08	0.09	0.05	0.18	0.06	0.01
C14:0	1.89	2.25	1.65	4.37	3.36	2.98	3.38	2.75	2.09	3.02	2.97
C14:1	0.69	1.05	1.10	1.66	2.09	0.61	3.10	1.72	2.24	1.37	1.42
C14:2	-	1.76	0.67	0.95	1.02	2.42	-	-	-	-	-
C16:0	22.61	28.16	19.15	30.06	32.51	32.94	28.27	27.75	22.24	29.18	29.06
C18:0	27.06	13.83	21.11	10.20	8.89	12.59	10.07	7.13	18.71	10.55	8.53
C18:1	22.94	29.26	18.92	29.13	29.22	35.28	29.51	35.29	17.68	34.30	35.13
C18:2	17.95	16.61	24.12	17.23	16.30	10.19	18.05	17.79	27.62	17.37	18.28
C18:3	6.51	6.80	12.78	5.70	6.14	2.70	7.16	7.40	8.96	4.12	5.63

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