Studies on the intermediary metabolism of the ox with special reference to the sympatho-adrenal system

by

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A study is made of the interrelationships of the concentrations of glucose, potassium, adrenaline, noradrenaline and the lower volatile fatty acids (VFA) in the blood of the ox.

An examination of biological and chemical methods for adrenaline and noradrenaline determination in the jugular vein plasma of the ox revealed that a fluorimetric method involving condensation with ethylenediamine was suitable. Polythene cannulae, implanted in the jugular vein were used to obtain serial blood samples without any psychic disturbance of the experimental animals. Under these conditions of blood sampling the mean normal concentration of adrenaline in bovine plasma was found to be 0.31 (range 0.03-0.7) µg/l. and that of noradrenaline 2.5 (range 0.4-4.6) µg/l.

Intravenous administration of insulin caused blood glucose concentration to fall, and the plasma concentrations of adrenaline and noradrenaline rose when blood glucose levels reached a critical level between 15 and 25 mg/100 ml. The increase in plasma adrenaline concentration was greater than that of noradrenaline. The increase in plasma noradrenaline concentration during hypoglycaemia is peculiar to the ox, and the physiological importance of noradrenaline relative to that of adrenaline was assessed by determining its ability to raise the blood glucose concentration. It was found that adrenaline:noradrenaline hyperglycaemic ratios for the ox ranged from 0.79 to 6.58, figures which are much lower than those of other species. It was concluded that in the ox the release of noradrenaline during insulin hypoglycaemia is physiologically significant.

Insulin administration caused a fall in plasma VFA levels,

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which was quantitatively indistinguishable from a fall that occurred following glucose administration. Arterio-venous studies suggested that this effect of insulin and glucose was not due to any enhancement of utilization of VFA by the extrahepatic tissues.

Other workers have shown that intravenous administration of sodium propionate or sodium butyrate would relieve hypoglycaemic convulsions in ruminant animals. The possibility that this may have been the result of sympatho-adrenal stimulation was investigated. Intravenous sodium propionate and occasionally intravenous sodium butyrate administration resulted in elevated plasma levels of adrenaline and noradrenaline. Sodium acetate administration caused a depression in plasma adrenaline and noradrenaline levels especially the adrenaline levels. Intravenous glucose administration also depressed plasma levels of adrenaline and noradrenaline. It was concluded that, with the possible exception of sodium propionate, the sympatho-adrenal system of the ox plays little part in producing the changes in blood glucose that result from intravenous injections of the sodium salts of the lower fatty acids. The rate of disappearance from the circulation of the injected acetate and propionate was inversely related to the initial plasma VFA and blood glucose levels respectively.

The intravenous administration of insulin, glucose, or the sodium salts of the lower fatty acids all produced hypokalaemia.

Studies on the intermediary metabolism of Bos indicus, a species of cattle indigenous to tropical areas and of Bos taurus, cattle indigenous to temperate zones, revealed that Bos indicus possessed higher normal blood glucose levels, a lower sensitivity to insulin, slower utilization of intravenously administered glucose and a lower plasma potassium concentration than Bos taurus. These differences were attributed to a higher degree of adreno-cortical activity in Bos indicus.

The results of these studies are compared to similar studies in non-ruminant animals, taking account of the differences in their dietary intake and mode of digestion. The comparative studies on the two species of Bos are discussed in relation to their physiological responses to their environment.

STUDIES ON THE INTERMEDIARY METABOLISM OF THE OX, WITH SPECIAL REFERENCE TO THE SYMPATHO-ADRENAL SYSTEM

A thesis submitted to the University of Glasgow for the degree of Doctor of Philosophy in the Faculty of Medicine

by

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INTRODUCTION

Dietary carbohydrate of ruminants is fermented in the rumen with the production of steam volatile fatty acids (VFA), particularly acetic, propionic and butyric, and these provide much of their energy supply, and are considered to replace to some extent glucose as an energy source (Lindsay, 1959). This fact tends to be reflected in the relatively low blood sugar values found in ruminant animals (Reid 1950a).

On account of the quantitative differences between ruminants and non-ruminants, the hormonal control of carbohydrate metabolism in the ruminant has received much attention.

Insulin is one of the hormones responsible for the maintenance of a constant blood sugar level. Amadon (1928) gave a total of 1280 units intravenously in two doses to a cow and blood sugar levels fell to 23.5 mg/100 ml. which produced coma at this level. Petersen et al. (1931) gave up to 1000 units in 200 unit doses and an 800 unit single dose intravenously to each of three cows and, although the blood sugar levels dropped to as low as 17 mg/100 ml. only slight muscular tremors were noted. Gowen & Lobey (1931) gave repeated intravenous injections of insulin to four cows in doses varying from 500 to 1200 units. In one animal they produced coma for 48 hr while other animals receiving larger doses showed no effects. The blood sugar dropped to very low levels although no figures are given. Brown et al. (1936) also gave repeated intravenous injections of 200 to

1000 units of insulin with occasional muscular tremors resulting. The blood sugar never fell below 10 mg/100 ml. Jasper (1953) gave insulin to cattle and, at dosages greater than 2 units/kg body weight increasing the dosage only prolonged the hypoglycaemia. Mild hypoglycaemic signs were noted in one cow receiving consecutive doses of 2, 5 and 10 units/kg body weight. Prolonged insulin hypoglycaemia resulted in convulsions usually occurring 33 to 36 hr after the initial hypoglycaemia. The blood sugar levels at which convulsions occurred varied from 5 to 21 mg/100 ml. He concluded that the duration of hypoglycaemia was of primary importance in determining the onset of convulsions rather than the degree of hypoglycaemia. The blood sugar levels did not reach a steady state at a minimum of 5 mg/100 ml. under maximum insulin influence, as was observed in a detailed study of insulin hypoglycaemia in sheep made by Reid (1951a). In one experiment Reid failed to detect any sugar in the blood after insulin administration but on only one other occasion did blood sugar levels fall below 5 mg/100 ml. He concluded from his work on insulin hypoglycaemia that sheep show a greater resistance to insulin than non-ruminants because:

(a) the rate of fall of blood glucose is slower than that of non-ruminants, and

(b) blood sugar values do not reach levels low enough to produce obvious hypoglycaemic signs.

From the work of Jasper (1953) this also appears to be true of the ox, although signs of hypoglycaemia were occasionally observed.

Insulin induced hypoglycaemic convulsions have been produced in sheep following adrenalectomy (Strand et al. 1934), where convulsions supervened after 50 min. Reid (1951b) similarly reports insulin-induced convulsions following adrenalectomy in the sheep but gives no details. Potter (1952) reported that insulin produced hypoglycaemic convulsions in sheep following section of the splanchnic nerves, but no work of this nature has been done in the ox. Splanchnic section stops the reflex liberation of catecholamines from the adrenal medulla (Duner, 1953) and it would thus appear that the liberation of catecholamines during insulin hypoglycaemia is partially at least responsible for the tendency of the blood sugar level not to drop below 5 mg/100 ml. and also protects the animal against hypoglycaemic convulsions. Setchell and Waites (1963) have shown that adrenaline-like substances are released during insulin hypoglycaemia in sheep. However, no direct evidence of increased catecholamine secretion has been obtained, and the relative proportions of adrenaline and noradrenaline released are unknown.

As hypoglycaemia increases catecholamine secretion, so hyperglycaemia depresses it (Duner, 1953). This observation led Duner to postulate an inverse relationship between catecholamine production and blood sugar levels. His work on hyperglycaemia has not been confirmed but the work on insulin hypoglycaemia of Armin & Grant (1959) with rabbits, and of Setchell & Waites (1963) with sheep would suggest that no such relationship exists, since catecholamine production is

increased only when blood sugar levels have dropped below a critical level.

One of the functions of adrenaline released during hypoglycaemia is to release glucose from the liver and help restore the blood sugar level. Garner (1952) in adult cattle, and Schultze (1959) in calves have shown that adrenaline will elevate blood sugar levels. Nothing is known of the hyperglycaemic action of noradrenaline in the ox.

The metabolism of acetic acid, the major fatty acid produced in the rumen, is closely related to that of glucose; Jarrett & Filsell (1961) have shown that prior administration of glucose facilitates the removal of injected acetate from the circulation. It might be expected therefore that insulin administration would affect VFA metabolism indirectly through its effect on glucose metabolism. Jarrett & Potter (1957) have shown that removal of injected VFA especially acetate is impaired in pancreatectomized sheep. McClymont (1951b) reported that hyperinsulinism did not affect mammary arterio-venous VFA differences. He quotes Reid as recording similar findings with respect of carotid arterial, jugular venous VFA differences. Reid (1950b) assumed that muscle is the main metabolically active component of the tissues of the head and deduced from his studies that insulin had no effect on the VFA uptake of muscle. His work thus supports the findings of Villee & Hastings (1949) who showed that, <u>in vitro</u> insulin had no effect on the uptake of acetate by muscle. It has been shown, <u>in vitro</u>, that insulin enhances the incorporation of acetate into fatty acids by the liver

(Bloch & Kramer, 1948), but this effect has not been demonstrated *in vivo*.

A study has been made of the ability of acetic, propionic and butyric acids to relieve hypoglycaemic convulsions in lambs and splanchnicotomized ewes following insulin administration. Propionic acid and butyric acid rapidly relieved hypoglycaemic convulsions and produced a rise in blood sugar levels, but acetic acid was ineffective (Potter, 1952). These experiments have been repeated using [carboxy-^{14}C] butyrate and [carboxy-^{14}C] propionate, and it was concluded that the rise in blood sugar produced by the injection of fatty acids was not due to gluconeogenesis from these acids (Ash *et al.* 1959). Jarrett *et al.* (1952) noted that following the intravenous injection of butyrate into sheep, their animals showed tachypnoea and suggested that the rise in blood sugar might be due to stimulation of the sympatho-adrenal system. Ash *et al.* (quoted by Lindsay, 1959) confirmed that the effect is due mainly to sympathetic stimulation since with butyrate, at least, the effect is much reduced by splanchnicotomy or treatment with adrenergic blocking agents. However, direct evidence obtained by measuring the plasma catecholamine content following these procedures has not been obtained.

The diet of ruminants compared with that of man and dog contains a large amount of potassium and relatively small amounts of sodium (Morrison, 1951). Much of the potassium is absorbed from the rumen into the blood (Parthasarathy & Phillipson, 1953). Anderson & Pickering

(1962) have demonstrated that the bovine kidney has an enhanced capacity to excrete potassium ions when compared with the kidney of the dog, and plasma potassium levels are maintained at levels similar to those found in carnivores. There is an apparent relationship between plasma potassium and blood sugar metabolism (Fenn, 1940a), but few studies on this relationship in the ruminant animal have been undertaken.

Insulin is known to cause a fall in plasma potassium (Harrop & Benedict, 1923). This was thought to be due to the reflex liberation of catecholamines, which themselves have a similar action (Dury et al. 1952). Later work, however, has shown that the actions of insulin and adrenaline are quite distinct (Dury, 1952). Grob et al. (1957) showed that there was a loss of potassium from the muscles during insulin hypoglycaemia and Farber et al. (1951) reported similar changes showing that the liver is possibly the main organ associated with the hypokalaemia.

The intravenous injection of glucose similarly causes a drop in plasma potassium concentration (Flock et al. 1938). Grob et al. (1957) presented evidence for the uptake of potassium by muscle during hyperglycaemia and suggested that this may partially explain the fall. However, Farber et al. (1951), in a similar study, reported a loss of potassium from muscle after administration of glucose. The question is complicated by the reflex secretion of endogenous insulin, resulting from the hyperglycaemia and the effect may be due to insulin per se, since the latter authors were unable to

show a hypokalaemia in diabetic subjects following glucose administration.

Setchell & McClymont (1955) reported that in sheep insulin causes a fall in plasma potassium levels of a similar order to those recorded in man.

The work presented here has studied the inter-relationships of carbohydrate, VFA, potassium and catecholamine metabolism in cattle. As indicated, the metabolism of these substances in cattle and other ruminants presents special physiological problems because of the nature of their diet and their mode of digestion.

Experiments are described in which the effect of insulin on the concentration in blood or plasma of these substances is studied. Arising out of these initial experiments, further experiments examined the relative hyperglycaemic actions of adrenaline and noradrenaline, and a detailed study was made of the effects of insulin and glucose on plasma VFA and potassium levels. Since the intravenous injection of some VFA are able to relieve hypoglycaemic convulsions and raise blood sugar levels, the possibility that this may be the result of sympatho-adrenal stimulation was examined.

Finally, there is a section on comparative aspects of these findings. The opportunity arose to perform some experiments on two types of cattle, Bos indicus, the humped animal of tropical areas, and Bos taurus, the animal indigenous to temperate zones. Linnaeus (1758), on morphological grounds separated Bos indicus and Bos taurus

into two species but the fact that they interbreed and produce fertile offspring indicates that the relationship between the two is less than a species difference, and they probably represent types or varieties of a common stock, now extinct. Physiologically they are quite distinct, Bos indicus being well adapted to a tropical environment (Findlay, 1950), having a different water metabolism (Phillips, 1961a), an improved digestibility of fibre (Phillips, 1961b), and having marked haematological differences (Evans, 1963). Comparisons were made, therefore, of certain aspects of their intermediary metabolism.

PART I

CHAPTER I

METHODS & MATERIALS

(1) Determination of catecholamines in plasma

The methods available for the quantitative determination of catecholamines in biological fluids are numerous, but few of them can be used for determining the concentrations in peripheral plasma. A study was made of the more commonly used methods.

(a) Biological method

Plasma amines were separated by the chromatographic method of Vogt (1952). Noradrenaline was assayed by the method of Brown & Gillespie (1957) and adrenaline by a method described by Gaddum et al. (1949). For noradrenaline assay, rats of approximately 200 g were used. For adrenaline assay, the uteri of virgin rats were used. Precautions were taken to ensure that the rats were not in oestrus since uteri from animals in oestrus were liable to show spontaneous activity. An automatic assay apparatus similar to that described by Lewis & Eaton (1957), was constructed which delivered 4μg of acetylcholine to the uterus every 3 min, allowed it to act for 15 sec and then delivered 5 ml. of the bathing solution to wash off the administered drug. Standards and plasma extracts were added to the bath immediately after the washing.

(b) Chemical methods

(i) The method of Euler & Floding (1955) with the modifications made by Robinson & Stott (personal communication), Price & Price (1957) and Euler & Lishajko (1961) was used. Readings were taken with a sufficiently sensitive fluorimeter using the following filter system:

Primary - filter combination (IF3) isolating the 436 mμ wavelength.

Secondary - yellow filter (Chance OY4) giving a cut-off at 510 mμ.

(ii) The method of Weil-Malherbe & Bone (1952, 1953) with the modifications of Aronow & Howard (1955), Millar (1956) and Renton & Weil-Malherbe (1956) was used. Primary filters were as above and secondary filters were Ilford 623 giving maximum transmission at 490 mμ, and IF9 giving a sharp cut-off at 600 mμ.

The anticoagulant used in the chemical methods was fluoride-thiosulphate (Weil-Malherbe & Bone, 1952), and in later experiments ethylenediamine-tetra-acetate (EDTA) and sodium thiosulphate (Renton & Weil-Malherbe, 1956). Blood was collected directly into the anticoagulant solution in an all-glass syringe, transferred to chilled centrifuge tubes and spun immediately for 10 min at 1750 x g.

Plasma was removed and stored in a deep-freeze cabinet (-25°C) until adsorption. Adsorption and elution of catecholamines was carried out by the method of Weil-Malherbe & Bone (1952) and eluates were kept at -25°C until the

condensation and extraction procedures. Compressed nitrogen was used to force the plasma through the aluminium oxide columns. This was done using a modification of the apparatus described by Bone (1953). The modification had an improved pressure regulator (Fig. 1.) and allowed the simultaneous adsorption of four samples.

A 'Locarte' fluorimeter (The Locarte Co., London) was used for the fluorescence determinations. The photomultiplier was set at maximum sensitivity and the light falling on the cuvette was reduced to a minimum by partial closure of the iris diaphragm.

In all the methods used standards were prepared by making a 1:1000 dilution of the commercial preparation (containing 1 mg per ml.) in 0.01 N-hydrochloric acid. This stock solution thus contained 1 g/ml. and was further diluted as necessary. The 1:1000 dilution was made by accurately measuring, with an 'Agla' micrometer syringe (Burroughs, Wellcome & Co.) 0.1 ml. into a 100 ml. volumetric flask and filling it up to the mark. The commercial preparations used were adrenaline chloride solution (Parke-Davis & Co. Ltd.) and noradrenaline bitartrate ('Levophed', The Bayer Products Co.).

(2) Determination of blood sugar

Blood sugar was determined by the method of King (1946). Blood was collected into heparin and proteins precipitated within 15 min of collection. Readings were made with a Beckman Model B spectrophotometer. (Beckman Instruments Inc.,

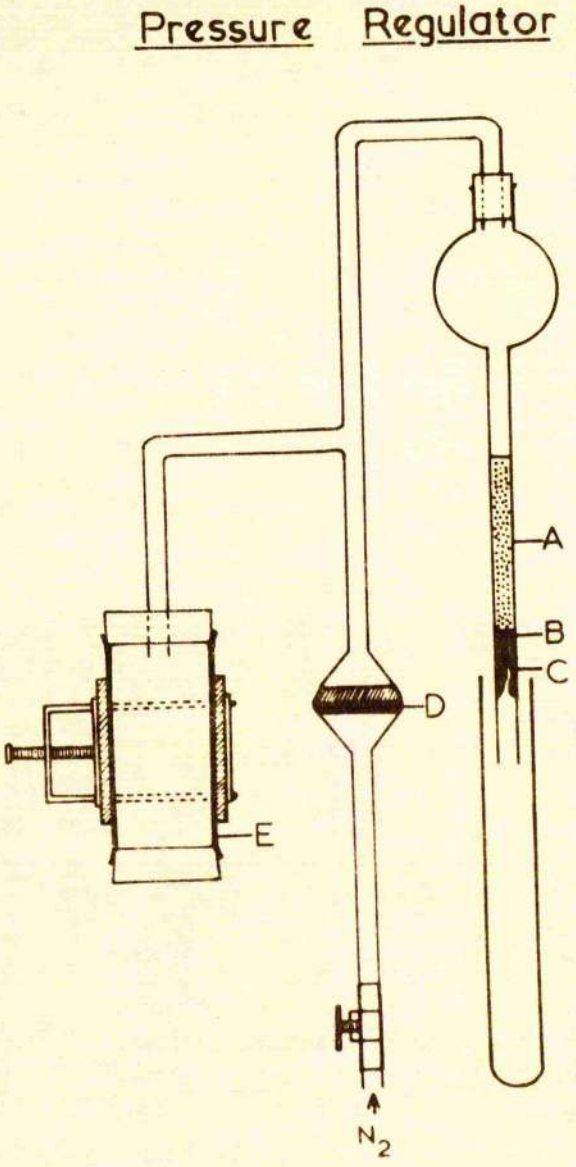


Fig. 1. Pressure regulator for adsorption of catecholamines.

A = alumina column

B = glass wool plug

C = constriction in tubing

D = no-return valve made of layer of mercury on sintered glass

E = wide bore rubber tubing compressed by a screw clamp, which can be used to increase the pressure within the system

N_2 = nitrogen from a cylinder supplying a rack of regulators

U.S.A.). The ability of the method to measure true blood glucose was tested by yeast fermentation studies on plasma and lysed blood. Samples of plasma and lysed blood were precipitated by the addition of 0.5 ml of 2/3 N H_2SO_4 and 0.5 ml. of 10% sodium tungstate. The supernatant liquid was fermented for 60 min at 37°C with washed fresh bakers' yeast. A control glucose solution in water was carried through the same procedure. Erythrocyte concentrations of sugar were determined by the method of Trimble & Maddock (1928).

(3) Determination of plasma volatile fatty acids

Plasma total volatile fatty acids were determined by the method of Scarisbrick (1952). Chemically pure water obtained by glass distillation of deionised water, prepared by a Deminrolit deioniser (Permutit Co. Ltd., London) was used for steam production. The steam volatile acids were titrated with standardized barium hydroxide solution using bromo-thymol blue as indicator, following the removal of carbon dioxide by the bubbling of nitrogen for 10 min. The industrial nitrogen contained acid impurities which were removed by passing it through a gas washing bottle containing weak sodium hydroxide solution, followed by a series of bottles containing pure water. The bottles contained bromo-thymol blue indicator to test the efficiency of the process.

(4)Determination of plasma potassium

Plasma potassium estimations were made on an EEL flame

photometer (Evans Electroselenium Ltd.) using a technique similar to that described by Duthie & McDonald (1960). Corrections for mutual interference were not made since the error introduced is small and, in plasma, constant. Erythrocyte concentrations of potassium were determined by the method described by Evans (1957). Deionised water was used for the 1:50 dilution of the plasma and for the preparation of standards. Two standards were used for the calibration of the instrument, one containing 10 μg/ml. and the other 5μg/ml.

(5) Determination of haematocrit

Haematocrit determinations were made in triplicate using Wintrobe tubes. The tubes were centrifuged for 30 min at 1750 x g. (Dacie, 1950).

The determination of volatile fatty acids and potassium in plasma and sugar in blood was done in duplicate.

(6) Experimental animals and sampling methods

Twenty-nine castrated male cattle were used. The animals of the Bos taurus type were of various breeds, mainly Hereford, of known pedigree, two British Friesians and one Jersey. The Bos indicus animals were all pure Boran of the same herd and were therefore a more homogeneous sample than the Bos taurus animals. Usually the animals were acquired as calves and subsequently hand-suckled and weaned. They were weaned at between 12 and 18 weeks of age, when a little concentrate feeding was given, otherwise the only supplementary feeding was good quality hay given at night when they were

penned. Good quality grazing was available during the day. The diet was therefore fairly uniform, a factor which is essential in experiments of this nature (Reid & Mills, 1961). Animals were considered to be suitable for use 12 weeks after weaning. The animals were weighed weekly and only those showing a steady weight gain over a period of 4 weeks were used. If the experimental procedure caused a loss in weight the animal was not used again until the above criterion had been fulfilled.

At 2 p.m. on the day before an experiment the animal was put into a pen without food or bedding but with access to water, and received no food until after the experiment. The length of starvation before the experiment began was therefore approximately 19 hr.

All the animals were trained to stand in wooden stocks without restraint. Serial blood samples were taken by means of a polythene cannula inserted in the jugular vein. The cannulae were inserted the day before the experiment, at a place away from the stocks, so that the animals did not associate the cannulation procedure with the stocks, and therefore remained quiet for the duration of the experiment. Depending on the type of experiment, either one jugular vein or both were used. The technique of cannulation was as follows:

All equipment was sterilized by boiling or by rectified spirit. The site over the jugular vein was infiltrated with 2.5 ml. of 5% procaine solution. It was then shaved and sterilized with rectified spirit. The vein was raised by

Fig. 2. Polythene cannula in the jugular vein for serial blood sampling.

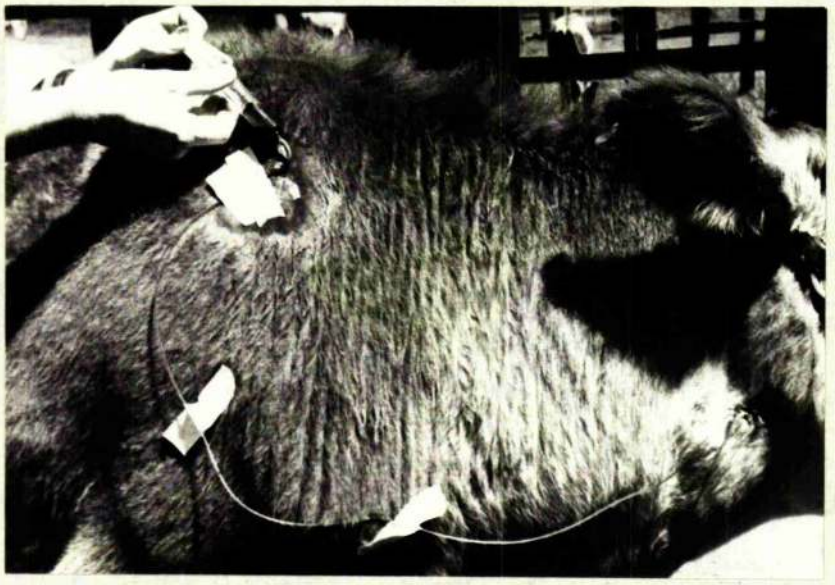


Fig. 3. Withdrawal of blood sample. Fixation of cannula to the skin of the scapula region allows minimal disturbance of the animal.

constriction central to the prepared site, and a 12 s.w.g. 2½ in. needle inserted into the vein until the blood was running freely. A polythene tubing cannula (1½ mm external diameter) was then inserted into the bore of the needle, and thence into the lumen of the vein. The constriction on the vein was released and the cannula inserted to about the level of the first rib. The needle was then withdrawn leaving the cannula within the vein, and rubber tubing tied on to the distal end of the cannula, which served as an adaptor for the syringes. The cannula was filled with normal saline containing heparin (25 units/ml.) and the end closed by a bulldog clamp, thus leaving the cannula permanently filled with an anticoagulent solution.

On the following day the cannula was attached to a shaved area over the scapula by a piece of plaster (Fig. 2).

By this technique blood samples could be taken without any disturbance to the animal (Fig. 3). In order to get a true sample of venous blood the first 2 ml. of blood and saline withdrawn were discarded. Blood could be withdrawn at the rate of 1 ml./sec. The cannulae were used also for the administration of fluids. Where the fluid administered could contaminate the subsequent blood samples, e.g. with glucose, the fluid was administered by a cannula in the other jugular vein.

Arterial blood samples were taken from a cannula in the carotid artery. The carotid artery was previously exteriorised by a technique similar to that described by McClymont (1950), except that the loop was established ventral to the

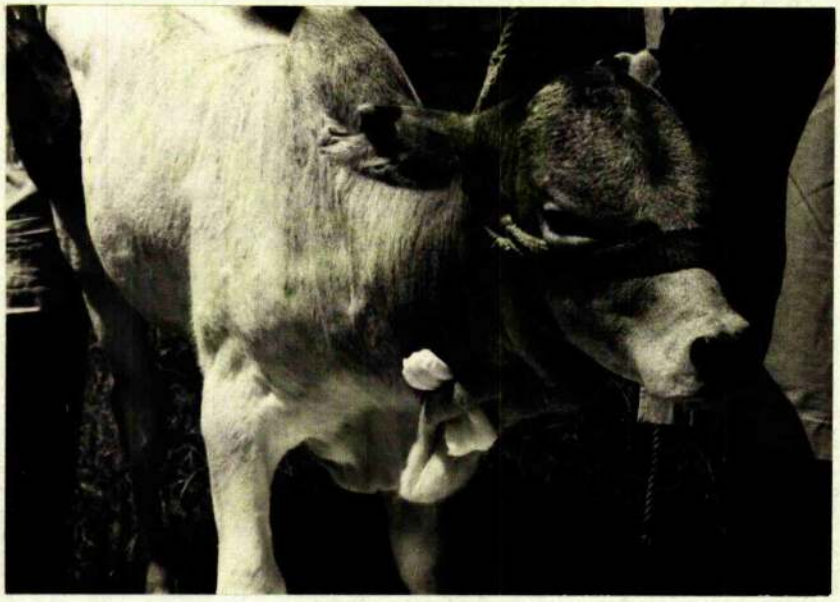


Fig. 4. Animal with exteriorized carotid artery.

Fig. 5. Cannulation of the ipsilateral jugular and carotid vessels.

jugular vein (Fig. 4). The best results were obtained with younger animals, i.e. animals that were less than 12 weeks old when the skin was relatively thin. Simultaneous arterial and venous blood samples could be taken by having cannulae in the carotid and jugular vessels (Figs. 5 and 6).

Infusions were made by having a reservoir suspended about 4 ft. above the animal and connected through a Murphy drip tube to a cannula in the jugular vein. The drip tube delivered twenty drops per ml. and the rate of flow was adjusted by a screw clip. By counting the drops the volume delivered in a given time could be calculated to the nearest 0.5 ml.

Insulin injection B.P. (A.B. Allen & Hanbury's Ltd. 80 units/ml.) was used.

All pH measurements were made on a Pye 'Master' pH meter, using phosphate buffers for standardization (Hawk et al. 1955). Stock solutions of the buffer components were kept in stock and the buffers prepared just before use.

(7) Statistical methods

Means and standard deviations were calculated, and differences were tested for significance by Student's t test (Snedecor, 1956). Lines of best fit were calculated by the method of least squares and the regressions were tested for significance by the analysis of variance (Snedecor, 1956).

Comparison of regression coefficients was by the method described by Fisher (1948). The significance of the distance

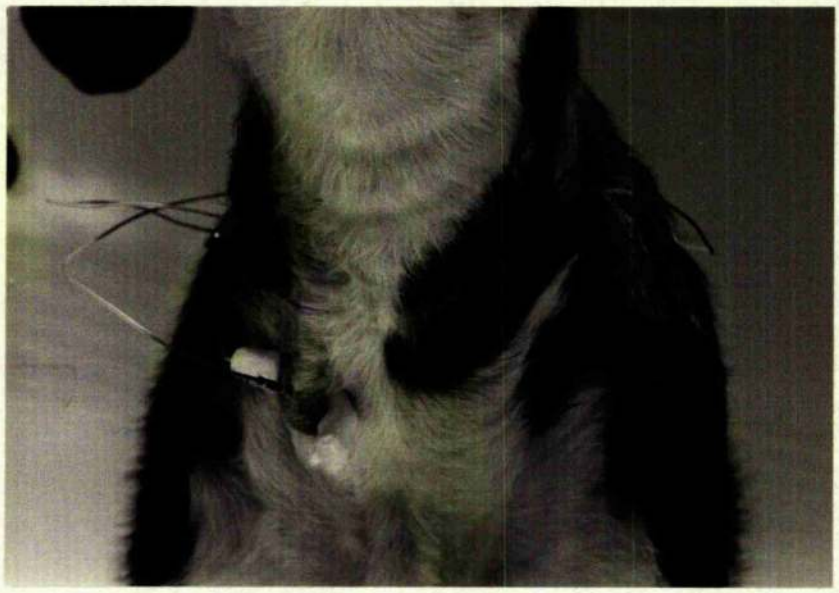


Fig. 6. Cannulation of the ipsilateral jugular and carotid vessels and contralateral jugular vein.

between regressions was tested by the method described by Quenouille (1950) and confidence limits by the method of Johnson (1950).

Table 1.

Recoveries of added adrenaline and noradrenaline from plasma and saline using biological assay methods

Amine	Medium	Amount added (ng)	Amount recovered (ng)	Recovery (%)	Mean recovery (%)
Adrenaline	Plasma	10	5	50	50
		20	12	60	
		50	20	40	
	Saline	10	4	40	40
		20	8	40	
		50	20	40	
Noradrenaline	Plasma	10	0	0	50
		20	10	50	
		50	25	50	
	Saline	10	<5	-	33
		20	5	25	
		50	20	40	

PART I

CHAPTER II

ASSESSMENT OF THE METHODS

(1) Catecholamines

(a) Biological method

With practice, the methods proved to be fairly straightforward, and little trouble was experienced in the assembly of the assay preparations. Rat preparations having a blood pressure greater than 80 mm Hg were discarded, since it was considered that pithing was incomplete, and some sympathetic activity remained. The virgin rat uterus would detect as little as 1 ng adrenaline, whereas the pithed rat preparation would not detect, with any certainty, less than 5 ng noradrenaline. From a sample of 10 ml. of plasma, therefore, assuming a 100% recovery, it should have been possible to measure concentrations greater than 0.1 μg/l. of adrenaline and 0.5 μg/l. noradrenaline.

Adrenaline and noradrenaline were added to plasma in quantities of 10, 20 and 50 ng and carried through the whole procedure of assay. Simple solutions in the same amounts and mixtures containing 20 ng of each amine were made up in 10 ml. of normal saline and similarly treated.

The results (Table 1) show that the mean recovery of amines from plasma was 50% for adrenaline and 50% for noradrenaline (25 samples). From saline solutions mean recoveries were 40% for adrenaline and 33% for noradrenaline. Plasma extracts showed no biological activity; in the

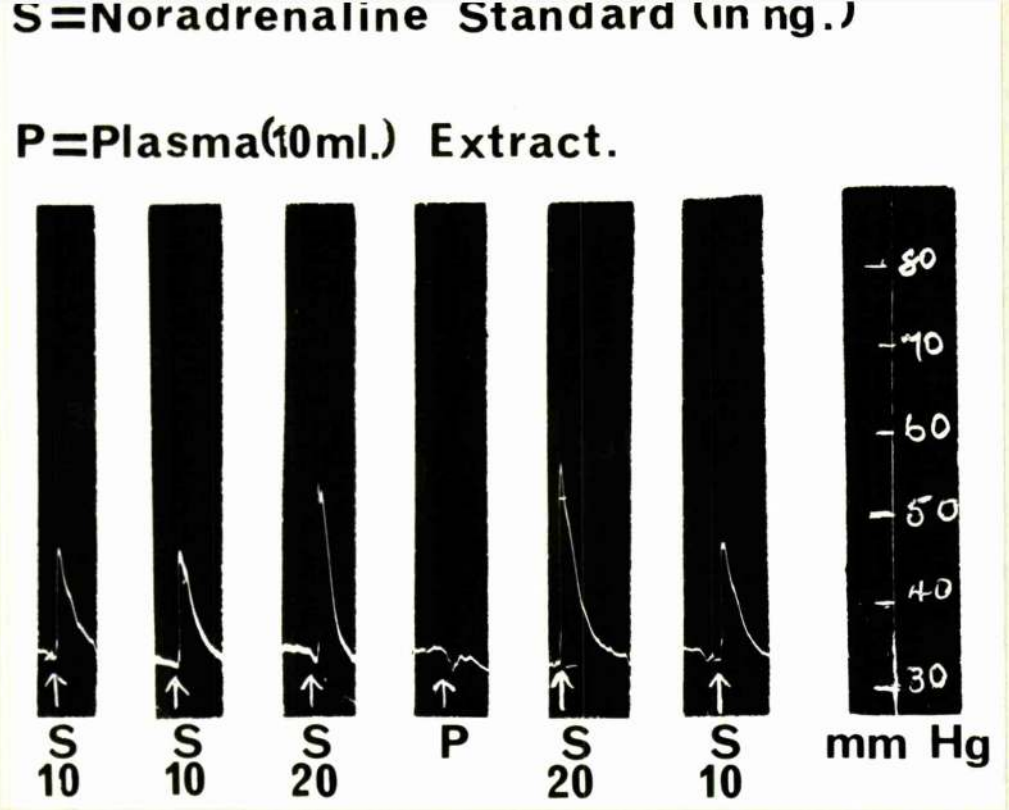


Fig. 7. Blood pressure tracing of a pithed rat showing the inability to detect any pressor activity in a plasma extract.

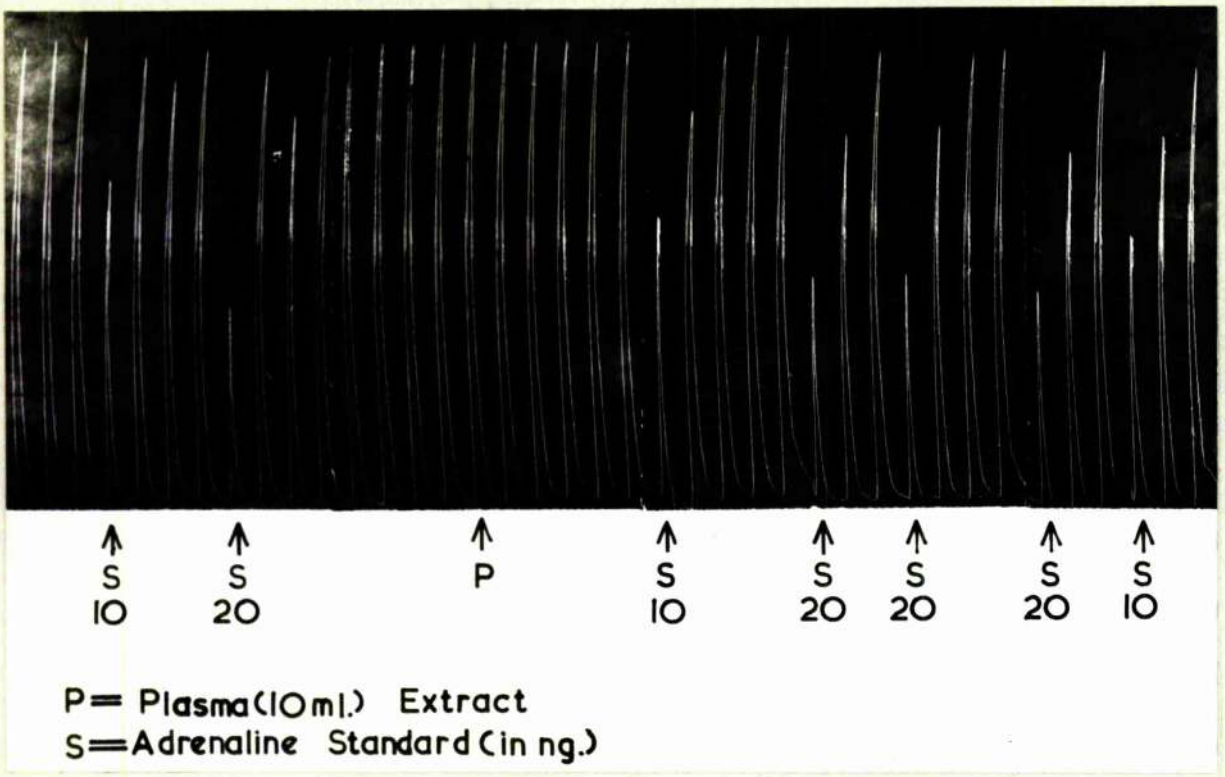


Fig. 8. Acetylcholine stimulated contractions of the virgin rat uterus showing that a plasma extract was unable to inhibit contraction.

noradrenaline assay there was sometimes a fall in blood pressure (Fig. 7) instead of a rise. Sometimes the eluting fluid itself showed biological activity and this had to be considered when calculating the results. Figs. 7 and 8 show the effect of an extract of bovine plasma on the pithed rat blood pressure, and the acetylcholine stimulated rat uterus respectively.

Since it was not possible to detect any catecholamines in bovine plasma, it must be assumed that the normal concentration of catecholamines in the plasma of the ox is lower than the limits of the method. Since losses were about 50% then it must be assumed that the normal bovine plasma concentration of adrenaline is less than 0.2 μg/l. and that of noradrenaline is less than 2.0 μg/l. This conclusion regarding the normal levels is similar to that reached by Holzbauer & Vogt (1954) in the dog, although by their technique these workers were able to demonstrate levels of adrenaline of less than 0.25 μg/l. of plasma.

This biological method, although highly specific, is not suitable for the determination of catecholamine concentration in peripheral plasma, since it would only detect increases and not decreases in peripheral plasma concentration. A decrease in adreno-medullary secretion could be detected by obtaining samples of adrenal vein blood which is rich in catecholamines. This, however, requires extensive surgical interference with subsequent slaughter of the animal, and its use for this type of experiment could be criticised on the grounds that it is non-physiological.

Because of this it was decided to examine the chemical methods.

(b) Chemical methods

Of the chemical methods available the most sensitive and widely used are the fluorimetric methods.

(i) Euler & Floding (1955) modified the method of Lund (1950) by using potassium ferricyanide instead of manganese dioxide as the oxidising agent, and used it for estimating the free catecholamines in urine. Various workers have applied this modification to the determination of plasma catecholamines and it has been widely used for this purpose. Many workers have found variation in the fluorescence of the extract blanks. Euler & Floding (1955) prepared extract blanks by omitting to add potassium ferricyanide. However potassium ferricyanide itself has some fluorescence and thus a true blank is not obtained. Price & Price (1957) attempted to overcome this by adding the potassium ferricyanide after the other reagents. Ascorbic acid prevents the catecholamine fluorescence from fading and this property has been used to prepare 'faded blanks', by omitting to add ascorbic acid. Ascorbic acid itself is fluorescent and so an extra reagent blank without ascorbic acid has to be prepared to apply a correction for the ascorbic acid fluorescence (Robinson and Stott, personal communication). Contrary to the findings of Euler & Floding (1955), both these groups of workers noted that the fluorescent product was unstable and therefore they took their readings at a specific time after the addition of the reagents. A recent publication by Euler & Lishajko (1961)

Table 2.

The fluorescence of sodium ascorbate, with and without the addition of ethylenediamine

Time (min)	Fluorimeter readings	
	Sodium ascorbate	Sodium ascorbate + ethylenediamine
1	18	20.0
2	19	19.0
3	22	20.0
4	26	21.0
5	36	20.5
6	45	19.0
7	55	24.5
8	68	25.5
9	77	25.0
10	87	24.0
11	97	24.5
12	113	24.0
13	121	24.0
14	133	25.0
15	146	24.5

has shown that the addition of ethylenediamine greatly improves the stability of the fluorescence.

On examination of the method it was noted that the fluorescence of the reagent blanks increased with time. The origin of this extraneous fluorescence was examined. The fluorescence appeared and increased following the addition of alkaline ascorbate solution. The fluorescence of sodium ascorbate solution was therefore examined, both with and without the addition of ethylenediamine. Table 2 shows the galvonometer readings of the fluorimeter using a Chance CY4 secondary filter. Sodium ascorbate was prepared by adding 9 ml. of 20% sodium hydroxide to 1 ml. of 2% ascorbic acid. Readings were taken every minute after mixing. In another experiment 0.2 ml. of ethylenediamine was added before the addition of the ascorbic acid and readings taken every minute. The increase in fluorescence was almost linear, and doubled in 5 minutes. Vogt (1954) may have noticed the effect because she added sodium hydroxide and ascorbic acid separately. No reason was given for this. Because ethylenediamine inhibited this rise in fluorescence, replicates both of plasma extracts and standard solutions were made using the modification of Euler & Lishajko (1961). Results were extremely poor; standard solutions showed a 100% variation, and plasma extracts very often gave readings lower than the blanks. The modification of Price & Price (1957) did improve the preparation of the blanks. The 'faded blank' described by Robinson & Stott very often failed to 'fade'.

The source of the variation could not be determined and it was felt that much work would be required to improve the method. Because of this the method was abandoned.

(ii) Natelson et al. (1949) described a reaction between adrenaline and ethylenediamine with the production of an unknown fluorescent compound. Weil-Malherbe & Bone (1952) adapted this reaction to the measurement of total catecholamines in plasma and later modified it for the separate measurement of noradrenaline and adrenaline (Weil-Malherbe & Bone, 1953). The reaction occurs with the catechol nucleus and therefore is likely to occur with all the catechols found in plasma. This lack of specificity has been the main criticism of this method (Valk & Price, 1956). Weil-Malherbe & Bone (1957) examined the catechol compounds in bovine plasma and found that adrenaline and noradrenaline were the only catechols present. Thus, although the method is not specific for adrenaline and noradrenaline, it can be used for their determination in bovine plasma.

This method has been widely used since it was first described, and some modifications have resulted. Valk & Price (1956) noted that the adrenaline derivative showed a greater fluorescence when the reaction was carried out in a solution containing acetic acid that had been passed through an alumina column than when it was performed in water or untreated acetic acid. Mangan & Mason (1957) confirmed this and found the increase in fluorescence to be about 30%, whereas Valk & Price (1956) found increases of 100-150%.

Table 3.

The effect of alumina-treated acetic acid on adrenaline fluorescence

Adrenaline (μg)	Fluorimeter reading (scale divisions)		
	Adrenaline + acetic acid	Adrenaline + acetic acid (alumina-treated)	Acetic acid (alumina-treated)
0.1	■ 100	98	3
0.1	97	105	-1
0.1	103	103	0
0.05	51	58	
0.05	50	52	

■ Full-scale deflection set with this sample

Table 4.

The effect of light on the fluorescence of the noradrenaline derivative

Time (min)	Fluorimeter readings
0	93.0
10	77.5
20	57.0
30	46.5
40	35.0
50	27.0
60	21.5
70	18.5
80	17.0
90	14.0

Nadeau et al. (1958) showed that alumina enhanced the fluorescence of the ethylenediamine-adrenaline product by 30% but the increase was only 17% when an equivalent amount of aluminium ions themselves were added. They deduced that the difference was due to impurities present in the alumina. Weil-Malherbe & Bone (1958) were unable to confirm their findings. The question of impurities is stressed by Miller & Elliot (1954) who reported that certain grades of alumina contained impurities that could not be washed off. Millar (1956) prepared his standards in acetic acid that had previously been passed through an alumina column. Richardson et al. (1956) passed a reagent blank through the entire procedure and thus had a measure of fluorescent impurities in the alumina.

Both the fluorescent impurities and the enhancing effect of alumina on the ethylenediamine-adrenaline product were examined. The alumina was prepared as described by Weil-Malherbe & Bone (1952) and in order to remove the fine particles which tended to clog up the columns, the water was decanted as the bulk of the alumina was settling. Large quantities of water were required for washing, which was continued until the washing water and distilled water gave identical readings in the fluorimeter. It was then considered that all fluorescent impurities had been removed. However, a reagent blank was carried through the whole procedure and the reading it gave was used to make a correction.

Table 3 shows the effect of alumina-treated acetic acid on adrenaline fluorescence. It can be seen that

alumina-treated acetic acid had no consistent effect on adrenaline fluorescence, and alumina-treated acetic acid itself gave no significant amount of fluorescence showing that all fluorescent impurities had been removed.

Aronow & Howard (1955) first noted that the blue-green fluorescence of the noradrenaline derivative was unstable in blue light and suggested that the condensation and extraction procedures should be carried out in a red-illuminated dark room. Gray et al. (1957) suggested that the cuvettes should be exposed to diffuse sunlight for 30 min.

The effect of light on the noradrenaline derivative was examined by performing the condensation and extraction procedures in a dark room illuminated by a 100-watt ruby-red light. Using an Ilford 623 filter, readings were taken, the tubes exposed to daylight, and further readings taken every 10 min for 90 min. Table 4 gives the results of such an experiment, and Figure 9 shows the relationship between the logarithm of the readings and time following exposure of the noradrenaline derivative to light. The decay of the fluorescence is exponential and after 90 min the fluorescence was still declining. It was thus decided that the whole condensation and extraction procedure should be performed in a dark room in order to get reliable noradrenaline results. Light had no effect on the adrenaline derivative.

Recoveries from aqueous solutions were variable and lower than those from plasma. This is in agreement with the findings of Mangan & Mason (1958) who postulated that plasma

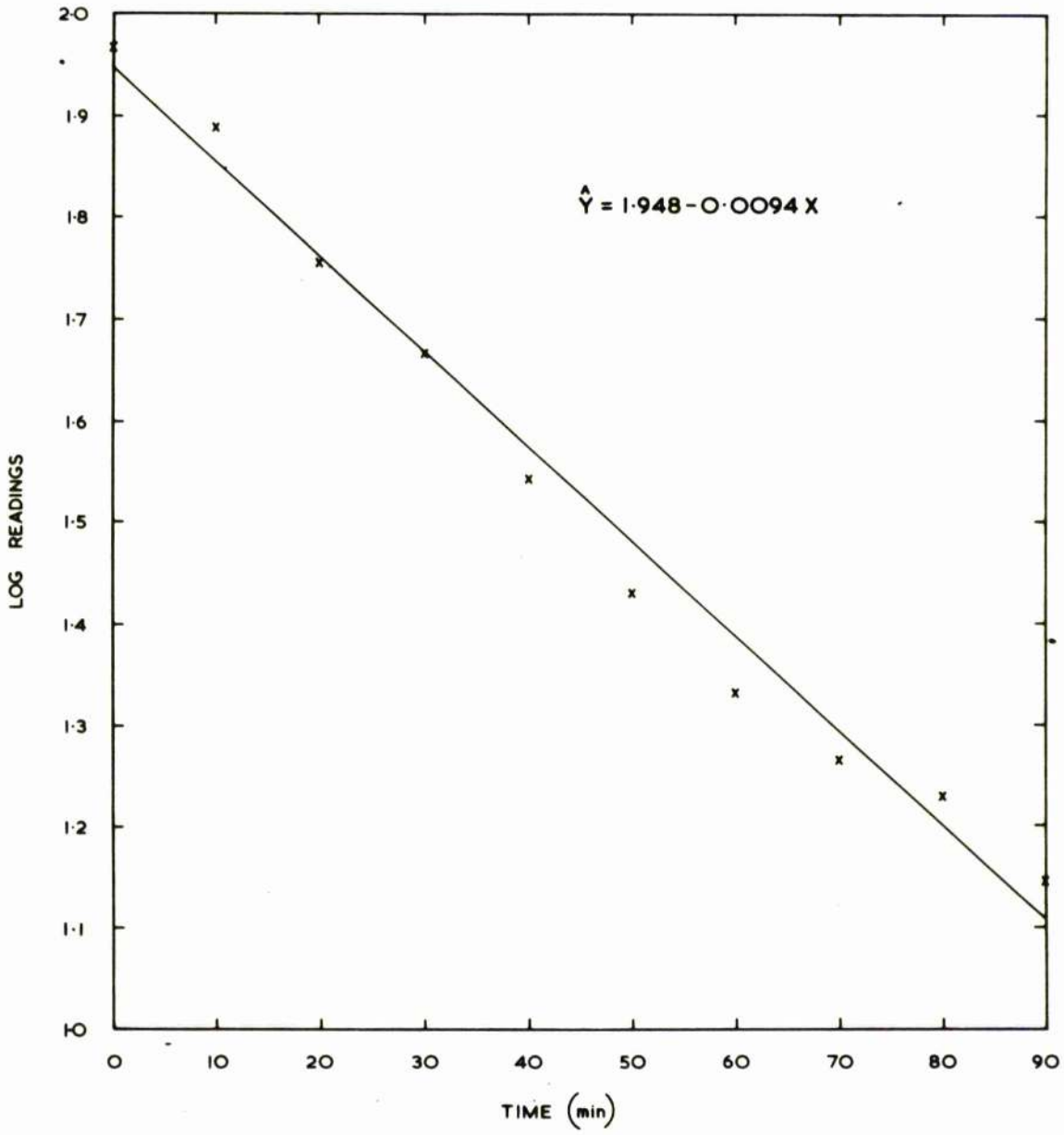


Fig. 9. The effect of light on the fluorescence of the noradrenaline ethylenediamine derivative.

Table 5.

Recoveries from plasma of added adrenaline and noradrenaline from plasma

Adrenaline			Noradrenaline		
Added (μg)	Recovered (μg)	Recovery (%)	Added (μg)	Recovered (μg)	Recovery (%)
0.1	0.098	98	0.1	0.098	98
0.1	0.092	92	0.1	0.073	73
0.1	0.095	95	0.1	0.086	86
0.05	0.047	94	0.05	0.040	80
0.05	0.057	114	0.05	0.042	84
0.05	0.058	116	0.05	0.052	104
Mean ± S.E.		101 ± 4.3			87.5 ± 4.7

Table 6.

Replicate analysis of a single sample (from two animals)

All concentrations expressed as μg/l. plasma

No.	Adrenaline	Noradrenaline
1	0.81	1.92
2	0.63	1.81
3	0.54	1.76
4	0.72	1.68
Mean ± S.E.	0.68 ± 0.06	1.79 ± 0.05

No.	Adrenaline	Noradrenaline
1	0.77	1.93
2	0.82	2.13
3	0.75	1.83
4	0.90	1.87
5	1.10	2.23
6	0.77	1.93
7	0.81	2.19
Mean ± S.E.	0.84 ± 0.06	2.02 ± 0.06

had some antioxidant property, which improved the stability of catecholamines in plasma.

Table 5 shows the recoveries from plasma of added adrenaline and noradrenaline. It will be seen that the standard errors of the mean recoveries were similar. The losses were irregular in amount so that it would be difficult to apply a constant correction to the results. Recoveries of added amines performed throughout this work consistently remained at about 100% for adrenaline and 80-90% for noradrenaline.

Table 6 shows the results from two experiments designed to test the replicability of determinations made on a single sample of plasma. For this, a large sample of blood was taken, the plasma removed and divided into equal portions. The blood was removed from an animal by the method described under the section on methods and materials and the results therefore give an estimate of the resting levels that might be found in the ox by this method of plasma catecholamine determination.

The results show that this method will measure, with an error of about $\pm$ 3-8%, the levels of adrenaline and noradrenaline in bovine peripheral plasma.

The method was further tested by stimulating the adrenal medulla and noting the changes in peripheral plasma catecholamines that ensue. This was done in two ways:

(1) By slow intravenous injection of potassium chloride solution. Table 7 gives the results. Initial levels tended to be low but there was a sharp rise in both

Table 7.

The effect of intravenous KCl (30 mg/kg) on plasma catecholamine concentrations

Time (min)	Plasma catecholamines (μg/l.)	
	Adrenaline	Noradrenaline
0	0.1	0.8
2.5	0.3	1.3
5	0.2	1.8
10	0.3	1.7
15	0.2	1.6
20	0.1	1.4
25	0.1	1.2

KCl injected at time zero.

Table 8.

The effect of intramuscular atropine and intravenous carbamylcholine on plasma catecholamine concentrations

Time (min)	Plasma catecholamines (μg/l.)	
	Adrenaline	Noradrenaline
-30	0.4	1.7
0	0.2	1.7
1	0.3	1.7
2	0.7	2.1
4	0.2	2.0
6	0.6	2.0
8	0.3	1.9

Atropine given at time -30 min

Carbamylcholine given at time zero.

Table 9.

Plasma catecholamine levels following intravenous administration of adrenaline and noradrenaline

Amine injected		Time after injection (min)						
		0	2	4	6	8	16	30
Adrenaline (5 μg/kg)	A	0.47	4.80	1.95	1.04			0.38
	NA	4.20	4.28	4.28	4.20			4.00
Adrenaline (10 μg/kg)	A	1.09	7.42	2.78	1.62			0.60
	NA	4.44	4.02	3.84	4.10			4.44
Adrenaline (10 μg/kg)	A	0.66	11.44	3.90		1.63	0.64	
	NA	1.14	1.41	2.10		1.77	1.14	
Noradrenaline (10 μg/kg)	A	0.50	0.70	0.79	0.83	0.43	0.48	
	NA	1.79	8.84	4.24	3.07	1.73	2.31	

A = Plasma adrenaline (μg/l.)

NA = Plasma noradrenaline (μg/l.)

Amines gives at time zero

adrenaline and noradrenaline 2½ min after injection. The noradrenaline levels reached a peak 5 min after injection, and then the concentration of both components decreased with time.

(2) Intravenous injection of carbamylcholine 30 min after the intramuscular injection of atropine. Table 8 gives the results. Atropine slightly depressed the initial levels. Carbamylcholine caused an immediate rise in the concentration of both components but a relatively greater one in the adrenaline levels. Noradrenaline levels remained slightly elevated 8 min after carbamylcholine injection, and there was a secondary rise in the adrenaline levels.

This showed that the method would detect changes in circulating levels of catecholamines, induced by adrenomedullary stimulants.

Four experiments were performed to follow the rate of disappearance from the circulation of injected adrenaline and noradrenaline. The experiment was a further test that the method could detect changes in circulating levels of catecholamines. Table 9 gives the results of these experiments. They show that there was a large rise in the concentration of the injected amine 2 min after injection and that the levels of adrenaline had returned to their initial value between 6 and 8 min after injection. The injection of noradrenaline caused a slight increase in the levels of adrenaline, but adrenaline injection had no significant effect

on noradrenaline concentration.

Weil-Malherbe & Bone (1953) were able to measure erythrocyte concentrations of catecholamines following lysis of washed red cells. This was not possible with bovine blood because bovine haemoglobin gave very high readings with the IF9 filter and therefore precautions against haemolysis had to be rigorous.

Weil-Malherbe (1961) has recently increased the specificity of the method by passing the alumina eluate over an ion exchange resin (Amberlite CG-50, type 2). This procedure gave much lower normal values than those reported in his earlier paper (Weil-Malherbe & Bone, 1953) and he concludes that other interfering substances are removed by the resin since his recoveries remained the same, i.e. 80 - 100%.

The ethylenediamine method for plasma catecholamine determination appeared to be the most reliable of the methods examined and was used in this work, with the modifications noted.

(2) Blood sugar

The method of King (1946) is supposed to measure true blood glucose and not any other reducing substances, such as glutathione, which are found mainly in the erythrocytes. The erythrocytes are preserved intact by mixing the blood with isotonic sodium sulphate solution before protein precipitation. The supernatant liquid obtained following protein precipitation was used to measure the concentration of non-glucose reducing

Table 10.

The effect of fermentation by yeast on the glucose concentration of supernatant liquid obtained from the protein precipitation of samples of blood (expressed as mg/100 ml. blood)

Sample	Time after addition of yeast (min)			
	0	30	60	90
A	46.2	8.4	4.6	3.6
A'	58.8	6.5	4.0	4.0
B	49.9	10.2	6.3	9.4
B'	61.4	7.3	4.2	6.5
Glucose solution	171	21.5	0.8	0.7

A B = Blood with intact erythrocytes

A'B' = Blood with lysed erythrocytes

substances in samples of blood in which the erythrocytes were preserved intact, as outlined above, and in samples in which the erythrocytes were lysed. Samples of supernatant liquid together with a glucose solution were fermented with yeast for 90 min and glucose concentrations measured. Table 10 gives the results of two typical experiments. The results show that the use of isotonic sodium sulphate does in fact preserve the red cells since the solutions of lysed blood had a higher glucose concentration than those of whole blood, and since this difference in concentration was fermentable lysis of the erythrocytes must have released glucose that was contained within them. It would appear, also, that either there is very little glutathione in bovine erythrocytes or that the method used is not affected by its presence. Non-carbohydrate reducing substances could be said to account for about 4 mg/100 ml. blood, i.e. about 6%. In this work corrections were not made for this amount but when examining the significance of actual blood glucose levels obtained, this amount had to be considered. The rise in reducing substances after 90 min in sample B,B' is unexplained, but could have been due to a build up of the metabolic products of fermentation. The method was taken as measuring true 'blood glucose'. Analysis of a single sample of blood divided into ten portions gave a mean value of 53.14 $\pm$ 0.58 (S.E.) mg/100 ml. which is an error of $\pm$ 1.1%. Recoveries of added glucose were 98%.

(3) Plasma VFA

The method used here (Scarisbrick, 1952) is specific for $C_2 - C_5$ acids since distillation occurs at pH 4. At this pH formic acid is not quantitatively distilled (Annison, 1954). Since acetic acid comprises 90 - 97% of total VFA in bovine blood (McClymont, 1951a) the results were expressed here as mg acetic acid/100 ml. plasma.

Analysis of a single sample divided into eight portions gave a mean value of 2.2 ± 0.01 (S.E.) acetic acid/100 ml. plasma, which is an error of ± 0.4%. Recoveries of added acetic acid were 96%.

In one experiment the distribution of VFA between erythrocytes and plasma was examined in two ways:

(i) Direct. Separation and washing of red cells which were subsequently lysed and the VFA concentration determined.

(ii) Indirect. By determination of the concentrations in whole blood and plasma, the intracellular concentration being determined from the formula.

$$C_c = \frac{100\,C_B - (100 - PCV)C_P}{PCV}$$

where

PCV = packed cell volume

C_c = erythrocyte concentration

C_B = whole blood concentration

C_P = plasma concentration

A correction of 12% was applied to the PCV estimation for

entrapped plasma (Jennings et al. 1954).

In a sample of blood the results were, (expressed as mg acetic acid/100 ml.)

Direct: C_c = 2.63

Indirect: C_B = 3.72

C_P = 4.70

PCV (corrected) 41.8%

by calculation C_c = 2.36

This shows reasonably good agreement between the two methods and gives C_P/C_c ratios of: (i) Direct 1.79, (ii) Indirect 1.99.

Annison (1954) in sheep found a C_P/C_c ratio of 1.48 which is similar to the ratios found here. However, his method measured formic acid as well and, if a correction is made for its presence, his ratio becomes 1.52.

(4) Plasma potassium

A single sample of plasma divided into eleven portions gave a mean value of 4.84 ± 0.01 (S.E.) m.equiv/l. plasma, which is an error of ± 0.2%.

For the determination of arteriovenous difference the accuracy of the method must be high, especially where the differences are likely to be small. It was considered that the errors of the methods for estimating plasma catecholamines and blood glucose were too great for calculated arteriovenous differences to be of any significance. With plasma potassium

and VFA, the method seemed sufficiently accurate for significant arteriovenous differences to be calculated. The importance of accurate methods for arteriovenous differences is stressed by Somogyi (1948) who points out that reports of negative arteriovenous blood glucose values have no meaning whatsoever. He suggested that the method for blood glucose determinations should be accurate to within 1 mg/100 ml. blood. He was discussing experiments on man where arteriovenous glucose differences are large. In ruminants they are small (Reid, 1950b) and so a high degree of accuracy becomes still more important. The accuracy achieved here, therefore, in blood glucose estimations falls far short of the ideal, and results would therefore be of little value.

Table 11.

The effect of various doses of insulin on the blood glucose concentration

(Expressed as mg/100 ml. blood)

Insulin given at time zero

Time (hr)	Dosage of insulin (units/kg body weight)					
	0.1	1	2	4	6	7.5
		Animal 6538 (*Bos taurus*)				
0		63.7	73.7	68.1	72.5	72.4
0.5		27.4	28.5	28.5	27.1	25.9
1		38.8	19.6	26.8	16.6	10.6
1.5		32.1	18.3	30.6	10.4	14.1
2		29.7	18.9	31.3	16.0	23.3
3		39.2	17.6	30.4	18.5	22.0
4		43.3	25.8	37.9	20.4	24.5
5		47.1	29.6	39.8	22.9	32.0
		Animal 6539 (*Bos taurus*)				
0		48.4	68.1	70.3	82.3	
0.5		28.4	24.5	22.9	27.2	
1		29.4	24.0	24.6	22.8	
1.5		29.1	26.4	26.3	27.4	
2		27.1	30.7	26.5	23.4	
3		27.7	25.7	29.5	19.9	
4		35.2	35.8	32.1	21.8	
5		45.3	30.7	37.4	26.1	
		Animal 6711 (*Bos indicus*)				
0	62.8		66.3	67.0	69.3	
0.5	36.3		32.7	29.0	30.5	
1	38.2		36.2	28.2	27.4	
1.5	44.0		38.0	27.7	25.5	
2	48.5		42.0	33.6	31.4	
3	52.6		37.6	32.1	30.8	
4	56.4		39.4	31.2	28.3	
5	55.8		39.4	31.5	26.3	

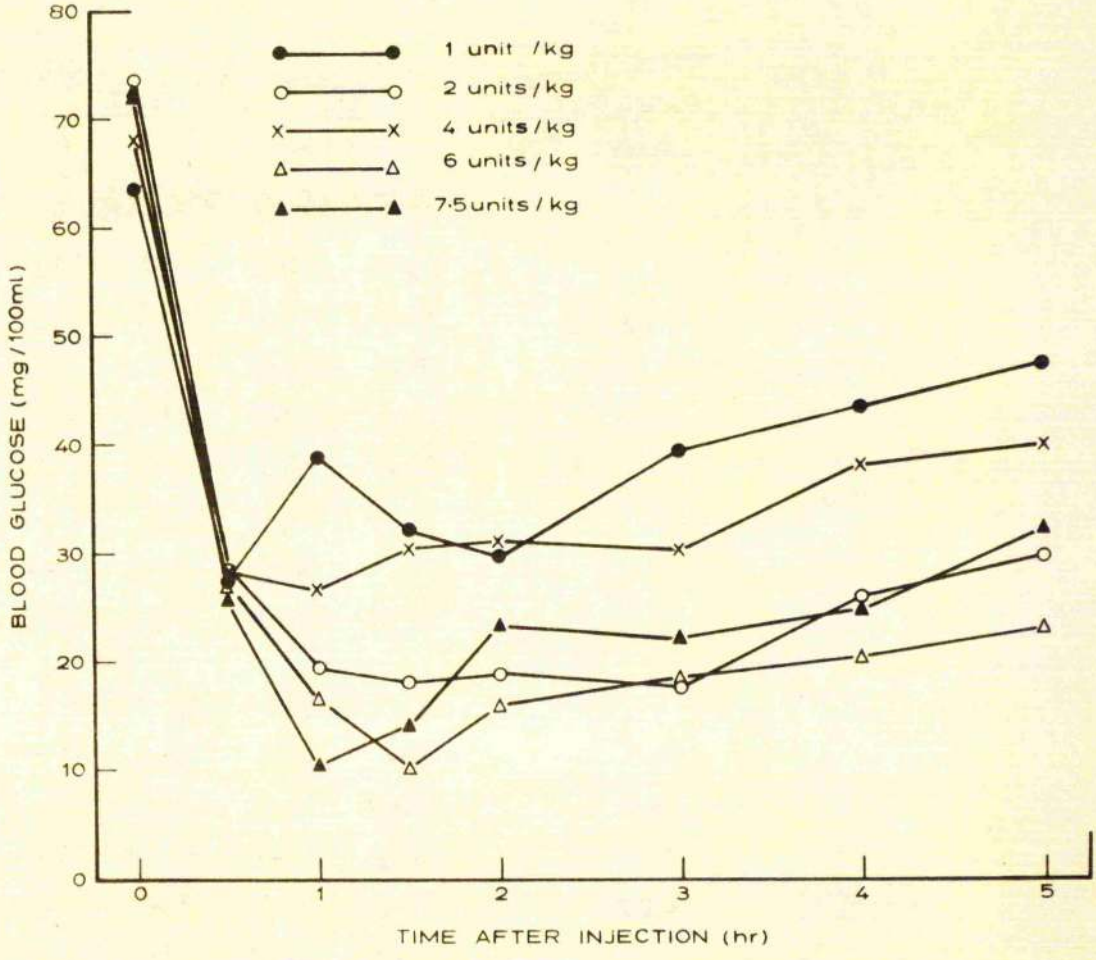


Fig. 10. The effect of various doses of intravenous insulin on blood glucose levels (Animal 6538).

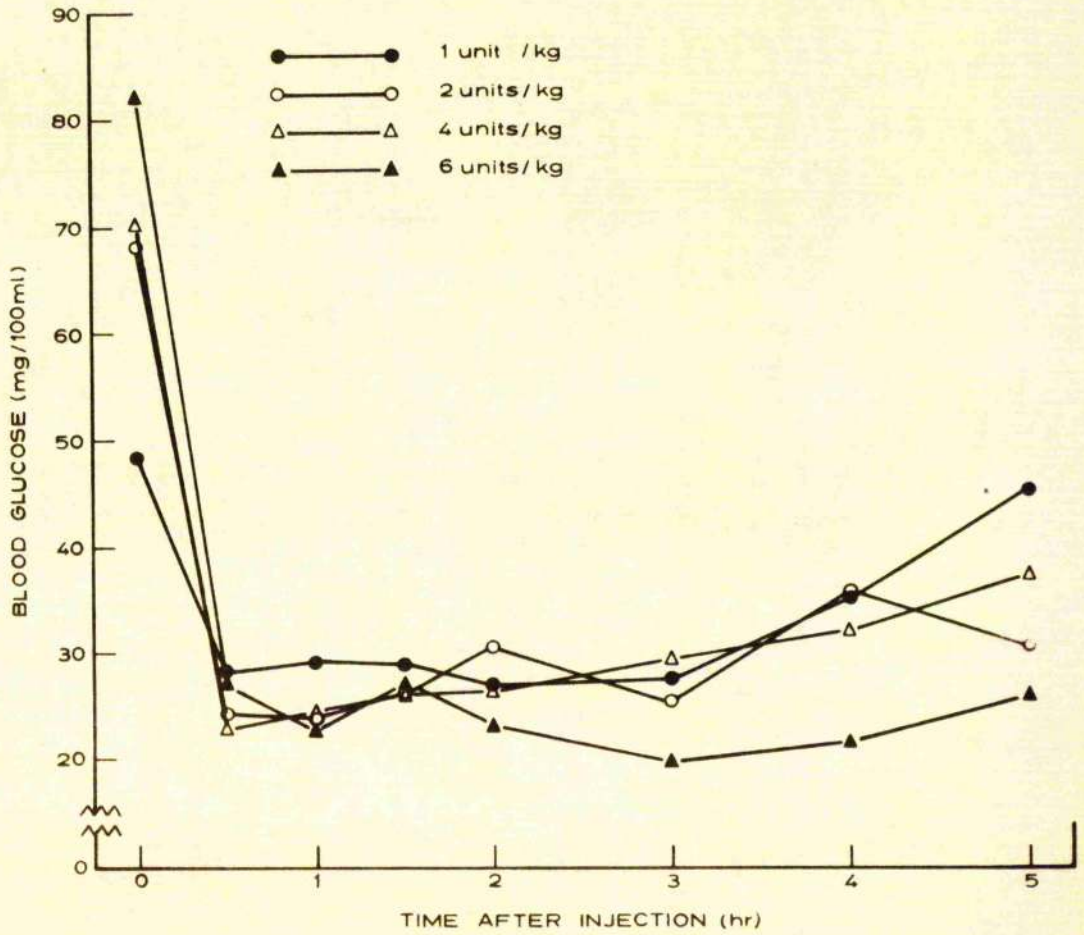


Fig. 11. The effect of various doses of intravenous insulin on blood glucose levels (Animal 6539).

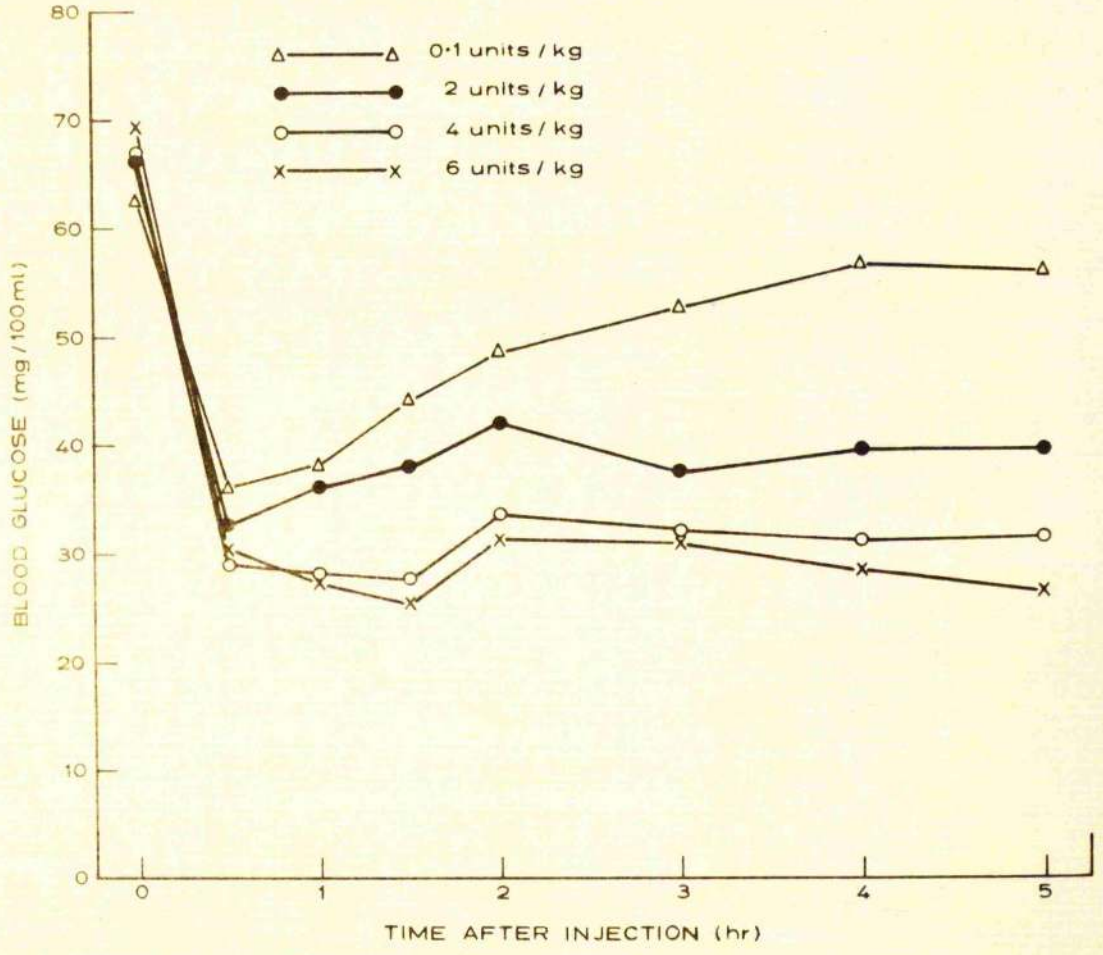


Fig. 12. The effect of various doses of intravenous insulin on blood glucose levels. (Animal 6711).

PART II

CHAPTER I

THE EFFECT OF THE INTRAVENOUS ADMINISTRATION OF INSULIN ON THE LEVELS OF VFA, POTASSIUM, ADRENALINE AND NORADRENALINE IN PLASMA AND GLUCOSE IN BLOOD AND ERYTHROCYTES

(1) The initial experiments studied the pattern of blood glucose response to various doses of insulin within the same animal. Three animals were used.

Insulin was given intravenously, and immediately after it was given blood samples were collected at intervals of $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, 3, 4 and 5 hr.

Table 11 and Fig. 10 show the blood glucose changes following insulin administration in Animal 6538 (Bos taurus) at dosages of 1, 2, 4, 6 and 7.5 units/kg body weight.

Table 11 and Fig. 11 show the results with animal 6539 (Bos taurus) at dosages of 1, 2, 4 and 6 units/kg body weight.

Table 11 and Fig. 12 show the results with Animal 6711 (Bos indicus) at dosages of 0.1, 2, 4 and 6 units/kg body weight.

The pattern of response differed somewhat in all three animals but they all had one feature in common, namely that the blood sugar level half-an-hour after injection was the same irrespective of dosage. Thereafter the values tended to remain at about the level reached at $\frac{1}{2}$ hr, the graphs having an L-shaped appearance. The main effect of an increase in dosage above 1 unit/kg was to prolong the duration of the

hypoglycaemia. At the end of 5 hr the blood glucose levels were still depressed in all the various tests, the degree of depression depending on the dosage, e.g. animal 6711 showed a depression of 7 mg/100 ml. with 0.1 unit/kg and 43 mg/100 ml. with 6 units/kg. Some of the important features of the results for the individual animals are given below:

Animal 6538. This shows very well the halt in the fall of the blood sugar values at ½ hr but does not demonstrate well the relationship between dosage and duration of hypoglycaemia. Some explanation of this might be found in the fact that the 2 units/kg and 6 unit/kg experiments were performed 96 and 48 hours respectively after a previous experiment; in both tests a massive dose had been given in the previous experiment. In these two experiments, therefore, it may be that if a greater time had been allowed to elapse between experiments for the carbohydrate metabolism to rectify itself, then the levels might not have been so low. Subsequent experiments, using insulin, were performed at intervals greater than 1 week.

Animal 6539. This demonstrated well both effects described above. There seemed to be an anomalous resting level of 48.4 mg/100 ml. in the experiment using 1 unit/kg. The sample had been allowed to stand for about 2 hr before precipitation so that some glycolysis might conceivably have occurred. However, similar experiments to test this point showed a fall of only about 5 mg/100 ml. No explanation is therefore available for this low level.

Table 12.

The effects of various doses of insulin on the levels of catecholamines, VFA and potassium in plasma and glucose in blood

Insulin given at time zero.

Animal No. 6711 (*Bos indicus*)

Time (hr)	Blood glucose (mg/ 100 ml.)	Plasma Adrena-line (µg/l.)	Plasma Nora-drenaline (µg/l.)	Plasma VFA (mg acetic acid/ 100 ml.)	Plasma potassium (m.equiv/l.)
Insulin dosage 0.1 unit/kg body weight					
0	62.8	0.2	2.0	1.9	5.8
0.5	36.3	0.1	2.0	1.0	6.0
1.0	38.2	0.3	2.5	2.3	6.3
1.5	44.0	0.1	2.0	1.1	5.7
2.0	48.5	0.1	1.5	1.4	5.9
3.0	52.6	0.5	1.0	1.6	5.7
4.0	56.4	0.3	1.8	1.6	5.6
5.0	55.8	-	-	2.6	5.4
Insulin dosage 2 units/kg body weight					
0	66.3	0.3	1.2	4.4	4.1
0.5	32.7	0.8	3.1	2.4	3.1
1.0	36.2	0.2	1.1	2.3	3.2
1.5	38.0	0.1	1.6	1.8	3.1
2.0	42.0	0.3	2.1	1.6	3.0
3.0	37.6	0.4	1.3	1.1	3.2
4.0	39.4	0.3	1.5	1.0	3.2
5.0	39.4	0.1	1.7	1.5	3.3
Insulin dosage 6 units/kg body weight					
0	69.3	0.2	1.9	5.7	3.8
0.5	30.5	0.3	1.9	2.4	3.1
1.0	27.4	0.5	2.1	1.0	3.0
1.5	25.5	1.1	4.0	2.1	2.9
2.0	31.4	0.5	2.5	2.1	2.6
3.0	30.8	0.2	2.0	0.7	2.8
4.0	28.3	0.5	3.0	1.3	2.7
5.0	26.3	0.3	2.4	1.0	2.5

Table 13.

The effects of various doses of insulin on the levels of catecholamines, VFA and potassium in plasma and glucose in blood

Insulin given at time zero.

Animal No. 6712 (*Bos indicus*)

Time (hr)	Blood glucose (mg/ 100 ml.)	Plasma Adrena-line (µg/l.)	Plasma Nora-drenaline (µg/l.)	Plasma VFA (mg acetic acid/ 100 ml.)	Plasma potassium (m.equiv/l.)
Insulin dosage 1 unit/kg body weight					
0	82.2	0.1	1.4	2.3	3.4
0.5	46.4	0.2	1.1	1.1	3.1
1.0	49.0	0.2	1.6	2.3	2.8
1.5	54.6	0.5	2.0	1.0	2.4
2.0	61.2	0.2	2.2	1.1	2.4
3.0	67.8	0.3	1.4	0.8	2.4
4.0	73.4	0.3	1.2	1.9	2.5
5.0	69.4	-	-	1.3	2.7
Insulin dosage 8 units/kg body weight					
0	76.7			2.8	3.8
0.5	31.3			0.8	3.6
1.0	30.7			1.8	2.7
1.5	30.3			1.9	2.4
2.0	32.4			1.8	2.3
3.0	32.8			1.8	2.4
4.0	40.8			0.5	2.4
5.0	39.4			0.8	2.6

Table 14.

The effects of various doses of insulin on the levels of catecholamines, VFA and potassium in plasma and glucose in blood

Insulin given at time zero.

Animal No. 6538 (*Bos taurus*)

Time (hr)	Blood glucose (mg/ 100 ml.)	Plasma Adrena-line (µg/l.)	Plasma Nora-drenaline (µg/l.)	Plasma VFA (mg acetic acid/ 100 ml.)	Plasma potassium (m.equiv/l.)
Insulin dosage 6 units/kg body weight					
0	72.5			2.6	4.1
0.5	27.1			1.8	3.0
1.0	16.6			0.8	2.9
1.5	10.4			1.6	3.0
2.0	16.0			2.6	2.9
3.0	18.5			1.0	3.0
4.0	20.4			1.3	3.2
5.0	22.9			1.3	3.3
Insulin dosage 7.5 units/kg body weight					
0	72.4	0.5	1.6	2.6	4.5
0.5	25.9	0.7	1.8	1.5	3.4
1.0	10.6	1.5	3.0	2.9	3.2
1.5	14.1	1.7	3.1	3.7	3.2
2.0	23.3	0.6	1.6	2.4	3.3
3.0	22.0	1.0	3.2	1.9	2.9
4.0	24.5	0.9	2.4	1.3	3.3
5.0	32.0	0.6	1.8	1.9	3.3

Table 15.

The effects of various doses of insulin on the levels of VFA and potassium in plasma and glucose in blood

Insulin given at time zero.

Animal No. 6539 (*Bos taurus*)

Time (hr)	Blood glucose (mg/ 100 ml.)	Plasma VFA (mg acetic acid/100 ml.)	Plasma potassium (m.equiv/l.)
Insulin dosage 4 units/kg body weight			
0	70.3	2.8	4.2
0.5	22.9	1.0	3.6
1	24.6	1.9	3.6
1.5	26.3	2.4	3.1
2	26.5	2.6	3.0
3	29.5	2.1	3.1
4	32.1	1.5	3.2
5	37.4	-	-
Insulin dosage 6 units/kg body weight			
0	82.3	3.4	4.0
0.5	27.2	2.6	3.5
1	22.8	1.6	3.3
1.5	27.4	1.6	3.1
2	23.4	-	3.1
3	19.9	0.8	2.8
4	21.8	1.1	2.9
5	26.1	0.3	3.0

Table 16.

The effect of insulin administration on the levels of catecholamines and VFA in plasma, glucose in blood and potassium in plasma and erythrocytes

Insulin given at time zero.

Animal No. 6595 (*Bos taurus*)

Insulin dosage 1 unit/kg body weight

Time (hr)	Blood glucose (mg/ 100 ml.)	Plasma Adrenaline (µg/l.)	Plasma Noradrenaline (µg/l.)	Plasma VFA (mg acetic acid/ 100 ml.)	Plasma potassium (m.equiv/l.)	Erythrocyte potassium (m.equiv/l.)
0	61.8	0.1	2.1	5.8	4.5	19.9
0.5	20.5	0.5	4.1	3.8	3.2	19.6
1.0	15.0	1.3	4.3	5.5	3.0	20.0
1.5	16.8	1.4	4.6	4.7	2.7	19.6
2.0	16.7	0.5	4.5	4.7	2.6	19.5
3.0	18.4	0.1	2.1	5.8	2.7	20.8
4.0	26.4	0.1	2.0	3.6	3.2	19.5
5.0	34.7	0.1	2.4	4.2	3.3	20.3

Animal 6711. This showed both effects well.

The mean initial levels of blood glucose (expressed as mg/100 ml. and ignoring the anomalous result) were:

Animal 6538 70.08 ± 4.15 (S.D.); Animal 6539 73.57 ± 7.64 (S.D.); Animal 6711 67.5 ± 1.69 (S.D.)

Although these figures only represent eleven estimations on three animals, they demonstrate that the resting blood glucose levels for these cattle were higher than the levels quoted for cattle by Ayyar & Nayar (1941). These workers reported a mean value of 46 mg/100 ml. with a range of 36-57 mg/100 ml. An analysis of more figures will be given elsewhere in this thesis.

(2) Having established the blood glucose response to varying doses of insulin within the same animal, a series of experiments were performed in which the effect of insulin administration on the concentration of VFA, potassium and catecholamines in plasma and of glucose in blood were analysed.

Ten experiments were performed on five animals. The following doses of insulin were given: 0.1, 1, 2, 4, 6, 7.5 and 8 units/kg body weight. The blood glucose responses in seven experiments have already been given in the previous section but are given again here for completeness. The results for individual animals are given in Tables 12 - 16.

Of the ten experiments, five were performed on two animals of Bos indicus type and five on three animals of

Bos taurus type. Plasma catecholamine levels were determined in six experiments. In one experiment (Table 16) erythrocyte concentrations of potassium were determined, but since insulin had no effect on the intracellular concentration of this element this experiment was not repeated.

Plasma adrenaline and noradrenaline

Animal 6711 (Table 12) showed fairly consistent resting levels and the major change occurred when the blood glucose levels fell to 25.5 mg/100 ml. (6 units/kg). There was a six-fold increase in the adrenaline levels and a two-fold increase in the noradrenaline levels. There were, however, smaller increases at other times which were transitory and occurred at blood glucose values of 38.2 mg/100 ml. (0.1 unit/kg) and 32.7 mg/100 ml. (2 units/kg). Increases in adrenaline levels were associated with increases in the noradrenaline levels, the increases in the adrenaline concentration being the greater.

Animal 6712 (Table 13) showed a slight increase in both components at a blood sugar level of 54.6 mg/100 ml. when given 1 unit/kg of insulin.

Animal 6538 (Table 14) showed an increase in plasma catecholamine concentration at a blood glucose level of 10.6 mg/100 ml. which was maintained until the blood glucose levels had risen to 32.0 mg/100 ml. except for a fall that occurred at a blood glucose level of 23.3.mg/100 ml.

Animal 6595 (Table 16) showed an increase in plasma catecholamine levels at a blood glucose concentration of

20.5 mg/100 ml. which was maintained for 1½ hr and then the levels returned to the initial values.

In all these experiments, increases in the adrenaline concentration were relatively greater than the associated increases in noradrenaline concentration.

The results demonstrated that at low blood glucose levels, both adrenaline and noradrenaline levels were elevated. The degree of change was variable, but in the experiment in which the lowest blood glucose levels were found (Table 14, 7.5 units/kg) the highest catecholamine values also occurred especially with respect to the adrenaline levels. The resting levels were fairly consistent: adrenaline 0.1 - 0.5 μg/l. noradrenaline 1.2 - 2.1 μg/l. From these experiments it was concluded that there was a level of blood glucose below which the sympatho-adrenal system was stimulated, and that this raised both the adrenaline and noradrenaline levels in plasma, but from these experiments it could not be decided what this critical level might be.

Plasma VFA

In all the experiments a fall in plasma VFA levels occurred after administration of insulin and this was most marked during the first half-hour. The subsequent changes varied considerably. In three experiments plasma VFA concentration continued to decrease 1 hr after insulin administration, whereas in the remaining five experiments levels had risen towards or even exceeded the initial level (Table 12, 0.1 unit/kg and Table 14, 7.5 units/kg). In every experiment

except one (Table 12, 0.1 units/kg) the concentration of VFA in plasma was lower than the initial levels 5 hr after insulin administration. In two control experiments (Table 19) there was practically no change in plasma VFA levels over 5 hr.

Since a fall within the first half-hour occurred in all the experiments, this effect was statistically examined. Examination of the data revealed that the fall in plasma VFA levels appeared to be related to the initial plasma VFA level, but since various doses of insulin were administered, the possible effect of dosage had to be considered, and a multiple regression analysis performed.

The dependent variable (Y) was the fall in plasma VFA levels at $\frac{1}{2}$ hr (expressed as mg acetic acid/100 ml.) the independent variables were the dosage of insulin (X_1, in units/kg) and the initial plasma VFA level (X_2, as mg acetic acid/100 ml.).

An analysis of variance of the regression gave the following results:

	df	S.S.	M.S.	F	p
REGRESSION	2	3.49	1.75	5.83*	<0.05
DEVIATIONS	7	2.12	0.30		
TOTAL	9	5.61			

$$\hat{Y} = -0.07 + 0.030X_1 + 0.446X_2$$

Test of each X after the effect of the other had been removed:

	df	S.S.	M.S.	F	p
$X_1 + X_2$	2	3.49			
X_2 alone	1	3.42			
X_1 after X_2	1	0.07	0.07	4.30	N.S.
DEVIATIONS	7	2.12	0.30		
$X_1 + X_2$	2	3.49			
X_1 alone	1	0.02			
X_2 after X_1	1	3.47	3.47	11.57*	<0.05
DEVIATIONS	7	2.12	0.30		

Since X_1 and Y are not related it is valid to ignore the effect of X_1 on the regression X_2Y. The regression X_2Y alone had a mean square of 3.42 with a variance ratio (F) of 11.29. Allowing an extra degree of freedom (8 and 1) this is highly significant ($p < 0.01$).

This revealed, therefore, that the fall in plasma VFA levels following insulin administration was related to the initial level and was independent of the dosage.

Plasma potassium

With one exception (Table 12, 0.1 unit/kg), insulin produced a fall in plasma potassium levels. The fall tended to be progressive until 2 hr after administration; thereafter it tended to increase slightly in most of the tests. Levels

had not returned to their initial value after 5 hr. The one exception (Table 12, 0.1 unit/kg) showed a rise in plasma potassium concentration followed by a fall at 1 hr, so that the level at 5 hr was slightly depressed.

A multiple regression analysis was carried out, as for the plasma VFA, relating the fall in plasma potassium level (Y) to the dose of insulin (X_1) and the initial plasma potassium level (X_2):

	df	S.S.	M.S.	F	p
REGRESSION	2	0.09	0.05	6.02	N.S.
DEVIATIONS	7	2.15	0.31		
TOTAL	9	2.24			

The multiple regression was, therefore, not significant.

The relationships between the following were examined:

(i) Fall in plasma VFA at 30 min and 4 hr, and fall in plasma potassium at 30 min and 4 hr.

(ii) Fall in blood glucose at 30 min and 4 hr, and fall in plasma potassium at 30 min and 4 hr.

(iii) The lowest blood glucose levels and the maximum fall in plasma potassium (Cheetham, 1963).

In no test was any statistically significant correlation found. It would have been preferable to compare the plasma concentrations of these substances 5 hr after insulin administration. However, a sample was missing in one experiment (Table 14, 4 units/kg), so the 4 hr samples were compared.

Blood glucose

The falls in blood glucose concentration 30 min after insulin can be summarized as follows:

Bos indicus			Bos taurus		
Animal No.	Dose units/kg body weight	Fall in blood glucose mg/100 ml.	Animal No.	Dose units/kg body weight	Fall in blood glucose mg/100 ml.
6711	0.1	26.5	6595	1	41.3
6712	1	35.8	6539	4	47.4
6711	2	33.6	6539	6	55.1
6711	6	38.8	6538	6	45.4
6712	8	45.4	6538	7.5	46.5

It is obvious from inspection that of the five animals studied (two Bos indicus and three Bos taurus) there was a marked difference in their glucose response to insulin over a similar range of doses, Bos indicus appearing more resistant to insulin than Bos taurus.

(3) The results of the previous two sections demonstrated that the major changes in concentration of blood glucose and plasma VFA and potassium occurred within the first 30 min after injection of insulin. The plasma catecholamine studies had suggested that there was a critical blood glucose concentration below which the sympatho-adrenal system was stimulated, but no clear cut evidence was obtained. Four experiments were performed, therefore, to study in more detail the events occurring within the first 30 min after intravenous

Table 17.

The effects of various doses of insulin on the levels of catecholamines, VFA and potassium in plasma and glucose in blood

Insulin given at time zero.

Animal No. 7439 (*Bos taurus*)

Time (min)	Blood glucose (mg/ 100 ml.)	Plasma Adrenaline (µg/l.)	Plasma Noradrenaline (µg/l.)	Plasma VFA (mg acetic acid/ 100 ml.)	Plasma potassium (m.equiv/l.)
		Insulin dosage 1 unit/kg.			
0	47.4	0.50	1.30	4.1	4.03
5	45.9	0.80	1.73	3.3	3.55
10	33.2	0.60	1.87	2.7	3.33
15	28.4	0.60	1.51	1.7	3.04
20	17.9	0.50	1.00	1.2	2.94
25	19.1	1.10	2.36	0.8	2.78
30	15.9	1.00	2.20	1.3	3.10
60	19.1	0.93	1.50	3.7	3.10
120	24.8	0.85	1.50	2.3	3.20
180	35.7	1.10	2.41	2.9	3.55
240	41.6	0.97	1.86	1.8	4.03
300	47.7	0.80	1.31	3.4	4.25
		Insulin dosage 4 units/kg.			
0	48.3	0.09	0.40	4.1	3.77
5	40.0	0.08	0.90	3.2	3.84
10	28.8	0.14	2.04	2.2	4.03
15	22.5	0.25	1.47	2.0	4.03
20	16.2	0.31	1.10	2.0	3.84
25	14.2	0.25	0.55	2.5	3.71
30	11.8	0.84	1.19	2.8	3.33
60	12.8	1.62	1.68	3.6	2.81
120	17.5	1.62	1.44	3.5	3.07
180	17.3	1.52	1.49	2.0	2.59
240	23.3	1.61	1.31	2.2	2.65
300	30.5	1.03	1.61	2.0	2.53

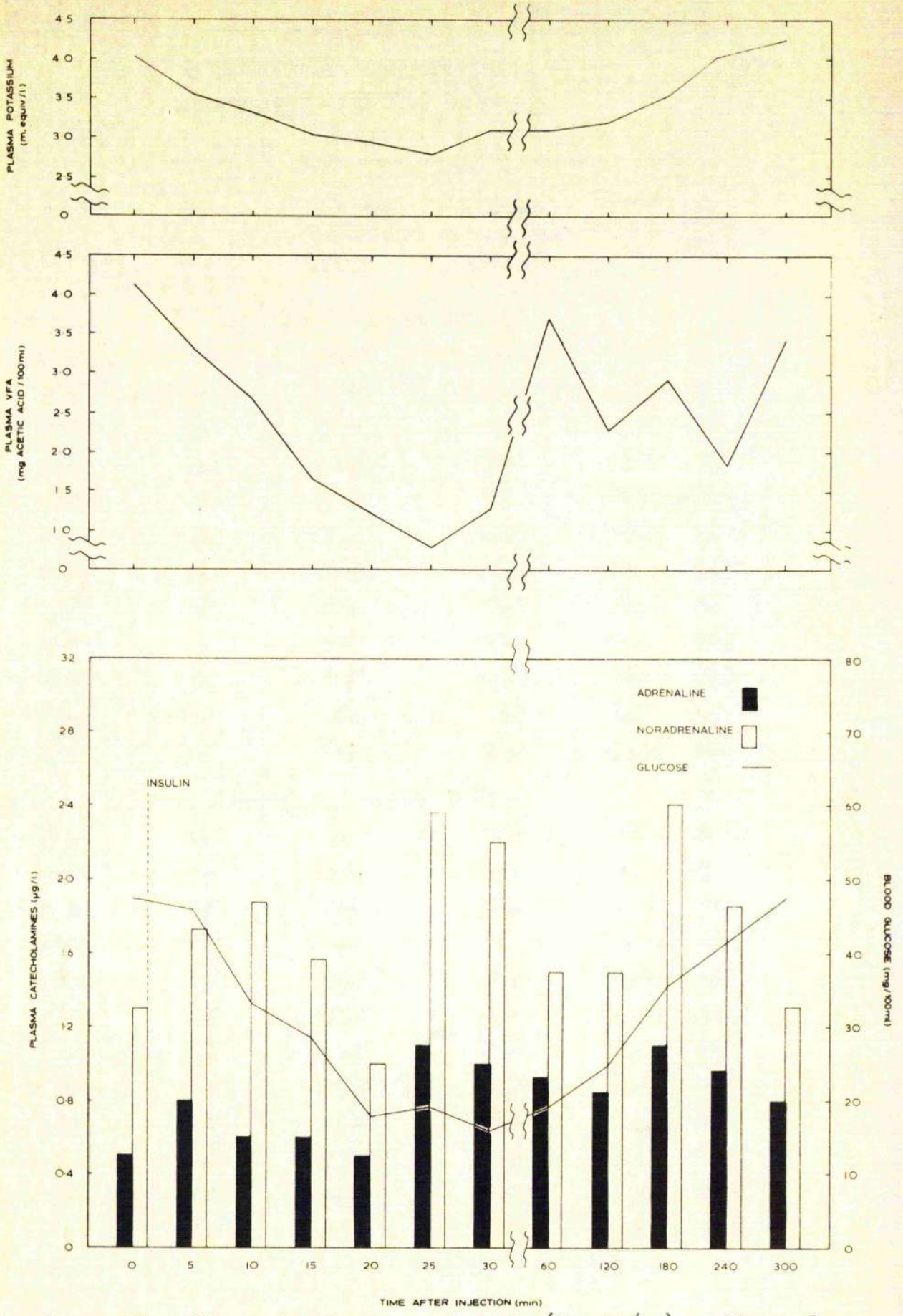
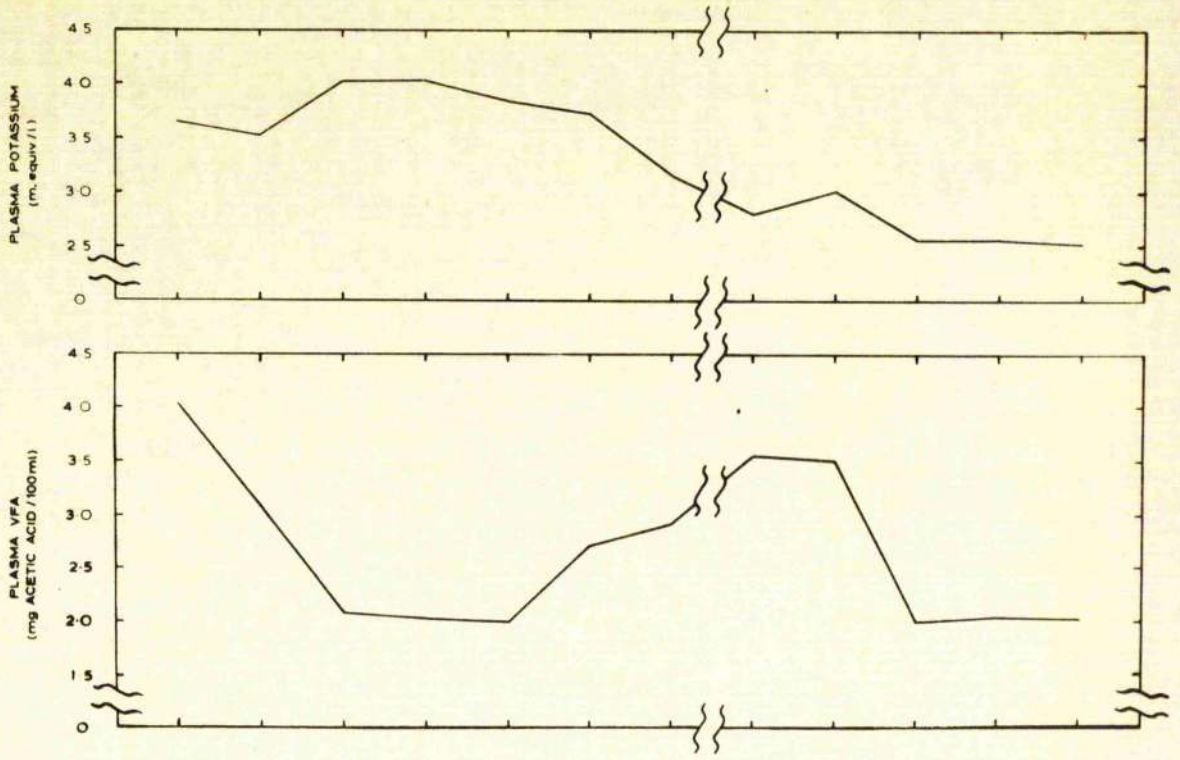


Fig. 13. The effect of intravenous insulin (1 unit/kg) on blood glucose, plasma VFA, potassium and catecholamine levels. (Animal 7439)



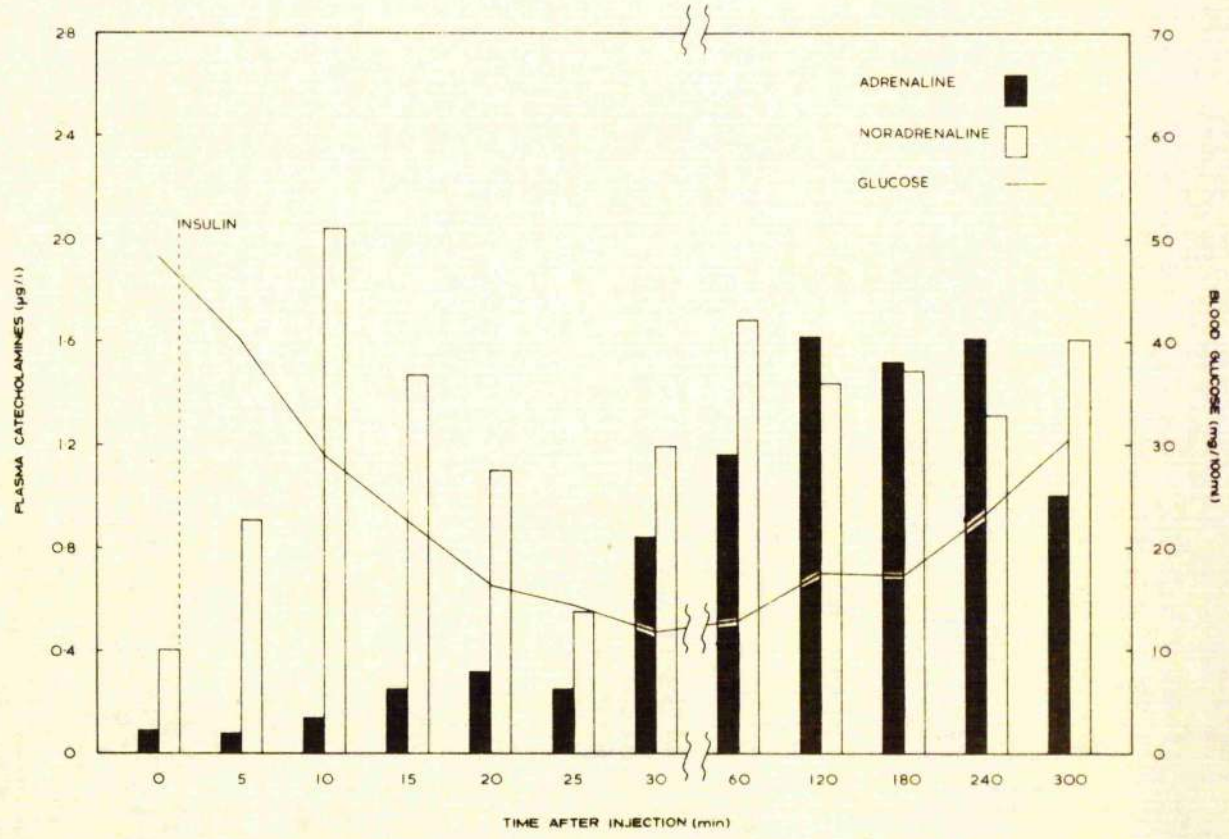


Fig. 14. The effect of intravenous insulin (4 units/kg) on blood glucose, plasma VFA, potassium and catecholamine levels. (Animal 7439)

Table 18.

The effects of various doses of insulin on the levels of catecholamines, VFA and potassium in plasma and glucose in blood

Insulin given at time zero.

Animal No. 7433 (*Bos indicus*)

Time (min)	Blood glucose (mg/ 100 ml.)	Plasma Adrenaline (µg/l.)	Plasma Noradrenaline (µg/l.)	Plasma VFA (mg acetic acid/ 100 ml.)	Plasma potassium (m.equiv/l.)
Insulin dosage 1 unit/kg body weight					
0	60.0	0.03	2.13	3.5	4.22
5	50.9	0.20	2.30	3.5	3.96
10	44.8	0.14	1.24	3.1	3.74
15	39.9	0.25	2.25	3.0	3.33
20	37.4	0.23	2.00	4.4	3.20
25	33.6	0.25	1.50	4.7	2.94
30	36.0	0.10	1.96	3.6	2.81
60	32.7	0.12	2.10	3.8	2.67
120	41.7	0.13	2.20	3.2	3.20
180	50.7	0.08	2.11	3.2	3.04
240	57.5	0.08	2.26	2.4	3.10
300	60.4	0.03	2.30	2.5	3.20
Insulin dosage 5 units/kg body weight					
0	58.9	0.19	1.45	2.5	4.22
5	57.2	0.13	1.43	2.5	3.45
10	48.0	0.08	0.97	2.5	3.84
15	41.5	0.10	2.58	2.5	4.60
20	35.1	0.16	2.70	2.0	3.71
25	32.8	0.12	2.01	2.2	3.36
30	29.5	0.28	2.13	2.1	3.52
60	25.5	0.27	1.61	2.9	3.07
120	28.0	0.15	2.20	1.9	3.01
180	32.2	0.16	1.84	1.5	3.04
240	35.1	0.19	1.40	1.2	3.10
300	37.9	0.26	1.82	1.3	3.20

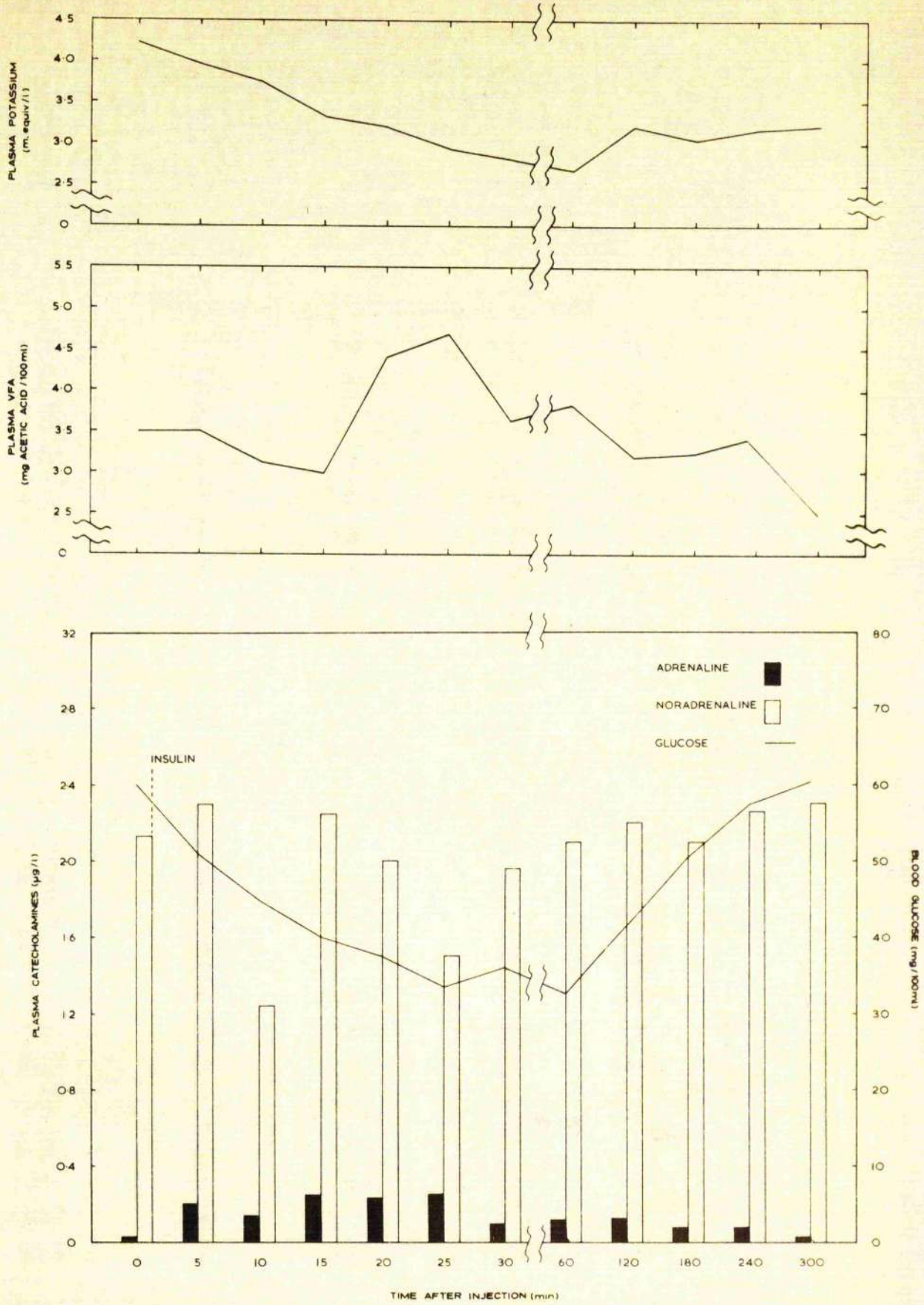


Fig. 15. The effect of intravenous insulin (1 unit/kg) on blood glucose, plasma VFA, potassium and catecholamine levels. (Animal 7433)

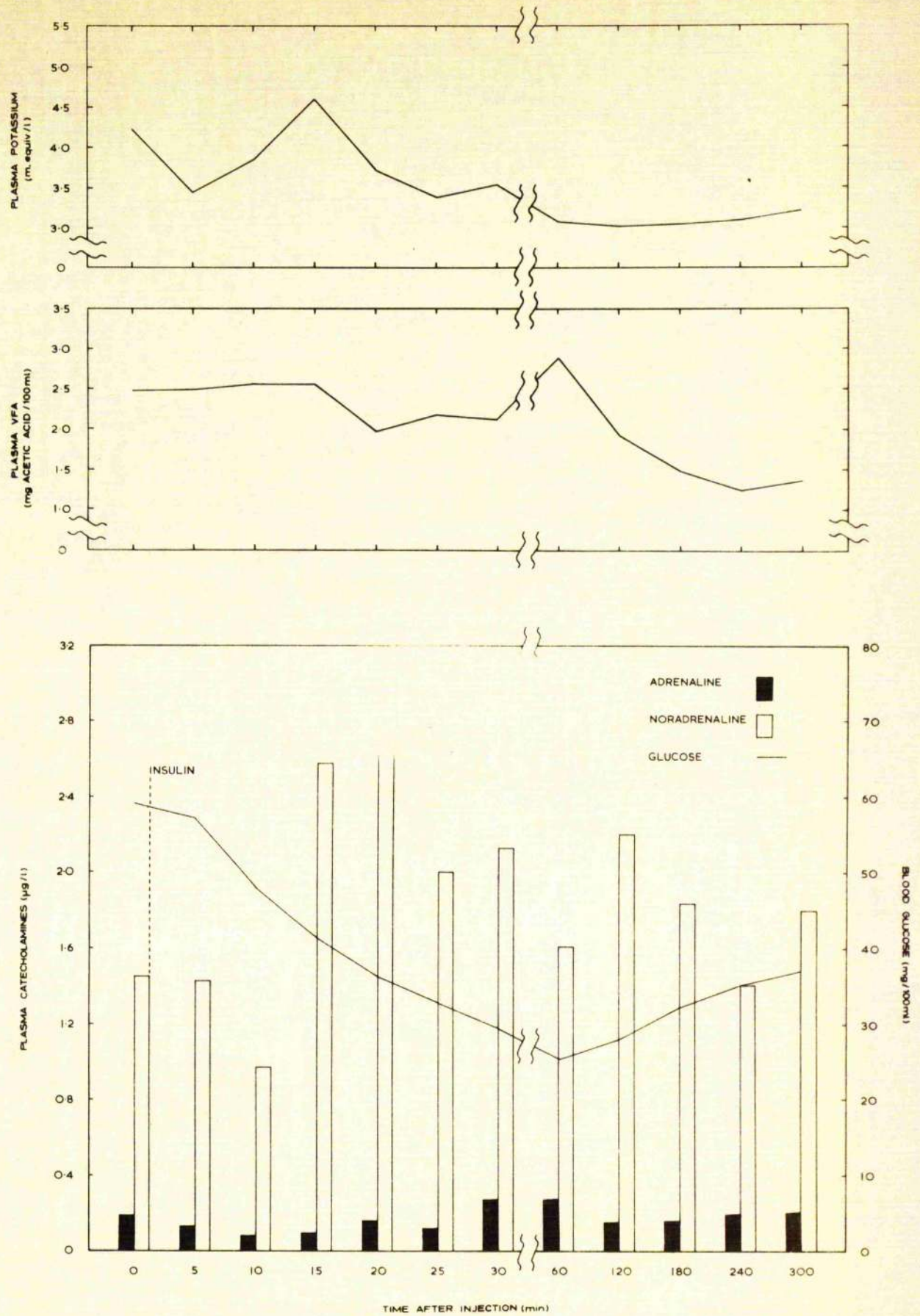


Fig. 16. The effect of intravenous insulin (5 units/kg) on blood glucose, plasma VFA, potassium and catecholamine levels. (Animal 7433)

insulin injection. Blood samples were taken every 5 min for 30 min and then at 60, 120, 180, 240 and 300 min. Two animals were chosen, one of Bos taurus type which was known to be sensitive to insulin and one of Bos indicus type which was known to be relatively more resistant. Two dosage levels were used; 1 and 4 units/kg body weight for Animal 7439 (Bos taurus) and 1 and 5 units/kg body weight for Animal 7433 (Bos indicus).

The results are given in Tables 17 - 18 and Figures 15 - 18.

Blood glucose

The results show the marked difference in response to insulin in the two animals, 7433 being considerably more resistant than 7439 to its action. In both animals the duration of hypoglycaemia was affected by the dose, 1 unit/kg body weight causing a hypoglycaemia which was terminated after 5 hr, and 4 and 5 units/kg body weight still showing a considerable hypoglycaemia after 5 hr.

Plasma adrenaline and noradrenaline

Animal 7439

Quite marked changes were produced in this animal. A dose of 1 unit/kg body weight caused no changes within the first 25 min except for a transient increase in the noradrenaline levels, with a peak at 10 min. After 25 min, at a blood glucose level of 19.1 mg/100 ml. there was a rise in both amines which persisted throughout the experimental

period and the values were still elevated, when the blood glucose values had returned to normal. At the higher dose (i.e. 4 units/kg body weight) the transient increase in noradrenaline levels occurring within the first 30 min was more marked than that at the lower dose. There was a marked increase in adrenaline levels at a blood glucose level of 11.8 mg/100 ml. following a slight increase at a level of 16.2 mg/100 ml. The increase in both amines was maintained throughout the experimental period, the adrenaline levels exceeding the noradrenaline levels on three occasions. When the blood glucose levels rose again to 30.5 mg/100 ml. after 5 hr there was a slight fall in the adrenaline levels, but the levels of both amines were considerably higher than their initial values.

Animal 7433

There were high noradrenaline and low adrenaline levels in this animal. At the dosage of 1 unit/kg body weight there was a slight fall in the noradrenaline levels at 10 min and thereafter little change. A rise occurred in the adrenaline levels. At the high dosage rate (i.e. 5 units/kg body weight) there was, once again, a slight fall in the noradrenaline levels at 10 min, followed by an increase. The noradrenaline levels remained somewhat elevated throughout the experimental period, though not significantly in most of the tests. There was a slight rise in adrenaline levels when the blood glucose concentration was 29.5 mg/100 ml. and remained elevated until blood glucose values had risen to 28.0 mg/100 ml.

Table 19.

The changes in certain blood constituents during the experimental period

Time (hr)	Animal 6712			Animal 6595				
	Blood glucose mg/100 ml.	Plasma VFA (mg Acetic acid/ 100 ml.)	Plasma potassium (m.equiv/l.)	Blood glucose mg/100 ml.	Plasma VFA (mg Acetic acid/ 100 ml.)	Plasma potassium (m.equiv/l.)	Plasma Adrenaline (g/l.)	Plasma Noradrenaline (g/l.)
0	73.8	1.3	4.37	63.4	4.2	4.50	0.62	2.20
0.5	69.2	1.4	4.58	63.9	4.1	4.45	0.44	2.21
1	69.0	1.3	4.63	63.7	3.7	4.80	0.62	2.40
1.5	70.4	-	5.06	64.0	3.4	4.71	0.62	2.24
2	70.4	1.6	4.86	60.7	4.7	4.59	0.70	2.60
3	68.2	2.1	4.83	63.9	3.2	4.86	0.72	2.62
4	68.0	1.6	4.73	60.9	3.9	4.87	0.72	2.62
5	-	2.1	4.91	-	-	4.59	-	-

Plasma VFA

Animal 7439

In both experiments there was an initial fall in plasma VFA levels, which was greatest at the lower dosage rate and which at 30 min was starting to rise again. There was no pattern of response that could be related to fluctuations in the levels of the other constituents measured.

Animal 7433

The results were not consistent with those obtained previously, there being an increase at 30 min in the 1 unit/kg experiment and only a slight fall in the 5 units/kg experiment. There was, however, a marked fall in both experiments from 60 min onwards. It would appear that any decline was superimposed on a high rate of entry of exogenous acetate from the gut. It was noticed that this animal ruminated during the experiments, even although it had not been fed for 24 hr.

Plasma potassium

If the experiments are taken together, the 1 unit/kg experiments both showed a fall over the first 30 min followed by a rise over the rest of the experimental period. With Animal 7439 the level at 300 min exceeded the initial level; in Animal 7433 it remained depressed. The high dosage rates both produced a rise in the plasma potassium within the first 30 min and this was associated with an increase in the noradrenaline levels. Thereafter there was a fall, the levels still being depressed after 300 min.

(4) In order to test the effect of insulin administration

Table 20.

The effect of insulin administration on plasma and erythrocyte glucose concentrations

Insulin given at time zero.

Time (hr)	Animal 6595			Animal 7437					
	5 units/kg			1 unit/kg			5 units/kg		
	E	P	E/P	E	P	E/P	E	P	E/P
0	24.3	70.1	0.35	31.4	91.5	0.34	27.6	89.6	0.31
0.5	11.3	28.7	0.39	35.4	46.1	0.77	25.8	28.9	0.89
1.0	16.3	19.0	0.86	29.1	63.8	0.46	15.0	26.7	0.56
1.5	11.7	13.6	0.86	30.1	60.0	0.50	22.4	26.1	0.86
2.0	11.5	12.3	0.93	36.7	58.8	0.62	19.3	28.5	0.68
3.0	14.8	15.6	0.95	46.0	64.2	0.72	22.1	36.5	0.61
4.0	16.6	23.0	0.72	39.6	77.6	0.51	23.5	42.8	0.55
5.0	19.8	21.3	0.93	46.7	73.3	0.64	21.9	46.2	0.47

E - Erythrocyte concentration (mg/100 ml.)

P - Plasma concentration (mg/100 ml.)

E/P - Erythrocyte plasma ratio

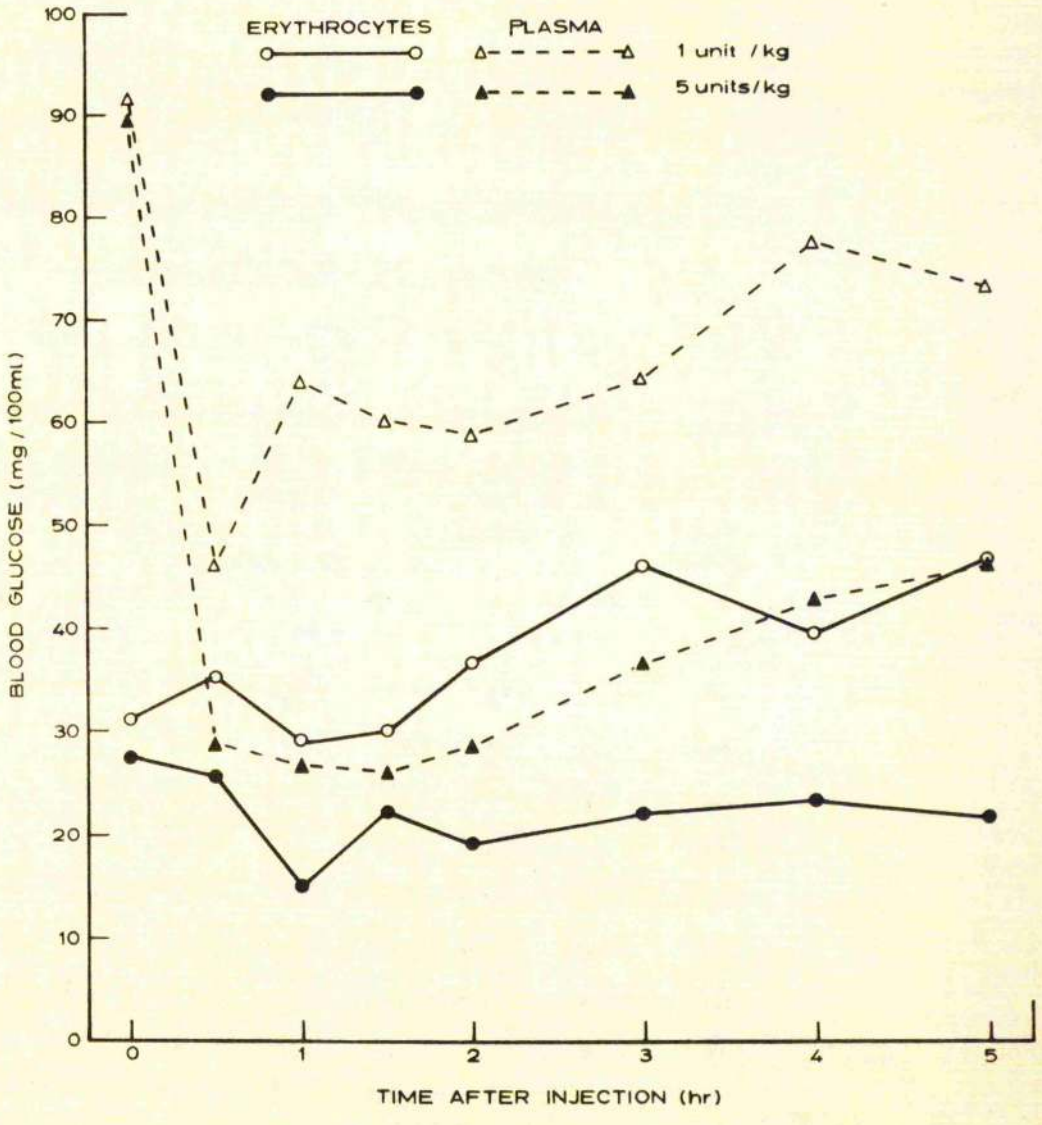


Fig. 17. The effect of various doses of intravenous insulin on plasma and erythrocyte glucose levels.

on the erythrocyte glucose concentration, three experiments on two animals were performed and both plasma and erythrocyte concentrations of glucose were measured. The results are given in Table 20 and Fig. 17. To Animal 7437, doses of 1 and 5 units/kg body weight and to Animal 6595 a dose of 5 units/kg body weight were given. The erythrocyte:plasma ratios (E:P) are also recorded in Table 20. The initial E:P values were all very similar (0.35, 0.34 and 0.31) and were higher than a mean value of 0.24 reported by Goodwin (1956). Insulin caused a rise in the ratio which was due to a large fall in the plasma glucose concentration associated with a relatively small fall or even a rise in the erythrocyte concentration. These results demonstrate that the erythrocyte glucose concentration is relatively resistant to large changes in plasma glucose concentration.

One can roughly determine the amount of glucose lost by the erythrocytes and the plasma to produce the falls in concentration noted. Animal 7437 weighed 250 kg and assuming that erythrocyte volume was 2.9% and plasma volume 2.8% of body weight (Howes et al. 1963), then the fall in concentration 30 min after the intravenous injection of insulin would represent a net loss of 4.24 g glucose from the plasma and 0.125 g glucose from the erythrocytes. This demonstrates, that in the ox the erythrocyte glucose is not a major source of glucose during insulin hypoglycaemia.

Conclusions

1. The degree of hypoglycaemia produced by intravenous insulin at dosage rates in excess of 1 unit/kg body weight

appeared to be independent of dosage, an increase in dosage prolonging the duration of the hypoglycaemia.

2. Plasma VFA and potassium levels both declined following injection of insulin. The fall in plasma VFA level was independent of dosage of insulin but was related to the initial levels.

3. A rise in both adrenaline and noradrenaline levels occurred when blood glucose levels fell to a critical level, which appeared to be within the range 15-25 mg/100 ml. At high dosage rates a transient rise in noradrenaline levels occurred 10-15 min after insulin administration.

4. Bos indicus appeared to be more resistant to insulin treatment than Bos taurus, and this was not associated with a more sensitive sympatho-adrenal system.

5. Erythrocyte concentrations of glucose changed much less than plasma glucose concentrations after intravenous insulin administration.

Table 21.

The effect of intravenous adrenaline and noradrenaline administration on blood glucose concentrations (expressed as mg/100 ml.)

Amines given at time zero.

Time (min)	Animal 7439				Animal 7436			
	2.5 µg/kg		10 µg/kg		2.5 µg/kg		10 µg/kg	
	A	NA	A	NA	A	NA	A	NA
0	55.0	60.0	55.0	50.8	63.9	61.9	67.1	53.9
5	69.1	66.9	87.5	84.9	79.2	66.5	100.0	84.4
10	72.2	67.8	93.5	91.7	83.4	70.7	161.9	86.4
15	64.4	64.9	96.8	88.2	86.3	69.7	183.2	86.8
20	75.5	65.1	97.0	87.8	89.1	66.5	159.8	85.2
25	74.5	66.4	99.4	83.3	86.5	66.7	111.9	81.4
30	74.7	65.6	94.4	77.4	87.8	65.9	110.0	78.2
45	69.5	61.3	95.9	70.0	73.0	67.4	101.3	69.3
60	58.4	62.4	91.4	65.1	74.7	70.5	93.4	68.9

Table 21a.

Increase in blood glucose (expressed as mg/100 ml.) 30 min after intravenous injection of adrenaline and noradrenaline

Animal	2.5 µg/kg		10 µg/kg	
	A	NA	A	NA
6270	18.0	7.0	41.0	23.0
6272	23.0	3.0	37.0	18.0
6273	20.5	5.0	27.0	19.0
6278	14.5	6.0	31.0	9.0
6279	4.0	7.0	15.0	25.0

A = Adrenaline

NA = Noradrenaline

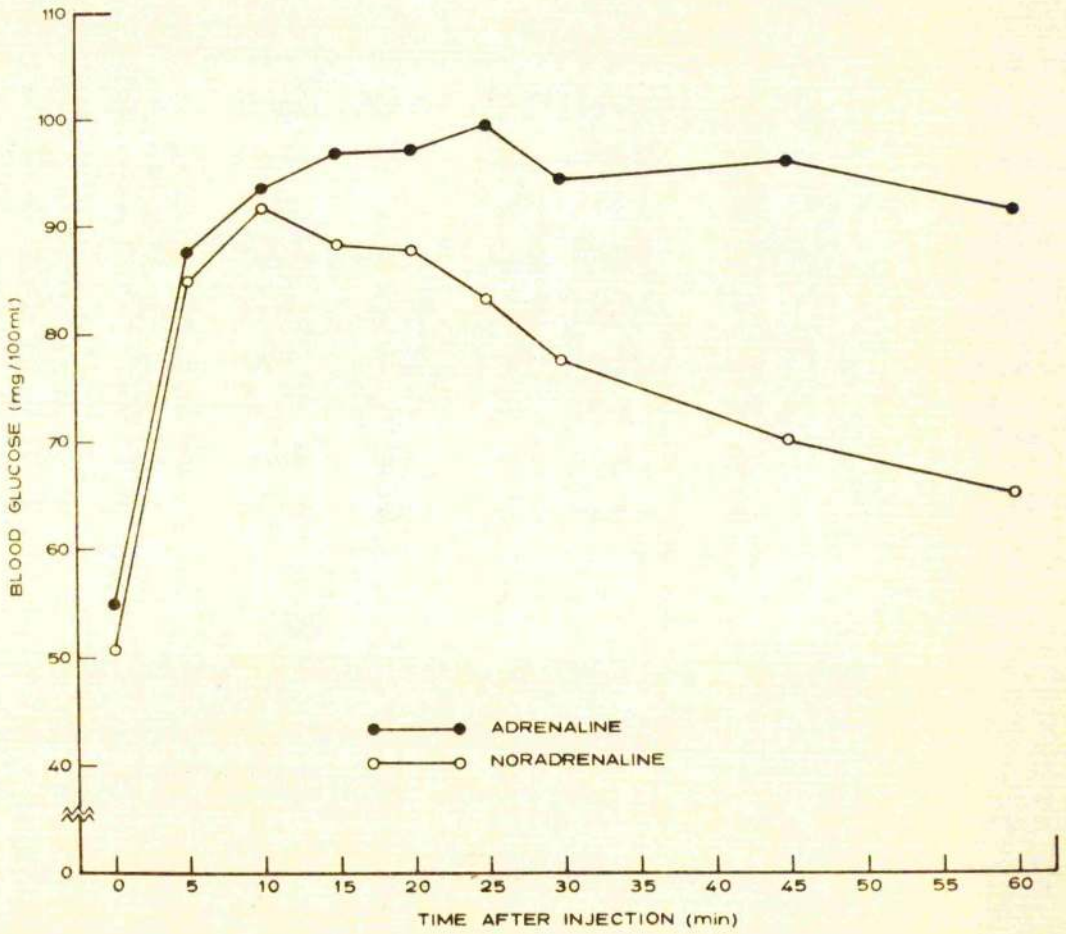


Fig. 18. The effect of intravenous adrenaline and noradrenaline on blood glucose levels.

PART II

CHAPTER II

THE EFFECT OF THE INTRAVENOUS ADMINISTRATION OF ADRENALINE AND NORADRENALINE ON BLOOD GLUCOSE LEVELS

Since insulin hypoglycaemia resulted in elevated levels of adrenaline and noradrenaline, and since both these amines have hyperglycaemic actions, it was important to study what their relative contributions towards the restoration of normal blood glucose levels might be during insulin hypoglycaemia.

(1) Eight experiments were performed on two animals. Doses of 2.5 and 10 µg/kg body weight of adrenaline or noradrenaline were given intravenously and blood samples taken at 5, 10, 15, 20, 25, 30, 45 and 60 min after injection. The results are given in Table 21, and Fig. 18 shows the blood glucose response to the administration of 10 µg/kg body weight of adrenaline and noradrenaline to Animal 7439. Animal 7439 was of Bos taurus type and 7436 of Bos indicus type. Unfortunately Animal 7433, the animal of the Bos indicus type used in the previous chapter, was not available so that the results cannot be directly related to previous results. However, No. 7436 had a similar insulin hypoglycaemia pattern to that of No. 7433.

Generally, the blood glucose rose rapidly for the first 10 min, and then became stabilized. Thereafter the curves for the two responses diverged, the adrenaline hyperglycaemia being more persistent than that of noradrenaline. With Animal 7436,

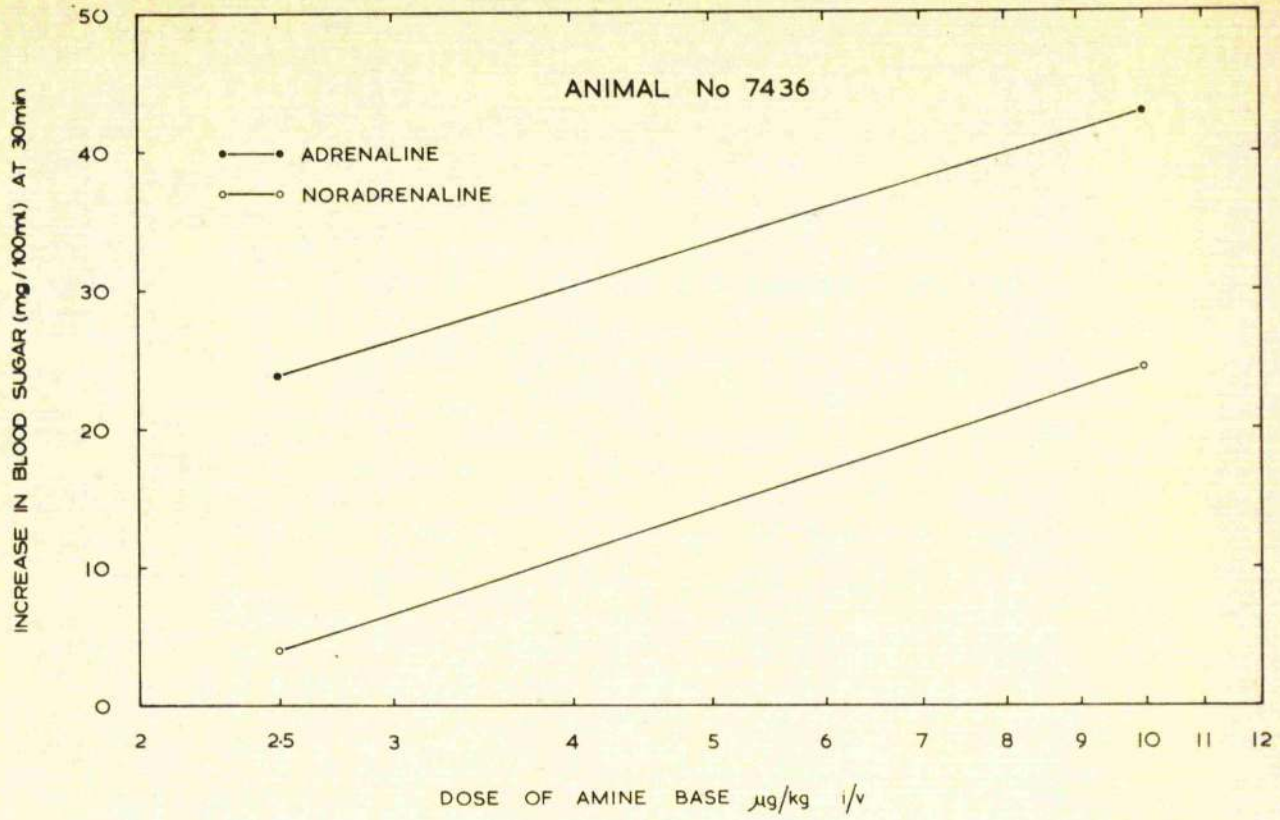
10 μg/kg body weight of adrenaline produced a very rapid rise until 15 min and then a rapid fall.

The determination of the relative hyperglycaemic potencies of adrenaline and noradrenaline (adrenaline: noradrenaline ratio) is difficult (Ellis, 1956). Graham & James (1952) determined the adrenaline:noradrenaline ratio by measuring and comparing the areas of the plots made on a constant scale of blood glucose responses above the fasting level. Application of this method to the results reported here gave the following values.

	A:NA 2.5 μg/kg	A:NA 10 μg/kg
Animal 7439	3.54	1.40
Animal 7436	2.87	2.26

This showed that the ratio, as measured by this method, varied with the dose of amine administered.

Ellis & Anderson (1951) point out that misleading figures are produced if the ratio is determined by comparing the effects on blood glucose of equal doses of each amine. More reliable results are obtained by comparing the doses of amine which produce equal hyperglycaemic effects, since the hyperglycaemic response is proportional to the logarithm of the dose (Chen & Clark, 1948). Therefore the increase in blood glucose at 30 min was plotted against the logarithm of the dose of amine base given. The results are shown in Fig. 19. They indicate that if the points for each animal are joined then almost parallel lines result. This suggests that the two amines had similar qualitative effects at the two



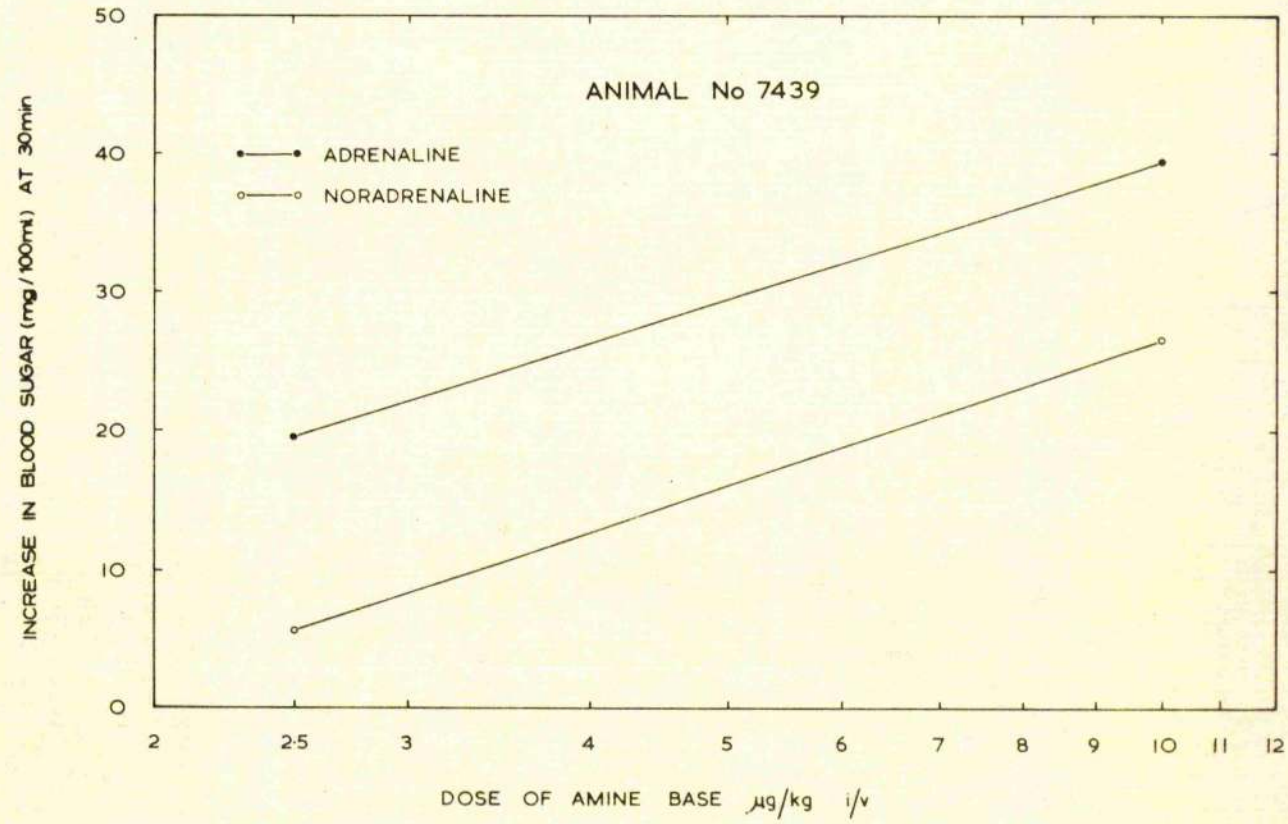


Fig. 19. The relationship between the increase in blood glucose 30 min after intravenous adrenaline and noradrenaline and the logarithm of the dose administered.

dosages. The assessment of the ratio A:NA from these graphs was practically independent of dosage and gave the following results.

	Animal 7439 Dosage for increase of 26.6 mg/100 ml.	Animal 7436 Dosage for increase of 24.3 mg/100 ml.
Adrenaline	4.08	2.57
Noradrenaline	10	10
A:NA	2.45	3.89

(2) Similarly from the results shown in Table 21a, ratios were deduced for five more animals of Bos taurus type and were as follows:

Animal	A:NA
6270	2.96
6272	6.58
6273	5.06
6278	1.43
6279	0.79

Conclusions:

(1) With one exception adrenaline had a greater hyperglycaemic action than noradrenaline. The adrenaline, noradrenaline hyperglycaemic ratio in seven animals ranged from 0.79 to 6.58.

Table 22.

The effect of intravenous sodium acetate administration on the concentration of glucose in blood and VFA, potassium, adrenaline and noradrenaline in plasma

Sodium acetate given at time zero.

Time (min)	Blood glucose (mg/ 100 ml.)	Plasma Adren- aline (µg/l.)	Plasma Nora- drenaline (µg/l.)	Plasma VFA (mg acetic acid/ 100 ml.)	Plasma potassium (m.equiv/l.)
			Animal 7432 (*Bos indicus*)		
0	88.6	0.50	2.59	2.2	4.06
5	97.9	0.65	1.97	45.3	3.39
10	98.0	0.56	3.33	31.5	3.36
15	98.8	Samples haemolysed		23.1	3.23
20	97.8			14.1	3.23
25	95.4			8.4	3.23
30	100.4			4.6	3.36
45	96.8			1.7	3.45
60	97.4			1.5	3.39
			Animal 6595 (*Bos taurus*)		
0	58.9	0.09	3.45	2.2	4.03
5	70.1	-	2.48	46.6	3.45
10	71.1	-	3.22	27.8	3.45
15	70.3	0.12	2.17	16.6	3.23
20	69.6	0.11	3.18	9.3	3.45
25	67.8	Samples haemolysed		5.2	3.55
30	64.6			3.6	3.33
45	61.9			2.4	3.42
60	65.0			3.0	3.52
			Animal 7439 (*Bos taurus*)		
0	57.3	0.13	4.13	8.3	4.09
5	69.5	-	4.62	51.7	3.71
10	70.1	-	4.00	37.3	3.68
15	71.2	-	5.00	29.8	3.61
20	70.6	-	5.00	22.7	3.64
25	70.8	Sample haemolysed		19.4	3.52
30	67.7	0.10	3.97	13.9	3.64
45	70.6			9.6	3.64
60	74.4			8.1	3.58

Table 23.

The effect of intravenous sodium acetate administration on the concentration of glucose in blood and VFA, potassium, adrenaline and noradrenaline in plasma

Sodium acetate given at time zero.

Time (min)	Blood glucose (mg/ 100 ml.)	Plasma Adren-aline (µg/l.)	Plasma Nora-drenaline (µg/l.)	Plasma VFA (mg acetic acid/ 100 ml.)	Plasma potassium (m.equiv/l.)
			Animal 7433 (*Bos indicus*)		
0	81.6	0.47	2.46	10.7	4.00
5	77.2	0.10	2.43	44.3	3.45
10	77.6	0.13	2.39	31.6	3.61
15	76.0	-	2.44	22.7	3.52
20	75.1	0.20	2.02	16.4	3.55
25	74.1	0.32	2.50	14.1	3.58
30	74.1	0.29	2.29	11.3	3.55
45	74.7			7.8	3.45
60	81.2			7.5	3.52
			Animal 6711 (*Bos indicus*)		
0	73.0	0.47	2.37	3.2	
5	86.3	-	2.47	49.9	
10	82.5	-	2.09	32.1	
15		0.12	3.34		
20	75.2	0.21	2.34	13.4	
25	71.8	0.22	3.60	7.9	
30	73.5	0.18	1.90	7.1	
45	66.6			3.9	
60	72.7			4.2	
			Animal 6712 (*Bos indicus*)		
0	82.1			6.5	3.96
5	84.5			64.6	3.68
10	82.1			49.6	3.74
15	79.0			36.0	3.58
20	80.0			23.0	3.64
25	79.2			16.7	3.64
30	78.3			9.9	3.64
45	81.7			8.9	3.74
60	83.2			5.3	3.68

PART II

CHAPTER III

THE EFFECT OF THE INTRAVENOUS INJECTION OF (i) SODIUM ACETATE, (ii) SODIUM PROPIONATE AND (iii) SODIUM BUTYRATE ON THE LEVELS OF GLUCOSE IN BLOOD AND VFA, POTASSIUM, ADRENALINE AND NORADRENALINE IN PLASMA

In these experiments sodium acetate and sodium propionate were administered at a dosage of 3.3 m.mole/kg body weight and sodium butyrate at a dosage of 2 m.mole/kg body weight. The injected solutions were prepared by dissolving the calculated amount of the sodium salt of the acid in as small a quantity of distilled water as possible, usually between 50 and 100 ml. The pH was adjusted to 7.4 with concentrated hydrochloric acid. This removed any effects that an injected alkaline solution might have on the sympatho-adrenal system. The solutions were then sterilized just before use by passage through a bacteriological filter.

(1) Sodium acetate

One experiment was performed on each of six animals and the results are set out in Tables 22 and 23. Plasma catecholamine and potassium estimations were made in five experiments. In three experiments haemolysis interfered with plasma catecholamine estimations.

Plasma adrenaline and noradrenaline

In every animal except one (Animal 7432, Table 22) there was a fall in the adrenaline levels to concentrations

below those that could be detected by the method, with little change in the noradrenaline levels. In some tests there was a slight but transient increase in the noradrenaline levels associated with the fall in the adrenaline levels. Within 15-30 min of injection detectable levels of adrenaline were found, usually less than the initial level.

Blood glucose

The results were variable and are shown in Fig. 20. In three animals (Nos. 7432, 6595 and 7439, Table 22) blood glucose levels rose and were still elevated after 1 hr. In two animals (Nos. 6711 and 6712, Table 23) there was a rise followed by a fall to below the initial level, and a return to the initial level or just above it after 1 hr. In the remaining animal there was a progressive fall after the acetate injection (Animal 7423, Table 23) but a return to just above the initial level after 1 hr. The changes in blood glucose concentration could not be correlated with any particular changes in the plasma catecholamine levels.

Plasma potassium

In all the experiments there was a fall in the plasma potassium levels, the falls being greatest in Animal Nos. 7432 and 6595 (Table 22). Both these animals showed a marked hyperglycaemic response. The animal showing the smallest fall was also the one whose blood glucose was least affected (Animal 6712, Table 23). Thus it was possible that there was a relationship between the blood glucose and plasma potassium levels.

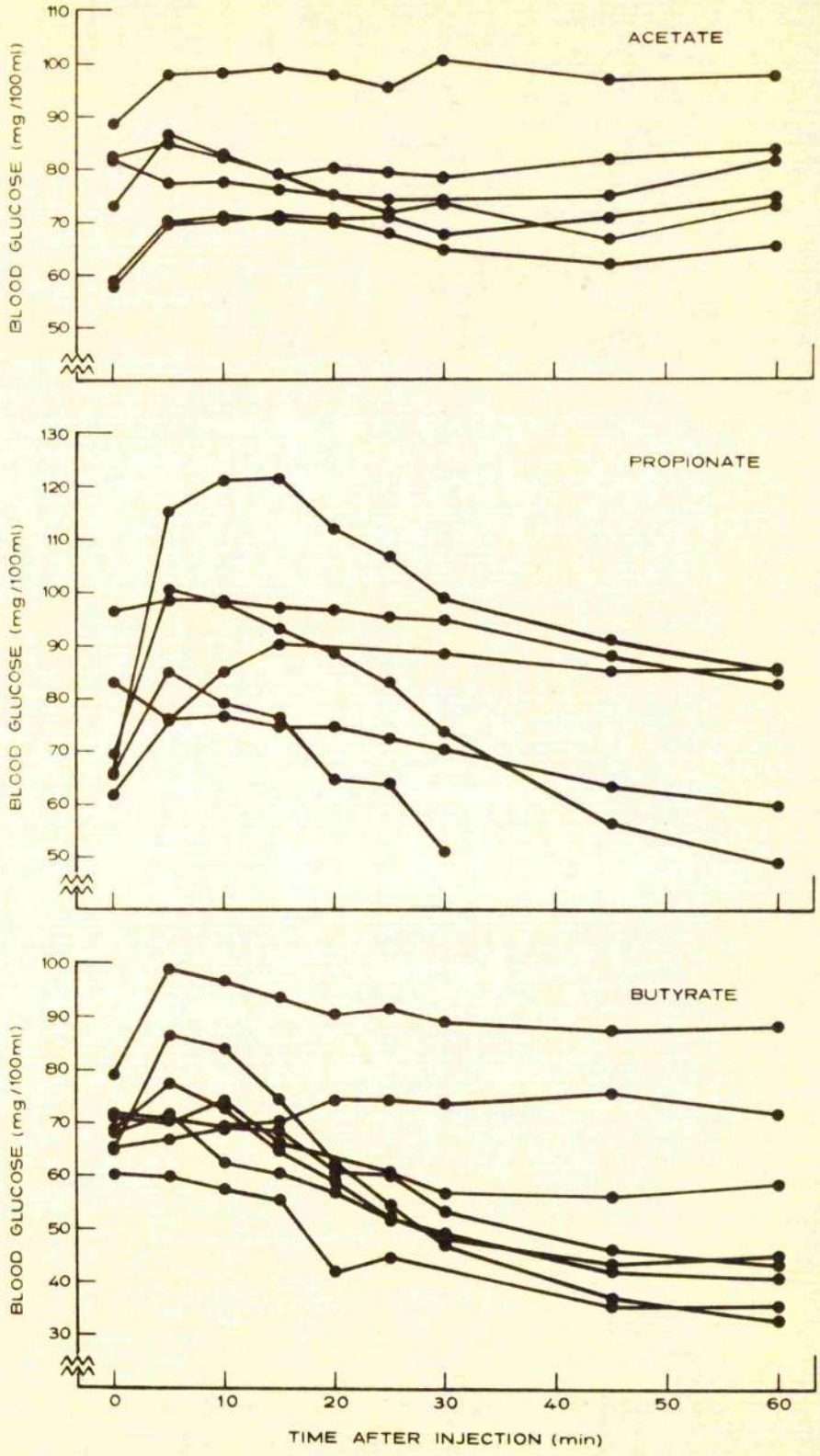


Fig. 20. The blood glucose response to intravenous administration of sodium acetate, propionate and butyrate.

Plasma VFA

The disappearance of acetate from the circulation after a single intravenous injection was exponential. As a measure of the rate of disappearance from the circulation, the half-time ($t_{1/2}$) was calculated. This is the time taken, in minutes for plasma acetate levels to reach half the value found 5 min after the injection of acetate and was determined using the formula

$$t_{1/2} = \frac{\log 2}{b}$$

where b = the slope of the regression line relating the log of the plasma acetate level with time. The slope was calculated statistically, thus:

$$b = \frac{\Sigma xy}{\Sigma x^2}$$

In order to obtain some information about the distribution of injected acetate, the volume of distribution (V) was determined from the formula

$$V = \frac{M}{C_Z}$$

where V is expressed in litres, C_Z is the theoretical concentration at zero time, expressed as mg acetic acid/l. of plasma, and M is the amount administered in mg. This volume was then expressed as a percentage of the body weight. The volume of distribution is an estimate of the volume of liquid that would be required to dilute the injected substance to give the concentration found at zero time. This concentration is determined by extrapolating back to zero, on a logarithmic scale, the line representing the disappearance

from the circulation of the injected substance. It is a theoretical value and is the concentration that would be found if mixing was instantaneous.

The acetate half-times and volumes of distribution obtained were:

Animal No. & type	$t_{1/2}$ (min)	V(% Body weight)
7439 (Bos taurus)	13.8	30.3
6595 (Bos taurus)	6.6	36.6
7432 (Bos indicus)	8.3	38.9
7433 (Bos indicus)	12.7	44.1
6711 (Bos indicus)	7.6	35.6
6712 (Bos indicus)	11.1	31.1

These half-times were used for certain statistical analyses. No correlations could be found between half-time, initial blood glucose and plasma potassium levels; no relationship was apparent between the pattern of blood glucose or plasma potassium responses. There was a highly significant positive correlation between the initial plasma VFA levels and the acetate half-times. An analysis of variance of this regression was:

	df	S.S.	M.S.	F	p
REGRESSION	1	36.65	36.65	23.34*	<0.01
DEVIATIONS	4	6.29	1.57		
TOTAL	5	42.94			

(2) Sodium propionate

Six experiments were performed on three animals, the results of which are shown in Tables 24 to 26. Plasma

Table 24.

The effect of intravenous sodium propionate administration on the concentration of glucose in blood and VFA, potassium, adrenaline and noradrenaline in plasma

Sodium propionate given at time zero.

Animal No. 6711 (*Bos indicus*)

Time (min)	Blood glucose (mg/ 100 ml.)	Plasma Adren- aline (µg/l.)	Plasma Nora- drenaline (µg/l.)	Plasma VFA (mg acetic acid/ 100 ml.)	Plasma potassium (m.equiv/l.)
0	69.2	0.20	3.10	8.3	4.67
5	100.5	0.65	4.55	65.8	3.58
10	97.9	0.59	4.30	43.9	3.39
15	93.3	0.47	3.26	28.0	3.23
20	88.7	0.22	1.84	16.0	3.13
25	83.0	-	1.41	11.2	3.13
30	73.9	-	1.65	7.3	2.97
45	56.6			5.5	2.94
60	49.0			6.8	2.91
0	65.3	0.56	3.85	5.7	3.93
5	85.1	1.18	4.81	59.1	3.20
10	79.0	1.90	4.03	43.1	3.20
15	76.4	1.33	5.39	26.4	3.20
20	64.6	1.16	5.16	19.0	3.20
25	63.8	1.20	3.65	12.5	3.20
30	51.2	0.66	3.51	6.3	3.33

Table 25.

The effect of intravenous sodium propionate administration on the concentration of glucose in blood and VFA, potassium, adrenaline and noradrenaline in plasma

Sodium propionate given at time zero.

Animal No. 6712 (*Bos indicus*)

Time (min)	Blood glucose (mg/ 100 ml.)	Plasma Adren- aline (µg/l.)	Plasma Nora- drenaline (µg/l.)	Plasma VFA (mg acetic acid/ 100 ml.)	Plasma potassium (m.equiv/l.)
0	83.1	0.27	4.63	6.3	3.45
5	75.9	0.71	5.90	36.6	2.81
10	76.5	0.44	5.34	27.1	2.69
15	74.5	0.47	3.73	16.7	2.56
20	74.5	0.52	3.83	11.5	2.53
25	72.4	0.38	3.77	7.5	2.58
30	70.4	0.28	3.82	5.2	2.49
45	63.4			5.7	2.49
60	59.7			5.5	2.57
0	96.3	0.33	3.37	7.9	3.96
5	98.4	2.92	3.28	71.8	3.07
10	98.2	1.68	2.39	55.4	3.04
15	97.2	1.75	1.14	41.3	2.94
20	96.8	1.37	1.69	31.1	2.94
25	95.3	0.46	2.62	19.6	2.97
30	94.9	1.33	1.19	12.8	3.10
45	88.0			7.0	3.07
60	82.7			8.4	3.10

Table 26.

The effect of intravenous sodium propionate administration on the concentration of glucose in blood and VFA, potassium, adrenaline and noradrenaline in plasma

Sodium propionate given at time zero.

Animal No. 6595 (*Bos taurus*)

Time (min)	Blood glucose (mg/ 100 ml.)	Plasma Adren- aline (µg/l.)	Plasma Nora- drenaline (µg/l.)	Plasma VFA (mg acetic acid/ 100 ml.)	Plasma potassium (m.equiv/l.)
0	65.5	0.13	3.75	4.2	4.00
5	115.5	1.67	6.36	32.9	3.29
10	121.2	0.85	6.36	21.1	3.29
15	121.6	1.63	4.56	12.5	3.23
20	112.0	1.73	6.40	7.5	3.20
25	106.6	1.36	5.60	5.3	3.07
30	98.9	1.50	6.36	4.7	2.97
45	91.2			3.7	2.94
60	85.6			4.1	3.10
0	61.7			5.3	4.19
5	75.5			148.2	3.45
10	85.0			99.1	3.49
15	90.1			42.1	3.20
30	88.6			6.8	2.94
45	85.4			5.0	2.91
60	85.4			8.7	2.91
90	84.5			7.1	2.85
120	79.4			2.9	2.78

catecholamine estimations were made in five experiments.

Plasma adrenaline and noradrenaline

In every experiment there was an increase in plasma catecholamine levels. In two animals (No. 6711, Table 24 and No. 6712, Table 25) it was transitory and returned to the initial levels within 30 min. In the other experiments the levels were still elevated even after 30 min. In most tests the adrenaline and noradrenaline levels varied together in the same way although in some experiments they varied independently.

Blood glucose

The results are shown in Fig. 20. In four experiments (Animal 6711 and 6595) there was a marked increase in blood glucose after sodium propionate injection; in Animal 6595 this remained elevated during the experimental period; in Animal 6711 it fell to below the initial level at the end of the experimental period. In the experiments on Animal 6712 there was a slow fall in blood glucose levels, although in one of the experiments there was a slight elevation lasting for about 20 min, followed by a slight fall. There was no apparent relationship between the increase in plasma catecholamine levels and the degree of hyperglycaemia.

Plasma potassium

In every experiment there was a marked fall in the plasma potassium levels following administration of the sodium propionate. In two experiments (Tables 24 and 25) the fall

was progressive, whereas in the other experiments the fall after 5 min was halted. These last two exceptions were the experiments in which there was only a transient increase in plasma catecholamine levels, so there appeared to be some relationship between plasma potassium levels and plasma catecholamines, especially the adrenaline component. The catecholamines appeared to have an elevating effect on the potassium concentrations counteracting the depressant effect of the injected propionate.

Plasma VFA

As with sodium acetate (p. 51) the half-times of injected propionate were determined, together with the volumes of distribution, which were as follows:

Animal No. & type	$t_{1/2}$ (min)	V(% Body weight)
6711 (Bos indicus)	7.9	34.8
6711 (Bos indicus)	7.6	28.6
6712 (Bos indicus)	8.6	58.0
6712 (Bos indicus)	12.5	33.2
6595 (Bos taurus)	5.5	11.6
6595 (Bos taurus)	7.4	45.0

There was a highly significant correlation between the half-times and the initial levels of blood glucose, as shown by the analysis of variance of the regression:

	df	S.S.	M.S.	F	p
REGRESSION	1	23.37	23.37	25.68***	<0.01
DEVIATIONS	4	3.65	0.91		
TOTAL	5	27.02			

Table 27.

The effect of intravenous sodium butyrate administration on the concentration of glucose in blood and VFA, potassium, adrenaline and noradrenaline in plasma

Sodium butyrate given at time zero.

Animal No. 6595 (*Bos taurus*)

Time (min)	Blood glucose (mg/ 100 ml.)	Plasma Adren- aline (µg/l.)	Plasma Nora- drenaline (µg/l.)	Plasma VFA (mg acetic acid/ 100 ml.)	Plasma potassium (m.equiv/l.)
0	64.9	0.18	3.42	2.3	3.96
5	86.4	0.32	3.83	20.5	3.33
10	84.1	0.91	4.64	8.6	3.26
15	74.7	1.30	4.38	3.5	3.33
20	62.5			3.9	3.33
25	54.8	0.50	4.01	3.2	3.26
30	47.2	0.17	3.62	3.3	3.42
45	37.3			2.5	3.45
60	32.9			1.0	3.58
0	65.4	0.20	2.50	5.0	4.16
5	66.7	0.20	2.40	26.1	3.58
10	69.0	0.15	2.40	16.2	3.84
15	68.4	0.25	2.65	9.2	3.58
20	60.7	0.25	2.65	5.5	3.33
25	60.3	0.20	2.40	4.6	3.07
30	53.5			3.8	3.10
45	46.6			2.9	3.23
60	43.6			4.0	3.33
0	60.1	0.15	2.77	2.0	4.09
5	59.7	0.20	2.80	25.5	3.39
10	57.6	0.30	2.80	13.9	3.45
15	55.7	0.20	2.70	7.6	3.39
20	42.1	0.20	2.70	4.3	3.33
25	44.8			3.6	3.26
45	35.7			2.0	3.58
60	35.8			2.0	3.58

Table 28.

The effect of intravenous sodium butyrate administration on the concentration of glucose in blood and VFA, potassium, adrenaline and noradrenaline in plasma

Sodium butyrate given at time zero.

Animal No. 7439 (*Bos taurus*)

Time (min)	Blood glucose (mg/ 100 ml.)	Plasma Adren- aline (µg/l.)	Plasma Nora- drenaline (µg/l.)	Plasma VFA (mg acetic acid/ 100 ml.)	Plasma potassium (m.equiv/l.)
0	68.0	0.41	3.01	No sample	No sample
5	71.8	0.26	3.16	18.6	3.33
10	62.5	0.13	2.50	10.2	3.13
15	60.6			4.3	2.97
20	57.1	0.18	2.50	2.4	3.01
25	52.1	0.19	2.50	2.2	2.94
30	49.5			1.8	2.94
45	42.2			1.3	3.04
60	41.1			1.4	3.07
0	68.8	0.30	1.64	2.4	4.19
5	77.4	0.70	2.86	14.1	3.64
10	72.7	0.33	2.24	6.7	3.52
15	64.7	0.33	2.21	3.3	3.45
20	58.9	0.15	2.21	2.2	3.45
25	52.3	0.43	2.73	1.7	3.52
30	48.4			1.6	3.45
45	43.9			2.2	3.52
60	45.4			2.5	3.45

Table 29.

The effect of intravenous sodium butyrate administration on the concentration of glucose in blood and VFA, potassium, adrenaline and noradrenaline in plasma

Sodium butyrate given at time zero.

Time (min)	Blood glucose (mg/ 100 ml.)	Plasma Adren-aline (µg/l.)	Plasma Nora-drenaline (µg/l.)	Plasma VFA (mg acetic acid/ 100 ml.)	Plasma potassium (m.equiv/l.)
			Animal 6712 (*Bos indicus*)		
0	79.1	0.72	1.79	3.7	3.29
5	99.0	0.72	1.78	32.1	2.91
10	96.9	0.34	2.10	23.1	2.69
15	93.8	0.72	1.82	14.4	2.56
20	90.6	0.68	1.93	6.8	2.81
25	91.7	0.68	1.93	5.9	2.69
30	89.3	0.34	2.10	4.6	2.65
45	87.7			4.5	2.65
60	88.4			3.9	2.62
			Animal 7433 (*Bos indicus*)		
0	71.2			5.9	3.55
5	70.1			19.0	3.33
10	74.5			12.1	2.94
15	66.2			8.6	2.69
20				4.7	2.94
25	60.9			2.9	3.04
30	57.0			2.2	3.23
45	56.5			1.6	3.26
60	58.7			2.0	3.13
			Animal 7436 (*Bos indicus*)		
0	71.9			4.1	4.06
5	71.0			19.8	3.64
10	69.5			10.6	3.71
15	70.4			8.3	3.93
20	74.7			3.1	3.96
25	74.7			2.0	3.90
30	74.0			1.8	3.90
45	75.9			1.8	3.74
60	72.1			1.1	3.55

There was a tendency for the hyperglycaemic response and the half-times to be related, in that Animal 6595 which had the greatest hyperglycaemic response also had the lowest half-time and Animal 6712 which had practically no hyperglycaemic response had the greatest half-time. The results for Animal 6711 were intermediate. The relationship, however, was not a linear one.

(3) Sodium butyrate

Eight experiments were performed on five animals, the results of which are set out in Tables 27 to 29. Plasma catecholamine determinations were carried out in six experiments.

Plasma adrenaline and noradrenaline

The results were variable. In two experiments (Table 27) there was no significant change. In two other experiments a slight rise occurred; in one experiment it was a progressive rise reaching a maximum after 15 min and being mainly in the adrenaline component (Table 27); in the other the increase was transient occurring at 5 min (Table 28), and was associated with a slight increase in blood glucose. In two other experiments (Tables 28 and 29) there was a fall in plasma catecholamines being slight in one experiment and quite marked in the other experiment.

Blood glucose

The blood glucose responses varied from a prolonged increase to a prolonged fall (Fig. 20). The plasma

catecholamine response did not appear to affect the blood glucose response. There was a correlation between the initial blood glucose levels and the change found after 60 min. The line of best fit and individual points are shown in Fig. 21. Analysis of variance of the regression relating change in blood glucose 60 min after sodium butyrate injection with the initial blood glucose concentration was:

	df	S.S.	M.S.	F	p
REGRESSION	1	147.00	147.00	21.3^{***}	<0.01
DEVIATIONS	6	41.31	6.89		
TOTAL	7	188.31			

$\hat{Y} = 74.42 + 0.33X$

Plasma potassium

In all the experiments there was an initial fall in plasma potassium concentration, the trend thereafter showing great individual variation but in no instance did the levels return to their initial value. There was no obvious correlation between the plasma potassium response and the plasma catecholamine or blood glucose response.

Plasma VFA

The calculated half-times and volumes of distribution for injected sodium butyrate were :

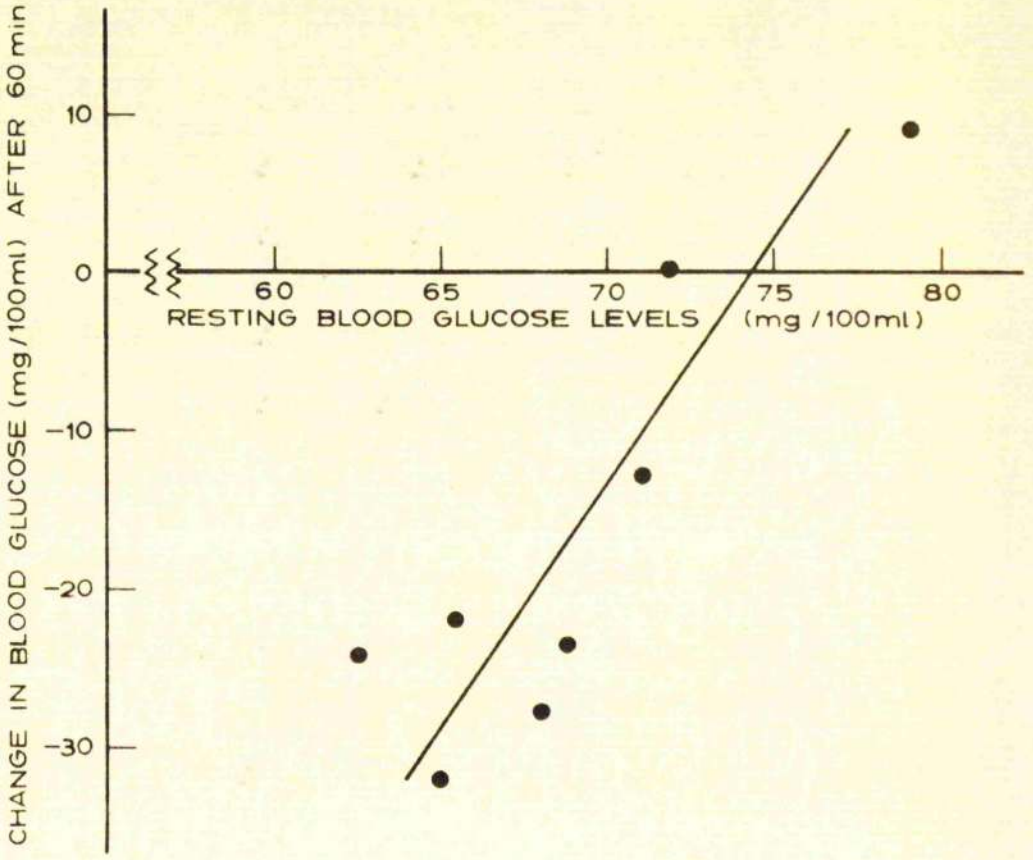


Fig. 21. The relationship between the change in blood glucose 60 min after intravenous butyrate administration and the initial blood glucose level.

Animal No. & type	$t_{1/2}$ (min)	V(% Body weight)
6595 (Bos taurus)	3.9	44.5
6595 (Bos taurus)	6.6	50.4
6595 (Bos taurus)	6.0	39.1
7439 (Bos taurus)	4.9	60.6
7439 (Bos taurus)	4.8	72.8
6712 (Bos indicus)	8.6	54.9
7433 (Bos indicus)	5.6	32.9
7436 (Bos indicus)	7.8	61.5

There were no correlations between these figures and initial blood glucose, plasma VFA or plasma potassium levels, or with the pattern of response of blood glucose or plasma potassium to intravenous sodium butyrate injection.

Conclusions:

1. Intravenous administration of sodium acetate caused a fall in circulating adrenaline levels but did not affect noradrenaline levels; sodium propionate caused a rise in both; sodium butyrate had no consistent effect.

2. Sodium acetate administration produced a rise in blood glucose levels in four experiments and depressed them in two experiments. Sodium propionate injection produced a rise in blood glucose levels in five out of six experiments. The magnitude of the rise was not related to the magnitude of the catecholamine response. Sodium butyrate produced changes in blood glucose concentration that were related to the initial blood glucose concentration, high initial values being associated with a hyperglycaemic response and low initial values being associated with a hypoglycaemic

response.

3. Plasma potassium levels fell following administration of the sodium salts of acetic, propionic and butyric acids.

4. Calculated half-times showed a positive linear relationship with the initial plasma VFA levels when sodium acetate was given and with initial blood glucose levels when sodium propionate was given.

5. Calculated volumes of distribution showed a wide range of variation.

Table 30.

The effect of the intravenous administration of glucose on the levels of glucose in blood and VFA, potassium, adrenaline and noradrenaline in plasma

Glucose given at time zero.

Time (min)	Blood glucose (mg/ 100 ml.)	Plasma Adren-aline (µg/l.)	Plasma Nora-drenaline (µg/l.)	Plasma VFA (mg acetic acid/ 100 ml.)	Plasma potassium (m.equiv/l.)
			Animal 6595 (*Bos taurus*)		
0	62.9	0.30	3.25	2.6	4.25
5	157.8	0.20	2.84	2.5	4.00
10	139.8	0.04	2.64	1.8	3.77
15	118.6	0.27	2.81	2.2	3.77
20	101.9	0.16	2.54	2.1	3.74
25	86.6	0.12	1.89	2.2	3.90
30	75.1	0.13	2.02	2.0	4.00
45	49.9			1.8	4.06
60	45.6			1.9	4.25
			Animal 7286 (*Bos taurus*)		
0	53.8			4.0	4.12
5	134.2			3.4	3.93
10	118.6			2.7	4.06
15	86.4			2.7	3.96
20	80.0			2.5	3.93
25	65.4			2.2	3.87
30	55.8			2.4	3.84
45	41.0			2.2	3.96
60	41.4			2.4	4.03
			Animal 7595 (*Bos taurus*)		
0	68.9	0.64	2.93	4.3	4.32
5	168.3	0.50	2.67	3.4	4.03
10	155.1	0.14	1.57	2.9	3.90
15	132.1	0.09	1.91	3.6	3.77
20	113.9	0.09	2.98	3.6	3.61
25	102.0	0.07	1.83	2.9	3.58
30	92.2	0.03	1.10	3.0	3.58
45	72.1			2.3	3.96
60	69.5			2.2	4.03

Table 31.

The effect of the intravenous administration of glucose on the levels of glucose in blood and VFA, potassium, adrenaline and noradrenaline in plasma

Glucose given at time zero.

Time (min)	Blood glucose (mg/ 100 ml.)	Plasma Adren-aline (µg/l.)	Plasma Nora-drenaline (µg/l.)	Plasma VFA (mg acetic acid/ 100 ml.)	Plasma potassium (m.equiv/l.)
Animal 6712 (*Bos indicus*)					
0	87.1			7.9	3.64
5	162.7			6.3	3.39
10	152.0			5.2	3.36
15	137.8			3.6	3.36
20	130.9			3.4	3.26
25	121.9			3.4	3.26
30	120.0			3.4	3.29
45	108.7			2.7	3.33
60	103.9			2.6	3.26
Animal 7433 (*Bos indicus*)					
0	83.5	0.71	2.00	5.7	3.58
5	169.2	0.66	1.82	3.3	3.45
10	160.4	0.53	1.50	3.1	3.39
15	145.8	0.84	2.45	4.3	3.33
20	135.2	0.10	1.93	4.0	3.39
25	126.2			2.8	3.20
30	116.6	0.17	1.85	2.9	3.20
45	100.7			3.4	3.17
60	90.3			3.4	3.13
Animal 7436 (*Bos indicus*)					
0	82.2	0.50	2.59	1.5	3.96
5	164.9	0.29	1.69	2.9	3.87
10	158.9	0.08	1.19	2.5	3.87
15	150.7	0.70	2.46	2.5	3.84
20	143.6	0.58	2.52	2.4	3.84
25	135.0	0.36	2.06	2.0	3.71
30	129.6			1.9	3.71
45	117.0			1.7	3.58
60	104.2			0.8	3.71

PART II

CHAPTER IV

THE EFFECT OF THE INTRAVENOUS ADMINISTRATION OF GLUCOSE ON THE LEVELS OF GLUCOSE IN BLOOD AND VFA, POTASSIUM, ADRENALINE AND NORADRENALINE IN PLASMA

Glucose was injected through a cannula inserted into one jugular vein and samples withdrawn from a cannula in the contralateral vein.

Glucose was administered at the rate of 0.18 g/kg body weight and the calculated amount was dissolved in the minimum quantity of distilled water and injected at a temperature of 39°C. The solution was less viscous at this temperature and therefore easier to inject.

One experiment was performed on each of six animals, the results being shown in Tables 30 and 31. In four of the experiments plasma catecholamine estimations were made.

Plasma adrenaline and noradrenaline

In every experiment there was a fall in both amines although the fall in the adrenaline component was much more pronounced. The fall in one animal (No. 6595, Table 30) was pronounced and prolonged. In one other experiment (Animal 7436, Table 31) it was transitory.

Blood glucose

From the changes in blood glucose following intravenous administration of glucose, it was possible to determine the half-time for the disappearance of glucose from the circulation by the method used for fatty acids in the previous chapter

(p.51). Similarly the volume of distribution could be determined from this data. These experiments were, in effect, intravenous glucose tolerance tests and the rate of utilization (k) was determined from the formula:

$$k = \frac{1}{t_{1/2}} \log_e 2$$

This specific rate constant, k, is numerically equal to the fraction of excess glucose removed per minute. It is more convenient to express this as a percentage (i.e. 100 k) which is known as the increment index (Duncan, 1956). The reason for assessing utilization rate by this means was so that the k values of the ox could be compared with those obtained for man and reviewed by Lundbaek (1962).

The calculated half-times, k values, and volumes of distribution are given in Chapter VI together with results from further experiments.

Plasma VFA

In every experiment, except one, there was a fall in plasma VFA levels after the intravenous administration of glucose. The exception (Animal 7436, Table 31) had the lowest initial plasma VFA level. In this animal there was a slight rise at 5 min followed by a progressive fall to below the initial level at the end of 1 hr. There was no obvious correlation between the degree of the change and the half-time for blood glucose or between the plasma VFA response and the plasma potassium response. The change in plasma VFA at 30 min was related to the initial level, as shown by the analysis of

variance of the regression, thus:

	df	S.S.	M.S.	F	p
REGRESSION	1	14.61	14.61	243.5***	<0.001
DEVIATIONS	4	0.22	0.06		
TOTAL	5	14.83			

In Chapter I it was noted that intravenous insulin administration also produced a fall in the plasma concentration of VFA and that the magnitude of the fall was related to the initial plasma concentration. In order to ascertain whether or not the effects of insulin and glucose on plasma VFA were quantitatively similar, the coefficients of the two regressions were compared. Since the variances of the two regressions did not show a statistically significant difference, a statistical comparison of the regression coefficients was valid. The difference between the regression coefficients gave a value for t of 0.368. The number of degrees of freedom were calculated from $n_1 + n_2 - 4$, which in this instance is 12. For twelve degrees of freedom $p<0.7$. The regressions were therefore not significantly different.

Plasma potassium

In all the experiments there was a fall in the plasma potassium levels, which were still below the initial levels at the end of 1 hr. In those animals which showed low half-time values, there tended to be an early recovery from the initial depression, whereas in the other experiments, where high half-time values were obtained, the fall tended to be continuous. It was not possible to demonstrate this effect

by statistical means.

In the one experiment which showed a persistent fall in plasma catecholamine levels, (Animal 7595, Table 30) there was a large fall in plasma potassium concentration, and the experiment in which there was no appreciable change in plasma catecholamines (Animal 7436, Table 31) there was only a slight fall in plasma potassium concentration.

Conclusions:

1. Intravenous glucose administration produced a fall in the levels of circulating catecholamines especially adrenaline.

2. Plasma VFA levels were depressed, the fall being proportional to the initial level and quantitatively similar to that produced by insulin.

3. Plasma potassium levels were depressed by intravenous glucose administration.

PART II

CHAPTER V

ARTERIOVENOUS STUDIES ON PLASMA VFA AND POTASSIUM LEVELS: THE EFFECTS OF THE INTRAVENOUS ADMINISTRATION OF INSULIN, GLUCOSE, ADRENALINE AND NORADRENALINE

In the experiments described in Chapter I (pp.36-38) it was noted that insulin produced a fall in plasma VFA levels, and in Chapter IV (pp. 60-61) it was shown that glucose produced quantitatively similar effects. In experiments not recorded in this thesis infusions of adrenaline and noradrenaline were also shown to depress plasma VFA levels. Similarly intravenous insulin or glucose administration had been shown to depress plasma potassium levels.

In order to examine the mechanism involved in these changes, simultaneous estimations of arterial and venous blood were made following insulin, glucose or catecholamine administration. Changes in the arteriovenous difference might reflect changes in the activity of the extra-hepatic tissues with respect to the substance being examined and give some indication of the possible site responsible for the changes noted; e.g. an increase in the arteriovenous difference would suggest an enhanced uptake by the extrahepatic tissues, and conversely a contraction of the arteriovenous difference might suggest that uptake had been inhibited.

The effects of insulin were studied in three experiments; insulin was given at the rate of 1 unit/kg body weight into the cannula in the right jugular vein. Samples were taken

Table 32.

The effect of intravenous insulin administration on the levels of VFA and potassium in arterial and venous plasma

Insulin given at time zero.

Time (min)	Plasma VFA (mg acetic acid/100 ml.)			Plasma potassium (m.equiv/l.)		
	A	V	A-V	A	V	A-V
	Animal 7604 (*Bos taurus*)					
-10	6.3	3.8	2.5	4.35	4.35	0
0	4.5	2.4	2.1	4.35	4.35	0
10	3.2	1.7	1.5	3.68	3.84	-0.16
20	3.0	1.7	1.3	3.33	3.49	-0.16
30	2.9	1.2	1.7	3.13	3.26	-0.13
40	3.2	1.7	1.5	2.94	3.10	-0.16
50	3.5	1.6	1.9	3.07	3.13	-0.06
60	4.6	2.5	2.1	3.07	3.10	-0.03
	Animal 7630 (*Bos taurus*)					
-10	3.3	2.0	1.3	3.84	3.96	-0.12
0	3.8	2.1	1.7	3.68	3.71	-0.03
10	3.1	1.9	1.2	3.33	3.42	-0.09
20	3.4	1.7	1.7	3.23	3.36	-0.13
30	2.8	1.8	1.0	3.07	3.01	+0.06
40	2.8	1.8	1.0	2.94	3.01	-0.07
50	2.5	1.4	1.1	2.94	2.94	0
60	2.4	1.4	1.0	2.85	2.81	+0.04
	Animal 7599 (*Bos taurus*)					
-10	2.8	1.1	1.7	4.41	4.51	-0.10
0	2.7	0.9	1.8	4.41	4.51	-0.10
10	1.9	0.7	1.2	3.84	4.03	-0.19
20	2.1	1.1	1.0	3.64	3.80	-0.16
30	2.0	1.1	0.9	3.58	3.71	-0.13

Table 33.

The effect of intravenous glucose infusion on the levels of VFA and potassium in arterial and venous plasma

Glucose infused from time zero until time 30 min.

Time (min)	Plasma VFA (mg acetic acid/100 ml.)			Plasma Potassium (m.equiv/l.)		
	A	V	A-V	A	V	A-V
	Animal 7599 (Bos taurus)					
-10	5.2	2.9	2.3	4.60	4.60	0
0	3.8	2.5	1.3	4.57	4.73	-0.16
10	4.8	3.0	1.8	4.92	4.73	+0.19
20	3.6	2.0	1.6	4.28	4.54	-0.26
30	2.4	1.5	0.9	4.41	4.60	-0.19
40	3.1	1.8	1.3	3.96	4.28	-0.32
50	2.6	1.5	1.1	4.00	4.32	-0.32
60	3.1	1.6	1.5	3.90	3.96	-0.06
	Animal 7630 (Bos taurus)					
-10	3.8	2.1	1.7	4.44	3.93	0.51
0	3.6	2.1	1.5	4.35	3.58	0.77
10	2.5	1.5	1.0	4.44	4.09	0.35
20	1.8	1.0	0.8	4.73	4.22	0.51
30	1.8	1.0	0.8	4.60	4.12	0.48
40	2.3	1.1	1.2	4.28	3.74	0.54
50	2.0	1.2	0.8	4.16	3.65	0.51
60	2.6	1.1	1.5	4.06	3.81	0.25
	Animal 7604 (Bos taurus)					
0	4.7	2.2	2.5	4.73	4.86	-0.13
5	5.2	3.1	2.1	4.41	4.51	-0.10
10	4.8	2.9	1.9	4.22	4.44	-0.22
15	3.6	2.0	1.6	4.38	4.57	-0.19
20	3.1	1.6	1.5	4.51	4.64	-0.13
25	3.6	1.9	1.7	4.28	4.38	-0.10
30	3.9	2.0	1.9	4.38	4.35	+0.03

A = Arterial

V = Venous

Table 34.

The effect of intravenous adrenaline infusion on the levels of VFA and potassium in arterial and venous plasma

Adrenaline infused from time zero to time 30 min.

Time (min)	Plasma VFA (mg acetic acid/100 ml.)			Plasma potassium (m.equiv/l.)		
	A	V	A-V	A	V	A-V
	Animal 7604 (Bos taurus)					
-10	4.3	2.8	1.5	4.35	4.12	+0.23
0	2.9	1.8	1.1	4.54	4.25	+0.29
10	1.7	1.0	0.7	4.41	4.48	-0.09
20	1.7	1.2	0.5	4.09	4.12	-0.03
30	2.0	1.3	0.7	3.74	3.87	-0.13
40	2.3	1.7	0.6	3.84	3.93	-0.09
50	2.4	1.9	0.5	3.74	3.55	+0.19
60	2.7	1.7	1.0	3.71	3.68	+0.03
	Animal 7596 (Bos taurus)					
-10	5.6	2.9	2.7			
0	4.8	2.4	2.4			
10	3.5	2.5	1.0			
20	3.8	2.7	1.1			
30	3.4	2.5	0.9			
40	2.8	2.1	0.7			
50	3.0	1.7	1.3			
60	3.5	2.0	1.5			

A = Arterial

V = Venous

Table 35.

The effect of intravenous noradrenaline infusion on the levels of VFA and potassium in arterial and venous plasma

Noradrenaline infused from time zero to time 30 min.

Time (min)	Plasma VFA (mg acetic acid/100 ml.)			Plasma potassium (m.equiv/l.)		
	A	V	A-V	A	V	A-V
	Animal 7596 (*Bos taurus*)					
0	5.5	2.7	2.8	4.84	4.70	+0.13
10	4.1	2.7	1.4	4.86	4.89	-0.03
20	3.8	3.1	0.7	4.76	4.86	-0.10
30	3.1	2.7	0.4	4.73	4.67	+0.06
40	3.9	2.5	1.4	4.54	4.41	+0.13
50	4.0	1.9	2.1	4.51	4.51	0
60	4.0	2.2	1.8	4.22	4.32	-0.10
	Animal 7590 (*Bos taurus*)					
-10	5.7	3.4	2.3	4.54	4.51	+0.03
0	5.2	3.0	2.2	4.57	4.67	-0.10
10	5.1	4.0	1.1	4.57	4.67	-0.10
20	4.4	3.1	1.3	4.60	4.67	-0.07
30	3.8	2.0	1.8	4.67	4.67	0
40	3.1	1.7	1.4	4.19	4.41	-0.22
50	3.5	2.0	1.5	4.00	4.16	-0.16
60	3.6	2.1	1.5	3.96	4.09	-0.13

A = Arterial

V = Venous

simultaneously from the right carotid artery and jugular vein at 10 min intervals for an hour thereafter an analysed for plasma VFA and potassium. The results are shown in Table 32.

Three experiments were performed in which the effects of glucose were studied. Glucose was given (0.18 g/kg) by infusion into the left jugular vein and samples removed simultaneously from the right jugular vein and carotid artery. The results of these experiments are shown in Table 33.

Similarly experiments were performed in which the effects of adrenaline and noradrenaline infusions were studied. Both amines were given at the rate of 0.6 μg/kg/min into the contralateral jugular vein and arterial and venous blood taken at 10 min intervals thereafter. The results are shown in Tables 34 and 35. In one experiment no potassium estimations were made.

All animals were Bos taurus type.

(1) Plasma VFA

Reid (1950b) has reported a linear relationship between arterial VFA levels and arteriovenous VFA differences. That this relationship exists in the ox was established by using all the 'resting values' from the present experiments, i.e. eighteen estimations from five animals and ten experiments. An analysis of variance of the regression gave the following:

	df	S.S.	M.S.	F	p
REGRESSION	1	3.08	3.08	32.0***	<0.001
DEVIATIONS	16	1.52	0.095		
TOTAL	17	4.60			

$\hat{Y} = 0.27 - 0.39X$

The regression, therefore, was highly significant and is shown graphically in Fig. 22.

(i) Effect of intravenous insulin injection

Examination of the results showed that the fall that occurred in the VFA levels in venous plasma also occurred in arterial plasma, and that the relationship between arterial VFA concentration and arteriovenous VFA differences still remained. An analysis of variance of this regression, i.e. all the results following the administration of insulin gave the following:

	df	S.S.	M.S.	F	p
REGRESSION	1	1.37	1.37	30.4^{***}	< 0.001
DEVIATIONS	13	0.58	0.045		
TOTAL	14	1.95			

$$\hat{Y} = 0.02 + 0.46X$$

The regression therefore was highly significant.

(ii) Effect of intravenous glucose infusion

Similarly after glucose administration, an analysis of variance of the regression gave the following:

	df	S.S.	M.S.	F	p
REGRESSION	1	2.57	2.57	98.8^{***}	< 0.001
DEVIATIONS	16	0.41	0.026		
TOTAL	17	2.98			

$$\hat{Y} = 0.20 + 0.38X$$

The regression was highly significant.

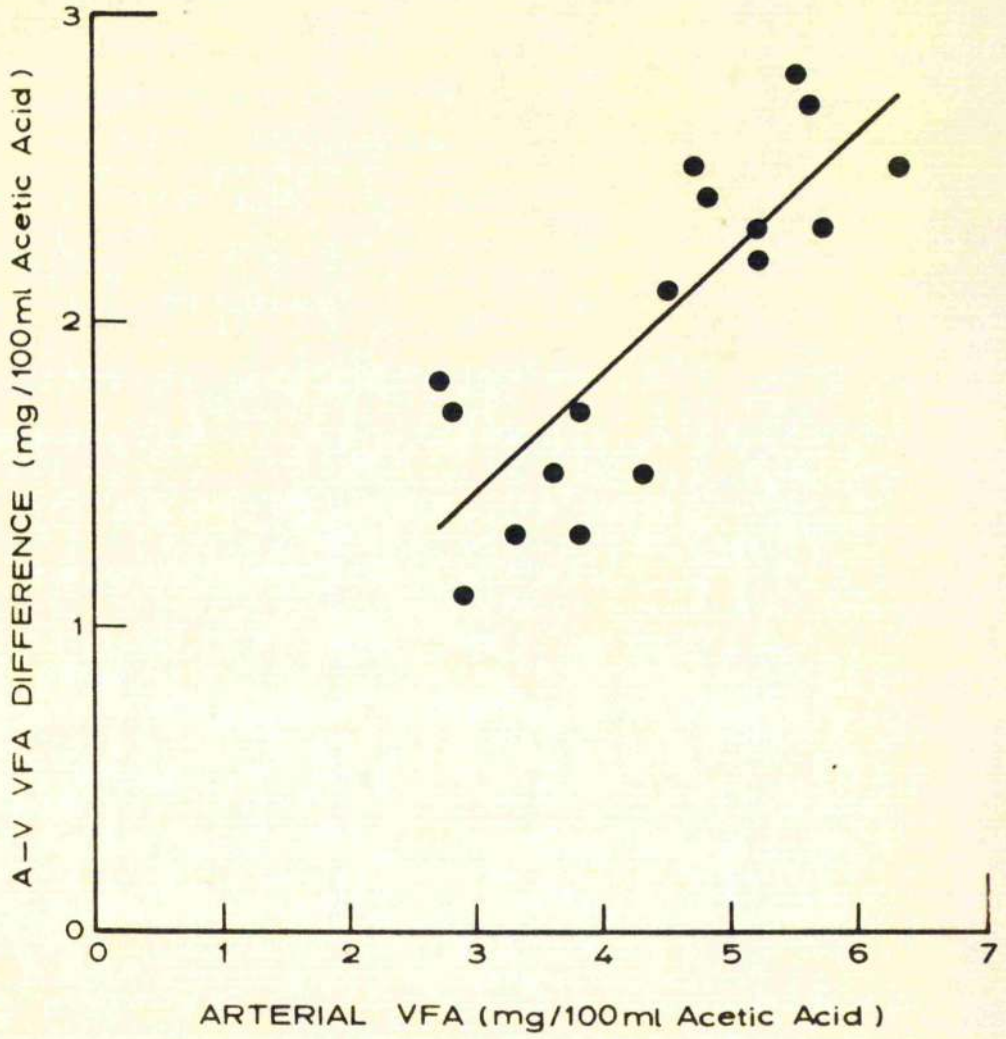


Fig. 22. The relationship between arteriovenous differences and arterial levels of plasma VFA.

(iii) Effect of intravenous adrenaline infusion

From estimations taken during adrenaline infusion analysis of variance of the regression gave the following:

	df	S.S.	M.S.	F	p
REGRESSION	1	0.22	0.22	27.5***	<0.01
DEVIATIONS	4	0.03	0.008		
TOTAL	5	0.25			

$$\hat{Y} = 0.25 + 0.21X$$

The regression was highly significant.

(iv) Effect of intravenous noradrenaline infusion

Although noradrenaline produced a fall in plasma VFA the arterial and venous changes were not proportionate and the regression of the arteriovenous differences on the arterial levels was not statistically significant. There was a marked contraction in the arteriovenous difference immediately following and during the infusion.

In order to compare the slopes of the insulin, glucose and 'resting level' regressions, their regression coefficients were compared. The 'resting level' regression used was derived from the pre-injection values obtained in the insulin and glucose experiments only and was as follows:

	df	S.S.	M.S.	F	p
REGRESSION	1	1.11	1.11	12.34***	<0.01
DEVIATIONS	9	0.8	0.09		
TOTAL	10	1.91			

$$\hat{Y} = 0.58 + 0.31X$$

The regression was highly significant.

Before comparing the regression coefficients the variance ratios (F) of the regressions were derived and found to be not significantly different.

(a) Insulin and glucose regressions

$$F = \frac{0.040}{0.026} = 1.73 \text{ N.S.}$$

(b) Insulin and 'resting level'

$$F = \frac{0.090}{0.045} = 2.0 \text{ N.S.}$$

(c) Glucose and 'resting level'

$$F = \frac{0.090}{0.026} = 3.46 \text{ N.S.}$$

(N.S. = Not significant)

The pooled regression is:

	df	S.S.	M.S.	F	p
REGRESSION	1	4.97	4.97	103.5***	<0.001
DEVIATIONS	39	1.87	0.048		
TOTAL	40	6.84			

Comparison of regressions:

	df	S.S.	M.S.	F	p
JOINT REGRESSION	1	4.97	4.97	103.5***	<0.001
DIFFERENCE BETWEEN REGRESSIONS	2	0.08	0.04	1.2	N.S.
SUM OF REGRESSIONS	3	5.05			
DEVIATIONS	37	1.79	0.048		
TOTAL	40	6.84			

∴ The joint regression was highly significant.

∴ The difference between the regressions was not significant.

A comparison of the regression coefficients of the adrenaline regression and the other regressions revealed that only the glucose and adrenaline regressions could be compared, since the variances of the 'resting level' and insulin regressions were significantly different from the adrenaline regression. The variance ratio of the adrenaline and glucose

regressions was derived and found to be:

$$F = \frac{0.026}{0.0078} = 3.33 \text{ N.S.}$$

The regression coefficients for the glucose and adrenaline regressions were 0.317 and 0.213 respectively. The difference between them was significant ($t = 2.16$ $p<0.05$).

Comparison of regression coefficients does not complete the possible comparison between the regressions. It is possible to have lines with the same slope but which are separate and distinct, i.e. the distance between them is significant. Before this can be done two conditions must be satisfied (a) the variances of the lines must be comparable and (b) the regression coefficients must be comparable. Both these conditions have been fulfilled, as described above, so the distance between the regressions could be determined.

(a) <u>Distance between the insulin and glucose regressions</u>

Overall regression coefficient = 0.398.

The two regressions are:-

Glucose $\hat{Y} = 1.39 + 0.398 (X - 3.16)$

Insulin $\hat{Y} = 1.34 + 0.398 (X_1 - 2.89)$

The distance between the lines is 0.06

Its standard error is ± 0.065

$$\therefore t = \frac{0.06}{0.065} = 0.923.$$

For 31 degrees of freedom $p > 0.3$. The distance therefore is not significant.

(b) Distance between the insulin and 'resting level' regressions

Overall regression coefficient = 0.365.

The two regressions are:-

'Resting level' $\hat{Y} = 1.85 + 0.365 \ (X - 4.05)$

Insulin $\hat{Y} = 1.34 + 0.365 \ (X_1 - 2.89)$

The distance between them is 0.09.

Its standard error is $\pm$ 0.102.

$$\therefore t = \frac{0.09}{0.102} = 0.88.$$

For 24 degrees of freedom $p > 0.3$. The distance therefore is not significant.

(c) Distance between the glucose and 'resting level' regressions

Overall regression coefficient = 0.351.

The two regressions are:-

'Resting levels' $\hat{Y} = 1.85 + 0.351 \ (X - 4.05)$

Glucose $\hat{Y} = 1.39 + 0.351 \ (X_1 - 3.16)$

The distance between the lines is 0.148.

Its standard error is $\pm$ 0.090.

$$\therefore t = \frac{0.148}{0.090} = 1.644.$$

For 27 degrees of freedom $p > 0.1$. The distance therefore is not significant.

The calculated regressions are shown in Fig. 23.

Summarizing this statistical examination, it can be stated that although a fall was produced in plasma VFA levels

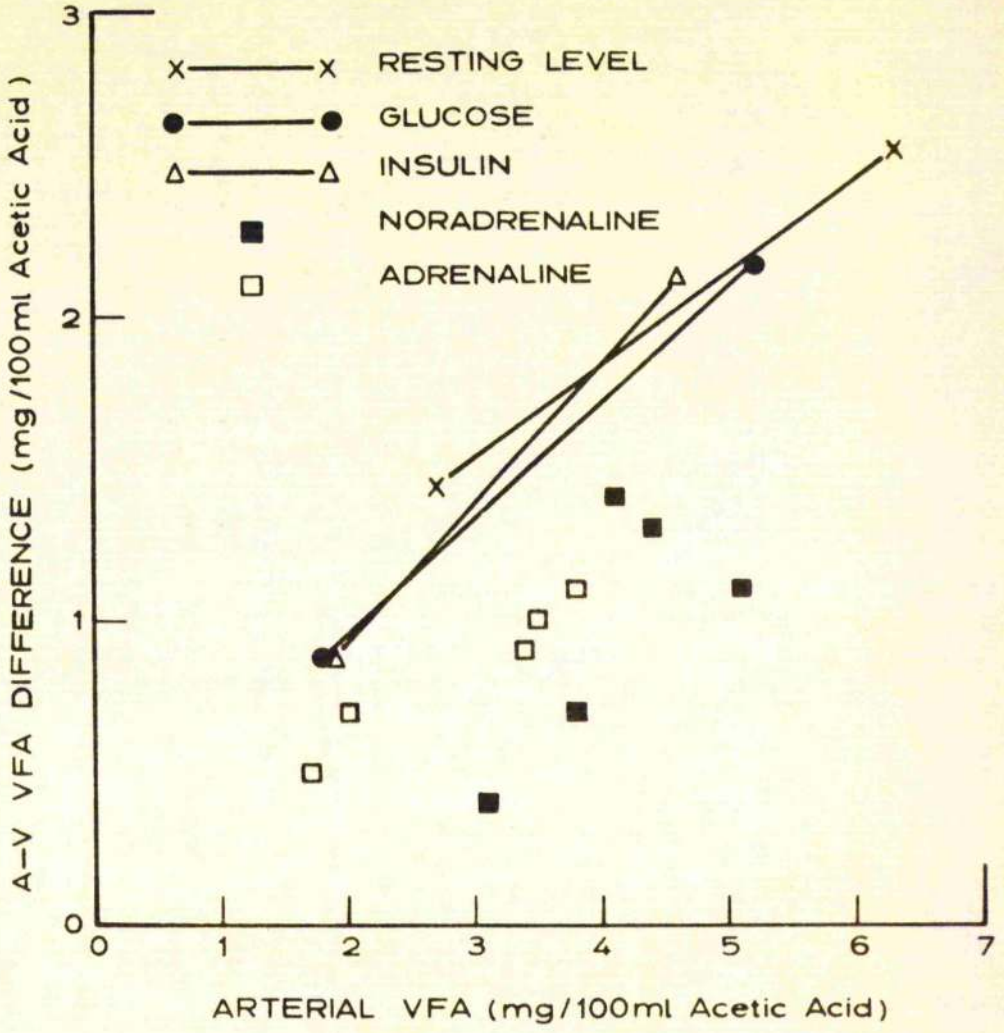


Fig. 23. The effect of insulin and glucose on the calculated regression lines relating arteriovenous difference to arterial plasma VFA levels. Individual points obtained during adrenaline and noradrenaline infusion have been inserted.

following glucose and insulin administration there was no significant change in the relationship between the arterial VFA levels and arteriovenous VFA difference, the fall occurring along the regression line towards the origin.

(2) Plasma potassium

(i) Effect of intravenous insulin injection

In two experiments (Table 32) insulin made the arterio-venous difference more negative as the arterial level fell and less negative when it began to rise again. In the remaining experiment there was an initial increase, but thereafter the course was somewhat erratic, in two instances positive differences being found although the levels continued to fall.

(ii) Effect of intravenous glucose infusion

The results were not consistent. In two experiments the immediate effect of glucose administration was to make the existing negative arterio-venous difference less negative. Thereafter the arteriovenous differences returned to the initial value or became more negative. In the remaining experiment there was a positive arteriovenous difference which was little affected by glucose infusion.

(iii) Effect of intravenous adrenaline and noradrenaline infusion

Infusions of adrenaline and noradrenaline converted positive arteriovenous differences into negative ones, which eventually became positive again.

Conclusions

(1) A linear relationship between arterial levels of plasma VFA and arteriovenous plasma VFA differences was established. Intravenous insulin or glucose administration had no statistically significant effect on this relationship. It was concluded that the fall in plasma VFA levels following insulin or glucose administration was not due to an enhancement of uptake from the circulation by the extrahepatic tissues. Infusions of adrenaline and noradrenaline altered the regression relating arterial levels of plasma VFA and arteriovenous differences. Adrenaline infusion significantly decreased the slope of the regression. Noradrenaline infusion resulted in a marked contraction of the arteriovenous differences of plasma VFA. It was concluded that the fall in plasma VFA caused by adrenaline or noradrenaline infusion was not due to an enhanced uptake of VFA from the circulation by the extrahepatic tissues.

(2) Insulin and catecholamines appear to increase the loss of potassium from the extrahepatic tissues. Glucose may have had an opposite effect but this was transitory and not established.

Table 36.

The blood glucose response of Bos indicus to 1 unit/kg body weight insulin given intravenously at time zero

(Blood glucose expressed as mg/100 ml.)

Animal No.	7432	7436	7433	7435	6712[m]	6711[mm]	7431	7681	7437	7683
Time (hr)										
0	59.2	76.5	60.3	68.8	82.2	66.3	76.0	69.5	66.7	91.8
0.5	28.9	29.1	25.4	25.7	46.4	32.7	37.1	35.1	33.4	47.0
1	26.5	33.5	29.3	26.6	49.0	36.2	31.0	24.8	43.9	35.8
1.5	29.7	33.3	31.9	25.2	54.6	38.0	35.8	27.7	43.9	32.5
2	33.7	37.0	32.8	28.0	61.2	42.0	37.5	28.8	43.1	31.7
3	43.0	48.1	40.8	33.2	67.8	37.6	39.2	29.4	49.2	34.6
4	47.7	62.7	50.9	41.9	73.4	39.4	39.4	28.3	55.8	39.3

m From Table 13

mm From Table 12 (2 units/kg)

Table 37.

The blood glucose response of Bos taurus to 1 unit/kg body weight insulin given intravenously at time zero

(Blood glucose expressed as mg/100 ml.)

Animal No.	6538*	6539**	6595	7428	7630	7439	7597	7628	7635	7686
Time (hr)										
0	63.7	68.1	50.9	57.0	63.2	67.7	61.0	69.8	57.7	57.9
0.5	27.4	24.5	16.5	23.0	19.3	21.4	19.8	28.0	21.9	13.2
1	38.6	24.0	14.3	17.7	18.5	24.4	18.8	25.5	21.7	12.0
1.5	32.1	26.4	12.9	17.9	17.5	30.5	20.5	25.1	23.2	27.3
2	29.7	30.7	14.1	16.9	20.2	30.7	17.5	25.3	22.5	17.6
3	39.2	25.7	13.4	23.9	24.9	35.8	17.1	29.8	26.1	
4	43.3	35.8	18.5	32.7	35.7	43.7	24.3	39.6	25.9	

* From Table 11

** From Table 11 (2 units/kg)

Table 38.

The plasma potassium response of Bos indicus and Bos taurus to 1 unit/kg body weight insulin given intravenously at time zero

Bos indicus

Animal No.	7432	7436	7433	7435	6712*	6711**	7431	7681
Time (hr)								
0	3.84	4.25	3.26	3.61	3.42	4.09	3.96	3.96
0.5	3.23	3.58	3.58	3.01	3.13	3.13	3.17	3.49
1	3.13	3.29	2.94	2.91	2.75	3.23	3.58	3.13
1.5	2.88	3.33	2.94	2.81	2.40	3.13	3.23	3.17
2	2.94	3.13	2.81	2.85	2.43	3.01	3.01	3.20
3	3.07	3.07	2.85	2.94	2.43	3.20	3.10	3.29
4	3.42	3.10	3.07	3.07	2.53	3.20	3.20	3.71

* From table 13

** From table 12 (2 units/kg.)

Bos taurus

Animal No.	6595*	7428	7430	7439	7597	7628	7635	7686
Time (hr)								
0	4.50	3.77	4.09	3.80	4.16	4.22	5.12	4.09
0.5	3.22	2.84	3.52	3.13	3.33	3.04	3.58	3.17
1	3.04	2.91	3.33	2.94	3.26	3.23	3.33	3.20
1.5	2.71	3.33	3.39	2.91	3.13	3.13	3.90	3.45
2	2.56	3.48	3.42	2.81	3.20	2.94	3.49	3.01
3	2.71	3.58	3.20	2.97	3.42	3.17	3.58	
4	3.17	3.90	3.77	3.13	3.68	3.26	3.64	

* From table 16

PART II

CHAPTER VI

COMPARATIVE ASPECTS OF THE CARBOHYDRATE AND POTASSIUM METABOLISM OF BOS INDICUS AND BOS TAURUS

The earlier experiments (p. 40, Chapter I) suggested that Bos taurus and Bos indicus might show certain differences in their carbohydrate and potassium metabolism. Accordingly some of the earlier observations were extended to a greater number of animals so that statistical analyses could be made.

(1) Blood glucose and plasma potassium response to insulin

Since 1 unit of insulin/kg body weight was sufficient to produce the maximum fall in blood glucose after 30 min, the original observations at this dosage were extended so that ten animals of each type were studied.

1 unit/kg was given to each animal after taking the initial blood sample, and at ½, 1, 1½, 2, 3 and 4 hr after insulin administration further blood samples were taken and analysed for blood glucose and plasma potassium. The blood glucose results are given in Tables 36 and 37, and the plasma potassium results in Table 38. Plasma potassium estimations were obtained for eight animals of each type. In some animals the results have already been given but they are given again here for completeness. Fig. 24 shows the mean changes in blood glucose concentration for Bos indicus and Bos taurus. The mean initial blood glucose concentration for Bos taurus was 61.7 ± 1.87 (S.E.) and for Bos indicus was 71.7 ± 3.18 (S.E.) mg/100 ml. The difference between the means was statistically

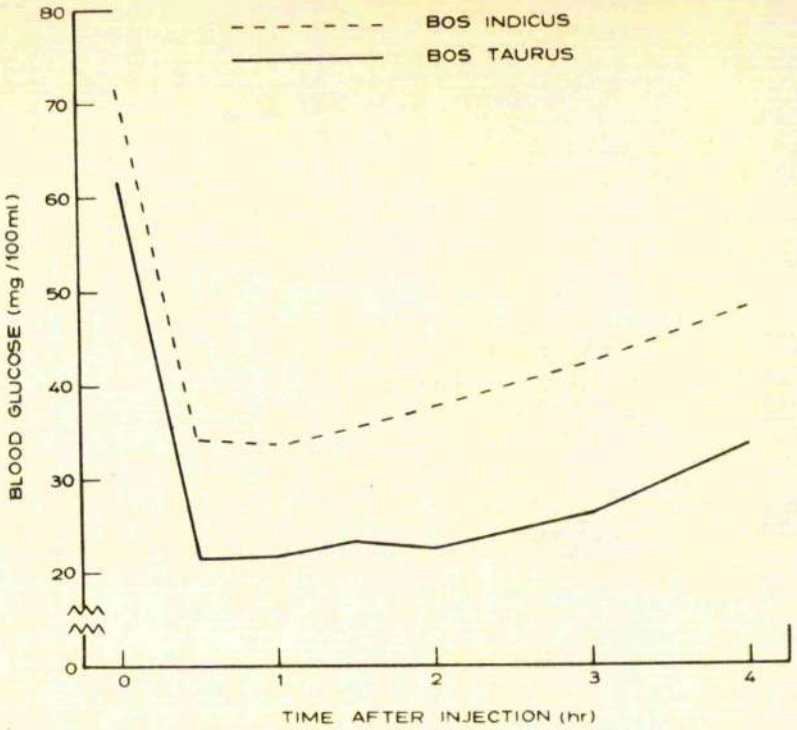


Fig. 24. The effect of intravenous insulin (1 unit/kg) administration on blood glucose levels of Bos taurus and Bos indicus. Each line represents the mean of ten animals

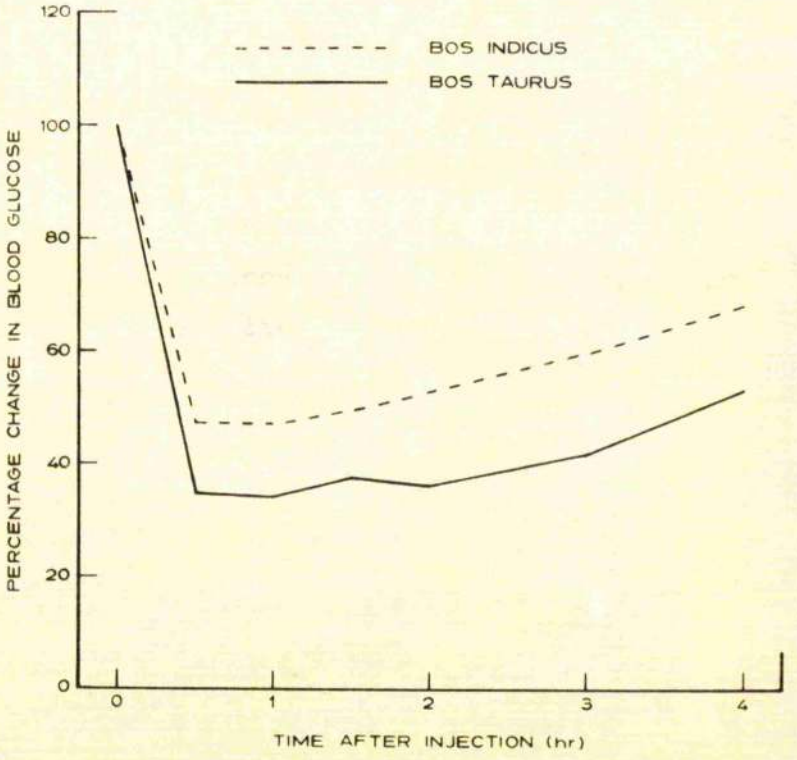


Fig. 25. The percentage change in blood glucose produced by the intravenous administration of insulin (1 unit/kg) in Bos taurus and Bos indicus. Each line represents the mean of ten animals.

significant ($t = 2.79$, $p < 0.02$).

In order to show that the difference between the hypoglycaemic responses was not just a reflection of the initial blood glucose values, the results were recalculated as a percentage of the initial value. The mean values are shown in Fig. 25 where it will be seen that there was still a difference between Bos indicus and Bos taurus, i.e. the difference was independent of the initial blood glucose level. Whether or not this difference was statistically significant was determined by calculating the line of best fit of the values from $\frac{1}{2}$ to 4 hr. The 99% confidence limits were then calculated and these together with the line of best fit are shown in Fig. 26. Since there is practically no overlap in the confidence limits then the difference in the degree of hypoglycaemia can be said to be statistically significant. The regressions relating percentage change in blood glucose, following insulin administration, with time were:

Bos taurus $\hat{Y} = 29.43 + 5.03X$

Bos indicus $\hat{Y} = 41.26 + 6.27X$

The 99% confidence limits are shown below.

Time (hr)	Bos indicus		Bos taurus	
	$\hat{Y}$	C.L.	$\hat{Y}$	C.L.
0.5	44.40	$\pm$4.25	31.95	$\pm$9.86
1.5	47.53	$\pm$2.86	34.46	$\pm$6.65
2	53.80	$\pm$2.64	39.49	$\pm$6.13
3	60.07	$\pm$3.45	44.52	$\pm$8.00
4	66.34	$\pm$5.16	49.55	$\pm$12.00

C.L. = 99% Confidence Limits.

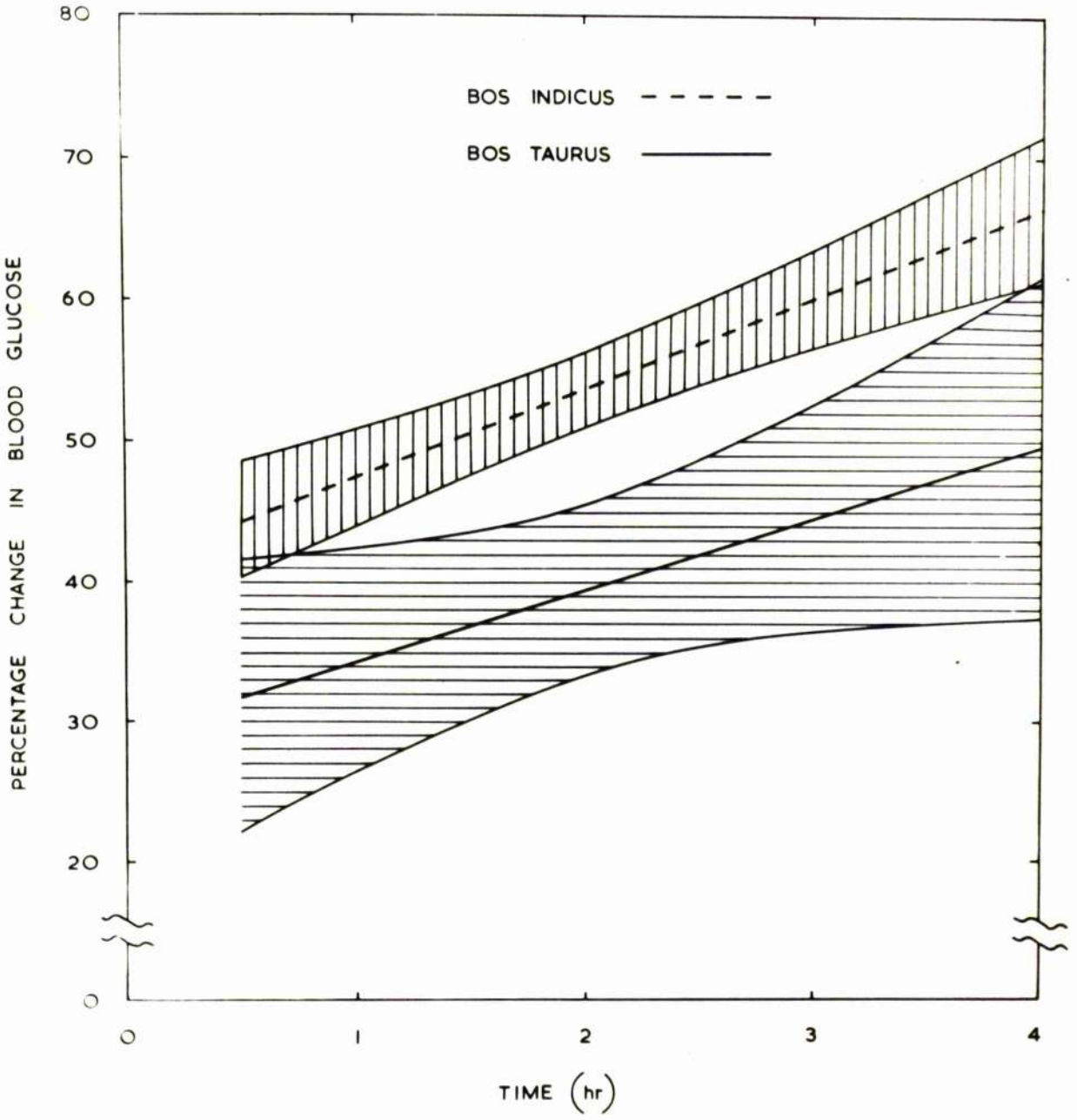


Fig. 26. The calculated regression lines from 30 min onwards of the percentage changes in blood glucose following insulin administration in Bos taurus and Bos indicus. Shaded areas represent the 99% confidence limits.

The rates of recovery from the hypoglycaemia between Bos taurus and Bos indicus were compared by analysing statistically the slopes of the two regressions. This gave a value for t of,

$$1.11 \quad (p < 0.4)$$

showing that there was no significant difference between the slopes, and demonstrating that the rate of recovery was similar in both types of animal.

The means of the initial plasma potassium levels for Bos indicus were 3.80 $\pm$0.12 (S.E.) and for Bos taurus were 4.22 $\pm$0.15 (S.E.)% m.equiv/l. The difference between the means was statistically significant ($t = 2.16$, $p < 0.05$). Because of this difference the changes were estimated as a percentage change of the initial level. There was a significant difference between the means of the percentage change after $\frac{1}{2}$ hr ($t = 2.49$, $p < 0.02$) and after 1 hr ($t = 2.90$, $p < 0.01$), Bos taurus showing the greater percentage depression. Thereafter there was no significant difference between the means of the two types of animal.

(2) Intravenous glucose tolerance tests

Estimates of glucose utilisation can be obtained by intravenous glucose tolerance tests. 0.18 g/kg body weight of glucose was injected intravenously and samples taken at 5, 10, 15, 20, 25, 30, 45 and 60 min thereafter. Results obtained in the previous chapter were used and six more experiments performed, making twelve experiments in all, of which six were with Bos indicus and six with Bos taurus

Table 39.

The blood glucose response to 0.18 g/kg body weight glucose given intravenously at time zero

Animal No.	Bos taurus			Bos indicus		
	7628	7635	7430	7437	7683	7425
Time (min)						
0	70.8	50.8	60.3	63.5	85.0	62.3
5	181.5	157.5	206.9	175.5	161.2	183.7
10	168.2	144.1	179.6	162.7	160.0	160.8
15	155.1	138.1	160.4	151.7	150.5	146.7
20	148.5	120.9	152.5	146.0	148.7	137.4
25	145.2	114.0	142.2	131.3	138.1	129.5
30	138.6	105.7	133.1	125.6	135.1	130.5
45	124.7	81.7	109.6	106.5	119.2	113.0
60	116.5		93.7	89.2	112.0	107.4

type animals. In all experiments the disappearance from the circulation was exponential. Half-times ($t_{1/2}$), specific rate constant (k) and volumes of distribution (V), were calculated as described before. The actual results from the extra experiments are shown in Table 39. The values of $t_{1/2}$, k and V are shown below:

	Animal No.	k (%)	$t_{1/2}$ (min)	V(% B.W.)
BOS TAURUS	6595	3.05	22.7	9.7
	7286	3.57	19.4	11.2
	7595	2.54	27.3	9.3
	7635	1.62	42.8	10.5
	7628	1.06	65.2	9.6
	7430	1.70	40.9	8.3
BOS INDICUS	7683	0.77	90.4	10.6
	7437	1.35	51.4	9.6
	7436	0.99	69.8	10.3
	7433	1.52	45.5	9.8
	7425	1.39	49.8	9.6
	6712	1.27	54.6	10.6

B.W. = body weight

An analysis of the half-times shows that there was a significant difference between the means of the two types of animal ($t = 2.43$, $p > 0.05$), Bos indicus showing the higher values. Similarly the k% values were significantly different. The volumes of distribution expressed as a percentage of body weight were fairly uniform and showed no difference between the two types of animal.

There was a significant correlation between the half-times and the initial blood glucose levels (Fig. 27). An

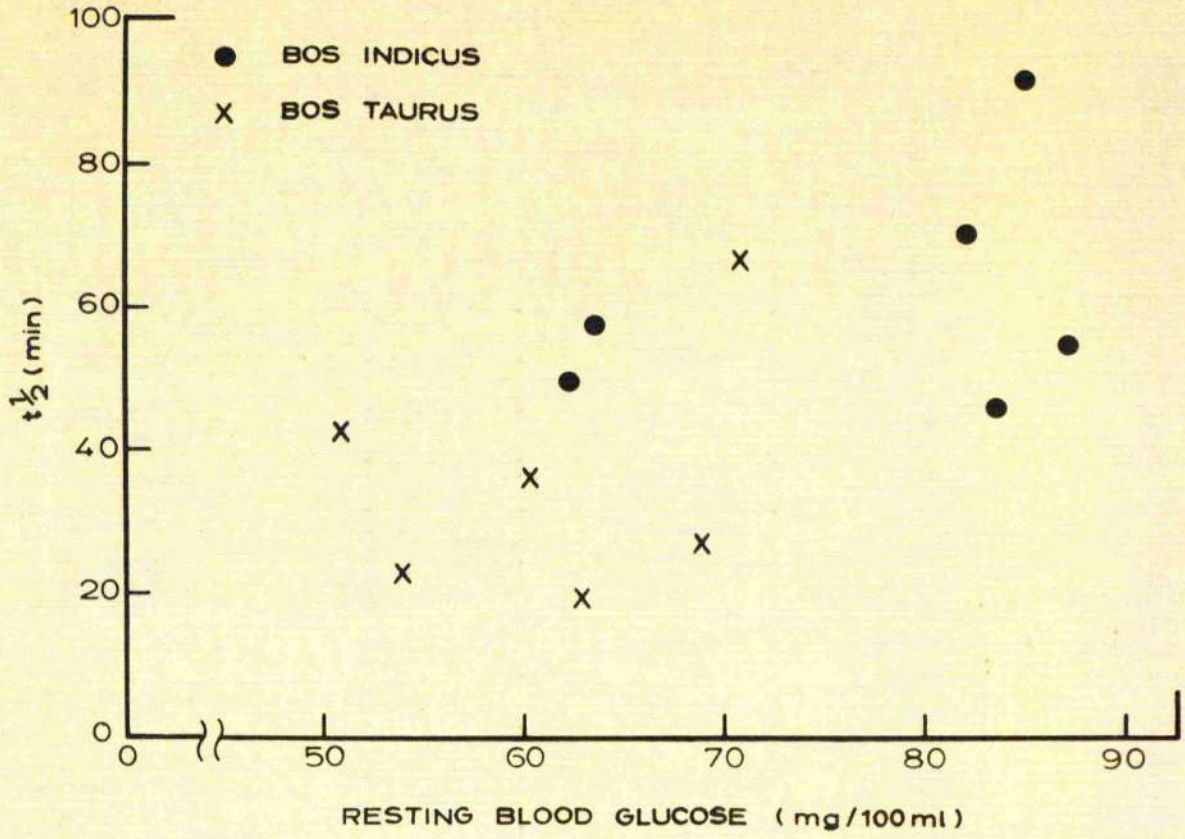


Fig. 27. Relationship between resting blood glucose levels before injection and glucose half-times.

Table 40.

Normal blood glucose concentrations of Bos indicus and Bos taurus

Bos taurus		Bos indicus	
No. of determinations	Mean concentration mg./100 ml.	No. of determinations	Mean concentration mg./100 ml.
3	73.6	9	69.4
6	69.7	6	76.9
16	58.8	2	73.9
8	60.6	7	65.3
1	68.1	4	74.2
2	59.0	2	77.4
5	66.2	2	88.4
3	65.9	1	68.8
2	70.0	1	76.0
2	61.8	2	65.1
1	57.0	1	62.3
1	61.0		
2	70.3		
2	54.2		

Table 41.

Normal plasma potassium concentrations of Bos indicus and Bos taurus

Bos taurus		Bos indicus	
No. of determinations	Mean concentration (m.equiv/l.)	No. of determinations	Mean concentration (m.equiv/l.)
1	4.22	7	3.65
1	4.45	4	4.18
16	4.24	2	3.80
6	3.96	7	3.68
1	4.32	4	4.00
2	4.00	2	3.84
1	4.16	1	3.61
4	4.40	1	3.96
5	4.35		
3	4.60		
4	4.01		
1	3.77		
1	4.16		
1	4.22		
1	5.12		

analysis of variance of this regression gave:-

	df	S.S.	M.S.	F	p
REGRESSION	1	1874.61	1874.61	6.88*	< 0.05
DEVIATIONS	10	2721.63	272.16		
TOTAL	11	4596.24			

A similar relationship existed between the K% values and the initial blood glucose values.

(3) Normal values for blood glucose and plasma potassium in Bos indicus and Bos taurus

Since a significant difference in blood glucose and plasma potassium values had been found in the experiments reported in Section 2, all the values obtained throughout this work were examined and analysed. The method of blood collection ensured that there was a minimum disturbance of the animal and so the normal blood glucose and plasma potassium values obtained should be uncomplicated by any sympathetic activity.

(i) Blood glucose: Fifty-four determinations on fourteen Bos taurus type animals and thirty-seven determinations on eleven Bos indicus type animals were made. The results are shown in Table 40. The mean blood glucose level for Bos taurus was 64.0 $\pm$1.57 (S.E.) mg/100 ml. and for Bos indicus was 72.5 $\pm$2.2 (S.E.) mg/100 ml. This difference was highly significant ($t = 3.21$, $p < 0.01$).

(ii) Plasma potassium: Forty-six determinations on fifteen Bos taurus type animals and twenty-eight determinations on

eight Bos indicus type animals were made. The results are shown in Table 41. The mean plasma potassium level for Bos taurus was 4.27 ±0.08 (S.E.) m.equiv/l. and for Bos indicus was 3.84 ±0.07 (S.E.) m.equiv/l. This difference between the means was highly significant ($t = 3.38$ $p < 0.01$).

Conclusions

1. Bos taurus showed lower blood glucose and higher plasma potassium levels than Bos indicus.

2. Bos indicus showed a greater resistance to insulin than Bos taurus in that the degree of hypoglycaemia was significantly less. There was no difference between members of the two breeds in the rate of recovery from hypoglycaemia.

3. For 1 hr after the administration of insulin plasma potassium levels fell to a greater extent in Bos taurus than in Bos indicus.

4. There was a significant difference in the rate at which injected glucose disappeared from the circulation between Bos indicus and Bos taurus, the half-time being greater for Bos indicus.

5. There was a significant correlation between the glucose half-times and initial blood glucose concentrations.

GENERAL DISCUSSION

Resting levels of plasma catecholamines in the ox.

Euler (1955) quotes figures for the resting levels of catecholamines in bovine peripheral plasma as being about 2.1 μg/l. for noradrenaline and 0.5 μg/l. for adrenaline, that is, adrenaline accounts for about 20% of the total catecholamines. The mean levels in the experiments described in this thesis were 2.5 μg/l. (range 0.4-4.6) noradrenaline and 0.31 μg/l. (range 0.03-0.7) adrenaline. The mean percentage of adrenaline was about 11% of total catecholamines. The levels found, therefore, were comparable to those described by Euler (1955) although the percentage of adrenaline was lower. About 75% of catecholamine production by the bovine adrenal medulla is adrenaline (Silver, 1960). The difference between the proportions of adrenaline in adreno-medullary secretion and in peripheral plasma is partially due to the fact that some of the circulating noradrenaline is derived from adrenergic nerve endings (Euler, 1955).

The effect of insulin administration on blood glucose levels.

The mean fall in blood glucose following the intravenous injection of 1 unit/kg body weight of insulin was 37.7 ± 5.7 (S.D.) mg/100 ml. for *Bos indicus* and 40.2 ± 4.6 (S.D.) mg/100 ml. for *Bos taurus*. These values represent the falls occurring during the first 30 min following insulin injection. Jasper (1953) recorded falls of 25-30 mg/100 ml. in cattle and Reid (1951a) falls of 17.1 - 24.7 mg/100 ml. in sheep.

Reid also noted that the rate of fall was slow when compared with the rate in non-ruminants, where falls of 50-60 mg/100 ml. are usually recorded. Thus it would seem that the high initial blood glucose levels reported in this work are also reflected in the relatively greater fall in blood glucose following insulin administration. Reid (1951a) described a 'plateau' effect of the blood glucose levels, whereby the levels tended to fall to, and remain at 5-10 mg/100 ml. without any signs of hypoglycaemic convulsions. Jasper (1953) failed to record this effect in cattle. The experiments recorded in this work demonstrate that a 'plateau' effect can occur in cattle although at a higher level than in sheep, namely 25-30 mg/100 ml. In any one animal the levels of blood glucose 30 min after insulin administration were identical at dosages above 1 unit/kg., and this level approximately represented the level of the 'plateau'. This suggests that dosages above 1 unit/kg saturate the tissues and produce a maximum effect on the blood glucose response. Increasing the dosage merely permits the state of saturation to be maintained over a longer time and prolong the hypoglycaemia. This saturation was noted by other authors in work reviewed by Reid (1951a).

The results of the experiments described here on erythrocyte glucose concentration during insulin hypoglycaemia are similar to those reported in man and in dogs by Trimble & Maddock (1928).

The relatively slow decline in blood glucose following insulin that is seen in ruminants may be explained by the

antagonist action to insulin that the short chain fatty acids possess (Lindsay, 1961). This could explain the small arteriovenous glucose changes that Reid (1952) reported following insulin administration in sheep. In man insulin produces a pronounced expansion of arteriovenous glucose differences (Somogyi, 1949). Long chain fatty acids also have an antagonist action towards insulin (Randle et al. 1963) but plasma levels of these acids in sheep are similar to those found in other species (Annison, 1960). Because of the slow rate of fall of blood glucose produced by insulin in sheep, Reid (1951b) considers that blood glucose levels closely reflect changes in the glucose levels of the brain. In dogs, there is such a rapid uptake of glucose after insulin administration by extrahepatic tissues other than the brain, and blood glucose levels fall so rapidly, that changes in brain glucose concentration lag behind changes in blood glucose concentration (Paschkis & Cantarow, 1943). Attempts, therefore, to correlate the degree of hypoglycaemia with clinical signs of hypoglycaemia in ruminants and non-ruminants are difficult. Ruminants can withstand the prolonged hypoglycaemia produced by intravenous insulin without showing any clinical signs of the hypoglycaemia. Reid (1951b) considers the critical blood glucose level is 5 mg/100 ml.; below this level signs of hypoglycaemia, e.g. convulsions, develop. However, in the experiments reported here hypoglycaemic convulsions occurred in two experiments at blood glucose levels of 17.6 and 15.6 mg/100 ml. and yet blood glucose levels lower than this had occurred in samples taken

earlier in the experiment. This is probably an example of a relatively rapid fall in blood glucose in which the brain glucose changes lag behind the blood glucose changes.

Sympatho-adrenal response to intravenous insulin administration.

The amount of glucose reaching the brain is dependent on the concentration of blood glucose and the cerebral blood flow. These factors may vary independently of each other, yet produce the same glucose level within the brain cells. Thus doubling the blood flow could reduce the critical blood glucose level for normal brain metabolism from 10 to 5 mg/100 ml. Jarrett & Potter (1953) showed that in adult sheep the blood glucose level could be maintained between 5 and 10 mg/100 ml. for 15-18 hr without any hypoglycaemic signs, whereas hypoglycaemic convulsions developed in splanchnicotomized sheep at a blood glucose level of 10 mg/100 ml. within 3-6 hr of insulin administration. In their experiments insulin was given intravenously.

Since splanchnicotomy denervates the adrenal medulla, it would appear that adrenomedullary secretion has a protective action, in that it delays the onset of clinical signs of hypoglycaemia. This can be achieved by two actions that the adreno-medullary secretion possesses: (1) the stimulation of release of glucose from the liver and (2) the stimulation of an increase in cerebral blood flow (Solokoff, 1959).

In man hypoglycaemic convulsions and coma occur 2-3 hr after large intravenous doses of insulin (Mayer-Gross & Walker,

1945). Hypoglycaemic signs occur in man, rabbits, mice, dogs and cats after 2-3 units of insulin/kg body weight (Reid, 1951a). The ruminant animal and especially sheep appear to be relatively resistant to insulin since they rarely show clinical signs of hypoglycaemia even when massive doses of insulin are given and even though blood glucose levels remain depressed for many hours. However, splanchnicotomy removes this resistance and convulsions are then more readily obtained. It would seem, therefore, that the adreno-medullary secretion of the ruminant animal is more efficient in protecting it against hypoglycaemic convulsions than is that of non-ruminants.

The brain of sheep resembles that of non-ruminants in depending on glucose as its main energy source (McClymont & Setchell, 1956), and quantitatively the brain of sheep utilizes glucose to the same extent as those of other species (Setchell, 1959). The brain metabolism of ruminants thus appears to be essentially the same as that of non-ruminants, and yet sheep are able to withstand prolonged hypoglycaemia without convulsions, and the normal blood glucose concentration of sheep is at a level that would cause convulsions in non-ruminants. This can possibly be explained by the fact that blood glucose levels give no indication of the concentration of glucose in different tissues. Blood glucose levels represent the balance between inflow of glucose into the blood and outflow from it, but give no indication of the relative proportions of outflow going to the brain and other tissues. These proportions differ in the ruminants and

non-ruminants both under normal conditions and after insulin administration. Thus, in the non-ruminants the uptake of glucose by tissues other than brain before and after insulin administration is much greater than in the ruminants. This can be inferred from the relatively small A-V blood glucose differences before and after insulin administration that Reid (1950b) recorded in sheep. The brain in both ruminant and non-ruminant species takes up a similar quantity of glucose from the circulation, but it is a relatively much greater proportion of the total blood glucose in ruminants than in non-ruminants. Hence ruminants can tolerate low normal blood glucose levels and withstand prolonged hypoglycaemia without having an apparent effect on brain metabolism. The greater efficiency of adrenomedullary secretion in ruminants noted above may similarly be explained along these lines. If glucose is added to the circulation during hypoglycaemia caused by insulin administration, then a greater proportion of this will be removed by tissues other than the brain in non-ruminants than in ruminants. Similarly, in ruminants, a large proportion of the glucose released by sympathetic activity is available for brain metabolism because of the low glucose uptake by the other extrahepatic tissues.

The concentration of blood glucose at which the hypothalamic neurones are stimulated to evoke an adrenomedullary secretion appears to be much lower in ruminants than in non-ruminants. In these present experiments it was 15-25 mg/100 ml., a level which is similar to that reported for sheep (14-20 mg/100 ml.) by Setchell & Waites (1963). Cannon et al. (1924)

reported levels of 70-80 mg/100 ml. for the cat; Houssay et al. (1924) and La Barre & Houssa (1932) values of 50 and 75 mg/100 ml. respectively for the dog, and Armin & Grant (1959) 50-70 mg/100 ml. for the rabbit. Except for the present work, all these figures were obtained by indirect methods of catecholamine assay. Since in non-ruminants, there is a rapid rate of fall of blood glucose following insulin administration, it is likely that the intracellular concentration at which the hypothalamic neurones are stimulated is much higher in non-ruminants than in ruminants and the difference is much greater than the blood glucose levels would suggest. In this respect, therefore, there appears to be a true difference in the brain metabolism of ruminants and non-ruminants. It is interesting to note that no sympatho-adrenal stimulation occurred in the insulin resistant animal, blood glucose levels never reached a low enough level and the resistance to insulin was therefore in no way associated with adrenomedullary hyperactivity.

By direct analysis of suprarenal venous blood during insulin induced hypoglycaemia, Duner (1954) observed an increase only in the adrenaline component of the adrenomedullary secretion, with no significant change in the noradrenaline output. Likewise, Holzbauer & Vogt (1954) could only detect a rise in the plasma adrenaline levels of the dog and of one man during insulin hypoglycaemia, and could not detect an increase in plasma noradrenaline levels. It was somewhat surprising therefore that Weil-Malherbe & Bone (1954a) should report a fall in the circulating levels of

plasma adrenaline with no change in noradrenaline levels. Millar (1956) using the ethylene-diamine method of estimation could only detect a rise in adrenaline levels in the dog and man. This was associated with increases in cardiac output and blood pressure. Goldfien et al. (1958) did, however, detect a slight rise in noradrenaline concentrations in the dog during insulin hypoglycaemia, also using the ethylenediamine method. In support of evidence for selective secretion of adrenaline from the adrenal medulla, Burn et al. (1950) found a decrease of both adrenaline and noradrenaline content of the suprarenal medulla of rats following insulin administration, with a decrease in the percentage of adrenaline. Their results have been confirmed by Hökfelt (1951) and and Outschoorn (1952), and in rabbits by Udenfriend et al. (1953). From these experiments it would appear that hypoglycaemia leads to a selective secretion of adrenaline from the adrenal medulla whilst noradrenaline output is more or less unaltered. Arguing teleologically, this is in accordance with what would be expected, since adrenaline is much more active than noradrenaline in releasing glucose from the liver (Ellis, 1956). In the experiments reported in this work a rise in both adrenaline and noradrenaline levels in plasma occurred although the increase in adrenaline was greater than noradrenaline especially under conditions of prolonged hypoglycaemia.

Adrenaline increases cerebral blood flow purely by its pressor action, but noradrenaline in normotensive animals is known to decrease cerebral blood flow by increasing cerebral

vascular resistance (Sokoloff, 1959). The effects of adrenaline and noradrenaline on the cerebral blood flow of the ox remains to be investigated.

The relative hyperglycaemic potencies of adrenaline and noradrenaline showed much lower adrenaline: noradrenaline ratios than have been found in other species. Since the ratio obtained varies with the method of determination, a direct comparison can be made only with the results of Ellis & Anderson (1951), who used a method similar to the one used here. Their experiments were performed on rats, and they obtained an adrenaline:noradrenaline ratio of 15. Thus the present findings may be physiologically significant, the released noradrenaline during insulin hypoglycaemia having a greater hyperglycaemic action in the ox than in other species.

The blood vessels of the sympathetically denervated rabbit's ear are extremely sensitive to the constrictor action of adrenaline and this preparation has been used by Armin & Grant (1959) as an index of changes in adrenaline-like activity of the blood. According to these workers the release of constrictor substance, presumed to be adrenaline, is inversely related to the blood glucose concentration at levels below the critical blood glucose level for sympathetic activity. When the blood glucose rises above the critical level 'adrenaline' release is reduced. Similarly glucose injection causes an immediate inhibition of 'adrenaline' release. Their findings were not borne out in the experiments described here, in which the fall in plasma catecholamine

levels occurred a long time after the blood glucose levels had risen above the critical level. In the experiments of Armin & Grant (1959) part of the constrictor activity of the denervated rabbit's ear was due to the hypoglycaemia per se and not to released catecholamines, since animals with denervated adrenal glands also displayed constrictor activity. Therefore, part of the lessening in constrictor tone associated with a rise in blood glucose levels may be due to the rise in blood glucose itself and not to a diminished catecholamine production. However, one would still expect a considerable amount of constrictor activity to be present if catecholamine production still continued.

Millar (1956) found that, although there was a considerable reduction in circulating adrenaline levels following intravenous glucose administration to hypoglycaemic dogs, the levels did not revert to the original levels, but were still elevated 30 min afterwards. Mayer, Gross & Walker (1945) found that intravascular and extravascular levels of glucose took a long time to equilibrate after intravenous glucose administration. They suggested that intracellular levels likewise would take longer still. This delay in equilibration of the glucose levels in the various bodily compartments might partially explain the results of Millar (1956) and those recorded in this thesis. The glucose levels in the glucose sensitive neurones in the hypothalamus which control sympatho-adrenal activity might not have increased to the extent that the blood glucose concentration would suggest.

The increase in noradrenaline levels occurring a few minutes after high doses of insulin could be the result of direct stimulation of the sympatho-adrenal system, as shown by Pereda et al. (1962), who reported an increase in blood pressure 2 - 9 min after injection of insulin in the dog, without hypoglycaemia. The effect was marked and immediate when small doses of insulin were administered into the carotid artery. However, blood pressure measurements were not made during the present experiments, and it is uncertain to what extent the increases in noradrenaline levels noted could cause a rise in blood pressure.

Sympatho-adrenal responses to intravenous glucose administration

The fall in plasma catecholamine levels following intravenous glucose injection supports the findings of Duner (1953) but is contrary to those of Weil-Malherbe & Bone (1954b) who found that the hyperglycaemia following ingestion of glucose in man was associated with an increase in plasma adrenaline. Duner's experiments demonstrated an inverse relationship between blood sugar and catecholamine output especially with respect to adrenaline production. No such relationship was apparent here and the fall in catecholamine levels did not occur until 20-30 min after injection in most animals. This delay might be expected because of the time taken for glucose to penetrate the cells of the hypothalamus (Mayer-Gross and Walker, 1945).

Sympatho-adrenal response to intravenous VFA administration.

In general the results of the experiments on the sympatho-adrenal response to intravenous injection of the lower fatty acids do not support the conclusion of Ash et al. (1959) that the hyperglycaemia following butyrate administration results from sympatho-adrenal stimulation. The catecholamine response to butyrate administration was equivocal since a rise in plasma catecholamines occurred in only two out of six experiments.

Reid & Mills (1961) deduced from their results that propionate injections did not have a sympatho-adrenal stimulating effect, which contrasts with the findings reported here. Their deductions were made because of the rapid rate of clearance of excess glucose after propionate administration, which was faster than that seen after adrenaline injection. However, other factors may promote the uptake of glucose from the circulation during elevated plasma catecholamines, as shown in these experiments where falls in blood glucose were noted whilst catecholamine levels were elevated. Reid & Mills (1961) do, however, comment that the rate of rise of blood glucose is so very rapid following propionate injection that direct conversion to glucose seems unlikely. Ash et al. (1959) postulated that only a small amount of glucose was derived directly from propionate and that most of the glucose was derived from sympatho-adrenal stimulation. This, however, is contrary to accepted theory on the gluconeogenic effects of propionate (Eckstein, 1933) which has been well demonstrated in ruminants by Johnson (1955), Clark &

Malan (1956), Armstrong & Blaxter (1957) and *in vitro* by Smith & Osborne-White (1961).

The depressant effect of acetate on circulating catecholamine levels, especially the adrenaline component, is difficult to explain. The short chain fatty acids are known to have a narcotic action (Samson *et al.* 1956) and barbiturates decrease plasma catecholamine levels (Hardy *et al.* 1959). This narcotic action, however, applies to all short chain fatty acids, in fact acetic acid is less potent than propionic or butyric acids in this respect. The rate of disappearance of injected acetate from the circulation, as measured by the half-time indicated that acetate remained in the circulation for a longer time than the other acids, and may therefore have had a greater depressant action on the central nervous system than propionate or butyrate. The reason for the different effects of the short chain fatty acids on catecholamine production, therefore, remains obscure. The difference may lie in their site of metabolism; propionate and butyrate are metabolized mainly by the liver, and acetate by the extrahepatic tissues (Leng & Armison, 1963). The liver is the main site of adrenaline degradation, and the process, o-methylation, requires a supply of adenosine triphosphate (ATP) (Axelrod, 1959). Under conditions of propionate and possibly butyrate administration, a supply of coenzyme A is required for propionate and butyrate oxidation the production of which utilizes ATP (Lipman, 1945). There may, therefore, be some competition for available ATP and propionate, and possibly butyrate metabolism may be at the expense of

catecholamine degradation, hence the rise in plasma catecholamine levels when propionate was administered.

The effect of intravenous VFA administration on blood glucose levels.

The changes in blood glucose concentration noted when lower fatty acids were injected intravenously have little physiological significance. The main purpose of these experiments was to demonstrate the part played by the sympatho-adrenal system. However, the changes in blood glucose are of interest, especially in respect of those found by other workers. Johnson (1955) observed increases in blood glucose levels after intravenous sodium acetate infusion, but Jarrett et al. (1952) observed no changes in the levels of total reducing sugar following single intravenous injections. Kronfeld (1957) found an increase in blood glucose concentration following sodium acetate infusion, associated with a contraction in the carotid jugular differences. Other workers have administered acetate by intraruminal infusion, but since some of the acetate is metabolized by the epithelium of the rumen (Pennington, 1952) the results are not comparable. However, intraruminal infusions of acetate invariably cause a fall in blood sugar in goats (Johnson, 1955; Schultz & Smith, 1951) and sheep (Clark & Malan, 1956; Armstrong & Blaxter, 1957), and this fall is usually attributed to the demand for oxidation products of sugar required for the metabolism of acetate (Armstrong & Blaxter, 1957). The changes in blood glucose following sodium acetate administration as recorded in Chapter III of this work are difficult to explain, since

acetate had no sympatho-adrenal stimulating action. There are two possible explanations of the mechanisms involved. Propionate metabolism is stimulated by acetate only in starved sheep (Pritchard & Tove, 1960) and not in fed sheep (Leng & Annison, 1963). This stimulation of propionate metabolism could result in a net glucose synthesis and a rise in blood glucose levels, and it is interesting to note that hyperglycaemia following acetate administration was found in animals showing low VFA levels, that is, those with a greater degree of starvation. There is, however, an exception to this (Animal 7439, Table 22).

The blood glucose levels of ruminants are relatively stable when compared with those of non-ruminants; they show no post-prandial hyperglycaemia or a hypoglycaemia after a fast of 24 hr. On account of this, blood glucose levels from animals accustomed to the experimental procedure may be said to reflect the endocrine balance of the animal in respect of carbohydrate metabolism more closely than in non-ruminants. The levels may, in fact, reflect the state of adrenocortical metabolism, high blood glucose levels reflecting a high rate of glucocorticoid secretion. With one exception (Animal 7432, Table 22), the change in blood glucose level following acetate administration was related to the initial blood glucose concentration, animals with low initial blood glucose levels showing a hyperglycaemia and those with high initial levels showing a hypoglycaemia. This one animal did show fairly high catecholamine levels and thus had rather high blood glucose levels (88.6 mg/100 ml.). In a subsequent ex-

periment (Table 36), the initial blood glucose level was 59.2 mg/100 ml. Lowering the blood glucose values by this amount (approximately 30 mg/100 ml.) would bring the observed blood glucose response more into line with the other results. Thus the blood glucose response to intravenous acetate administration may be dependent on the state of adrenocortical activity. Since acetate cannot give rise to a net glucose synthesis if metabolized in the Krebs cycle (Weinman et al. 1957) then some other mechanism must be involved. In the present experiments as discussed later, evidence is produced that insulin promotes the utilization of acetate. Jarrett & Potter (1957) showed that acetate removal was prolonged in diabetic ketotic sheep and the effect on propionate and butyrate removal was less marked. Also prior administration of glucose facilitates the removal from the circulation of injected acetate (Jarrett & Filsell, 1961). Therefore insulin, or glucose may enhance the utilization of acetate. With the sudden administration of acetate into the circulation there may be an uptake of insulin from the circulation. This fall in plasma insulin would depress the uptake of glucose from the circulation and lead to a rise in blood glucose concentration. The animals having high blood glucose levels are also resistant to the effects of insulin so little effect would be noticed in them.

Propionate given intravenously to both goats (Johnson, 1955) and sheep (Jarrett et al. 1952; Reid & Mills, 1961) and by intraruminal infusion to goats (Johnson, 1955; Schultz & Smith, 1951) and sheep (Clarke & Malan, 1956; Armstrong &

Blaxter, 1957) produces a marked hyperglycaemia. It will also relieve insulin convulsions as effectively as glucose when given intravenously (Potter, 1952). In the experiments reported in this work the degree of hyperglycaemia varied and was inversely related to the initial blood glucose levels. The lack of any hyperglycaemic effect of propionate at the high initial blood glucose levels is difficult to explain, especially in view of the raised plasma catecholamine levels. The direct relationship between blood glucose levels and half-times of injected propionate does suggest that in animals with high blood glucose levels there is some inhibition in the uptake and, possibly, utilization of propionate. High blood glucose levels are also associated with diminished glucose utilisation, and it would thus appear that propionate uptake or utilization is dependent on glucose uptake or utilization. If the high blood glucose levels are associated with high adrenal steroid production, then one might expect that the gluconeogenetic pathways would be facilitated, and propionate administration under these circumstances would lead to a large increase in blood glucose production. If the high blood glucose levels are due to a diabetes of pancreatic origin, rather than adrenocortical origin, then the work of Jarrett & Potter (1957) does to some extent support these findings, in that their pancreatectomised and alloxan diabetic sheep did show a slower rate of removal of injected propionate and a more diminished hyperglycaemic response than control animals.

Since a sympatho-adrenal response to butyrate administration could not be conclusively demonstrated, then

some other explanation must be found for the hyperglycaemic action that butyrate administration occasionally produces and also for its ability to relieve insulin convulsions, which Potter (1952) has demonstrated. Jarrett et al. (1952) reported a rise in blood sugar but Johnson (1955) recorded a variable response following single intravenous injections of sodium butyrate. Kronfeld (1956) found that the effect of butyrate depended on the initial blood glucose level, lower levels resulting in an elevation and higher levels resulting in a fall in blood glucose. He administered butyrate by infusion, and concluded that a plethora of body glucose inhibited 'gluconeogenesis from butyrate'. The results presented here, although on fewer animals, in fact show the reverse effect when butyrate is given by single injection. Using isotopes, Black et al. (1961) in cattle and Annison et al. (1963) in sheep have shown that gluconeogenesis from butyrate is minimal and probably does not occur at all. Butyrate can inhibit the uptake of glucose by muscle (Randle et al. 1962) which would tend to result in elevated blood glucose levels. However, an important product of butyrate metabolism, β-hydroxybutric acid (Annison et al. 1963) is known to cause an increased secretion of insulin (Brahmachari & Raghupathy Sarma, 1961) which would tend to lower blood glucose levels. β-hydroxybutyrate also inhibits the uptake of glucose by muscle (Randle et al. 1963). The blood glucose response to intravenous butyrate administration would therefore be the net result of these various effects. In animals resistant to the hypoglycaemic effects of insulin

the inhibitory action on glucose uptake of butyrate and β-hydroxybutyrate would predominate and a rise in blood glucose values result, and similarly in animals sensitive to the hypoglycaemic action of insulin, butyrate administration would result in a hypoglycaemia. This may explain the results obtained here since animals showing high blood glucose levels were markedly resistant to insulin administration and showed rises in blood glucose levels following butyrate administration. This may also explain the hyperglycaemic action that butyrate has if administered during insulin hypoglycaemia. Under these conditions further release of insulin from the pancreas would have no effect due to the fact that the tissues were 'saturated' with insulin. The inhibitory action, therefore, of butyrate and β-hydroxybutyrate on membrane transport would prevail causing a rise in blood glucose levels. Such an effect occurring during hypoglycaemic convulsions would be beneficial to the animal.

Acetate utilization

Since the disappearance of injected acetate from the circulation was exponential, it was possible to calculate the acetate half-time and use this as an index of rate of utilization. The acetate half-times, thus calculated, are similar to those found by Reid (1958) in sheep maintained on a good quality diet. The inhibition of utilisation that occurred at the higher plasma VFA levels is contrary to his findings since he showed that feeding, which results in elevated plasma VFA levels increased the rate of utilization

of injected acetate. In all the experiments reported in this thesis the time of feeding relative to the time of experiment was constant and so the post-prandial state should have been common to all experiments. The correlation, therefore, between plasma VFA levels and the half-time of injected acetate would suggest that, in these experiments, the plasma VFA levels found reflected the utilization of acetate and not the prandial state of the animal. The inhibition of acetate utilization may have been due to an inhibitory effect on liver oxidation of acetate that propionate and butyrate possess (Leng & Annison, 1963). If the higher VFA levels are due to absorbtion of VFA from the gut, then the amounts of propionate and butyrate absorbed will be higher at the higher plasma VFA levels.

Glucose utilization

The increment index is the rate of clearance per minute from the circulation of injected glucose and is usually expressed as a percentage. It has been used in man to detect diabetes, diabetic subjects having lower values than normal subjects. The values for the increment index of glucose obtained in these experiments can be compared with those of Reid (1958) who showed that in sheep diet and fasting can markedly influence the increment index. The values from Bos taurus animals were similar to those for sheep kept on a chaff diet supplemented with cracked maize, whereas the values from Bos indicus were similar to those for sheep kept on a poorer diet. He calculated increment indices from the data

of other workers, and for cattle he obtained values of 0.7 (Goetsch et al. 1956) and 3.0 (Bell & Jones, 1945) and much higher figures from the data of Holmes (1951), whereas from the results of other workers he calculated figures for man of 3.6, 3.7 and 3.7. These last figures do not compare with figures given by Lundbaek (1962) who used the increment index to diagnose diabetes in man. His mean value for 64 non-diabetic subjects was 1.72 and he set the lower limit for non-diabetic subjects at 1.05, whereas true diabetic subjects had values of less than 0.95. Only one animal in this series had a value of less than 0.95, but the means for Bos taurus of 2.26 and for Bos indicus of 1.22 obtained in these experiments suggest that cattle show a similar rate of removal of injected glucose to man. This is contrary to the findings of Reid (1958) who from his own work and that of other workers concluded that the rate of removal of injected glucose was significantly lower in ruminants than in non-ruminants.

Carbohydrate and potassium metabolism of Bos indicus and Bos taurus

A comparison of the carbohydrate metabolism of Bos indicus and Bos taurus revealed that Bos indicus possessed higher resting blood glucose levels, a lower sensitivity to insulin, and a slower rate of clearance of injected glucose than Bos taurus.

Ayyar & Nayar (1941) found mean blood glucose values of 46 mg/100 ml. in cattle whereas in the work reported here the mean for Bos indicus was 72.5 $\pm$2.23 (S.E.) mg/100 ml. and

for Bos taurus 64.0 ±1.57 (S.E.) mg/100 ml. The difference between these means was statistically significant. It is not possible to obtain absolute blood glucose values from the data of Blincoe & Brody (1951), but there was no significant difference between the two cattle types. Evans (1963) reported mean blood glucose levels of 47.74 ±0.85 (S.E.) mg/100 ml. for Bos indicus (Brahman) and 41.96 ±0.39 (S.E.) mg/100 ml. for Bos taurus (Hereford). The difference between the means was statistically significant and supports the differences recorded in this work although absolute values are much lower. However, from another group of Bos taurus (Hereford) animals he obtained a mean value of 46.22 ±0.54 (S.E.) mg/100 ml. which was not significantly different from the Bos indicus animals. Reid (1950a) found a mean of 39.1 ±3.37 (S.E.) mg/100 ml. for sheep in England and from his review of the literature on the normal blood glucose values of ruminants and non-ruminants, the figures reported here would lie in the lower range of non-ruminant values, and upper range of ruminant values, particularly cattle; such high levels are rarely found in sheep. The reason for the high values found here is not known. The composition of the diet is known to affect the carbohydrate metabolism of sheep (Reid, 1958). Diets producing large amounts of the carbohydrate precursor, propionic acid, might be expected to show a trend toward the non-ruminant state, but the diet in these experiments was unlikely to produce large amounts of propionic acid.

Decreased sensitivity to insulin and high resting blood glucose levels are seen in animals with hypersecretion

of growth hormone or adrenocortical steroids (De Bodo & Altszuler, 1958). The rate of fall of blood glucose following insulin in untreated acromegalic man is relatively slow when compared with normal control subjects (Fraser et al. 1962). In the experiments recorded in this thesis however, the rate of fall in the two species of animal was almost identical. This would suggest that adrenal hyperactivity is responsible for the features of carbohydrate metabolism that Bos indicus exhibits. Again, glucose tolerance is either not affected by, or is enhanced by growth hormone (De Bodo & Altszuler, 1958) whereas, in the work reported here Bos indicus type animals had significantly greater glucose half-times than Bos taurus. The question of a diminished glucose tolerance in response to adrenal corticoid hormones is somewhat controversial (De Bodo & Altszuler, 1958). However, the only studies that have been performed on ruminants are those of Bassett (1963) who showed that in sheep there was a definite decrease in glucose tolerance after cortisol acetate administration. He further demonstrated a relationship between glucose half-times and blood glucose levels. Such a relationship was found in this work, which suggests that the high blood glucose levels of Bos indicus type animals are a result of diminished glucose utilisation. Differences in the carbohydrate metabolism of the two species, therefore, appear to be attributable to differences in adrenal corticoid production or metabolism. Further evidence in support of this is the significantly lower plasma potassium levels found in Bos indicus. Adrenal

corticoids are known to depress plasma potassium levels (Fenn, 1940a). Reasons for these differences might be due to a true species or, in this case, sub-species difference, or the two types may be basically similar and the adrenal hyperactivity might be related to some other factor. In this work the management of all the animals was identical. However, Bos indicus and Bos taurus do differ in their response to their environment, the former being more heat-tolerant than the latter (Findlay, 1950). Similarly, their thermoneutral zones are different. Kibler & Brody (1950) estimated, roughly, that the thermoneutral zone of Bos indicus lies between 50° and 80°F. and that of Bos taurus between 40° and 60°F. These figures have not been confirmed completely but Blaxter & Wainman (1961) in carefully controlled experiments on two Bos taurus steers obtained figures of 40° and 44.6°F. for the critical temperature. The upper end of the thermoeutral zone is more difficult to determine but the estimate of Kibler & Brody (1950) for Bos taurus may be a little low. Examination of the data of Beakley & Findlay (1955) shows that the onset of thermal polypnoea occurs between 60° and 68°F. and similarly the onset of sweating occurs between these temperatures (McLean, 1963). Meteorological data taken from the Annual Report of the East African Agriculture and Forestry Research Organisation for 1960 shows the environmental conditions underwhich these these experiments were performed. The data presented below are taken from that report.

Month	Monthly means of daily values			Relative humidity (%)
	Max. (°F)	Min. (°F)	Mean (°F)	
January	72.0	52.6	62.3	51
February	74.8	52.7	63.8	40
March	72.1	54.0	63.1	54
April	69.5	54.4	62.0	67
May	68.3	51.6	60.0	63
June	67.7	48.9	58.3	55
July	66.2	47.0	56.6	58
August	69.0	47.3	58.2	54
September	70.4	49.1	59.8	53
October	71.8	51.9	61.9	48
November	70.7	52.9	61.8	53
December	72.1	53.0	62.5	49

It can be seen that in 4 months of the year, June to September, the ambient temperatures fell below the critical temperature of Bos indicus, and that from September to March the ambient temperature approached the upper part of the thermoneutral zone of Bos taurus. This table gives no idea of the length of time for which these temperatures were maintained. The animals of Bos taurus type were never seen to be heat stressed although they did seek the shade during the day. Animals of Bos indicus type were frequently seen to be shivering especially just after dawn. It would appear, therefore, that Bos indicus was 'cold-stressed' relative to Bos taurus or that Bos taurus was 'heat stressed' relative to Bos indicus. It is difficult to determine if Bos indicus was cold-stressed for any length of time, but the observation of shivering, especially during the winter months, would suggest that this was so. The fact that Bos taurus showed plasma potassium and blood glucose levels that are within the normal range described for cattle suggests that

Bos indicus was the type that was 'stressed'. Blincoe & Brody (1951) simulated, to some extent, the conditions described here. They kept animals of both types at various ambient temperatures for several weeks. Examination of their results does not reveal any rise in blood glucose or fall in plasma potassium levels with decreasing ambient temperatures. Their conditions allowed for acclimatization whereas under the conditions in East Africa 'cold-stress' was sporadic and for short periods of time and complete acclimatisation would be difficult. Adrenocortical activity is sensitive to ambient temperature; MacFarlane & Robinson (1957) have demonstrated a decrease in the excretion of 17-ketosteroids and 17-ketogenic steroids with increasing ambient temperature. In man there seems to be a difference in 17-ketosteroid excretion between the different ethnic groups; Negro (Barnicot & Wolffson, 1952), Indian (Friedman, 1954) and Malay (Lugg & Bowness, 1954) all have a lower 17-ketosteroid excretion than European races. This seems to be a true species or type difference since Barnicot & Wolffson (1952) could find no change in the 17-ketosteroid excretion of a Nigerian who came to live in Great Britain, a situation which might, to some extent, parallel the circumstances described here.

If the findings reported in this thesis reflect adrenocortical activity, then whether they are a true species difference or caused by different responses of the two types of cattle to the same environment remains an open question.

As noted earlier, other workers did not find significant differences in the blood glucose levels of the two types.

Similarly, the plasma potassium levels of Bos indicus and Bos taurus reported by other workers show no significant difference between the types. This would suggest that the differences observed in this thesis do in fact represent differences in adrenocortical activity and are not merely a species difference, since it should have been noted by the other workers.

Evans (1963) recorded values of 3.80 ±0.18 (S.E.) and 3.72 ±0.10 (S.E.) m.equiv/l. for the plasma potassium levels of Bos indicus (Brahman) and Bos taurus (Hereford) respectively. Blincoe & Brody (1951) quoted levels of 4.86 and 4.78 m.equiv/l. for Bos indicus and Bos taurus respectively. The values recorded in the present work are intermediate between these figures. Fisher (1960) reviewed the literature on the plasma potassium levels of the ox, and they appear to vary between 4.0 and 5.0 m.equiv/l. Thus the values for Bos taurus recorded in this thesis lie within the normal range whilst those for Bos indicus are somewhat lower than this, but similar to levels reported by Evans (1963).

The effect of insulin administration on plasma potassium levels.

Insulin produced falls in plasma potassium slightly greater than those recorded for man. Harris et al. (1938) reported falls of 11 to 35% whereas in the work reported here falls of 13.8 to 43.1% with a mean of 29.2% were found. Setchell & McClymont (1955) reported falls of 28 to 53% in

sheep. The cause of this fall is still in doubt. Kestens et al. (1963) have shown that potassium is taken up by the liver in response to insulin. Andres et al. (1962) have shown that insulin promotes the transfer of potassium from plasma to the muscles of the forearm in man. This confirms the finding that in vitro insulin promotes the uptake of potassium by muscle (Zierler, 1959). In the experiments of Andres et al. (1962) very small doses of insulin were given intra-arterially (0.2 units). Grob et al. (1957) administered 20 units of insulin intra-arterially and obtained evidence of an increased loss of potassium from the forearm muscle of man. Farber et al. (1951) reported similar findings and also produced evidence that the liver was primarily responsible for the fall in plasma potassium following insulin administration. The experiments recorded in this thesis would tend to confirm the latter authors' findings, although the work of Andres et al. (1962) is difficult to refute since by simultaneously measuring blood flow they were able to measure the actual uptake of potassium by the muscles, whereas arterio-venous measurements only suggest that an uptake or loss has occurred.

The lack of change of erythrocyte potassium levels in face of falling plasma levels confirms the findings of Kerr (1928) in dogs.

The effect of glucose administration on plasma potassium levels.

The fall in plasma potassium levels following glucose administration was first described by Flock et al. (1938)

in dogs. They obtained falls of from 13 to 25% occurring during an infusion lasting 3 hr. In these present experiments falls ranged from 6.8 to 17.1% with a mean of 11.4% following a single injection of glucose. This fall, therefore, is of a similar order to those found in the dog, bearing in mind the differences in method of administration.

The arteriovenous studies with glucose were equivocal and did nothing to clarify previous work in man. Grob et al. (1957) provided evidence of an uptake of potassium by muscle whereas Farber et al. (1951) demonstrated an increased loss of potassium following oral glucose administration. In vitro potassium is taken up by muscle along with glucose (Fenn, 1940b). Assuming that in vivo glucose has this effect, then secretion of endogenous insulin, which possibly has the opposite effect would mask it. In one experiment there was an immediate positive arteriovenous difference which may have been produced by glucose alone since secretion of endogenous insulin does not occur until ten minutes after the intravenous injection of glucose (Garcia-Fernandez & Candela, 1963).

Although it has been postulated that potassium metabolism and carbohydrate metabolism are related (Fenn, 1940b), plasma potassium levels are influenced by many factors so that in vivo such a relationship is difficult to demonstrate. However, the work of Andres et al. (1962) has shown that glucose uptake and potassium uptake by muscle are independent of each other. This tends to be borne out in the experiments reported here in which changes in plasma potassium and blood glucose levels

were not related in time.

No conclusions can be drawn from the effects of adrenaline and noradrenaline on plasma potassium arterio-venous differences. Grob et al. (1957) were unable to differentiate an effect due to blood flow changes from one due to uptake of potassium, since adrenaline injection (0.1 mg) converted negative arteriovenous differences into positive ones.

These studies show that the plasma potassium changes following insulin or glucose administration are similar to those observed in non-ruminants. The fact that ruminants have relatively low normal blood glucose levels and a high dietary intake of potassium has little apparent effect on their responses to these treatments.

The effect of intravenous VFA administration on plasma potassium levels.

The hypokalaemic effect of intravenous fatty acid administration has not been described previously. It is known that organic acids when administered will cause a fall in plasma potassium, but this is due to the resultant changes in the carbon dioxide tension of the blood (Simmons, 1962). It is unlikely that in these experiments there were any changes in carbon dioxide tension since the injected solutions had been adjusted to pH 7.4. The sodium ion produces no changes since injection of sodium chloride in similar proportions has no effect on plasma potassium.

The volume in which the injected fatty acids were distributed was greater than the extracellular fluid volume of cattle (Hix et al. 1959), which suggests that there was an uptake of the fatty acid by the tissues. It is unlikely that either sodium would move in or chloride move out in exchange for the fatty acid radical as the cell membrane is only slightly permeable to sodium or chloride ions (Conway, 1957). Potassium is freely permeable, and it is suggested that the fatty acid radical enters the cell with potassium ions, thereby causing a lowering of the plasma potassium levels. Examination of the data shows that potassium levels usually started to return to their initial level after plasma VFA had been cleared from the circulation, except in the propionate experiments where elevated blood glucose levels could conceivably have kept the plasma potassium levels depressed.

The effect of insulin or glucose administration on plasma VFA levels.

The depressant effect of insulin or glucose on plasma VFA levels has not previously been described. McClymont (1951b) in his studies on hyperinsulinism in cattle took blood samples every 2 hr and would therefore have missed this effect. The falls in plasma VFA produced by insulin and glucose are statistically indistinguishable from each other and the mechanism may therefore be similar. Both treatments result in enhanced glucose utilisation and the effect may be secondary to this. However, glucose will cause reflex release of insulin from the pancreas and the depression of

plasma VFA levels may be due to insulin *per se*. There seems some doubt, however, as to the time of release of insulin from the pancreas following intravenous glucose administration. Garcia-Fernandez *et al.* (1963) were able to show a significant increase in plasma insulin activity only 10 min after intravenous glucose administration. In the present experiments the depression of plasma VFA levels was apparent after 5 min. This would suggest that the fall in plasma VFA levels was a result of the enhanced glucose utilization and not a direct effect of insulin.

Differences in the plasma VFA levels of the common carotid artery and the external jugular vein will only reflect changes in uptake by the tissues of the head since almost all the blood draining the tissues of the head is carried by the external jugular vein and in these experiments the internal jugular vein had been ligated at the time of exteriorization of the carotid artery. The external jugular vein drains chiefly muscle tissue, skin and brain, and most of the difference between the concentration of VFA in the plasma of the carotid artery and external jugular vein is a reflection of the assimilation of VFA by these tissues. However, the brain of sheep and probably other species does not metabolize plasma VFA (McClymont & Setchell, 1956), and so the main tissues concerned are muscle, skin and possibly fat. Reid (1950b) using sheep and McClymont (1951b) with cattle have shown that the uptake of plasma VFA is proportional to the arterial concentration of VFA, a fact verified in this work. Any enhancement of uptake by the tissues of the head would affect

this relationship, that is, for a given arterial level the A-V difference would increase, and the slope of the regression relating A-V plasma VFA differences to arterial plasma VFA levels would increase. Similarly any inhibition of uptake would produce opposite effects. The results suggest that following insulin or glucose administration there is no change in the slope of this regression; the fall occurs primarily in the arterial levels and the uptake by the tissues remains in proportion to these levels. The change in plasma VFA levels therefore is not due to an enhancement of uptake by the tissues of the head but must occur elsewhere, e.g. the liver, heart, or kidney. There are three possibilities: (1) that there is an increased uptake, possibly in the liver, (2) that there is a diminished release of endogenous acetate, or (3) that there is a decrease in VFA absorption from the gut.

Since acetate is the main component of plasma VFA in the ox (McClymont, 1951a), the fall in plasma VFA would almost certainly involve a fall in acetate. Jarrett & Filsell (1961) have shown that acetate tolerance is increased by prior administration of glucose, which suggests that glucose, or the reflex liberation of insulin produced by glucose, enhances the uptake of administered acetate. Bloch & Kramer (1948) reported that insulin stimulated the incorporation of acetate into fatty acids in liver slices *in vitro*, but this has not been demonstrated *in vivo*. Annison & Lindsay (1961) reported that insulin causes a decrease in endogenous acetate entry, and Annison & White (1962) reported a reduced entry of endogenous acetate along with raised concentration of blood

glucose in starved sheep. These workers suggest that free fatty acids (FFA) are the major source of endogenous acetate, and the inhibition of endogenous acetate entry reflects the fall in plasma FFA levels produced by insulin and glucose (Annison, 1960). In support of this they show that the inhibitory effects of insulin and glucose are most marked during starvation when plasma FFA levels are high. This contrasts with the experiments described here in which the fall in plasma VFA was shown to be proportional to the initial plasma VFA level, that is, it was most marked when plasma VFA levels were high, as occurs following feeding.

There remains the possibility that VFA absorption is inhibited. Hill (1954) has shown that insulin inhibits ruminal movements but it has not been shown that inhibition of ruminal movements is associated with a decrease in VFA absorption. It is of interest to note that falls in plasma FFA levels produced by glucose and insulin are due to changes in FFA production and not to changes in uptake since under all conditions the relationship between plasma FFA concentration and turnover remains the same (Armstrong et al. 1961).

The effect of catecholamine administration on plasma VFA A-V differences.

The doses of adrenaline and noradrenaline infused were within physiological limits. Silver (1960) reported that adrenaline production in the ox during splanchnic nerve stimulation varies from 0.30 to 0.67 μg/kg/min and noradrenaline

production from 0.39 to 0.61 μg/kg/min during the 2nd to 30th week of life.

Adrenaline and noradrenaline infusions caused a marked contraction of the arteriovenous plasma VFA differences and the slope of the adrenaline regression was diminished. Large alterations in blood flow might bring about these effects. Muscle is probably the most active tissue with respect to VFA metabolism in the head, and large changes in muscle blood flow could lead to alterations in muscle VFA uptake. Adrenaline increases muscle blood flow on account of its vasodilator action (Skinner & Whelan, 1962), and noradrenaline constricts muscle blood vessels, thereby causing a fall in muscle blood flow (Barcroft & Konsett, 1949). This fact would suggest that blood flow in itself has little effect on the uptake of VFA by muscle, since in both instances the effect on muscle VFA uptake, as assessed by arteriovenous plasma VFA differences, was qualitatively similar. If the changes in muscle blood flow produced by these amines also occur in cattle, then one might postulate that adrenaline and noradrenaline themselves inhibit muscle uptake of plasma VFA. If this were the only action of adrenaline and noradrenaline then administration of these amines would result in a rise in plasma VFA concentration. Since a fall in plasma VFA occurred there must have been an inhibition of release or active uptake of plasma VFA by some other tissues.

There is also the possibility that the effect of adrenaline is to inhibit the uptake of VFA metabolically, whilst the inhibition produced by noradrenaline is secondary

to the vasoconstriction.

Experiments with isolated perfused limbs at varying rates of perfusion would be useful in examining the effects of blood flow *per se* on VFA uptake, and could also be used to examine the possibility of an active inhibition by these amines.

GENERAL SUMMARY AND CONCLUSIONS

(1) An examination of biological and fluorimetric methods for the determination of the levels of adrenaline and noradrenaline in bovine peripheral plasma revealed that the fluorimetric ethylenediamine condensation method of Weil-Malherbe & Bone (1952) was satisfactory and would detect gross changes in the peripheral plasma levels of these amines.

(2) Intravenous insulin was administered to cattle in doses varying from 0.1 to 7.5 units/kg body weight. The resulting degree of hypoglycaemia at doses of 1 unit/kg and above was independent of dosage, but the duration of hypoglycaemia was related to the dosage. Blood glucose levels 30 min after intravenous insulin administration were identical within the same animal and tended to remain at this level for several hours. Clinical signs of hypoglycaemia were not produced.

(3) When blood glucose levels fell below a critical level following intravenous administration of insulin, the plasma levels of circulating adrenaline and noradrenaline were elevated and remained elevated until blood glucose levels had approached their initial level. The increase was mainly in the adrenaline levels, especially during prolonged hypoglycaemia. High doses of insulin produced transient increases in noradrenaline levels 10 min after administration, without marked hypoglycaemia.

(4) An increase in plasma noradrenaline levels during insulin hypoglycaemia had not been described before, and its

physiological significance was examined by determining the relative hyperglycaemic potencies of adrenaline and noradrenaline. The hyperglycaemic adrenaline:noradrenaline ratios showed a mean of 3.31 with a range of 0.79 to 6.58. This is lower than ratios that have been determined in other species, and it was concluded that the increase in noradrenaline that occurs in cattle during insulin-induced hypoglycaemia is of some physiological significance.

(5) Intravenous administration of sodium acetate produced various changes in blood glucose levels depending on the initial blood glucose level but always depressed circulating plasma adrenaline levels. Intravenous administration of sodium propionate produced marked changes in blood glucose and elevated plasma adrenaline and noradrenaline levels, particularly the former. Intravenous administration of sodium butyrate produced only slight rises in blood glucose followed by marked falls. The changes in blood glucose produced by sodium butyrate were related to the initial blood glucose levels.

It was concluded that with the possible exception of sodium propionate, the sympatho-adrenal system plays little part in producing the changes in blood glucose that result from intravenous injections of the sodium salts of the lower fatty acids.

(6) The rate of disappearance of intravenously administered sodium acetate was related to the initial plasma VFA level and that of sodium propionate was related to the initial

blood glucose levels.

(7) Intravenous glucose administration depressed plasma adrenaline and noradrenaline levels especially the former.

(8) Insulin or glucose produced a fall in plasma VFA levels, the reduction occurring within 30 min after administration. The degree of fall was related to the initial level of plasma VFA, and the effects produced by insulin or glucose were quantitatively indistinguishable. Simultaneous measurements of carotid and jugular plasma VFA levels during insulin or glucose administration revealed that the fall noted was not due to enhancement of uptake by the tissues of the head, the fall occurred primarily in the carotid plasma VFA levels.

(9) Simultaneous measurements of VFA levels in carotid and jugular plasma during adrenaline or noradrenaline infusions revealed gross changes in the relationship between arterial levels and arteriovenous differences of plasma VFA.

(10) Intravenous insulin or glucose administration depressed plasma potassium levels in a manner quantitatively similar to changes described in non-ruminant species, and it was concluded that although ruminants ingest relatively large amounts of potassium when compared with non-ruminants, this has little effect on their carbohydrate potassium interrelationships. Measurement of carotid and jugular plasma potassium did not suggest a possible site of action for this effect.

(11) Intravenous administration of the sodium salts of acetic, propionic or butyric acids produced a fall in plasma potassium levels.

(12) A comparison of certain aspects of the carbohydrate metabolism of Bos indicus and Bos taurus revealed that Bos indicus possessed higher resting blood glucose levels, a lower sensitivity to insulin, and a greater glucose half-time than Bos taurus. Bos indicus also had lower plasma potassium levels than Bos taurus. It was concluded that these findings reflect differences in the levels of adrenocortical activity in the two species, which may be related to their heat tolerance.

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