7

GROWTH HORMONE
AND THE
FUEL OF MUSCULAR EXERCISE

by

C. C. FONSEKA
M.B.B.S. (Ceylon)

Thesis presented for the Degree of Doctor of Philosophy of the University of Edinburgh in the Faculty of Medicine

November 1966

CONTENTS

Chapter		Page
	INTRODUCTION	1
1.	THE FUEL OF MUSCULAR EXERCISE	5
2.	GROWTH HORMONE AND FAT MOBILISATION	14
3.	ANALYTICAL METHODS	18
4.	THE EXPERIMENTS	25
	(1) The Effect of Exercise on Plasma Growth Hormone Levels in Human Adults	25
	(2) Investigation of the Variability of the Initial Fasting Value of Plasma G.H. Concentration in Human Adults	35
	(3) The Effect of Feeding on Plasma G.H. Levels during Exercise	40
	(4) The Effect of Long-continued Exercise on Plasma G.H. Levels in Human Adults	48
	(5) The Influence of Physical Activity on a Treadmill on the Response of Adipose Tissue to Growth Hormone in Rats	55
5.	SUMMARY AND CONCLUDING REMARKS	68
	ACKNOWLEDGEMENTS	73
	APPENDIX	
	REFERENCES	

INTRODUCTION

This thesis sets out an account of a study of the role of Growth Hormone (G.H.) in the mobilisation of fuel for muscular exercise. A few words may be in order to explain how this study came to be undertaken.

In 1907 Fletcher and Hopkins from Cambridge published a celebrated paper in the Journal of Physiology entitled: "Lactic Acid in Amphibian Muscle". Few papers in the history of physiology can have had so great an influence. It was an epoch-making paper in the strict sense of that phrase. It led to the belief, held almost universally until a mere decade ago, that carbohydrate was the "prime", if not indeed, the sole fuel of muscle. Nobody explicitly denied that fat could yield energy to muscle, but the qualifying phrase "provided it is first converted to carbohydrate" was always added or implied. Based on this premise, the disposal of ingested glucose was accounted for as follows. After absorption from the gut, glucose was first used to cover the immediate metabolic needs of the tissues. Any excess above this need was soon converted into triglycerides and stored as such. Only a small portion of the excess glucose was converted into glycogen, the stores of which were small and relatively constant.

One of the first series of experiments to cast doubt on the sequence outlined above was carried out by Douglas and Koch at Oxford in 1951. They showed that if a subject were given glucose /

glucose shortly before a period of exercise, the glucose was not immediately utilized. The rise of R.Q. during the exercise was found to be much less than would have been expected if the muscles had used the ingested glucose as fuel. Here was one of the earliest inklings that muscle may not accord to glucose the preferential treatment it was widely believed to.

In 1956 Dole, and at the same time Gordon, recognised the great metabolic significance of the non-esterified fatty acid (NEFA) fraction of the blood plasma. By 1958 the NEFA were widely regarded as a direct source of energy for the working cell.

In 1960 a paper from the Physiological Laboratory of the University of Edinburgh by Basu, Passmore and Strong reported for the first time a consistent and marked <u>rise</u> in the concentration of plasma NEFA during a period of moderate exercise carried out in the post-absorptive state. They interpreted this as an increased rate of mobilisation of NEFA from the fat depots to meet the energy needs of the muscles. They went on to ask: "What is the physiological stimulus which promotes this mobilisation ?" They speculated that because injections of G.H. (Raben and Hollenberg, 1959) and adrenaline (Dole, 1956) both raise the plasma concentration of NEFA, these hormones may be among the many natural stimuli for the mobilisation of fat.

Meantime, Koch had become Professor of Physiology in the University of Ceylon and the present author joined his staff in /

in 1962. Inevitably he became interested in Koch's work with Douglas and was eager to extend this work during his period of study leave in the United Kingdom. To do that Edinburgh University seemed the place to go to and in 1963, when Professor Koch explored this possibility, Dr. Passmore replied: "I read Douglas and Koch at intervals and have frequently thought about the need for a further step forward. So far as I know, the subject is just as you left it over ten years ago. However, we know now that NEFA are an important fuel of muscle and that their level in the plasma rises during exercise in the post-absorptive state. It would be interesting to see how glucose given before the exercise affects this rise. It is also known that pituitary Growth Hormone (G.H.) mobilises NEFA very rapidly in the blood. We have coming to Edinburgh soon a man who has a new technique for assaying G.H. in the blood. I am sure the time is now opportune to go back to your old experiments. It would be very nice to have someone from Ceylon to be at the centre of the work." As it happened, in early 1964 Dr. W.M. Hunter, a pioneer in the radio-immunoassay of G.H., and the present author both came to Edinburgh. To him the author is grateful for instruction in the technique of the radio-immunoassay of G.H. in the blood. The experiments on human subjects reported in this thesis were the product of this fortuitous concourse of circumstances. An account of these has already been published (Hunter, Fonseka and Passmore, 1965). The experiments on rats embodying the results of the author's /

author's own special work stemmed directly from the insights and experience gained from the human experiments.

CHAPTER 1

The Fuel of Muscular Exercise

To talk of the fuel of muscular exercise is to imply that the body is a machine. Thermodynamically speaking, a machine is a contrivance for transforming one form of energy into another and the body undoubtedly conforms to this definition. It was, however, not in the thermodynamical sense that the analogy between the body and a machine was first drawn. Rene Descartes (1569 - 1650) was probably the first man to regard the bodies of men and animals as machines. Animals he regarded as automata governed entirely by the laws of physics; men differed from animals in having a soul which resides in the pineal gland (p. 583, History of Western Philosophy, Bertrand Russell). Descartes's thinking was doubtless influenced by the discovery of the circulation in 1628 by his distinguished contemporary, William Harvey, and also by the spectacular successes of the early 17th century engineers. The concept of the body as a machine has been found to be both vivid and valid in all physiological processes involving energy utilisation. The relation between work, heat and the oxidation of organic substances in the animal body was appreciated by physiologists long before physicists formulated the first law of thermodynamics. In fact, it was not a physicist but a ship's doctor, Robert Mayer, who in 1842 first apprehended the truth of the principle of the conservation of energy. What is /

is more, the foremost journal of physics of that time, Poggendorf's "Annalen", actually rejected Mayer's paper reporting his discovery (quoted by Max Kleiber in "The Fire of Life", 1961). Rubner, in 1892, established beyond any manner of doubt that the animal body was no exception to the principle of the conservation of energy. It is of melancholy interest that what Rubner firmly established in 1892, was originally conceived a century earlier in Lavoisier's fertile head, before the more sanguinary enthusiasts of the French Revolution cut it off. It is a common-place today that aspects of the body such as mass, temperature, energy and movement can most intelligibly be described only in the language of physics. To talk of the fuel of muscular exercise, therefore, is not merely to talk metaphorically.

Theoretically, the human machine should be able to derive energy from a variety of sources, since any form of energy may be completely converted to any other form. In fact, however, the body is only able to utilise the energy inherent in specific molecular configurations of certain ingested substances. Other forms of energy such as electricity cannot fuel the body because the body does not possess the appropriate energy-coupling mechanisms.

Prout in 1834 classified the many components of natural foods into three groups: the albuminous group, the saccharine group and the oleaginous group - in modern language, proteins, carbohydrates and fats respectively. In 1842 Liebig clearly appreciated /

appreciated that the body derives its energy from the oxidation of these three groups of organic compounds. He believed, however, that muscular work was exclusively associated with the breakdown of protein. Soon Liebig's doctrine that mechanical work was the result of protein breakdown in muscle became widely accepted and protein came to be regarded as the special fuel of muscular exercise. Playfair (1865) voiced the common feeling when he proclaimed that "the common experience of mankind is in favour of the nitrogenous ingredients of food being the source of dynamical work".

In 1866 Pettenkofer and Voit carried out experiments which showed that exercise was not associated with significant protein breakdown. Nor was that all. In 1865 Fick and Wislicenus climbed to the top of Faulhorn (6,000 ft.) whilst consuming a diet low in protein, and showed that the output of nitrogen in the urine corresponded to a breakdown of tissue protein quite insufficient to account for the energy used in their climb. These and many other experiments have conclusively established that protein is by no means the exclusive fuel of muscular exercise.

In 1896 Chauveau reported an experiment in which the resting R.Q. of 0.75 of a subject rose to 0.95 after 45 minutes of exercise. After an hour's rest the R.Q. returned to the basal level. Upon this experiment Chauveau formulated the hypothesis that muscular exercise was accomplished at the expense of carbohydrate and that fat when used was first changed to carbohydrate.

Zuntz /

Zuntz et al (1911) tested Chauveau's hypothesis and in a paper in Oppenheimer's Handbuch upheld the view that not only carbohydrate but also fat could be oxidized during exercise. There was, however, no compelling evidence in support of any of these views. Moreover, by then, what might be characterised as the "lactic acid era" had been inaugurated by Fletcher and Hopkins (1907) and under A. V. Hill's leadership, this school of thought was beginning to dominate thinking in this field. Between 1924 and 1926 A. V. Hill and his school, on the basis of R.Q. measurements during exercise, concluded that carbohydrate was the only substance oxidized during muscular exercise.

At the end of 1926 Eggleton and Eggleton showed that when a muscle is excited a substance they called "phosphagen" breaks down and in the presence of oxygen is rapidly restored. Next, beginning in 1930, Lundsgaard published a series of papers on phosphagen, on the basis of which he proposed the hypothesis that "phosphagen is the substance directly supplying the energy for the contraction while lactic acid formation in the normal muscle continually provides the energy for its resynthesis".

In a famous review entitled "The Revolution in Muscle Physiology", A.V. Hill (1932) critically summarised the state of knowledge at the time. With almost prophetic conviction, he predicted that during the next few years further discoveries will no doubt be made requiring drastic changes of concept. He was right. For in that very year Meyerhof and Lohmann found that, at pH 8.0 to 9.0, the addition of adenosine-triphosphate /

triphosphate (A.T.P.) to muscle extract containing polysaccharide led to a reaction in which phosphagen and adenylic acid were formed. They postulated that this reaction was the <u>immediate</u> source of the energy for the resynthesis of phosphagen, and that the formation of lactic acid supplied the energy for the resynthesis of A.T.P. In 1934, however, Lohmann found that the phosphate interchange was reversible; at pH 7.0, adenylic acid reacted with phosphagen to form A.T.P. and creatine. The hypothesis then became that the initial reaction in contraction was the breakdown of A.T.P. and that the recovery process began with the resynthesis of A.T.P. by phosphagen. At a later stage in anaerobic recovery the phosphagen was resynthesised by the energy of formation of lactic acid and the final recovery was accomplished by oxidative reactions. This, of course, is essentially the modern view. A.T.P. is now regarded as an energy shuttle which uses the free energy of food to synthesise its high energy phosphate bonds and then releases the energy in the bonds to be used in vital processes.

In 1942 Gemmill summarised the knowledge concerning the fuel of muscular exercise thus: "From a survey of the literature it is obvious that the use of carbohydrate is of primary importance as a fuel of muscular exercise in man ...... The evidence that protein is used during exercise indicates that it is of secondary importance, probably to supply carbohydrate or carbohydrate intermediates. The results of experiments on fat utilisation during muscular work have demonstrated that this /

this substance is used indirectly. There is no experimental evidence at the present time for the direct utilisation of fat by mammalian muscle."

Nor was this surprising. For, up to 1942 when Schoenheimer's book "The Dynamic State of Body Constituents" appeared, adipose tissue was regarded simply as an inert store of fat. For that very reason it was largely ignored. In 1948, Wertheimer and Shapiro wrote an article for the Physiological Reviews on the general theme "The Physiology of Adipose Tissue" which did much to make adipose tissue acceptable to physiologists as a subject worthy of proper study. They concluded: "Adipose tissue is a tissue with a special structure and a special type of cell ...... Deposition and mobilisation of fat in adipose tissue is an active process involving the metabolism of tissue. All these metabolic activities are regulated by nervous and endocrine factors."

After 1948 there was, in a well-worn phrase, an explosion of interest in adipose tissue. In 1954 Hausberger et al presented clear-cut evidence that adipose tissue itself is the major site of conversion of carbohydrate into fat. Because this process was essentially unidirectional, mechanisms regulating the rate of conversion of carbohydrate into fat were investigated. The crucial problem at that stage concerned the identity of the substance that was actually mobilised from adipose tissue and how it was transported to and oxidised by the various organs. The solution to this problem came in 1956 when /

when Dole and at the same time Gordon recognised the metabolic significance of the non-esterified fatty acids (NEFA) of the blood plasma. They showed that although the NEFA comprise only 5% of the total fatty acids in plasma, they have an extremely rapid turnover and respond consistently to a variety of physiological stimuli. The recognition of the physiological significance of NEFA in 1956 must rank in the history of physiology as an event comparable in importance to the discovery of lactic acid in amphibian muscle in 1907. It inaugurated what might be called for short the NEFA ERA - in which we live.

By 1958 adipose tissue was widely regarded as a major energy source and NEFA as the principal form in which mobilised fat is transported to tissues for utilisation.

In 1959 Carlson and Pernow catheterised both femoral veins in their subjects and exercised one leg only on a bicycle for short periods of time. In every subject the NEFA concentration in the plasma from the exercising leg was lower than that from the resting leg.

In 1960 Friedberg et al showed that seven minutes after the start of exercise the mean plasma NEFA in seven subjects fell from 0.85 to 0.63 m.mole/l. In the same year Basu, Passmore and Strong reported an experiment in which during a period of one hour's moderate exercise in the post-absorptive state, the mean concentration of NEFA in ten healthy subjects rose from 0.76 to 1.44 m.mole/l. They made no measurements

on /

on the plasma before 15 minutes but at 15 minutes four of the ten subjects had a plasma NEFA concentration below the resting level. Thirty minutes after the walk had commenced the plasma NEFA concentration in every subject was higher than the resting level. In discussing their results they took into consideration the findings of Carlson and Pernow (1959) and Friedberg et al (1960) referred to above. They concluded that all these observations were consistent with the view that during exercise the muscles utilise NEFA from the plasma as a source of energy. On the premise that the plasma NEFA concentration at any given moment is the resultant of its rate of utilisation and its mobilisation, they explained the fall in NEFA concentration early during exercise (Carlson and Pernow, 1959; Friedberg et al, 1960) as the reflection of the excess of utilisation over mobilisation. The rise in the NEFA concentration later during exercise which they found, they attributed to excess of mobilisation over utilisation. The general validity of this interpretation is now beyond dispute.

In 1961 Armstrong et al showed that in dogs the turnover of ^{14}C palmitate was proportional to the concentration of NEFA in the plasma. They inferred therefrom that the only requirement for utilisation of fat was stimulation of depot fat mobilisation. In 1963 Friedberg et al found that during exercise the half-life of NEFA is decreased. The evidence of Armstrong et al (1961) and Friedberg et al (1963) taken together yield the conclusion that a rise in the plasma NEFA level /

level during exercise implies greatly increased utilisation.

Ever since the introduction of calorimetry and the measurement of net chemical changes, it had been evident that fat must be an important source of energy during exercise (Lusk, 1917). There was, however, no proof of direct fatty acid oxidation by muscle. In view of the evidence discussed above, there can no longer be any reasonable doubt that fatty acids are directly utilised by muscle. In the case of heart muscle, this has been proved (Gordon, 1957; Ballard et al 1960).

The rate of turnover of NEFA in the blood may be 40 times greater than that of glucose (Dole, 1958). A molecule of NEFA provides on oxidation more than three times as much energy as a molecule of glucose. These facts challenge the traditional concept that carbohydrate is necessarily the sole or even the prime fuel of muscle. In fact in current physiological theory, fat has supplanted carbohydrate as the prime fuel of muscle. So the wheel has turned full circle, and each proximate principle has had its day.

CHAPTER 2

Growth Hormone and Fat Mobilisation

The human machine, although it is constantly running, is refuelled only three times or so a day, i.e. at meal-times. Since absorption is normally completed in about four hours after a meal, in the intervals between exogenous refuelling - amounting to some 12 hours a day - the energy needs of the body must be supplied from endogenous sources, mainly fat. All available evidence suggests that Growth Hormone (G.H.) plays an important role in the mobilisation of fat as a fuel.

Interest in the pituitary gland as a possible regulator of the mobilisation of depot fat arose when Lee and Schaffer (1934) showed that giving a crude growth promoting exact from the anterior pituitary to a normal rat resulted not only in the deposition of protein-containing tissue but also in a loss of body fat. Lee and Ayres (1936) then showed that the hypophysectomised rat lost more protein and less fat than control animals. This suggested that in the absence of the pituitary the process of fat mobilisation was impaired.

Young (1945) confirmed the observations of Lee and Schaffer in both normal rats and dogs. In addition, he made the fundamental observation that "the non-fasting animal treated with pituitary extract resembles the untreated fasting animal in basic metabolic pattern". Subsequent work with purified G.H. preparations by Li, Simpson and Evans (1948, 1949) /

1949) supported Young's observations. The question that posed itself at this stage was: does the pituitary secrete a specific fat mobilising substance in response to a need for fuel ? Of the many individual substances of pituitary origin which have an influence on fat metabolism, G.H. received most attention. The underlying theory was that G.H. promoted growth probably by "burning" fat for energy purposes, thus sparing protein for growth. In support of this theory was the fact that rats treated with diabetogenic extract, which probably contained G.H. and maintained on a constant diet put on weight (Young, 1945).

In a review of the evidence Young (1953) wrote: "There is as yet no evidence of which I am aware to show that G.H. is secreted during starvation by the pituitary gland of an adult animal, but it seems to me probable that this is so. So far the methods available for the estimation of G.H. are not adequate for this task." And so they remained until 1963.

In 1956 Li and Papkoff reported the presence of comparatively large amounts of G.H. in the adult anterior pituitary gland, suggesting that the hormone may indeed have a regulatory function in normal metabolism in adults. In the same year Dole (1956) and Gordon (1956) recognised the role of NEFA in the transport of lipid for utilisation by the tissues. This concept enabled a closer inspection of the influence of G.H. on fat mobilisation and it was soon found that the most immediate effect that followed injection of G.H. was a marked rise in plasma NEFA (Raben and Hollenberg, 1958; Raben 1959; Engel et /

et al, 1958; Knobil and Greep, 1959; Wilgram et al, 1959). Thus a mechanism was found to explain how G.H. actually mobilised fat. Moreover, Raben and Hollenberg (1958) found that the fat-mobilising effect of G.H. was produced by smaller doses of the hormone than were required to produce any other measurable response. This suggested that fat mobilisation may well be an important physiological action of G.H.

By 1960 there was good inferential evidence to believe that in adults G.H. was - as Young suspected in 1953 - secreted in situations in which fat was mobilised and utilised, e.g. fasting. The only thing that was needed to clinch the issue was a sufficiently sensitive and specific method for the assay of G.H. in the blood. This came when Hunter and Greenwood (1962, 1964) and Roth et al (1963a) developed a radio-immunoassay for the estimation of G.H. in the plasma. Here at last was a highly sensitive and highly specific method for the estimation of G.H. The conditions were now right and the scene was set to put Young's prediction to the test. When Roth et al (1963a) reported after a series of preliminary studies that the G.H. concentration of the blood was increased in response to fasting, exercise and hypoglycaemia, Young's brilliant prediction of a decade earlier was confirmed. The fat-mobilising action of G.H. was no longer in doubt.

In early 1964 when the work reported in this thesis began, the entire literature on the role of G.H. in the mobilisation of fat as fuel for exercise based on actual measurement of G.H. in /

in the blood consisted of two papers. Roth et al (1964) had reported small increases in the plasma concentration of G.H. in two subjects after they had walked 8 km. Hunter and Greenwood (1964b) had found high concentrations of G.H. in the plasma of three subjects after they had played squash for two to three hours. Thus in undertaking to investigate the role of G.H. during moderate exercise in human adults we were really embarking on a voyage of discovery.

CHAPTER 3

Analytical Methods

1. Analysis of Blood

(a) Non-esterified Fatty Acids (NEFA)

These were estimated by the method of Dole (1956) with minor modifications. The exact procedure is described below.

Principle: The NEFA are extracted from the plasma by an extraction mixture consisting of heptane (10 parts), isopropyl alcohol (30 parts) and N sulphuric acid (1 part). Heptane is a non-polar solvent which quantitatively extracts the NEFA. Isopropyl alcohol brings heptane and water present in the plasma into a single phase. This one phase system is then converted to two phases by the addition of further heptane and water. An aliquot of the upper phase which contains the NEFA is titrated against standard alkali.

Procedure: As soon as possible after separation of plasma, 5 ml. of the extraction mixture are added to 1 ml. of plasma in a glass-stoppered tube and shaken vigorously for 30 seconds. After allowing to stand for at least ten minutes the system is divided into two phases by mixing into it an additional 2 ml. of heptane and 3 ml. of distilled water. The phases separate rapidly forming a sharp interphase. A 3 ml. aliquot of the upper phase is transferred to a conical centrifuge tube containing 1 ml. of the titration mixture (thymol blue in ethanol) and titrated against approximately 0.02 N NaOH. Blank titrations using 1 ml. of heptane were always carried out.

The /

The procedure is simple but very time-consuming. It has a coefficient of variation of about 5% on replicate samples. It has a sensitivity sufficient for accurate titration of fatty acids in 1 ml. of plasma.

(b) Human Growth Hormone (H.G.H.)

Plasma H.G.H. was estimated by the radio-immunoassay method of Hunter and Greenwood (1964b) with minor modifications (Hunter et al, 1965).

Principle: In essence the method is based upon the ability of H.G.H. (as standards or unknowns) to inhibit the *in vitro* reaction of I^{131} labelled H.G.H. with its specific antibody.

Requirements:

(i) A purified H.G.H. preparation: This was obtained from acetone dried human pituitaries by the method of Raben (1959).

(ii) A method for labelling the purified H.G.H. with I^{131}: The method used was that of Hunter and Greenwood (1964b) with minor modifications.

(iii) Antiserum to H.G.H.: This was obtained from adult rabbits by the method described by Read and Bryan (1960).

(iv) A method of differentiating between H.G.H. bound to antibodies from unbound H.G.H. after chemical union between antigen and antibody: This was done by cellulose acetate electrophoresis in 0.0[illegible]M-barbitone buffer, pH 8.6, at a constant output voltage of 400 V for 3½ hours.

Method: /

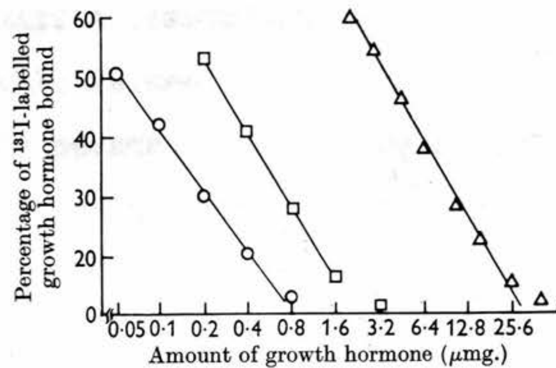


Fig. 1. Standard assay curves for growth hormone: comparison of the amounts of growth hormone required to decrease the amount of ^{131}I-labelled growth hormone bound to immune γ-globulin. △, ^{131}I-labelled growth hormone (2 μmg.) and antiserum (1:20 000 dilution); □, ^{131}I-labelled growth hormone (0·2 μmg.) and antiserum (1:200 000 dilution); ○, ^{131}I-labelled growth hormone (0·05 μmg.) and antiserum (1:700 000 dilution).

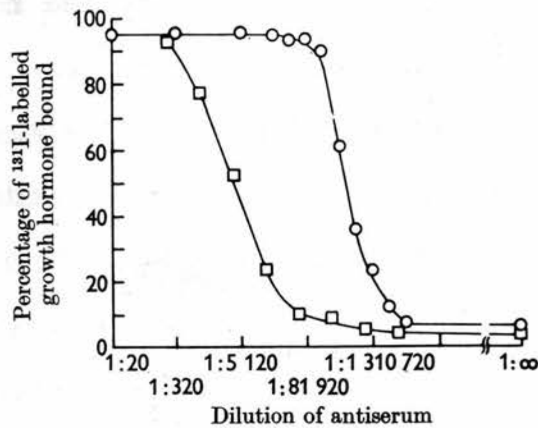


Fig. 2. Titration of rabbit antisera to growth hormone with ^{131}I-labelled growth hormone: the relationship between the percentage of the radioactivity of ^{131}I-labelled growth hormone (0·2 μmg.) bound to the immune γ-globulin and the antiserum dilution. □, Rabbit antiserum VII; ○, rabbit antiserum III.

(From Hunter & Greenwood (1964b) Biochem. J., 91, 44)

Figures 1 and 2

Method:

(i) Titration of Antiserum: A fixed amount of labelled H.G.H. is incubated with serial dilutions of antiserumIII. After the system has equilibrated aliquots of the incubates are separated by electrophoresis and the percentage of the labelled H.G.H. bound to antibody is plotted against antiserum dilution (Fig. 2).

(ii) Construction of a standard curve: Keeping the amounts of labelled H.G.H. and of antiserum constant, increasing quantities of standard H.G.H. are added to different tubes. This results in a progressive reduction in the percentage of the labelled hormone bound to the fixed amount of antibody present. The plot of the percentage of the labelled H.G.H. bound to antibody against the log of the amount of H.G.H. added to each tube yields a straight line which serves as the standard (Fig. 1).

(iii) Estimation of H.G.H. concentration in unknown samples of plasma: The unknowns are incubated together with the standards. The percentage of the labelled H.G.H. bound to antibody is determined. The values of the unknowns are read off from the standard curve. *

Critical Evaluation of the Radio-immunoassay of H.G.H.: Implicit in the use of an in vitro immunochemical method for measuring a protein hormone in the peripheral blood is the assumption that the hormone present in the plasma is in the same form as that extracted from the pituitary gland and used as /

* The HGH values appearing in the body of the thesis are the means calculated from estimations carried out at two or more different dilutions of the plasma. (Dr. Hunter carried out most of the HGH estimations at the M.R.C. Clinical Endocrinology Research Unit Laboratories, Edinburgh).

as standards. This assumption may not be valid. One method of checking the specificty of an immunochemical method is to have a specific biological assay to be run in parallel with the immunochemical method. Unfortunately none of the biological assays of H.G.H. available is sufficiently sensitive or specific to permit of such a direct check. Even so, there is good inferential evidence to believe that the radio-immunoassay of H.G.H. is highly specific. This evidence has been obtained:

(i) by comparing the behaviour of unknowns with standard solutions of H.G.H. in their ability to compete with labelled H.G.H. for sites on the antibody.

(ii) from the effect of animal sera on the assay.

(iii) from the results of assays in normal and pathological plasma.

The radio-immunoassay of H.G.H. is not only highly specific, but also highly sensitive. The lower limit of sensitivity for purified H.G.H. is 0.07 μmg/ml. and this concentration is measured with a maximum percentage error of ± 50% (Hunter and Greenwood, 1964b).

(c) Blood Glucose

This was estimated using the Technicon Antoanalyser (Wootton, 1964).

Principle: The method depends on the reduction of yellow potassium ferricyanide to colourless ferrocyanide by glucose in the presence of cyanide as sensitising agent. This chemical reaction can occur only in protein-free plasma. The Autoanalyser /

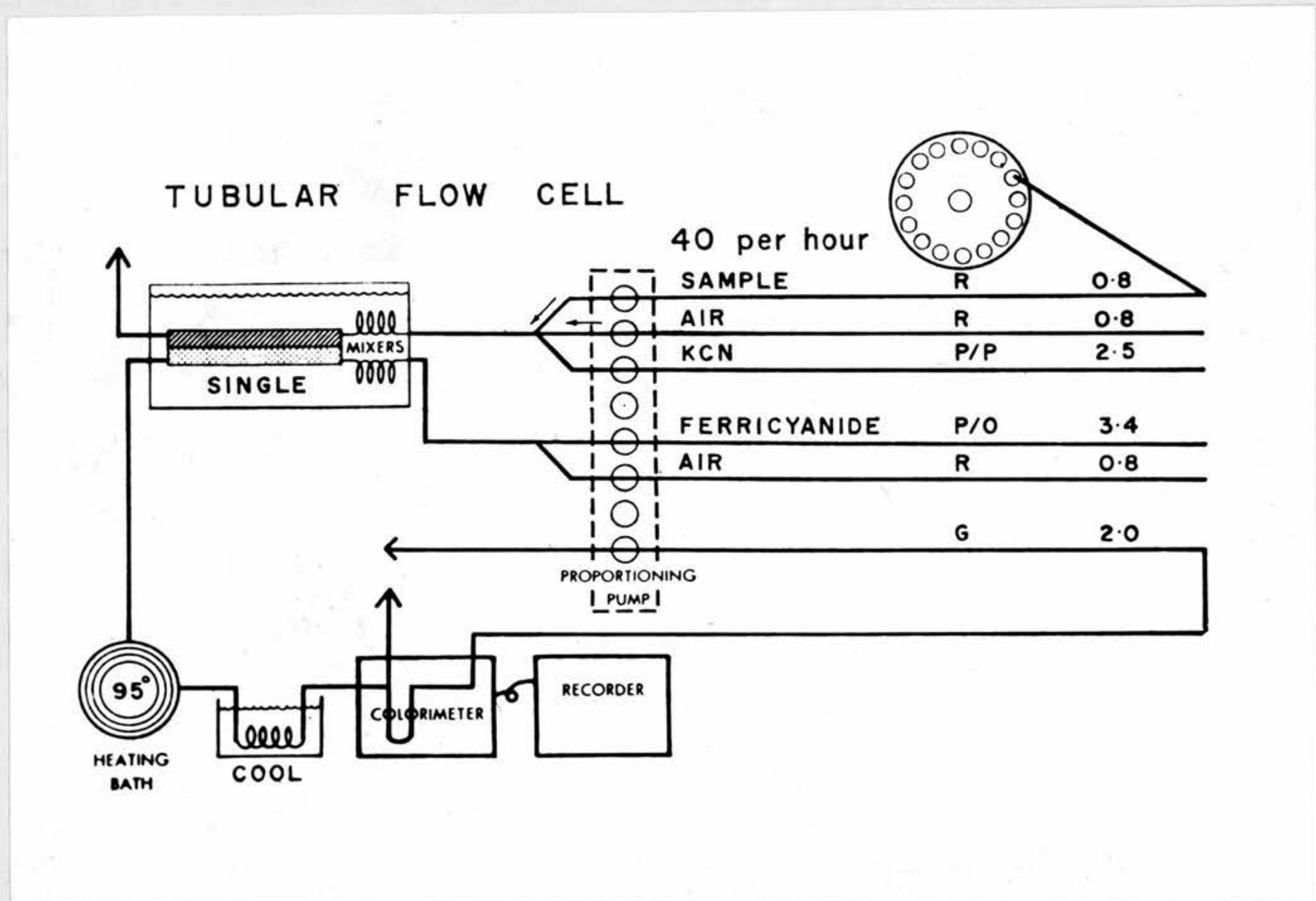


Figure 3

The numbers on the pump lines refer to flow in ml/min. The letters are code references to different sizes of pump tubing.

analyser uses dialysis to separate the proteins from the plasma. The reactions occur in a continuously flowing stream of liquid. A multichannel pump operates continuously to draw up a number of separate streams of reagent which are combined as required. The samples to be analysed are aspirated successively into one of the liquid streams. The necessary chemical reactions are performed, the mixture is eventually passed through a flow colorimeter and the result obtained as a peak on a recorder chart. Suitable standards are included among the unknown samples so that a direct comparison can be made. The basic design of the Autoanalyser is illustrated in Figure 3.

Although the reduction of yellow potassium ferricyanide to colourless ferrocyanide by glucose is a very non-specific reaction, most interfering substances are removed by the dialysis process and the results are within 5 mg. per 100 ml. of those obtained by the glucose-oxidase method.

2. The Metabolic Mixture

This was computed by the procedure devised by Passmore and Johnson and first published in full by Consolazio et al (1963). This procedure renders unnecessary the tedious arithmetic and the artificiality of calculating the non-protein R.Q. involved in the time-honoured method of Zuntz and Schumberg (1901)

The three independent variables which reflect the composition of the metabolic mixture are the O_2 used, the CO_2 produced and the nitrogen excreted in the urine. The data are /

$$\text{Carbohydrate (g.)} \quad . \quad = 4{\cdot}12\ CO_{2m} - 2{\cdot}91\ O_{2m} - 2{\cdot}54\ U_n.$$
$$\text{Fat (g.)} \quad . \quad . \quad . \quad = 1{\cdot}69\ O_{2m} - 1{\cdot}69\ CO_{2m} - 1{\cdot}94\ U_n.$$
$$\text{Protein (g.)} \quad . \quad . \quad = 6{\cdot}25\ U_n.$$
$$\text{Energy (Cal.)} \quad . \quad . \quad = 3{\cdot}78\ O_{2m} + 1{\cdot}16\ CO_{2m} - 2{\cdot}98\ U_n.$$

1		2	3	4	5	6	7	8	9
O_{2m} litres	125·1	**−2·91**	−364·0	**+1·69**	+211·4	...	...	**+3·78**	+472·9
CO_{2m} litres	97·0	**+4·12**	+399·6	**−1·69**	−163·9	...	...	**+1·16**	+112·5
U_n g.	1·44	**−2·54**	−3·7	**−1·94**	−2·8	**+6·25**	9	**−2·98**	−4·3
		CHO	32 g.	**Fat**	45 g.	**Protein**	9g.	**Energy**	581 Cal.

The data are from an experiment in which the subject in the post-absorptive state walked on a treadmill for two hours at 4 m.p.h. The values for O_{2m} and CO_{2m} were based on four measurements of the respiratory exchanges throughout the walk.

Figure 4

are rearranged in the form of two equations relating the total oxygen used and the total carbon dioxide produced in terms of the amounts of the three proximate principles oxidized. A third equation relates the metabolised protein with the urinary nitrogen (Fig. 4). When the data are so arranged the components of the metabolic mixture can each be calculated from the three basic measurements. The labour of routine calculations is reduced by coding the various equations in a work sheet as shown in the figure.

The oxygen and carbon dioxide contents of the samples of expired air collected using a Douglas bag or a Max-Planck respirometer were determined with the Lloyd modification of the Haldane apparatus (Lloyd, 1958) using chromous chloride solution as oxygen absorbant (Dahlstrom and Wahlund, 1949). Urinary nitrogen was determined by the Kjeldhal method using the catalyst recommended by Jacobs (1959).

3. Determination of the Body Fat Content of Rats

McCance and Shipp (1933) have pointed out that any method for the estimation of fat in biological material must of necessity be a compromise. In the method used in this work the dried, homogenised, weighed carcass was repeatedly extracted with petroleum ether. The extracted carcass was dried to constant weight and the difference taken as representing fat.

This method is open to both theoretical and technical objections. The chief theoretical objection is that, besides fat, petroleum ether extracts material which, from a metabolic point /

point of view cannot be regarded as fat. What this method measures is therefore more accurately called "petroleum ether extract".

The main technical objection to this method is that petroleum ether is not a very good solvent for fats. McCance and Shipp (1933) have shown, however, that using the Soxhlet method a single extraction removed the whole of the fatty acids. In the experiment reported in this thesis, each carcass was reduced to a homogeneous mass and repeatedly extracted until there was no colour change in the added petroleum ether. There is no reason to suppose that this would have failed to completely extract almost the whole of the petroleum ether soluble material.

CHAPTER 4

The Experiments

1. The Effect of Exercise on Plasma Growth Hormone Levels in Human Adults

Roth et al (1963a) first suggested that the mechanism responsible for stimulating secretion of Growth Hormone (G.H.) appeared to be sensitive to muscular exercise. In 1964 they reported small increases in the plasma G.H. concentration in two subjects after they had walked 8 km. Hunter and Greenwood (1964a) found high concentrations of G.H. in the plasma of three subjects after they had played squash for two to three hours. In view of these findings, the present experiment was designed to study the effect of a standardised period of exercise on the plasma G.H. levels in human adults.

METHODS

Subjects: Eight healthy students and three research workers whose ages ranged from 19 to 54 years acted as subjects. These included two females.

Procedure: The subjects came to the laboratory between 8 and 9 a.m. having had no breakfast. They then rested for 30-60 minutes. The men walked on a treadmill at 4 m.p.h. on the level and the women at 3 m.p.h. up an incline of 3.5 per cent for two hours. Resting samples of expired air were collected using a Douglas bag. In each 30 minute period of exercise, the volume of expired air was measured for 5 minutes and /

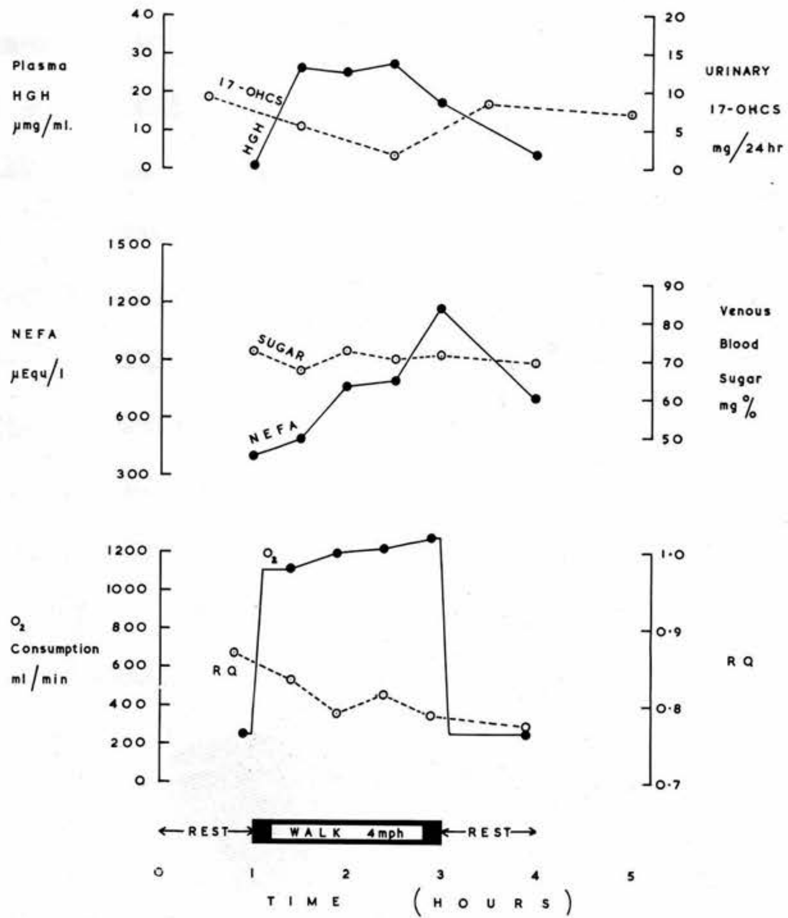


Figure 5

and an aliquot collected using a Max-Planck respirometer. The walk was interrupted for about 1-2 minutes whilst samples of blood were taken from a forearm vein by venepuncture.

Analytical Procedures: Plasma H.G.H. was estimated by the radio-immunoassay method of Hunter and Greenwood (1964b). Plasma non-esterified fatty acid (NEFA) was determined by the method of Dole (1956). Blood sugar was estimated by a method using the Technicon Autoanalyser and depending upon reduction of ferricyanide. The expired air was analysed for oxygen and carbon dioxide in the Lloyd-Haldane apparatus, duplicates agreeing within ± 0.02 per cent. Urinary N was obtained by micro-Kjeldhal analysis. Urinary 17-hydroxy-corticosteroids (17-OHCS) were estimated using the method of Appleby et al (1955) by Dr. R.A. Harkness of the Medical Research Council Clinical Endocrinology Research Unit, Edinburgh.

RESULTS

The results from the eleven subjects are given in Tables 7-13 in the Appendix.

Figure 5 shows the results on one subject M.Y.S., a 29 year old male. During the first hour of the walk, the plasma G.H. concentration rose and this was associated with a steadily rising NEFA concentration in the blood and a falling R.Q. During the second hour, however, the plasma G.H. concentration fell despite continued fat mobilisation as evidenced by a rising NEFA and a falling R.Q. The blood sugar level was well maintained. The 17-OHCS secretion was increased towards the end /

end of the walk (normally there is a steady fall in urinary 17-OHCS in the resting state).

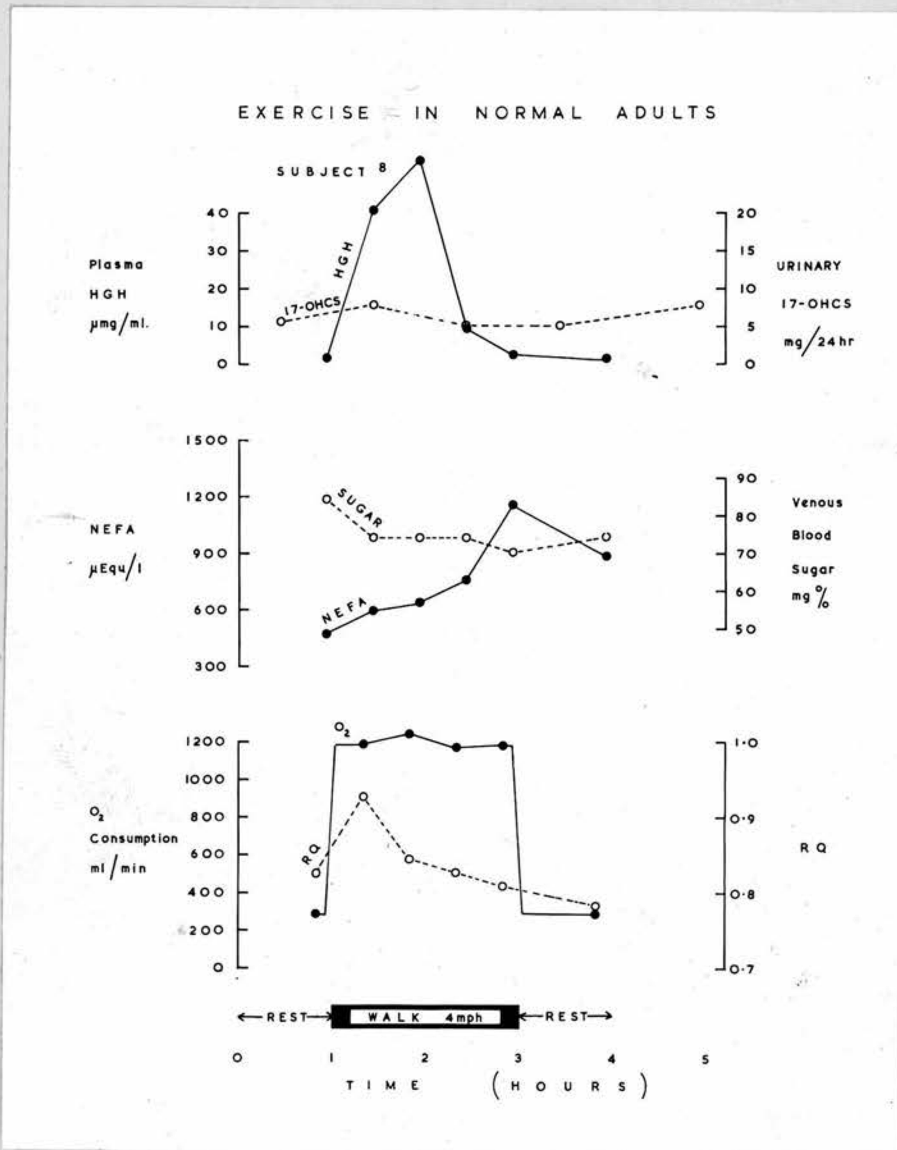


Figure 6

Figure 6 summarizes the data for D.S., a 19 year old male. The G.H. level in the plasma rose 50-fold after one hour of walking. The precipitous fall in its concentration during the second hour of walking was remarkable.

Figure /

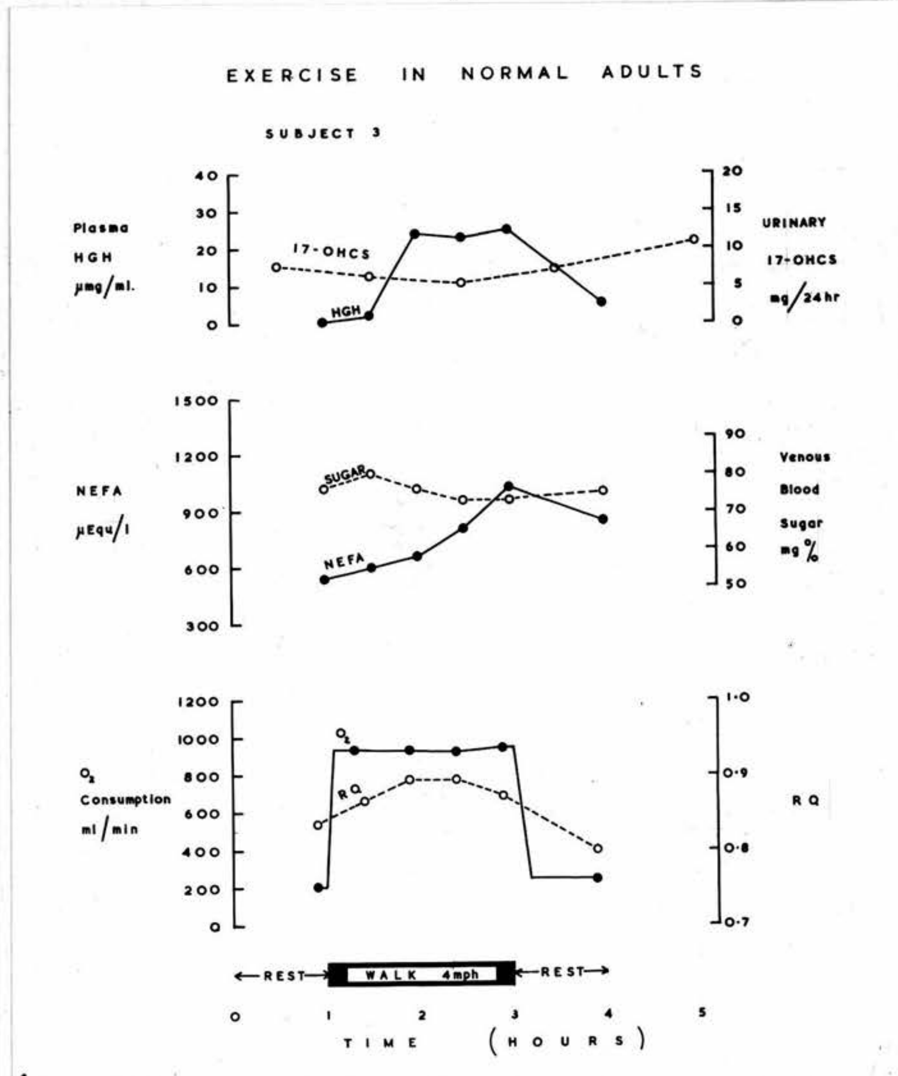


Figure 7

Figure 7 depicts the results obtained on W.M.H., a 37 year old male. The general pattern was the same as that shown for the first two subjects except that there was no fall in the plasma G.H. level during the second hour of the walk.

The data for these three subjects typified that for six of the /

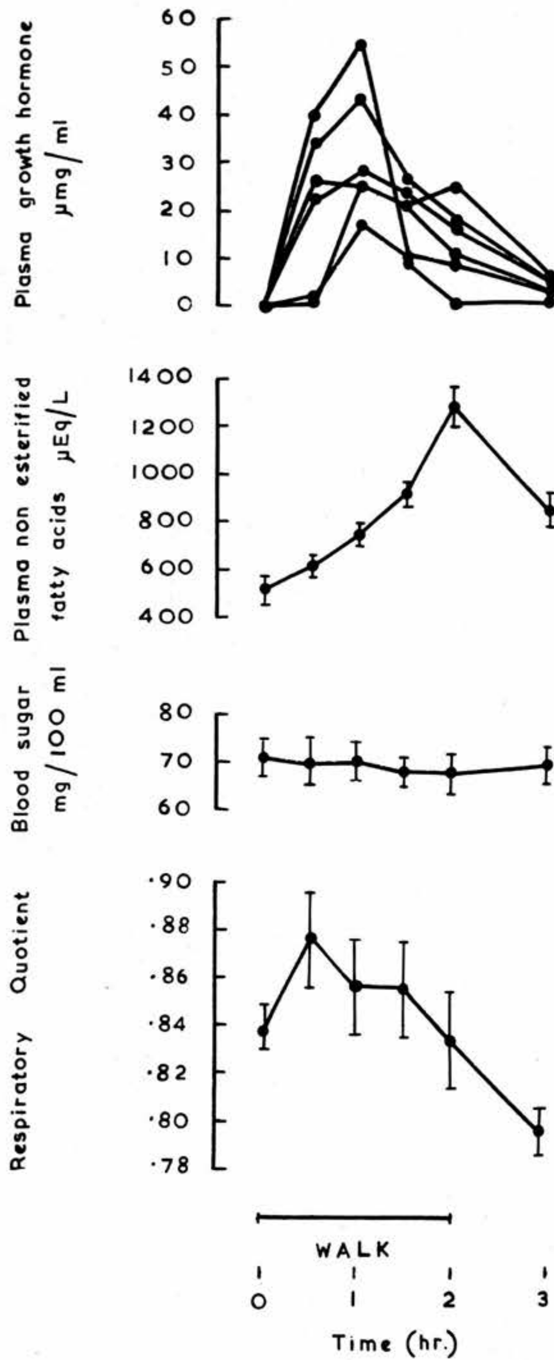


Figure 8

the eleven subjects studied and Figure 8 summarises the data for these six subjects. The association of a steadily rising concentration of NEFA in the plasma, a falling R.Q. and a marked increase in G.H. in the plasma with peak concentrations ranging from 17 to 53 μ mg/ml. is evident. The pattern of secretion of G.H. in three of the six subjects showed a peak in one hour. In all but one subject, there was a fall in the plasma G.H. concentration during the second hour of exercise.

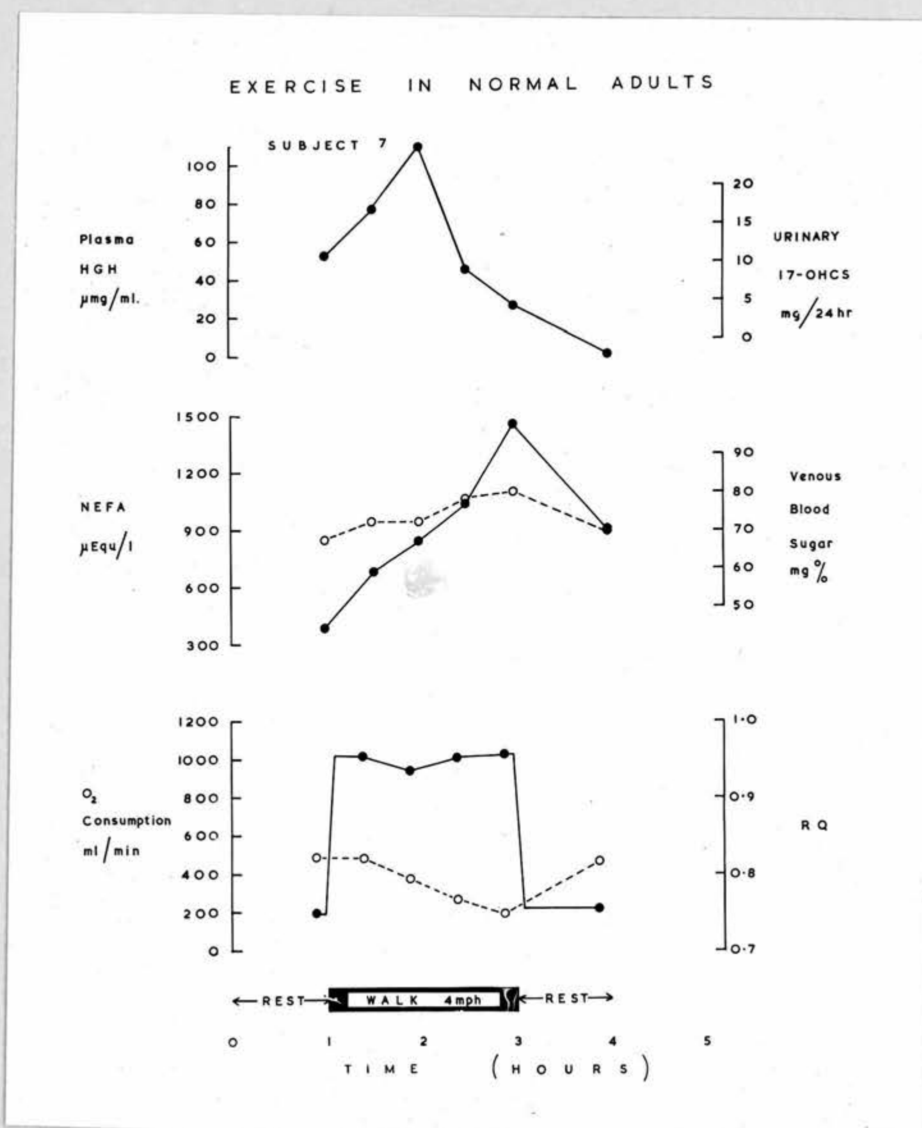
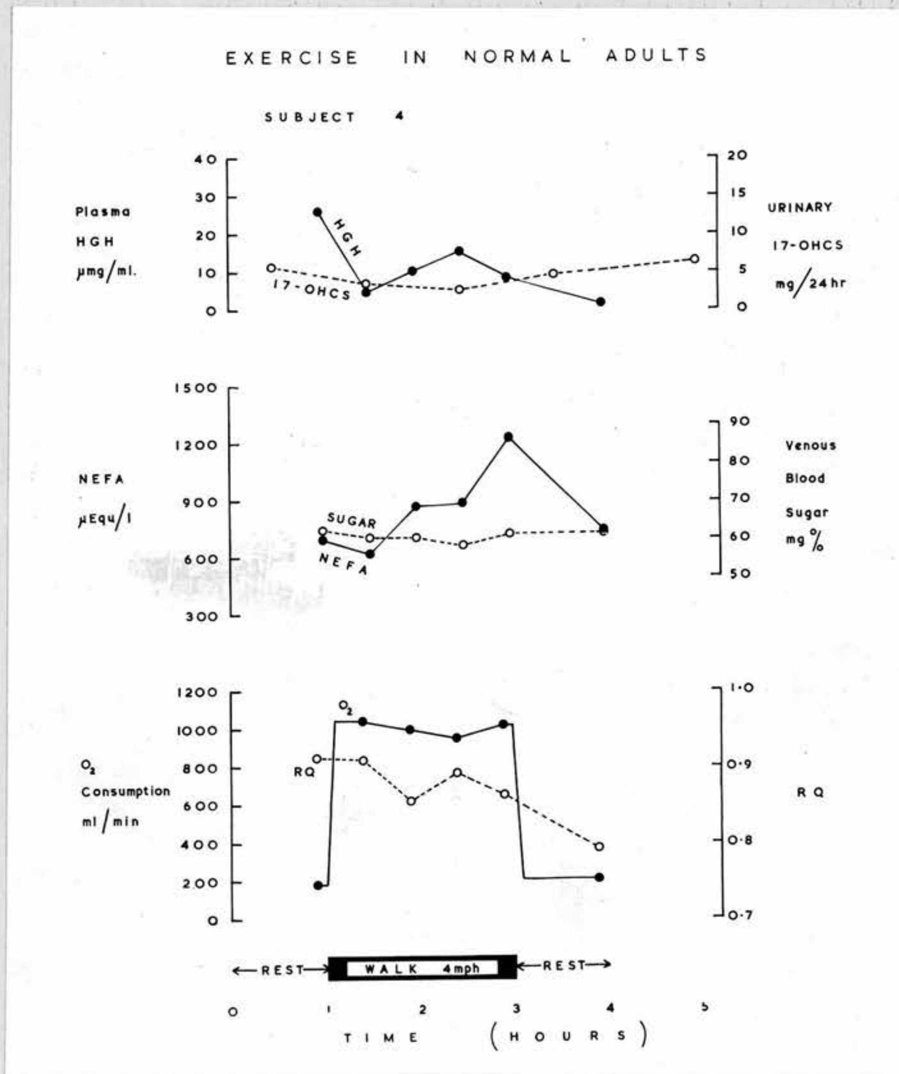


Figure 9

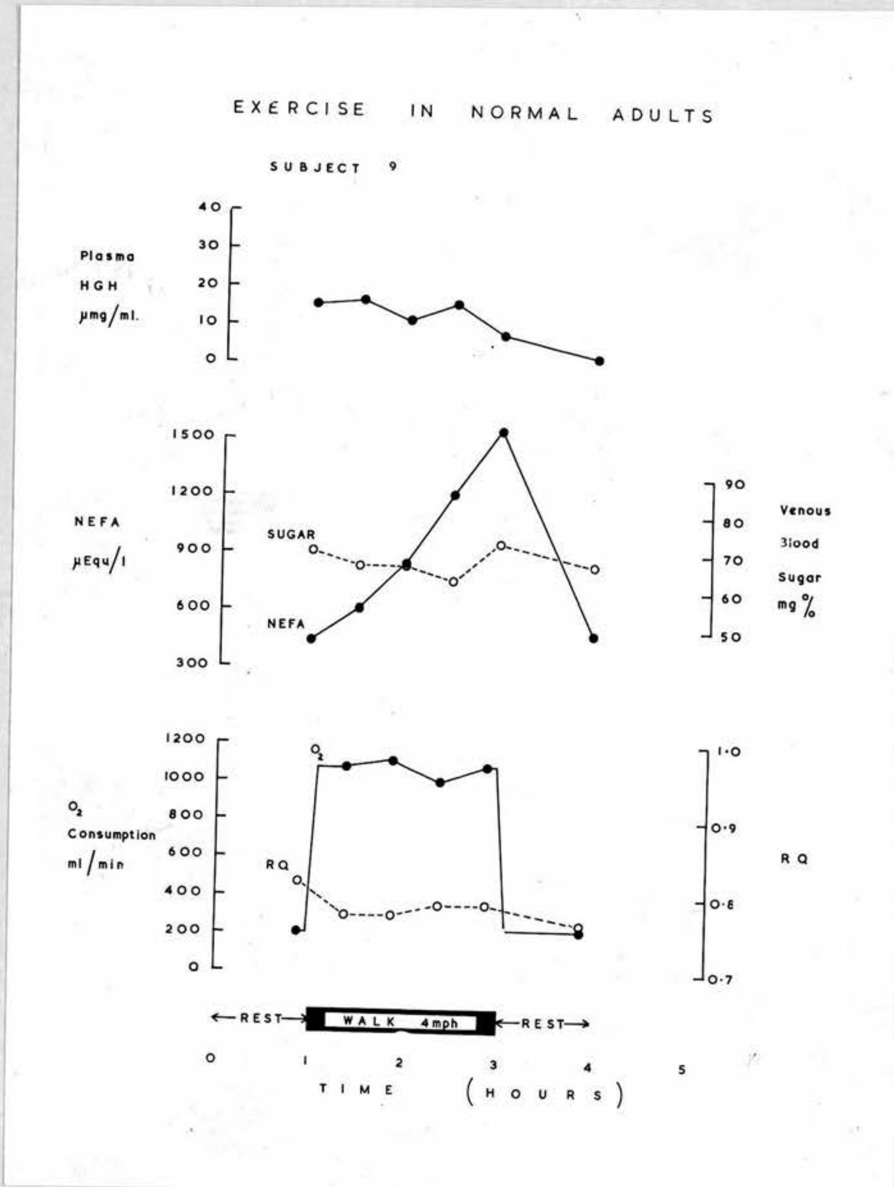
Figure 9 shows the data for D.H., a 19 year old male. The striking feature in this subject was a very high (53 µ mg/ml.) initial plasma G.H. level which rose to a peak and then fell during the second hour of the walk. Another subject, G.R., a 20 year old male, showed a similar marked rise from a high initial value and then a fall during the second hour of the walk.



<u>**Figure 10**</u>

Figure 10 showing the results for D.W., a 22 year old male, typifies the results in two of the subjects of this series. They /

They showed a high initial G.H. concentration in the plasma which first fell and then rose to fall again during the last part of the walk.



<u>**Figure 11**</u>

Figure 11 depicts the data for subject G.L., a 19 year old female. She had a high initial plasma G.H. concentration which remained high until the last half hour of the walk when the usual /

usual fall occurred.

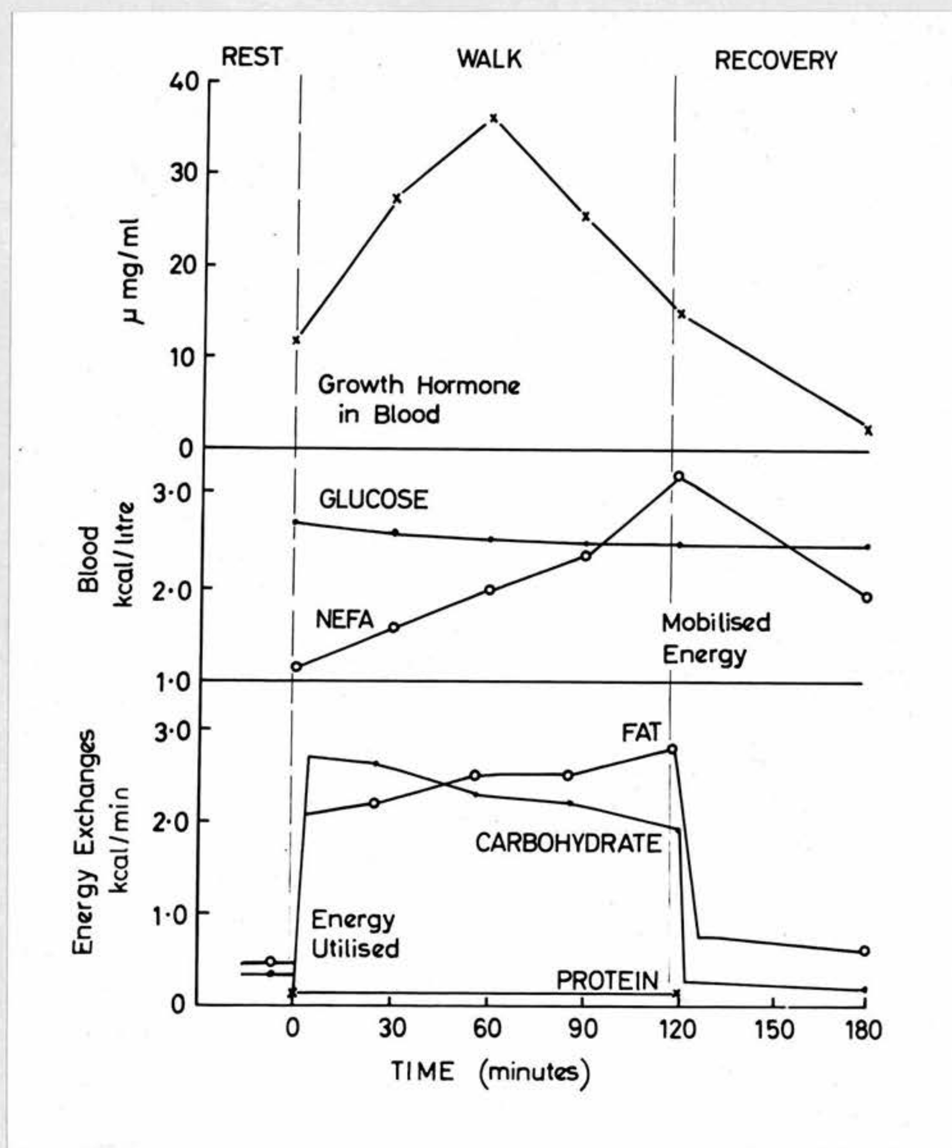


Figure 12

Figure 12 summarises the mean results for the whole series. In this, the proportion of energy derived from carbohydrate, fat and protein as calculated from the respiratory exchanges and urinary N determinations are shown. The proportion of energy derived from fat progressively increased during the walk. This /

This was associated with a marked rise in the plasma NEFA concentration (representing mobilised energy). The blood sugar concentration showed no significant change. The plasma G.H. concentration rose during the first hour of walking and then fell markedly despite continued fat mobilisation and utilisation.

Table 1 shows the G.H. levels in the plasma of the eleven subjects when rested for 30 - 60 minutes after arrival in the laboratory in the post-absorptive state.

Subject	Initial G.H. concentration (µ mg/ml.)
R.P.	8.4
M.Y.S.	0.4
W.M.H.	0.3
D.W.	26
K.W.M.	0.6
C.B.	1.2
D.H.	53
D.S.	1.1
G.L.	15
A.B.	1.2
G.R.	26

Table 1

DISCUSSION

In adults G.H. is secreted both during fasting and during exercise (Roth et al, 1963a, b, 1964). These workers found a value of 9 µ mg/ml in an adult following a 72 hour fast, and values of 5 and 15 µ mg/ml respectively in two subjects at the end /

end of a five mile hike. Each of these conditions involves an energy deficit. Subjects in the present experiment came to the laboratory in the post-absorptive state, i.e. after an overnight fast. This represents an accumulated energy deficit of about 500 kcals. In six of the subjects, the initial G.H. level in the plasma was less than 1 μ mg/ml. For these, at least, an overnight fast was not an adequate stimulus for G.H. secretion.

One hour's walking at 4 m.p.h. involves an energy expenditure of about 300 kcals (Passmore and Draper, 1965). This energy deficit was associated with secretion of G.H. in all the subjects. This suggests that the <u>rate</u> at which a supply of energy is required is more important than a cumulative energy deficit as a stimulus to G.H. secretion in adults.

A wholly unexpected finding in this experiment was the marked fall in the plasma G.H. level during the second hour of the walk despite continued fat mobilisation and utilisation as evidenced by a rising NEFA concentration in the plasma and a falling R.Q. This fall could mean that the rise in plasma NEFA and the rise in plasma G.H. during the first hour of exercise were merely an association and not causally related at all. Alternatively, G.H. might only initiate fat mobilisation and other fat-mobilising agents - which are legion - then sustain it. Or the effect of G.H. on NEFA release might last <u>longer</u> than the presence of the hormone in the blood stream. Each of the possibilities that suggested themselves to account for /

for the inexplicable fall of the plasma G.H. concentration during the second hour of the walk despite continued fat mobilisation, formed the basis of a hypothesis which was put to the test in experiments described below.

The other intriguing finding in this experiment was the extraordinary variability of the initial fasting value for plasma G.H. concentration (Table 1). In six subjects, the hormone was undetected, i.e. less than 1 µ mg/ml; in the others the values varied from 2 to 53 µ mg/ml. A survey of the literature showed that other workers using the same method of assay of G.H. had also found high initial fasting values in some subjects reporting to the laboratory after an overnight fast. Because the mechanism responsible for stimulating G.H. secretion is known to be sensitive to muscular exercise, the possibility that the small amount of exercise involved in reaching the laboratory had provoked G.H. secretion in these subjects suggested itself. This hypothesis was tested in experiment 2.

2. Investigation of the Variability of the Initial Fasting Value of Plasma G.H. Concentration in Human Adults

Because the initial fasting value of plasma G.H. concentration of adults coming to the laboratory after an overnight fast was very variable (Table 1), the present experiment was designed to see whether the small amount of exercise involved in reaching the laboratory might have caused the elevated levels in those subjects who showed them.

METHOD /

METHODS

Subjects: In addition to the eleven subjects studied in experiment 1, twelve other healthy students whose ages ranged from 19 to 24 years were included.

Procedure: The plasma G.H. concentration in all subjects was determined after they had rested for 30 - 60 minutes since reporting to the laboratory in the post-absorptive state. All but one of the subjects who gave high values on reporting to the laboratory were then sampled whilst they were still in bed after waking up in the morning.

RESULTS

From a total of 44 samples taken from 23 subjects at the end of the initial rest period, 27 gave G.H. values of less than 2 μ mg/ml. On the other hand, 17 values from nine individuals showed elevated levels ranging from 2 to 53 μ mg/ml (Table 2).

Table 2

Subject	Plasma G.H. concentration (μ mg/ml)	
	In Laboratory	In Bed
R.P.	8.4, 14 6.0, 17	<1 <1
W.M.H.	<1, <1, <1 <1, 4.3, 4.4 33	<1
D.W.	26	<1
D.H.	53	<1
G.L.	15	1.5
G.R.	2.6	<1
C.C.	6.2	<1
J.K.	18	<1
W.M.	14, 1.7, 13	<1

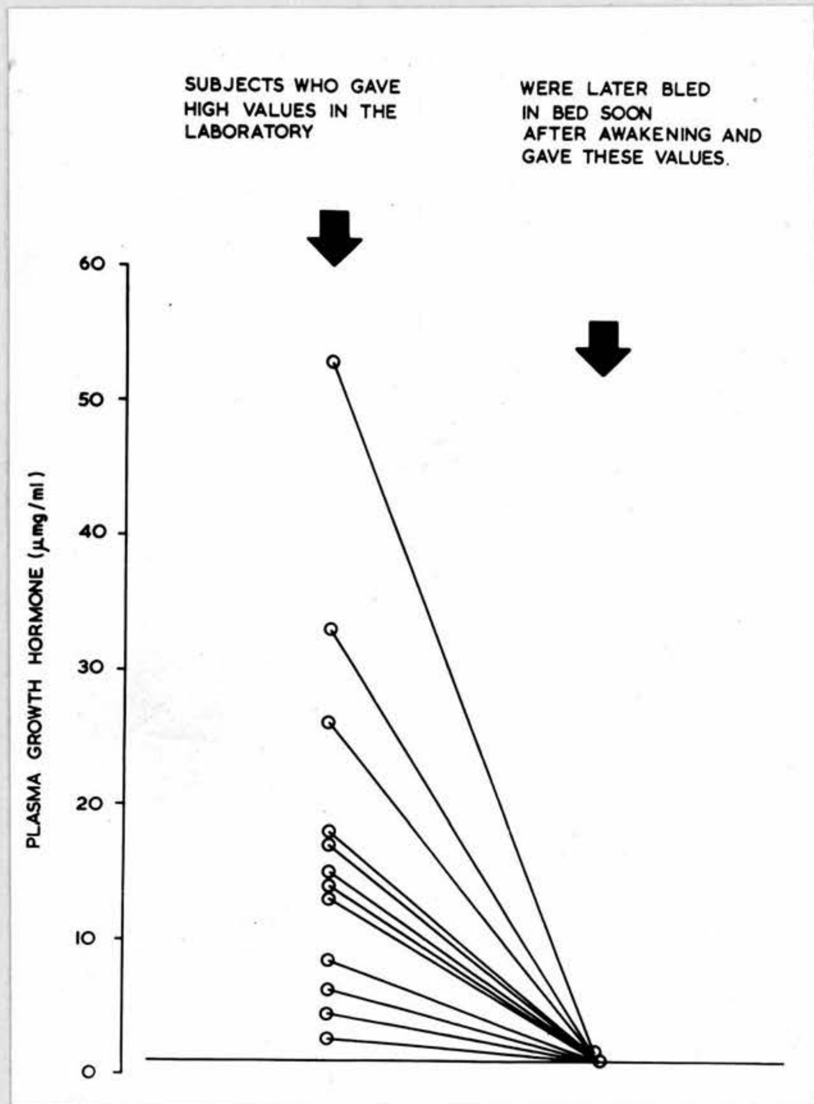


Figure 13

Figure 13 is a comparison of the values of plasma G.H. concentration found after one hour's rest in the laboratory with those found in the same subjects before rising in the morning. All values in bed were less than 2 μ mg/ml.

DISCUSSION

In three centres (Roth et al, 1964; Frantz et al, 1964; Unger et al, 1965) samples taken from healthy adults on reporting /

porting to the laboratory in the fasting state gave values of plasma G.H. concentration below the threshold of sensitivity (1 - 2 µ mg/ml) in two thirds of the subjects. The rest showed widely varying elevated levels. The mechanism responsible for the secretion of G.H. is demonstrably sensitive to muscular exercise. Therefore, a possible explanation of the high values of plasma G.H. shown by some subjects on reporting to the laboratory after an overnight fast is that there are subjects in whom a small amount of exercise is sufficient to produce secretion of G.H. That this cannot be the whole explanation, however, is evident from the fact that the same subject, e.g. W.M.H. in the present series, shows widely different values on reporting to the laboratory under quasi-equal conditions. On the other hand, all subjects sampled in bed before rising gave values below 2 µ mg/ml for their plasma G.H. concentration. The conclusion that the present data warrant is that a low threshold of sensitiviey to a stimulus associated with exercise or some other factor (or factors) is responsible for the high initial fasting value of plasma G.H. concentration shown by some subjects on reporting to the laboratory. What other factors might conceivably be concerned ? Greenwood and Landon (1966) have recently reported an experiment in which the role of "emotional stress" in relation to G.H. secretion was investigated. A medical student was injected with saline but was told that he had received a large dose of insulin and would presently experience severe hypoglycaemic symptoms.
This /

This was followed by a rise in plasma G.H. to 5.2 μ mg/ml. Saline injections in four other subjects without prior suggestion produced no rise in plasma G.H. level. The hypothesis they were testing was that "emotional stress" was capable of producing secretion of G.H. Glick et al (1965) tested the same hypothesis and reported an elevation of plasma G.H. after major surgery which was not suppressed even by giving glucose. In the present series of 23 subjects, nine showed high initial plasma G.H. concentrations. These included seven medical students and two research workers (R.P., W.M.H.). None of the medical students had far to travel to reach the laboratory but all of them were acting as subjects in a scientific investigation for the first time in their lives. The "emotional stress" implicit in such a situation may well have been an important contributory factor in producing elevated initial levels of plasma G.H. On the other hand, R.P. was the most experienced subject in the series and showed high levels of plasma G.H. on reporting to the laboratory on each of four separate occasions. Significantly he had to travel some 13 miles by car or train to reach the laboratory. In his case, the exercise involved in reaching the laboratory was probably the more important factor. That the above explanation is altogether too facile is evident from the values obtained for the initial plasma G.H. concentration in the other research worker, W.M.H., on reporting to the laboratory under quasi-equal conditions. Out of a total seven samples taken on seven /

seven separate occasions, four had values below the threshold of sensitivity and three had values well above it including one of 33 µ mg/ml. Clearly the problem is complex.

3. The Effect of Feeding on Plasma G.H. Levels During Exercise.

Roth et al (1963a) were the first to show that G.H. secretion can be abruptly suppressed by giving glucose. In experiment 1, which involved moderate exercise in the post-absorptive state, a rise of up to 50-fold in plasma G.H. concentration was shown to be associated with increased fat mobilisation and utilisation. A cause and effect relationship between these was assumed and it was considered that the secretion of G.H. during exercise might be governed by the need for mobilising fat as fuel. The present experiments were designed to test this suggestion. If the secretion of G.H. was in fact governed by the need for depot fat mobilisation, then it should not be secreted when there is no such need. Two subjects took part in experiments designed to see the extent to which this prediction corresponded with reality.

Procedure: Two subjects R.P. (54 years) and M.Y.S. (29 years) each walked 8 miles:

(i) twice in the fasting state

(ii) twice with glucose taken orally in 25 g doses, immediately before and at half-hourly intervals during the walk.

(iii) once with protein (casein) taken in 25 g doses

half /

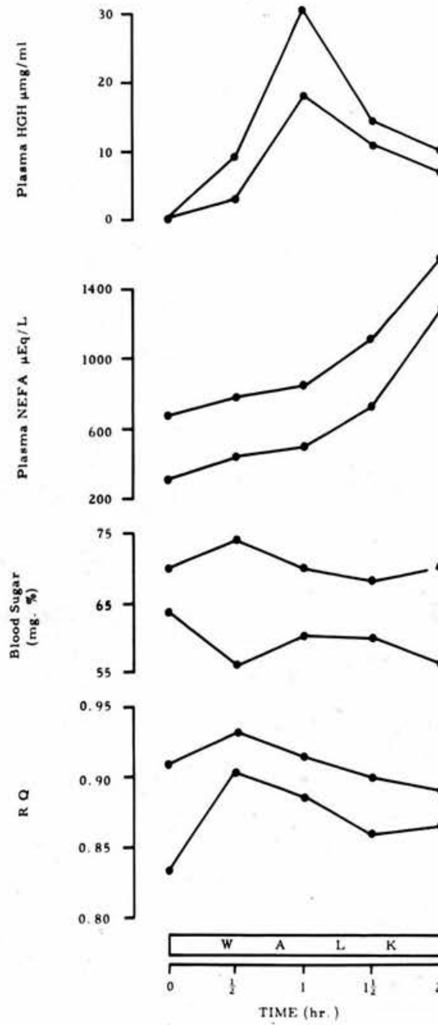


Figure 14

half an hour before, immediately before and at half-hourly intervals during the walk.

(iv) R.P. alone walked 8 miles whilst fat emmulsion (prosparol) was given by nasogastric tube into the lower third of the duodenum half an hour before and at half-hourly intervals during the walk.

RESULTS

Figure 14 shows the data from duplicate experiments on one subject (M.Y.S.) who walked 8 miles in the fasting state. After an initial rise, the R.Q. fell during the walks indicating an increased utilisation of fat. The plasma NEFA concentration rose throughout the walks and the plasma G.H. concentration showed the usual marked rise during the first hour and the fall during the second hour of the walk.

Figure 15 depicts the data obtained for the same subject during duplicate experiments when glucose was taken immediately before and at half-hourly intervals during the walk. The R.Q. remained high indicating that carbohydrate was the major fuel used throughout the walk. The blood sugar level remained almost unchanged. The plasma NEFA concentration did not rise above the resting value and the plasma G.H. was almost undetectable throughout the walk.

Figure 16 summarises the results of the duplicate experiments depicted in figures 14 and 15. Similar results were obtained /

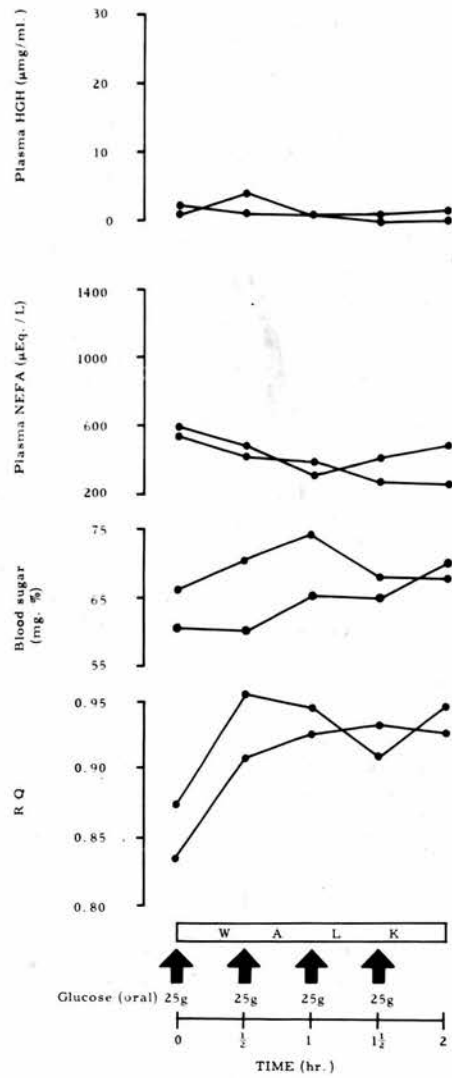
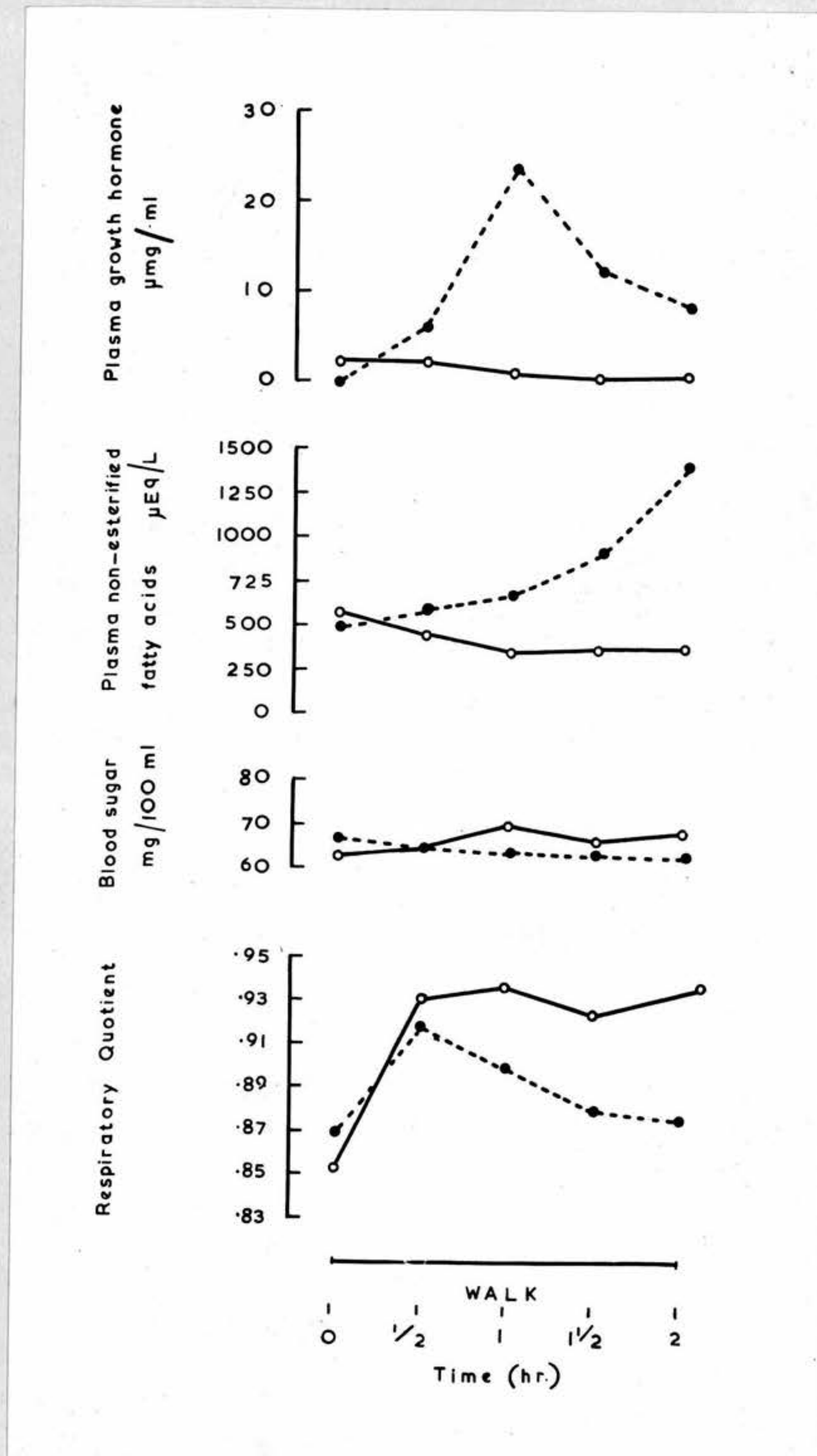


Figure 15



<u>**Figure 16**</u>

obtained in duplicate experiments in the second subject, R.P.

Figure /

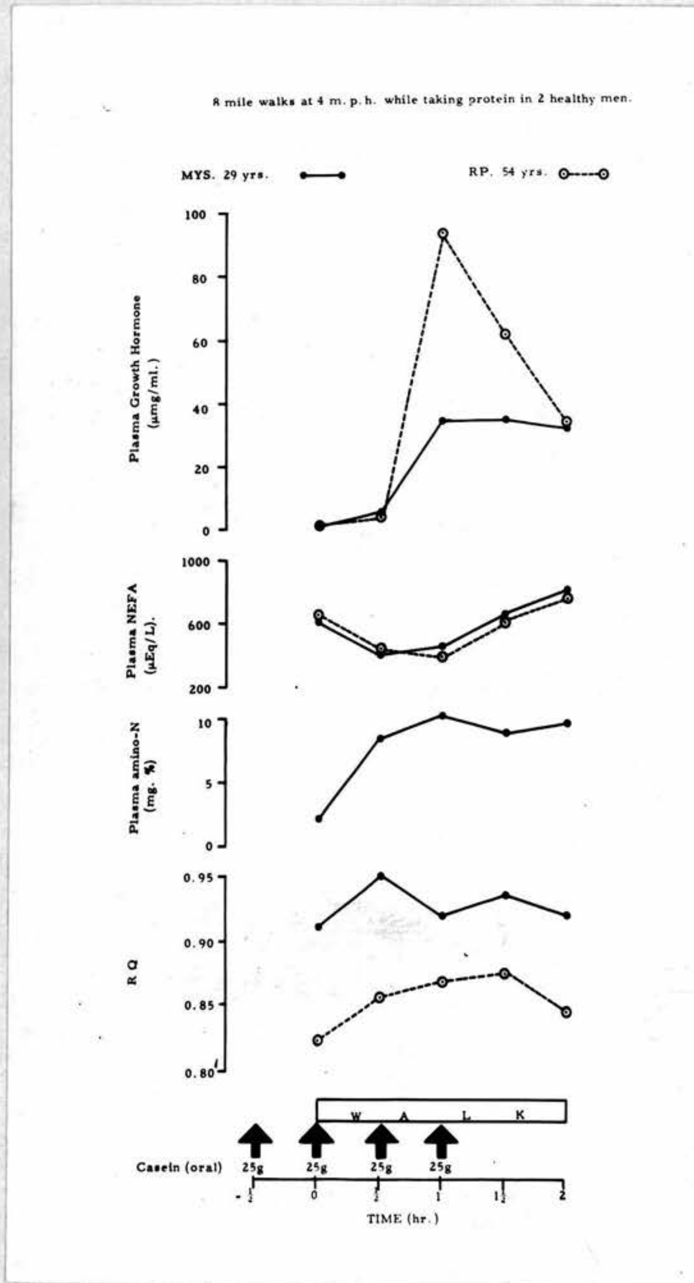


Figure 17

Figure 17 shows that when the same two subjects walked whilst taking casein the R.Q. did not fall. The plasma NEFA after an initial slight fall rose a little above the resting value towards the end of the walk. There was no suppression of /

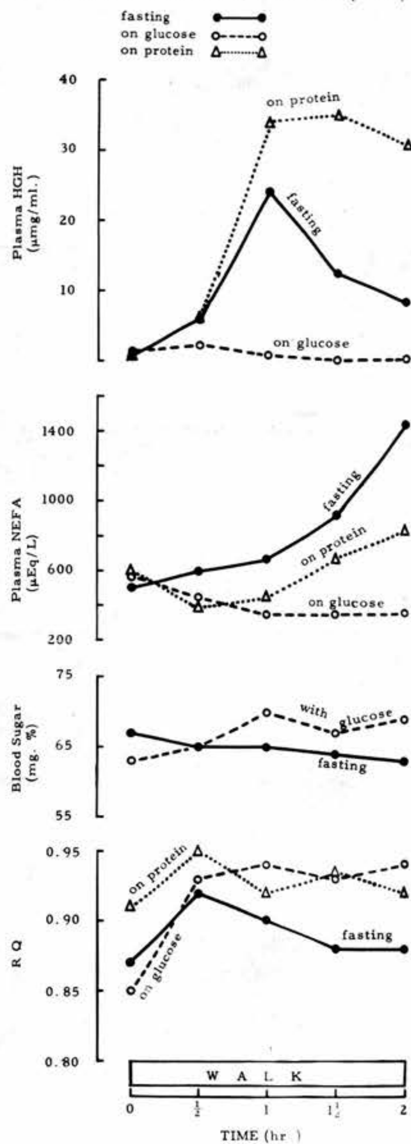


Figure 18

of G.H. secretion in either subject. In both subjects the urinary N was no higher than during the walks in the fasting state. In one subject (M.Y.S.) plasma amino N determinations were carried out on samples taken immediately before and at half-hourly intervals during the walk. Table 3 shows that there was a more than two-fold increase in amino N in the plasma over the fasting concentration throughout the walk. (Plasma amino N was determined by the method of Albanese & Irby (1955) which is based on the iodimetric titration of copper complexed to the amino acids.)

Plasma Amino N (mg/100 ml)				
Before walk	30 mins. walking	60 mins. walking	90 mins. walking	120 mins.
3.9	8.4	10.3	8.9	9.8

Table 3

Figure 18 summarises the data for 8 mile walks in M.Y.S. in the fasting state when taking glucose and when taking protein. The figure is self explanatory.

Figure 19 shows that in both subjects exogenous glucose suppressed or reversed the fall in R.Q. and the rise in plasma NEFA concentration that occurs when a subject walks in the fasting state. It also completely suppressed the secretion of G.H. associated with exercise in the fasting state. Protein /

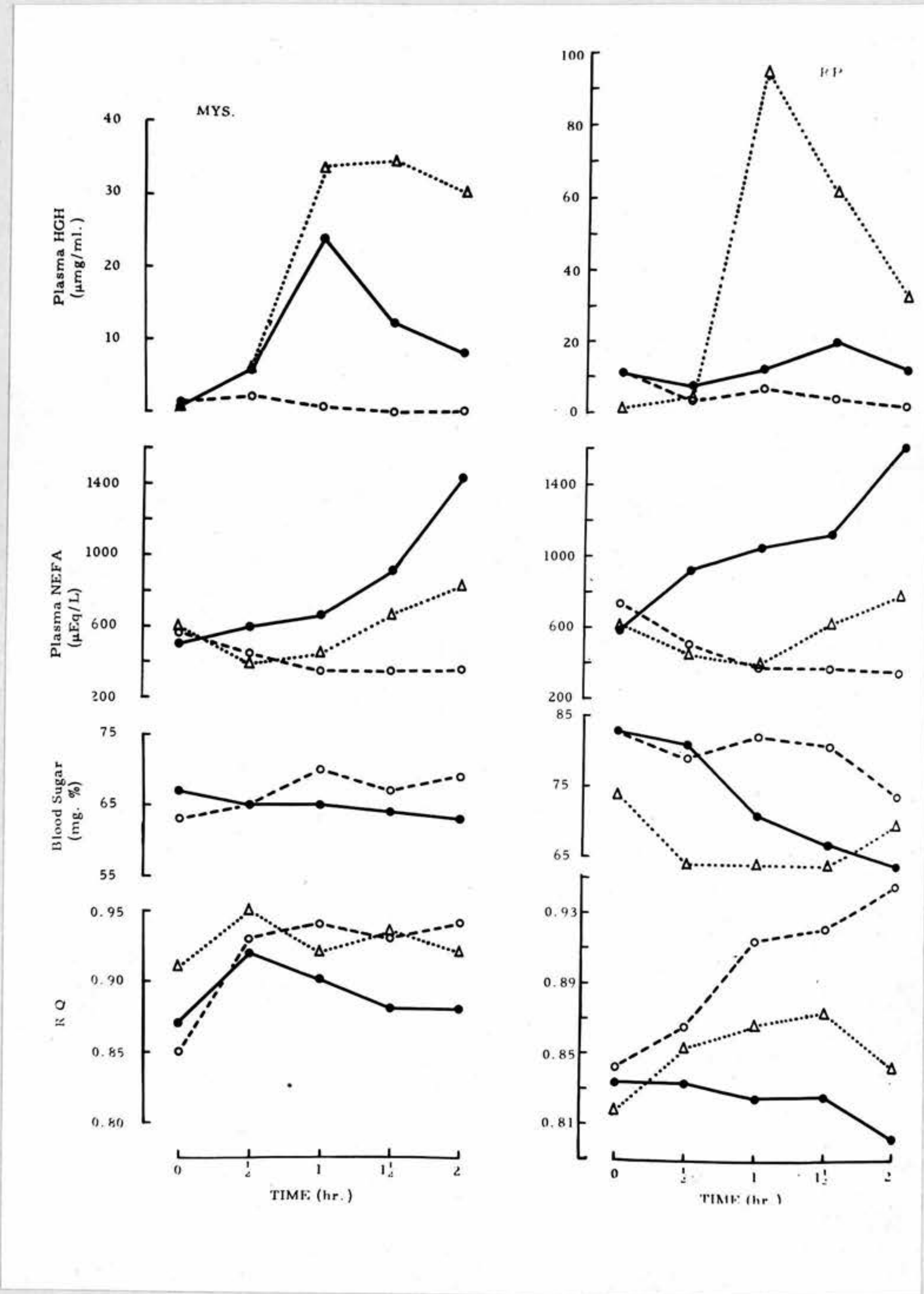


Figure 19

tein suppressed the fall in R.Q. and partially suppressed the rise in NEFA. It did not, however, prevent the secretion of G.H. /

G.H. at all.

In the experiment in which R.P. walked whilst a fat emulsion was being introduced by a nasogastric tube into the lower third of his duodenum, the changes in R.Q., plasma NEFA concentration and G.H. concentration were similar to those found when he walked in the fasting state.

DISCUSSION

Exogenous carbohydrate abolished the mobilisation and combustion of stored fat and the secretion of G.H. thought to be responsible for this. Ingested fat was without effect and this is in accord with the current concept that fat is utilised only after its uptake by adipose tissue and subsequent release as fatty acids. The results from walks taken during fasting and whilst carbohydrate or fat was taken are entirely consistent with the view that in adults G.H. secretion is governed by the need for mobilising fat as fuel.

Ingestion of protein did not suppress the rise in G.H. induced by exercise although it partially suppressed the rise in plasma NEFA concentration. Now it has been known from the time of Pettenkofer and Voit (1866) that muscular exercise does not increase protein metabolism. In the present walks after casein ingestion too the protein did not appear to be used as fuel during the walk as judged by the output of urinary N. The need for fat mobilisation, therefore, continued to operate and this probably explains why G.H. secretion was not suppressed by the ingestion of protein.

As /

As to the partial suppression of the rise in NEFA concentration induced by casein, it is interesting that Gordon (1957) has found lower NEFA levels than during fasting at the same time of day when he infused the amino acids leucine, glutamic acid or alanine into healthy subjects. How much of this represents a reflection of the utilisation of amino acids for energy purposes is not certain.

There is evidence that injection of G.H. promotes amino acid uptake by tissues (Kostyo and Engel, 1960; Peckham and Knobil, 1960). Rabinowitz et al (1965) have shown that the intravenous infusion of arginine monohydrochloride produces a rise in the serum concentration of not only G.H. but also of insulin. They have argued that although G.H. antagonises the action of insulin on glucose uptake by tissues, there is reason to believe that it may act synergistically with insulin to produce anabolic effects, i.e. nitrogen retention and enhanced protein synthesis. In one experiment on M.Y.S. a rise in G.H. secretion was found at a time when the blood amino nitrogen concentration was elevated as a result of protein ingestion. It is probable that there was also an associated rise in plasma insulin concentration at that time in M.Y.S. (as there was in all six of the healthy subjects studied by Rabinowitz et al).

The data of the experiments in which the subjects walked whilst ingesting protein imply that carbohydrate was the principal fuel utilised during these walks. The principal fuel /

fuel cannot have been fat because there was a partial suppression of the rise in plasma NEFA and the fall in R.Q. normally associated with fat mobilisation and utilisation. Nor could protein have been the chief fuel, not only because the weight of historical evidence from the time of Pettenkofer and Voit (1866) is against it, but also because the urinary N output during these experiments was no higher than that during exercise in the fasting state. The fact that insulin increases glucose turnover whereas G.H. is probably without direct effect on carbohydrate metabolism is in consonance with this interpretation. It is conceivable that the secretion of G.H. (associated as it was with a partial suppression of plasma NEFA concentration) in this situation may have served to direct amino acid incorporation into protein at a time when amino acids were freely available.

4. The Effect of Long-continued Exercise on Plasma G.H. Levels in Human Adults

One wholly unexpected and at the time quite inexplicable finding in experiment 1, was the marked fall in the plasma G.H. level during the second hour of exercise despite evidence of continued fat mobilisation and utilisation (Fig. 12). One hypothesis that suggested itself to account for this finding was that the effect of G.H. on NEFA release lasted longer than the presence of the hormone in the blood stream. If this was indeed so, then a further rise in the plasma G.H. level might be anticipated if the need for increased fat mobilisation continued /

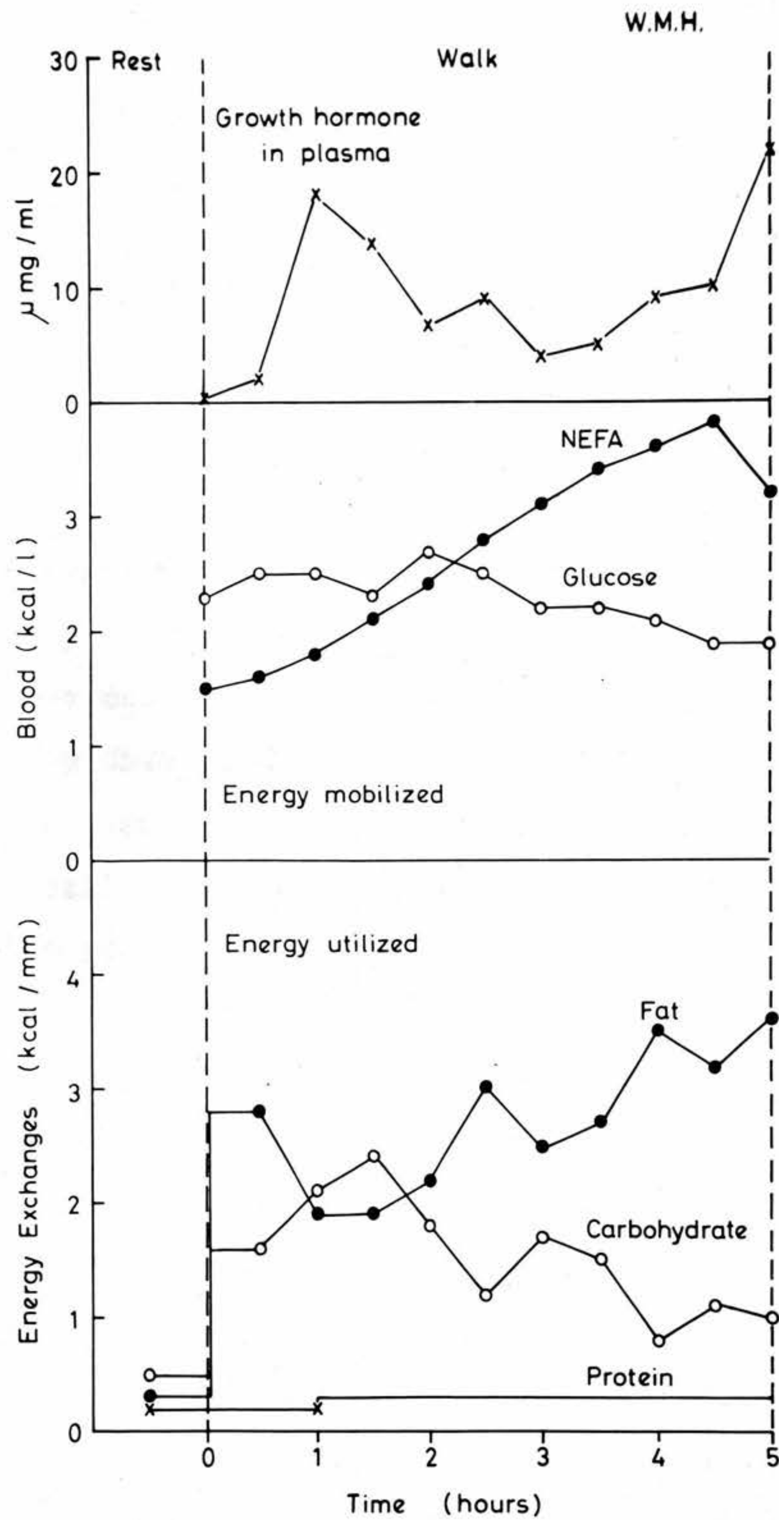


Figure 20

tinued to operate. Thus the experiment that suggested itself was one involving more prolonged walking in the post-absorptive state with a view to finding out whether the G.H. level which fell during the second hour rose again.

(a) The effect of a five hour walk on the plasma G.H. level in a healthy adult.

Procedure: A 37 year old healthy male (W.M.H.) acted as subject. He walked for five hours at 4 m.p.h. in the post-absorptive state. Samples of expired air were collected immediately before and at half-hourly intervals during the walk. The walk was interrupted for 1 - 2 minutes at half-hourly intervals whilst samples of blood were taken from a forearm vein through an indwelling Gordh needle. The blood was analysed for G.H., NEFA and glucose. The energy derived from carbohydrate, fat and protein immediately before and at half-hourly intervals during the walk was calculated from respiratory exchanges and urinary N.

Results: Figure 20 shows that there was a steady rise in the plasma NEFA level during the walk (except at the very end). The blood glucose level was well maintained. The plasma G.H. level rose during the first hour of the walk and, as usual, fell during the second hour and then rose again in the fourth hour to reach a high value at the end of the fifth hour. The proportion of energy derived from fat progressively increased and that derived from carbohydrate decreased during the walk.

Comment /

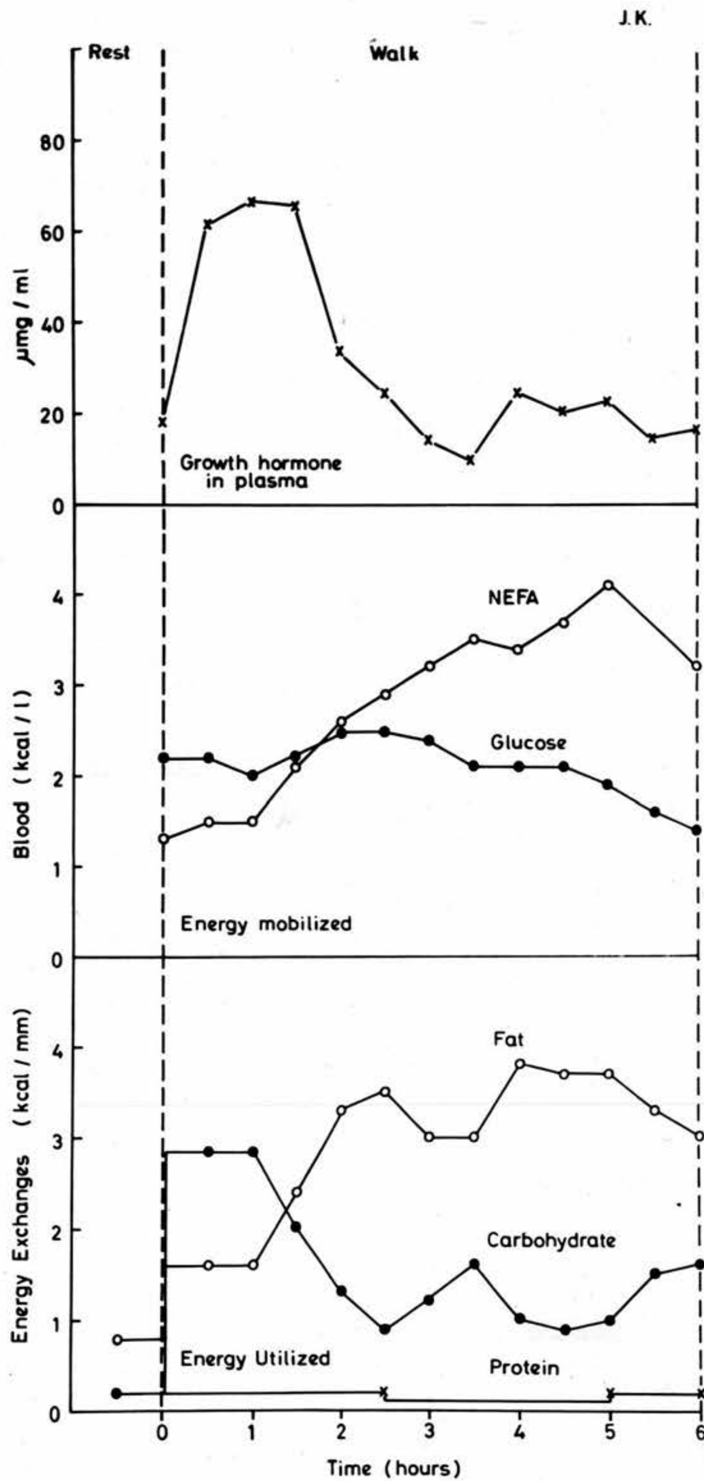


Figure 21

Comment: As was anticipated, the plasma G.H. level which fell during the second hour of exercise rose again when the walk was continued for longer than two hours. This suggested that G.H. was concerned not only with the initiation of fat mobilisation but also with its maintenance. The tantalisingly high value for plasma G.H. concentration obtained at the end of the fifth hour inevitably raised the question of what might have happened if the subject had continued to walk for longer than five hours. Would the plasma level of G.H. have kept on rising or would it have fallen again as in the second hour of a two hour walk ? The next experiment in this series was carried out to gain further evidence on this point.

(b) The effect of a six hour walk on the plasma G.H. level in a healthy adult.

Procedure: Under the same protocol as in the previous experiment, a 19 year old healthy male (J.K.) set out to walk for six hours at 4 m.p.h. in the post-absorptive state. He walked quite steadily for five hours. Thereafter, however, he gradually became unsteady and collapsed at the end of the sixth hour but quickly revived after receiving glucose.

Results: Figure 21 summarises the results obtained from this subject. There was a gradual fall in the blood sugar level as the walk progressed and it had reached 38 mg% when he collapsed. The plasma NEFA concentration rose steadily during the walk except at its very end. After a high early peak the G.H. level fell and then showed a smaller second peak at /

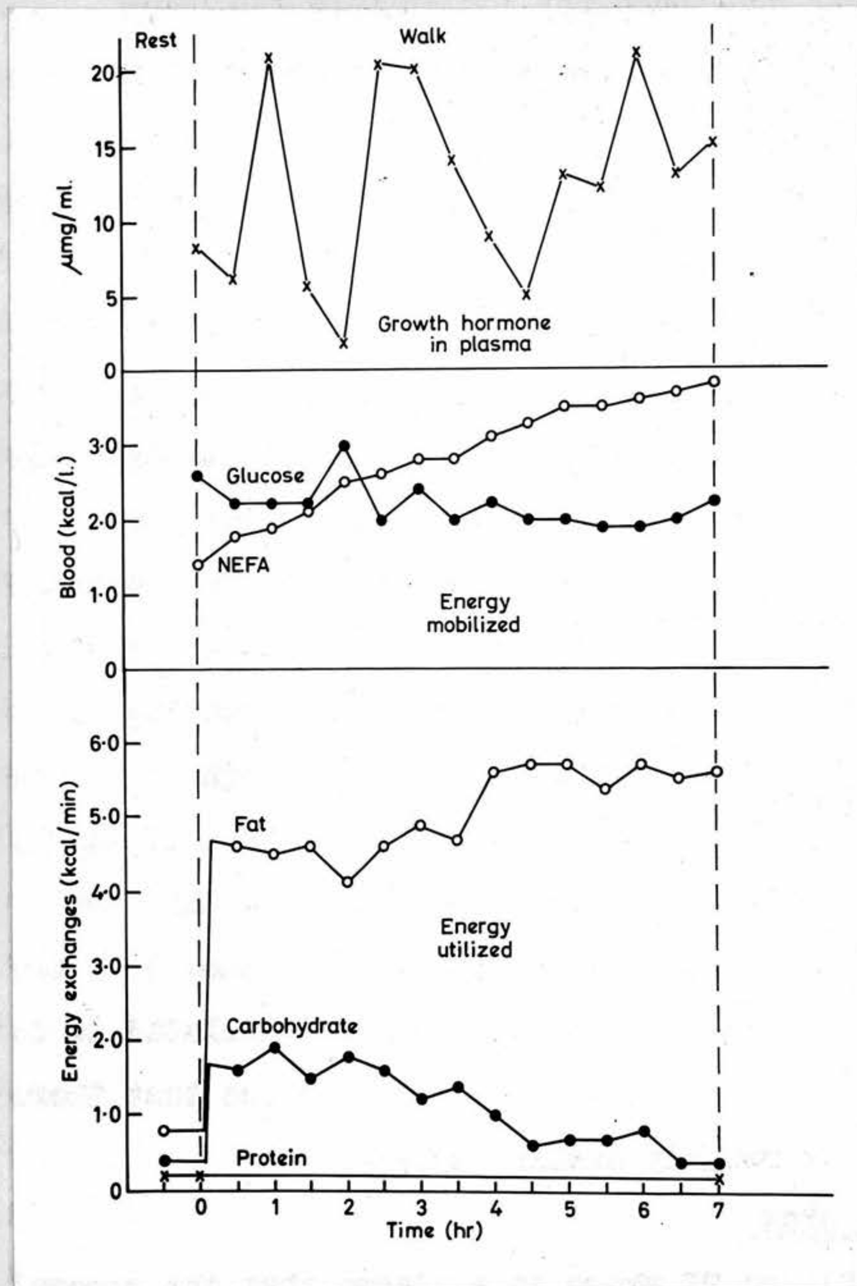


Figure 22

at the end of the fourth hour.

Comment: The results of this subject confirm those of the previous subject. The general pattern of results is essentially the same for both subjects. Even so, it seemed desirable to carry out another experiment in this series.

(c) The effect of a seven hour walk on the plasma G.H. level in a healthy adult.

Procedure: Under the same protocol as in the previous experiments in this series, a 20 year old male (C.C.) walked for seven hours at 4 m.p.h. in the post-absorptive state. He accomplished this task with comparative ease.

Results: Figure 22 depicts the data obtained for this subject. There was a steady rise in the plasma NEFA concentration to about three times its resting value. The blood sugar level was well maintained throughout the walk. There was a steady increase in the proportion of energy derived from fat as the walk progressed. Despite this continued fat mobilisation and utilisation, the plasma G.H. level rose and fell periodically during the walk unrelated to the rise of the NEFA concentration. The figure shows that there were three peaks of roughly similar height.

DISCUSSION

Figure 23 shows at a glance that the general pattern of results obtained from the three long walks has been essentially the same for the three subjects. The central theme that emerges from these results is that despite continued fat mobilisation /

EX BIBL. UNIV. EDINBURGEN

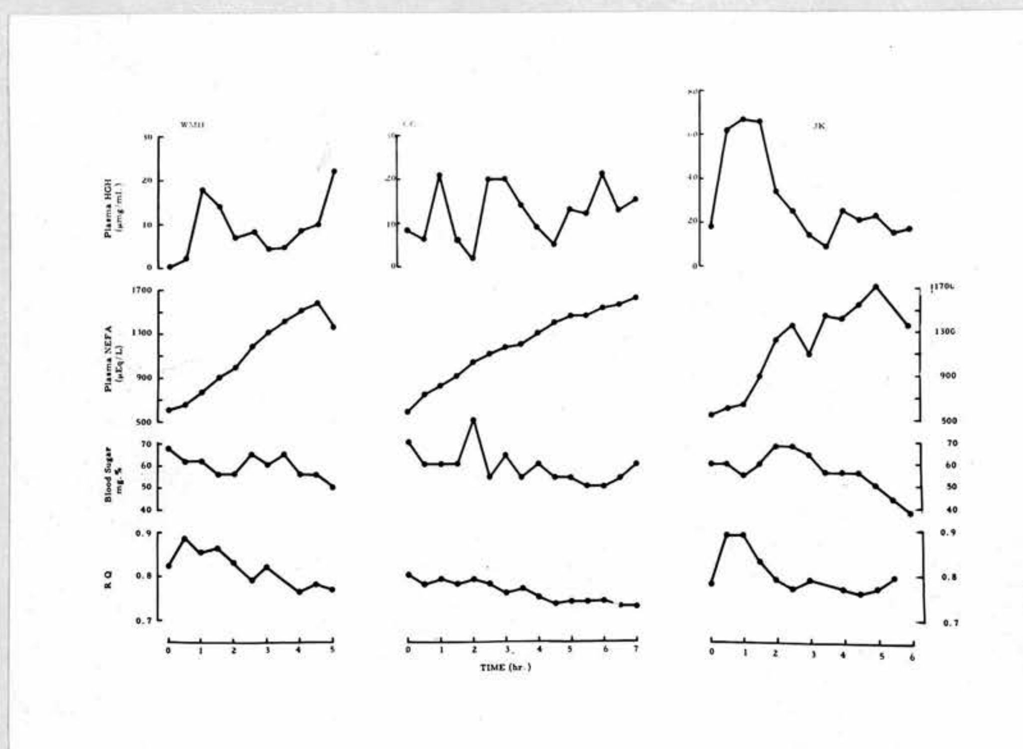


Figure 23

mobilisation and utilisation (as evidenced by a rising NEFA concentration and a falling R.Q.), the plasma G.H. level rises and falls periodically during the walks. If there is a causal relationship between the secretion of G.H. and rise in plasma NEFA concentration, why does the hormone level in the plasma rise and fall periodically instead of rising steadily and coincidentally with NEFA ? One possible explanation of this might be that G.H. is secreted not continuously, but in a series of intermittent "bursts" because its effect on NEFA release lasts longer than its presence in the blood stream. In fact, Rabinowitz et al (1965) have reported /

reported an experiment in which the plasma NEFA concentration continued to rise for 35 minutes after the infusion of G.H. had stopped. It would seem therefore that G.H. mobilises NEFA by a series of "triggering" actions.

Although the general pattern of data for the three subjects was essentially similar, there were significant differences in detail as regards the total performance. The 20 mile walk of W.M.H. went smoothly and uneventfully. Soon after the walk had ended, however, he suffered a vaso-vagal attack and vomited. J.K. walked 24 miles. He was very unsteady during the sixth hour of walking and collapsed with a blood sugar level of 38 mg% at the end of the sixth hour. C.C. who walked 28 miles accomplished the task with relative ease. Nor did he appear to be exhausted after the walk had ended. Comparisons may be odious but it was natural to inquire what particular attribute or attributes enabled C.C. to accomplish an arduous task with comparative ease. None of the three subjects was an athlete. However, two weeks before he undertook the 28 mile walk, C.C. had spent two weeks in military training in ice and snow for six to seven hours a day at a military camp in the Cairngorms near Inverness. Was this a case in which the fallacy of post hoc ergo propter hoc might be ignored ? There was, moreover, internal evidence from the data collected during the walk to account for C.C.'s superior performance. His R.Q. indicated that he mobilised and utilised fat more efficiently than W.M.H. and J.K. His plasma /

plasma NEFA concentration rose steadily throughout the walk, unlike that of W.M.H. and J.K. which showed a significant fall towards the end of their walks. His R.Q. showed that there was a steady increase in the proportion of energy he derived from fat which reached almost 100% towards the end of the walk. If, as seems probable, the increase in plasma NEFA concentration resulted principally from the secretion of G.H. one would, a priori, expect C.C. to have shown the "best" secretory pattern of G.H. during the walk. A glance at figure 23 shows that C.C.'s curve of G.H. secretion shows three peaks of similar height implying a more sustained level of hormonal action. The impression that C.C.'s pattern of G.H. secretion was somehow "better" than that of the other two subjects was thus difficult to avoid. Once that impression was gained it was but a short step to associate that pattern with the physical training he underwent two weeks before he did his 28 mile walk. Thus the possibility that physical training in some way influenced either the pattern of secretion of G.H. or the response of adipose tissue to G.H. or both came to be considered. There was some evidence that the reactivity of adipose tissue to adrenaline was increased by physical training (Parizkova and Stankova, 1964). Because of the established fat mobilising action of G.H. it seemed reasonable to ask whether physical training made adipose tissue more reactive to G.H. also. This hypothesis was put to the test in an experiments on rats.

5. The Influence of Physical activity on a Treadmill on the Response of Adipose Tissue to Growth Hormone in Rats

Introduction: Perhaps a few words are called for to justify yet another experiment on rat adipose tissue. In the introduction to the volume devoted to "Adipose Tissue" (1965) of the American Physiological Society's Handbook Series, H.E. Wertheimer, the doyen of studies on adipose tissue, felt constrained to warn: "Today the study of adipose tissue is still a popular subject ...... What may well be an exaggeration is the over-emphasis placed on studies of rat adipose tissues. There is enough experimental evidence today to suggest that conclusions based on rat adipose tissue must be interpreted with caution because such results do not necessarily apply to other mammals. Moreover, epididymal fat, which is used for so many experiments may have a unique metabolic pattern and cannot be considered as a 'typical' adipose tissue. It is known today that there are considerable quantitative differences between the various adipose tissues." To justify an experiment on rat adipose tissue in the face of that warning there must be compelling reasons indeed.

Speculation on the results of the experiments which involved long-continued walking led to the formulation of the hypothesis that physical training might make adipose tissue more reactive to G.H. To design an experiment to test this hypothesis was easy enough in principle. However, to do such an experiment using humans as subjects presented immense practical /

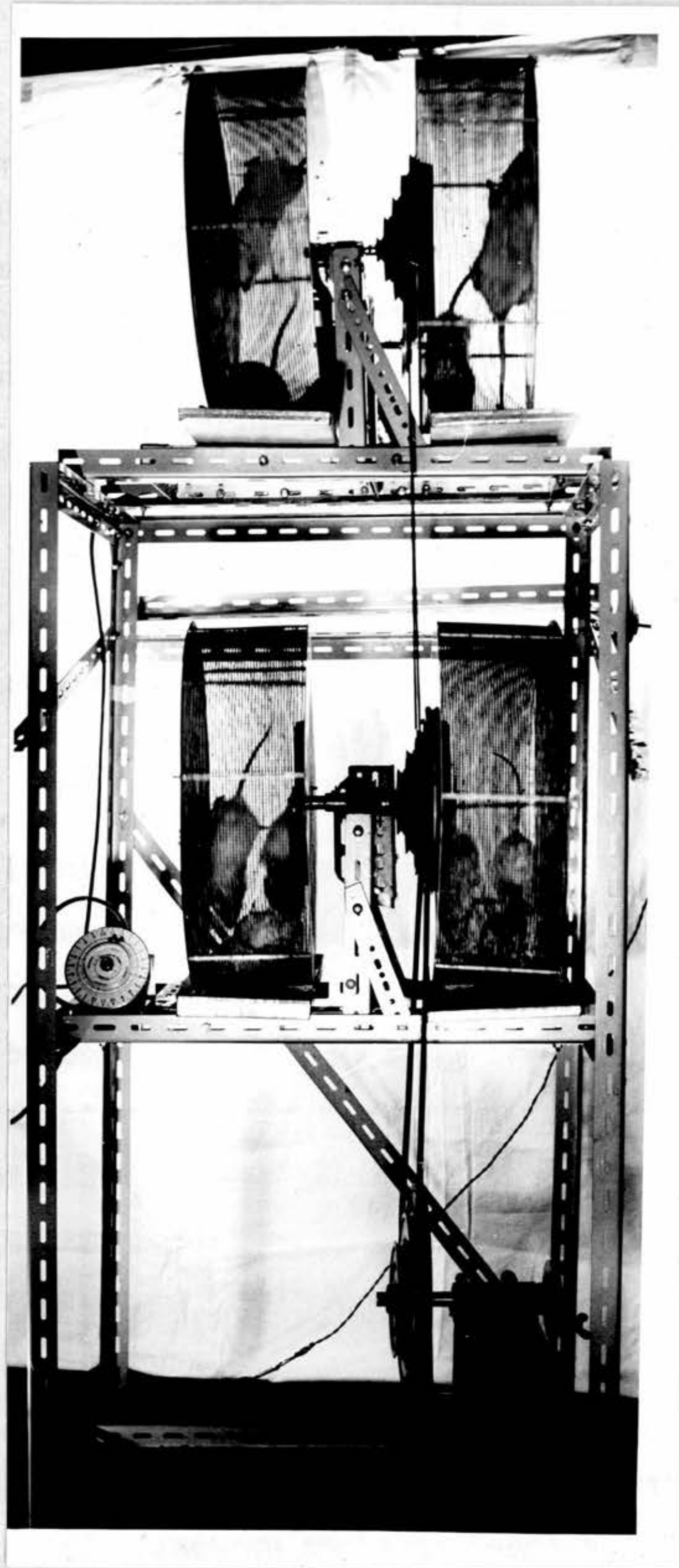


Figure 24

practical difficulties. If the experiment were to yield a sufficient number of observations to enable a meaningful statistical analysis of the results, there was no practical alternative in this laboratory to using the rat. If it was not feasible to heed Westheimer's warning on the over-emphasis placed on studies of rat adipose tissue, it was at least possible to show deference to his warning that epididymal fat cannot be regarded as a 'typical' adipose tissue. By doing the experiment in vivo, the injected G.H. could be permitted to act on those tissues on which it may also act in real life.

METHODS

(a) Treadmill

The treadmill used in this investigation was built in the mechanical workshop of the Physiology Department of Edinburgh University. The treadmills (figure 24) consist primarily of a battery of four revolving drums connected to a motor by means of pulley wheels and belt. Up to three rats can be exercised in each drum at a time. A digital counter connected to each pair of drums records automatically the number of revolutions in a given time. The motor can be switched on and off automatically by a pre-set timer. Several speeds varying from 4 m/min. to 20 m/min. are available. For the experiment reported here which involved essentially endurance walking, the speed used was gradually increased from 8 m/min. to 16 m/min.

(b) Animals /

(b) Animals

The observations were made on healthy male albino Parton rats. They were offspring of several females of the same random breeding.

(c) Growth Hormone

Bovine G.H. (NIH - G.H. B9 Bulk) was a gift from the Endocrinology Study Section of the N.I.H. The preparation had a growth promoting potency of 1.17 U.S.P. units/mg. Its contaminating activities included 0.001 μ/mg. of TSH activity. ACTH activity had not been assayed.

(d) Plan of Experiment

From birth onwards, the rats received standard food pellets supplemented with fresh milk twice a week ad libitum. They were kept in cages, three animals to a cage. Their weight gain was followed by weighing them once a week.

Two groups of twelve rats each were trained during two consecutive periods. Both groups commenced training on about the 100th day of life and were trained for about 15 weeks. Each group was given a preliminary period of about two weeks to accustom the animals to the treadmill. During this period the amount of exercise was gradually increased from 5 minutes at 8 m/min. morning and afternoon to 60 minutes at 16 m/min. morning and afternoon. This final rate of exercise was maintained for the remainder of the training period. The rats were exercised on every day of the week except Sundays. The control groups lived in cages without training.

On /

On the final day, all rats after a 12 hour fast (from 10 p.m. on previous night to 10 a.m. on final day) were injected intraperitoneally with G.H. calculated on the basis of 0.5 mg or 1 mg per 100 g body weight and dissolved in 1 ml of 0.9% saline adjusted to pH 8-9 with 0.1 N NaOH. Six rats from each group (trained and controls) were each injected with 1 ml of 0.9% saline adjusted to the same pH. Six hours after injection (total fasting time = 18 hours) the rats were anaesthetised with ether, killed by decapitation and blood collected into heparinised tubes.

Soon after decapitation, the carcasses were opened and the adrenals were dissected out and weighed. The carcasses were then put in cold storage and subsequently analysed for total lipid content.

NEFA estimations were done in duplicate on 1 ml aliquots of plasma by the method of Dole.

(e) Expression of Results

The mean values of NEFA obtained have been expressed in three ways:

(i) µ-equivalents/litre of plasma (1 m.mole = 1000 µ-eq).

(ii) µ-eq/l per g fat - to allow for the different fat contents of individual rats.

(iii) µ-eq/l per cent fat to allow for the different body weights of individual rats.

(f) Statistical Analysis of Results

The 't' test was employed. A difference between two mean values was regarded as significant if P was no greater than 0.05.

RESULTS /

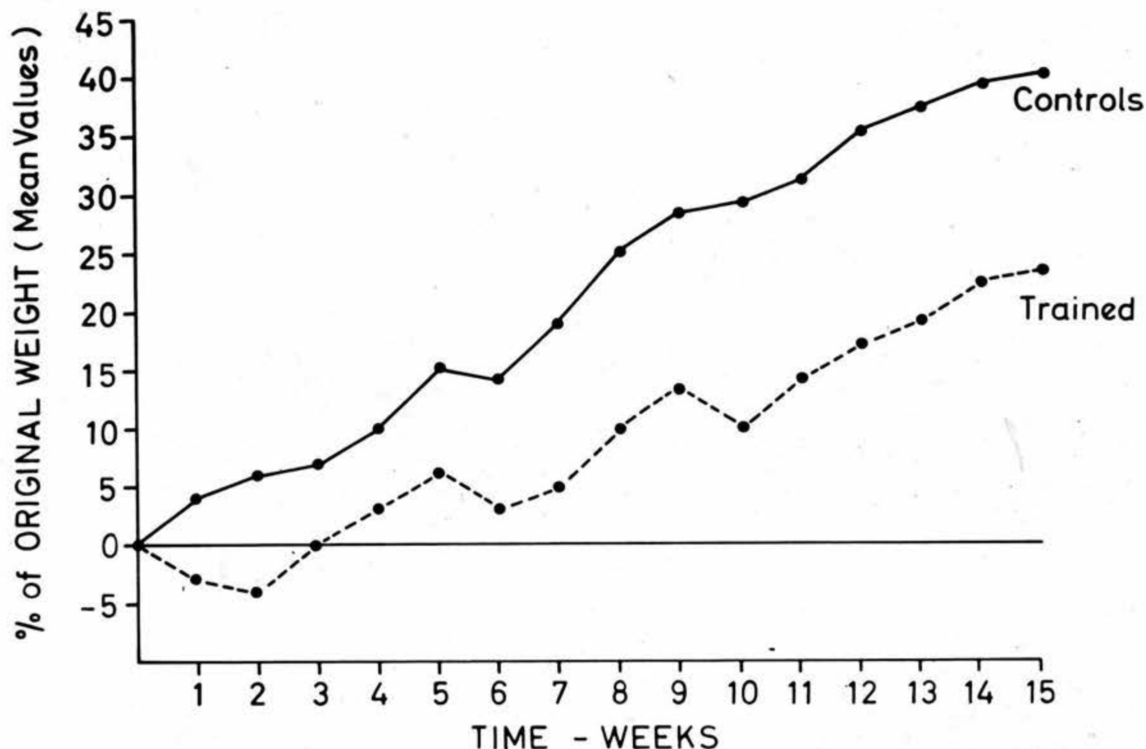


Figure 25

RESULTS

Figure 25 shows the mean weight gain curves of trained and control animals. To allow for the different initial weights of individual rats, the curves have been plotted as the percentage change of initial body weight against time. The trained rats gained less weight than the controls especially in the first three weeks. The initial mean weights of trained and control rats did not differ significantly (Fig. 26a). Their final weights, however, were significantly different /

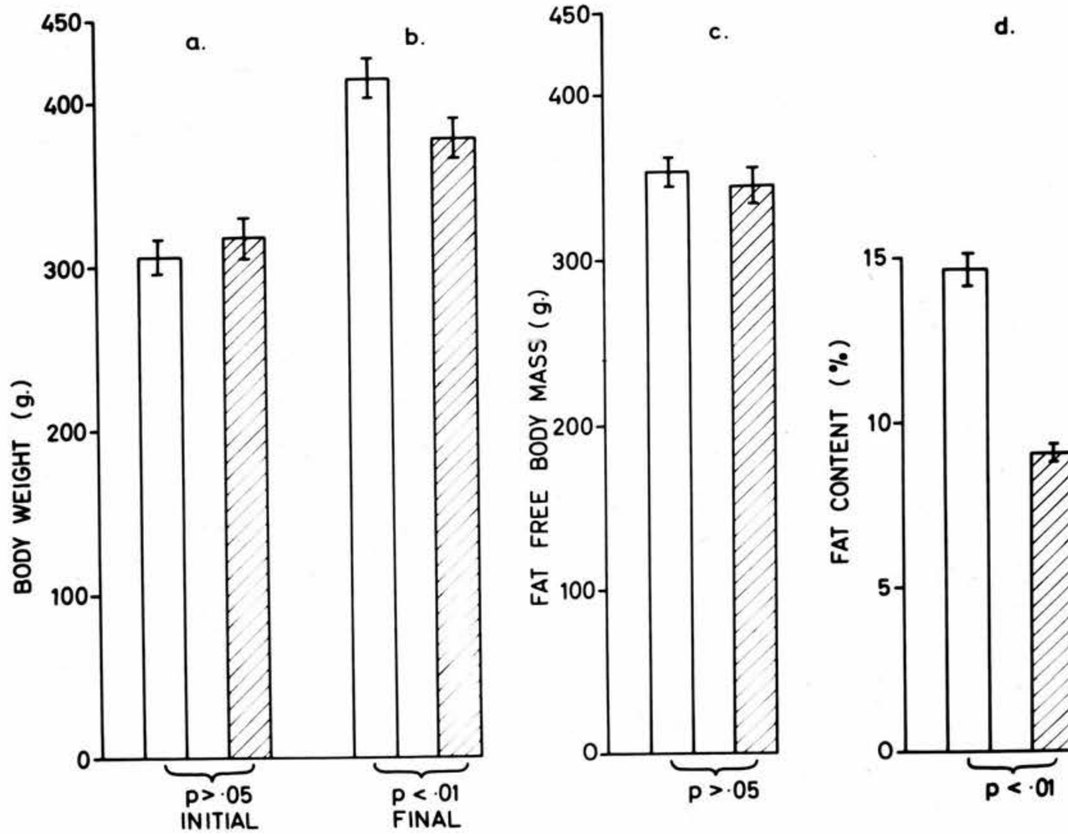


Figure 26

different (Fig. 26b). This difference was due not to a significant difference in their lean body mass (Fig. 26c) but to a marked difference in their fat content (Fig. 26d).

Table 4 shows the effect that training had on the weight of the adrenals.

The /

Table 4: Effect of training on adrenal of rat.

Adrenal Wt. (mg)	Number of Rats	
	Controls	Trained
30 - 39	5	3
40 - 49	6	2
50 - 59	2	4
60 - 69	4	6
70 +	1	3
Total	18	18

The adrenals of the trained rats 58 mg (S.D. ± 14) were significantly ($P < 0.01$) heavier than those of the controls 49 mg (S.D. ± 10)

Table 5: Caloric intake per 100 g body weight in 24 hrs by trained rats and controls determined between the 190th and 200th day of life.

Group	Exp. No.	No. of Rats	Food intake (g)	Hours	Intake/ 100 g/ 24 hrs.	Caloric value (kcals)	Average (kcals)
Controls	1	12	2877	205	6.3	25.2	25.6
	2	12	3176	214½	6.5	26.0	
Trained	1	9	2272	205	7.5	30.0	30.2
	2	11	3133	214½	7.6	30.4	

Table 5 shows that the caloric intake per 100 g body weight of the trained rats was about 20% higher than that of the /

the controls despite the fact that they gained in weight only about half as much as did the controls.

Summary: The trained rats differed from the controls not only in their percentage content of fat, but also in their mobilisation of NEFA per gram fat in response to a 18 hour fast with and without the injection of G.H. at two dose levels (Table 6). The trained rats always mobilised larger amounts of NEFA per gram fat than did the controls.

Table 6: Mean values with their standard errors for release of NEFA (expressed in 3 ways) by trained rats and controls when injected with saline or G.H. at 2 dose levels.

Treatment	Group	No. of rats	Plasma NEFA					
			µeq/l	P	µeq/l/g fat	P	µeq/l% fat	P
Saline	C	6	635±71	>.05	13±2	<.05	49±6	<.01
	T	6	642±38		23±3		81±7	
G.H. 0.5 mg/100 g	C	6	883±73	>.05	14±3	<.02	60±9	<.01
	T	6	887±63		30±4		113±8	
G.H. 1 mg/100 g	C	6	1022±81	>.05	24±3	<.05	92±10	<.01
	T	6	1028±54		41±5		145±10	

Figure 27 shows the results obtained for the two groups when the amount of NEFA mobilised was related to the percentage fat content of the rats. In both groups at the dose level of 1 mg/100 g /

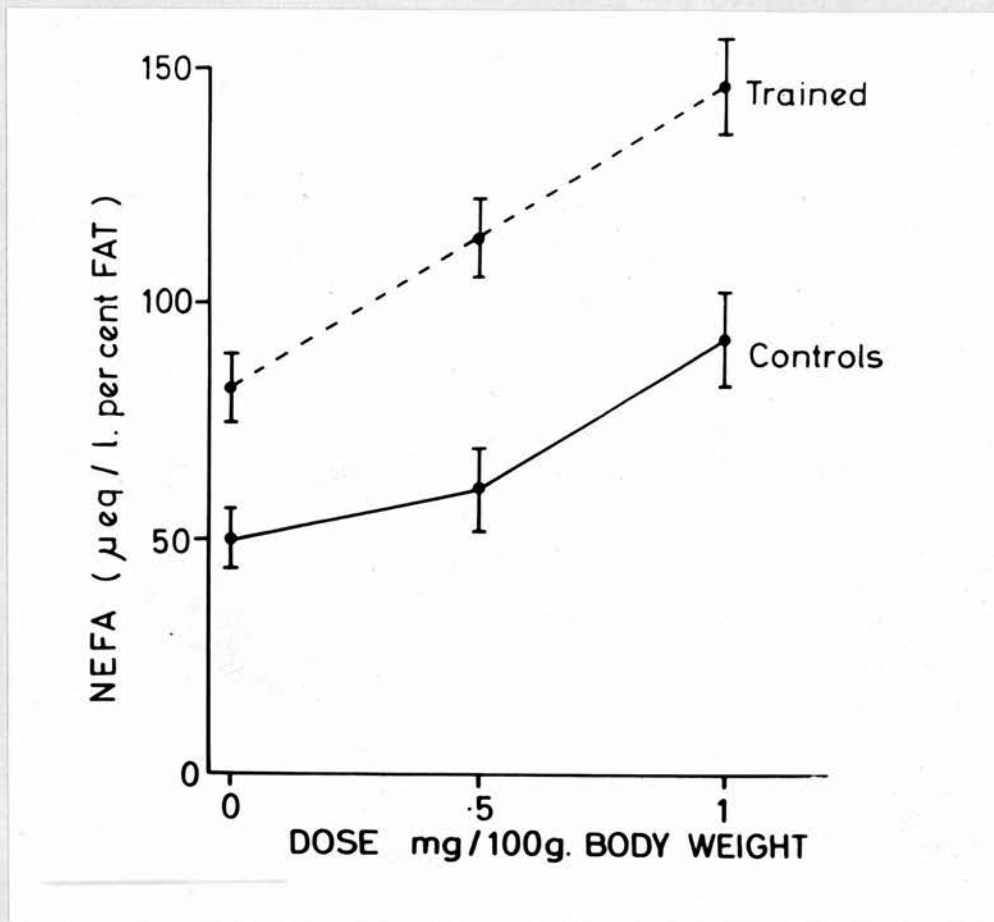


Figure 27

1 mg/100 g body weight G.H. evoked a response which was significantly different from that of rats not injected with G.H. At the dose level of 0.5 mg/100 g body weight, however, the response obtained was significantly different from the uninjected rats only in the case of the trained animals. This suggests that the adipose tissue of trained rats was more reactive to injected G.H. than that of untrained rats.

DISCUSSION

The point this experiment set out to investigate was whether /

whether physical training of rats makes their adipose tissue more reactive to injected G.H. than adipose tissue of untrained rats. In order to establish this point, it is necessary first of all to show that the physical activity imposed on the experimental rats in fact constituted a sufficiently intense biological stimulus. Three parameters are to hand which indicate that the stimulus was an adequate one. The first is the weight of the adrenals which was significantly heavier in the trained group (Table 4). The second is the shift in body composition shown by the rats subjected to training, viz. a proportional decrease in body fat in spite of a calorie intake 20% in excess of that of the controls (Table 5). The third is the shape of the body weight curve (Fig. 25). The trained rats showed a marked reduction in weight gain due to fat depletion as a result of treadmill exercise. Mayer (1960) reported a drastic reduction in weight gain during growth in genetically obese mice induced by treadmill exercise. What is true of genetically obese mice may not apply to normal rats, but there is evidence, albeit from another mammal, that the shift in body composition induced by physical training occurs not only in the obese but also in the lean (The Kentucky Physical Fitness Experiment, Jokl, 1964).

Having established that the experimental rats were truly physically trained, it is necessary to show that their adipose tissue was in fact more reactive to G.H. than that of the untrained /

trained rats. Scrutiny of Table 6 in conjunction with Figure 27 reveals that the conclusion that training makes rat adipose tissue more sensitive to injected G.H. is warranted only when the NEFA mobilised is expressed in terms of µ-eq/l per cent fat. When the NEFA mobilised is expressed as a function of the number of grams of fat in individual rats, although it is clear that the trained rats released more NEFA than the controls at the dose level of 0.5 mg/100 g body weight, the difference of this value from that of uninjected rats does not reach statistical significance in either group. Moreover, it might be contended that only an <u>in vitro</u> experiment in which adipose tissue is directly exposed to the action of G.H. can establish beyond doubt the point in question. Unfortunately, however, at the time this study began, there was considerable question as to whether G.H. is active <u>in vitro</u> at all (Raben, 1965). Also, as already explained in the introduction to this experiment, an <u>in vivo</u> experiment commended itself if only to avoid using the epididymal fat pad.

To sum up: the judgement on this experiment must be that it has yielded evidence to suggest that the physical training of rats makes their adipose tissue more reactive to G.H. but that with the numbers studied the conclusion cannot be regarded as having been firmly established.

There is some evidence that an increased capacity for NEFA mobilisation occurs during a period of physical training (Parizkova et al, 1963). The role of catecholamines in NEFA release /

release has been extensively investigated. The release of NEFA from adipose tissue *in vitro* (Gordon and Cherkes, 1958; Reichl, 1959) and the effect of catecholamines on this process in various physiological conditions has been demonstrated (White and Engel, 1958; Werke et al, 1962). Parizkova and Stankova (1964) explored the question whether physical training made rat adipose tissue more reactive to adrenaline and concluded that it did. The results of the present experiment suggest that physical training of rats makes their adipose tissue more reactive to G.H. also.

It is not surprising that the adipose tissue of an animal adapted to a greater intensity of muscular work should respond to fat mobilising hormones more readily than adipose tissue of an untrained animal. It is probable, too, that a trained animal is also capable of utilising NEFA as a source of energy to a greater extent than an untrained one. Drummond and Black (1960) have proved this in migratory birds adapted in the course of phylogeny to intense muscular work. The results of the present experiment as well as those of Parizkova and Stankova (1964) are consistent with the view that an increased capacity to mobilise fat in response to the action of fat-mobilising hormones could develop during ontogeny as part of the adaptation to intense and sustained muscular activity.

Extrapolation of biological data from one species to another must of course be done with extreme caution. It would be prudent therefore to confine the interpretation set out /

out above to the population actually sampled and not extend it over the animal kingdom in general. Nevertheless, it would be surprising if future research invalidated the general thesis that, amongst other things, physical training makes the adipose tissue of an animal more reactive to fat-mobilising hormones such as G.H.

CHAPTER 5

Summary and Concluding Remarks

The following conclusions emerged from the experiments reported in this thesis:

1. In adults in bed before rising in the morning, plasma G.H. levels are consistently below the threshold of sensitivity of present methods of detection ($<$ 2 u mg/ml).
2. During a two hour period of moderate exercise in the post-absorptive state, a rise of up to 50-fold in the plasma G.H. level occurs during the first hour of exercise in association with evidence of fat mobilisation. During the second hour, there is a marked fall in the plasma G.H. concentration despite continued fat mobilisation.
3. During long continued moderate exercise in the post-absorptive state, the plasma G.H. level rises and falls periodically.
4. Ingestion of glucose at intervals during a two hour walk in the post-absorptive state abolishes the secretion of G.H. Neither protein nor fat suppresses the secretion of G.H. in similar circumstances.
5. Physical training of rats for endurance walking seems to make their adipose tissue more reactive to injected (bovine) G.H.

The first indication of pituitary involvement in growth regulation came in 1887 when Minskowski associated acromegaly with /

with enlargement of the pituitary gland. In 1921 Evans and Long injected a simple saline extract of the anterior pituitary lobe into young rats and produced experimental gigantism. This was the first time that the injection of any extract of the anterior pituitary had been shown experimentally to affect an animal and the credit for the discovery of Growth Hormone (or "Somatotropin" as he prefers to call it) belongs to Evans. Most of the subsequent work which eventually led to the isolation of the pure hormone was also done in his laboratory (Li and Evans, 1944).

There is evidence that the foetal pituitary is not essential for foetal growth (Seckel, 1960). That the maternal pituitary is not essential for foetal growth has been shown by the delivery of a normal infant by a patient who was hypophysectomised in the thirteenth week of pregnancy (Kaplan, 1961). In the early neonatal period growth occurs in the absence of the pituitary (Simpson, Asling and Evans, 1951). Hypopituitary dwarfism is not manifest until about three years of age. The period of growth from this time to the onset of puberty is one in which G.H. undoubtedly plays a part because hyperpituitary gigantism and hypopituitary dwarfism first becomes clinically manifest during this period.

Since the accredited function of G.H. was to promote growth, it was tacitly assumed that G.H. was probably not present in abundance in pituitaries of healthy adults because they had stopped growing. The discovery of comparatively large /

large amounts of G.H. in the adult anterior pituitary gland (Li and Papkoff, 1956; Raben, 1959; Wilhelmi, 1960) suggested that the hormone may have a function to perform in adults. The association of the action of G.H. with increased fat mobilisation has been well established by many studies (Lee and Schaffer, 1934; Best and Campbell, 1936; Levin and Faber, 1952; Greenbaum and McLean, 1953). The discovery of Raben and Hollenberg (1959) that injected G.H. caused a rise in plasma NEFA at the expense of depot fat provided the probable mechanism by which G.H. mobilised fat. The development of a highly sensitive and specific radio-immunoassay of G.H. in the plasma (Hunter and Greenwood, 1962, 1964b; Roth et al, 1963) provided the essential tool required to explore its physiological role.

The studies of Roth et al (1963a, b, 1964), Hunter and Greenwood (1964a) and the results of experiments reported in this thesis strongly suggest that in adults G.H. plays an important role in initiating and maintaining the mobilisation of fat in situations involving the utilisation of fat for energy purposes. The physiology of G.H. in the adult is almost entirely explicable in terms of its lipolytic action. Thus, during exercise in the post-absorptive state - a situation in which there is, teleologically speaking, a need for depot fat mobilisation, - G.H. is secreted. On the other hand, when glucose is taken during exercise and utilised as fuel, G.H. is not secreted at all because there is no need for fat mobilisation /

mobilisation. The inference that the secretion of G.H. during exercise is governed by the need for mobilising fat as fuel is therefore inescapable.

There is some evidence to suggest that G.H. may not be essential for fat mobilisation during exercise. Basu et al (1960) have reported an experiment in which two patients with well marked clinical evidence of hypopituitarism showed increases in plasma NEFA concentrations on exercise similar to normal subjects. In these patients, evidently, other factors must have been responsible for fat mobilisation. Goodman and Knobil (1961) found that an increase in plasma NEFA occurred during fasting in adrenalectomised and hypophysectomised dogs, though the magnitude of the response was smaller than in intact animals. Catecholamines promote NEFA release (Dole, 1956). Nictonic acid (which blocks the action of catecholamines) suppresses the rise of NEFA during exercise (Carlson et al, 1963). This suggests that the presence of catecholamines as permissive agents is a requirement for NEFA release. Ingestion of glucose at intervals during a two hour walk in the post-absorptive state suppresses the rise of plasma NEFA concentration and the associated secretion of G.H. that occur during exercise in the fasting state (see Experiment 3 above). This could mean that catecholamines, if secreted at all, are not importantly responsible for the rise in plasma NEFA that occurs in adults during moderate exercise in the fasting state.

Known /

Known fat mobilising agents are numerous. Amongst these G.H. is probably an important one. The fact that it is not essential for fat mobilisation during exercise does not mean that its fat mobilising action is trivial when present.

The growth promoting action of G.H. in young animals is indisputable. There is good inferential evidence that in adults G.H. participates in the physiological regulation of fat mobilisation and the provision of energy in response to metabolic demands. Thus both growth (an anabolic process) and fat mobilisation (a catabolic process) are brought about by one and the same biologically active substance.

Raben (1965) has pointed out that it is possible to offer a unitary explanation for these apparently contrasting physiological actions of G.H. As Roth et al (1963a) first showed, G.H. is secreted in response to hypoglycaemia, deoxyglucose (an inhibitor of intracellular glucose utilisation) muscular exercise and fasting. These are all situations in which fatty acids are needed as a source of energy because of actual or impending intracellular glucose deprivation. Thus, the fat mobilising action of G.H. appears to be calorie-providing and thereby glucose-sparing. But to spare glucose is, in the last resort, to spare protein, for the energy equivalent of the glucose that is spared would otherwise have to come from amino acids. In the context of the over-all body economy, therefore, the lipotropic action of G.H. even when mobilising fat /

fat as fuel for muscular exercise is in effect anti-catabolic. When so viewed, the essentially anabolic nature of G.H. becomes apparent.

ACKNOWLEDGEMENTS

It is a pleasure to thank Professor D. Whitteridge F.R.S. for affording me facilities in his Department and for his sustained interest in my general welfare.

To Dr. R. Passmore, I am permanently indebted. When I began my period of apprenticeship under him, I was wholly innocent of the practice of the scientific method. From him I learnt the importance of cogitating about a problem until you can clearly define it, the technique of constructing a solution to the problem and of translating it into experimental results and - not least - the importance of presenting the results in intelligible language. Of his personal kindness to me I do not speak. I will only say that my apprenticeship with him was an education in the deepest sense of that word.

For instruction in the technique of radio-immunoassay of Growth Hormone and for his unfailing encouragement, I am most grateful to Dr. W.M. Hunter.

From Mr. D. Shirling, I received the greatest possible technical help and I sincerely thank him. The technical staff of the Physiology Department, Edinburgh University, were always helpful.

APPENDIX

Tables 7 - 16

Table 7: The sex, age, height and weight of subjects who took part in the experiment.

Subject	Age	Sex	Height (cm)	Weight (kg)
R.P.	54	M	182	63
M.Y.S.	29	M	171	63
W.M.H.	37	M	175	57
D.W.	22	M	163	55
K.W.M.	20	M	176	64
C.B.	20	F	168	49
D.H.	19	M	166	54
D.S.	19	M	179	70
G.L.	19	F	159	63
A.B.	20	M	162	57
G.R.	20	M	183	68

Table 8: The oxygen, consumption (in ml/min) determined by analysis of expired air.

Subject	Periods					
	Prel.	Walk				Recovery 60 min
		30 min	60 min	90 min	120 min	
R.P.	242	1075	1104	1037	955	263
M.Y.S.	182	1047	929	1019	1024	190
W.M.H.	204	934	932	924	944	221
D.W.	181	1037	992	943	1018	199
K.W.M.	225	1110	1190	1211	1268	246
C.B.	-	913	916	955	995	-
D.H.	190	1011	931	1006	1023	215
D.S.	288	1199	1240	1171	1183	290
G.L.	196	1067	1103	992	1007	208
A.B.	226	1148	1163	1123	1146	247
G.R.	275	1163	1200	1131	1145	271
Mean	221	1064	1064	1047	1064	235

Table 9: The respiratory quotient determined by analysis of expired air.

Subject	Periods					
	Prel.	Walk				Recovery 60 min
		30 min	60 min	90 min	120 min	
R.P.	0.793	0.785	0.774	0.775	0.765	0.749
M.Y.S.	0.831	0.903	0.885	0.859	0.865	0.821
W.M.H.	0.853	0.867	0.894	0.893	0.871	0.800
D.W.	0.912	0.908	0.854	0.891	0.861	0.790
K.W.M.	0.867	0.832	0.788	0.813	0.786	0.772
C.B.	-	0.936	0.932	0.928	0.897	-
D.H.	0.832	0.905	0.907	0.901	0.868	0.809
D.S.	0.826	0.928	0.844	0.827	0.809	0.783
G.L.	0.816	0.772	0.772	0.786	0.786	0.760
A.B.	0.835	0.790	0.814	0.809	0.774	0.802
G.R.	0.795	0.818	0.792	0.752	0.747	0.815
Mean	0.836	0.858	0.841	0.839	0.821	0.790

Table 10: The NEFA levels obtained by analyses of blood (in μ eq/1).

Subject	Periods					
	Prel.	Walk				Recovery 60 min
		30 min	60 min	90 min	120 min	
R.P.	740	1300	1380	1430	1980	1520
M.Y.S.	670	780	850	1120	1590	1000
W.M.H.	540	600	660	810	1030	850
D.W.	690	620	870	880	1220	740
K.W.M.	390	490	760	790	1170	700
C.B.	690	750	860	980	1340	1060
D.H.	390	680	840	1030	1450	900
D.S.	470	590	640	760	1150	880
G.L.	430	600	830	1200	1530	470
A.B.	330	500	670	1030	1400	630
G.R.	390	480	790	1030	1380	530
Mean	521	672	832	1005	1385	843

Table 11: The figures obtained by the analysis of blood for the content of glucose (in m.M/1).

Subject	Periods					
	Prel.	Walk				Recovery 60 min
		30 min	60 min	90 min	120 min	
R.P.	5.0	5.2	4.0	3.7	3.3	3.4
M.Y.S.	3.6	3.1	3.3	3.3	3.1	3.6
W.M.H.	4.2	4.4	4.2	3.8	3.8	3.9
D.W.	3.6	3.3	3.3	3.2	3.4	3.4
K.W.M.	4.1	3.7	4.1	3.9	3.9	3.8
C.B.	3.9	4.8	4.7	4.2	4.7	4.7
D.H.	3.9	3.7	4.1	3.9	3.7	3.7
D.S.	4.7	4.1	4.1	4.1	3.9	4.1
G.L.	3.9	3.7	3.7	3.4	4.0	3.7
A.B.	3.7	3.3	3.3	3.2	3.1	3.1
G.R.	3.7	4.0	4.0	4.3	4.4	3.9
Mean	4.0	3.9	3.9	3.8	3.8	3.8

Table 12: The figures obtained by analysis of blood for the content of Growth Hormone (in μ mg/ml).

Subject	Periods					
	Prel.	Walk				Recovery 60 min
		30 min	60 min	90 min	120 min	
R.P.	8.4	3.7	7.3	23	14	2.7
M.Y.S.	0.4	3.0	18	11	10	2.0
W.M.H.	0.3	2.2	24	23	25	5.3
D.W.	26	4.7	10	15	8.3	1.1
K.W.M.	0.6	26	25	27	17	3.5
C.B.	1.2	24	45	26	12	2.2
D.H.	53	74	110	46	27	3.0
D.S.	1.1	40	53	8.8	2.0	1.0
G.L.	15	16	11	15	6.9	0.8
A.B.	1.2	23	28	25	16	2.5
G.R.	26	85	74	60	24	4.1
Mean	12.1	28.3	36.9	25.5	14.7	2.6

Table 13: The Metabolic Mixture: The relative amounts of carbohydrate, protein and fat utilised during the walk calculated from the total utilisation of oxygen and output of carbon dioxide and urinary nitrogen using the assumptions of Zuntz (1897). The calorie value of the metabolic mixtures and the percentage of calories derived from fat is given.

Subject	Metabolic Mixture			Calorie value of mixture (kcal)	% of calories derived from fat
	Carbo-hydrate (g)	Fat (g)	Protein (g)		
R.P.	33	46	6	583	73
M.Y.S.	83	23	5	576	37
W.M.H.	78	21	6	535	36
D.W.	84	23	4	572	37
K.W.M.	56	46	5	673	64
C.B.	99	13	5	548	22
D.H.	91	20	5	573	32
D.S.	84	34	8	683	46
G.L.	36	46	3	601	71
A.B.	50	45	7	643	65
G.R.	38	50	7	688	68
Mean	66.5	33.4	5.5	606.8	50.0

Table 14: Basic data for the subject (W.M.H.) who walked 20 miles at 4 m.p.h. in the post-absorptive state.

Periods		O_2 (ml)	CO_2 (ml)	NEFA (μ eq/l)	Glucose (mg%)	G.H. (μ mg/ml)
Rest		217	175	610	63	0.4
Walk (hours)	½	965	779	660	67	2
	1	893	762	770	68	18
	1½	974	840	900	63	14
	2	923	766	1000	72	7
	2½	960	757	1190	68	9
	3	964	790	1320	59	4
	3½	965	779	1420	59	5
	4	976	742	1520	58	9
	4½	996	779	1580	51	10
	5	1036	797	1360	51	22
Recovery (hours)	¼	-	-	1100	55	14
	½	253	178	1060	59	7
	1	277	210	900	61	3

Table 15: Basic data for the subject (J.K.) who walked 24 miles at 4 m.p.h. in the post-absorptive state.

Periods		O_2 (ml)	CO_2 (ml)	NEFA (μ eq/1)	Glucose (mg%)	G.H. (μ mg/ml)
Rest		267	208	550	60	18
Walk (hours)	½	977	868	610	60	62
	1	975	865	640	55	67
	1½	974	811	900	60	66
	2	1016	802	1100	68	34
	2½	972	745	1230	68	25
	3	938	739	1360	64	14
	3½	(1002)	(805)	1450	56	8.9
	4	1056	812	1420	56	25
	4½	1005	766	1550	56	21
	5	1038	798	1710	50	23
	5½	1070	851	-	42	15
	6	(1002)	(805)	1360	38	17

Table 16: Basic data for subject (C.C.) who walked 28 miles at 4 m.p.h. in the post-absorptive state.

Periods		O_2 (ml)	CO_2 (ml)	NEFA (µ eq/l)	Glucose (mg%)	G.H. (µ mg/ml)
Rest		316	253	580	70	8.2
Walk (hours)	½	1376	1075	740	60	6.2
	1	1399	1105	810	60	21
	1½	1363	1059	900	60	5.6
	2	1301	1029	1030	80	2
	2½	1364	1061	1100	54	20.1
	3	1360	1039	1160	64	20.0
	3½	1329	1025	1190	54	14
	4	1454	1091	1290	60	8.9
	4½	1400	1029	1390	54	5.0
	5	1424	1055	1450	54	13.0
	5½	1357	1004	1450	50	12.0
	6	1453	1081	1520	50	21.0
	6½	1333	972	1550	54	13.0
	7	1345	979	1610	60	15.0

REFERENCES

Appleby, J.I., Gibson, G., Norymberski, J.K. and Stubbs, R.B. (1955). Biochem. J., 60, 453.

Armstrong, D.T., Steele, R., Altszuler, N., Dunn, A., Bishop, J.S., and De Bodo, R.C. (1961). Amer. J. Physiol., 201, 9.

Ballard, F.B., Danforth, W.H., Naegle, S. and Bing, R.J. (1960). J. clin. Invest., 39, 717.

Basu, A., Passmore, R. and Strong, J.A. (1960). Quart. J. exp. Physiol., 45, 312-317.

Best, C.H. and Campbell, J. (1936). J. Physiol., 86, 190.

Carlson, L.A., Habel, R.J., Ekelund, L.G. and Holmgren, A. (1963). Metabolism, 12, 837.

Carlson, L.A. and Pernow, B. (1959). J. Lab. clin. Med., 53, 833-841.

Chauveau, A. (1896). C.R. Acad. Sci., Paris, 122, 58-64 and 113-120.

Consolazio, G.F., Johnson, R.E. and Pecora, L.J. (1963). Physiological Measurements of Metabolic Functions in Man. McGraw Hill, New York.

Dahlström, H. and Wahlund, H. (1949). Scand. J. clin. Lab. Invest., 1, 86.

Dole, V.P. (1956) J. clin. Invest., 35, 150-154.

Dole, V.P. (1958) Arch. Internal Med., 101, 1005.

Douglas C.G. and Koch, A.C.E. (1951) J. Physiol., 114, 208-221.

Drummond /

Drummond, G.I. and Black, E.C. (1960). Annu. Rev. Physiol., 22, 169.

Eggleton, P. and Eggleton, G.P. (1927). Biochem. J., 21, 190.

Engel, H.R., Hallman, L., Siegel, S., and Bergenstal, D. (1958). Proc. Soc. exp. Biol. (N.Y.), 98, 753.

Evans, H.M. and Long, J.A. (1921). Anat. Rec., 21, 61.

Fick, A. and Wislicenus (1865). Phil. Mag. (4), 31, 485.

Fletcher, W.M. and Hopkins, F.G. (1907). J. Physiol., 35, 247-309.

Frantz, A.G. and Rabkins, M.T., (1964). New Eng. J. Med., 271, 1375.

Friedberg, S.J., Harlan, W.R., Trout, D.L. and Estes, E.H. (1960). J. clin. Invest., 39, 215-220.

Gemmill, C.L. (1942). Physiol. Rev., 22, 48.

Glick, S.M., Roth, J., Yalow, R.S. and Berson, S.A. (1965). Recent Prog. Hormone Res., 21, 241.

Goodman, H.M. and Knobil, E. (1959). Endocrinology, 65, 451.

Gordon R.S. (1957). J. clin. Invest., 36, 810.

Gordon, R.S.Jr., and Cherkes, A. (1956). J. clin. Invest., 35, 206.

Gordon, R.S.Jr. and Cherkes, A. (1958). Proc. Soc. exp. Biol., (N.Y.), 97, 150.

Greenbaum, A.L., and McLean, P. (1953). Biochem. J., 54, 507.

Greenwood, F.C. and Landon, J. (1966). Nature, 210, 540.

Hausberger, F.X., Milstein, S.W. and Rutman, R.J. (1954). J. Biol. Chem., 208, 431.

Hill /

Hill, A.V. (1932). Physiol. Rev., 12, 56-67.

Hill, A.V. (1965). Trails and Trials in Physiology. Arnold, London.

Hunter, W.M., Fonseka, C.C. and Passmore, R. (1965). Quart. J. exp. Physiol., 50, 405-416.

Hunter, W.M., Friend, J.A.R. and Strong, J.A. (1965). J. Endocrin., 34, 139.

Hunter, W.M. and Greenwood, F.C. (1962b). Biochem.J., 85, 39P.

Hunter, W.M. and Greenwood, F.C. (1964a). Brit.Med.J., 1, 804.

Hunter, W.M. and Greenwood, F.C. (1964b). Biochem. J., 91, 44.

Jacobs, S. (1959). Nature, 183, 262.

Jokl, E. (1964). Nutrition, Exercise and Body Composition. Thomas, Illinois.

Kaplan, N.M. (1961). J. Clin. Endocrin., 21, 1139.

Kleiber, Max (1961). The Fire of Life. Wiley, New York.

Knobil, E. and Greep, R.O. (1959). Recent Prog. Hormone Res., 15, 1.

Kostyo, J.L. and Engel, F.L. (1960). Endocrinology, 67, 708.

Lee, M. and Ayres, G.M. (1936). Endocrinology, 20, 489.

Lee, M.O. and Schaffer, N.K. (1934). J. Nutrit., 7, 337.

Levin, L. and Farber, R.K. (1952). Recent Prog. Hormone Res., 7, 399.

Li, C.H. and Evans, H.M. (1944). Science, 99, 183.

Li, C.H. and Papkoff, H. (1956). Science, 124, 1293.

Li, C.H. Simpson, M.E. and Evans, H.M. (1948). Growth, 12, 39.

Li, C.H., Simpson, M.E. and Evans, H.M. (1949). Growth, 13, 171.

Lloyd /

Lloyd, B.B. (1958). J. Physiol., 143, 5P.

Lundsgaard, E. (1930). Biochem. Z., 217, 162; 220, 1, 8; 227, 51.

Lundsgaard, E. (1931). Biochem. Z., 230, 10; 233, 322.

Lusk, G. (1917). The Elements of the Science of Nutrition.

Mayer, J. (1960). Science and Medicine of Exercise and Sports, chapter 16. Harper, New York.

McCance, R.A. and Shipp, H.L. (1933). Spec. Rep. Ser. Med. Res. Counc. (Lond.), No. 187.

Minskowski, O. (1887). Berlin klin. Wochschr., 21, 371.

Parizkova, Jana and Stankova, Libuse (1964). Brit. J. Nutrit., 18, 325.

Parizkova, J. Stankova, L., Sprynarova, S and Vambarova, M. (1963). Cs. Fysiol., 12, 332.

Passmore, R and Draper, M.H. (1965). in Newer Methods of Nutritional Biochemistry, edited by A.A. Albanese, vol.II, 41.

Peckham, W.D. and Knobil, E. (1962). Proc. Am. Endocrine Soc., 44th Annual Meeting. Precirculated Abstract No. 62.

Pettenkofer, M.von, and Voit, E. (1866). Z. Biol., 2, 537.

Playfair, L. (1865). Proc. Roy. Instn. G.B., 4, 431.

Raben, M.S. (1959). Recent Prog. Hormone Res., 15, 71.

Raben, M.S. (1965). in Handbook of Physiology, Section 5. American Physiological Society, Washington D.C.

Raben, M.S. (1965). Diabetes, 14, 6 (Editorial).

Raben /

Raben, M.S. and Hollenbergh, C.H. (1959). J. clin. Invest., 38, 484-487.

Rabinowitz, D., Klassen, G.A. and Zierler, K.L. (1965). J. clin. Invest., 44, 51.

Read, C.H. and Bryan, G.T. (1960). Ciba Found. Coll. Endocrin., 13, 68.

Reichl, D. (1959). Physiol. Bohemoslov., 8, 514.

Roth, J., Glick, S.M., Berson, S.A. and Yalow, R.S. (1963a). Metabolism, 12, 577.

Roth, J., Glick, S.M., Berson, S.A. and Yalow, R.S. (1963b). Science, 140, 987.

Roth, J., Glick, S.M., Berson, S.A. and Yalow, R.S. (1964). Diabetes, 13, 355.

Schönheimer, R. (1942). The Dynamic State of Body Constituents. Harvard University Press, Cambridge.

Seckel, H.P.G. (1960). Amer. J. Dis. Child., 99, 115.

Simpson, M.E., Asling, C.W. and Evans, H.M. (1951). Yale J. Biol. Med., 23, 2.

Unger, R.H. Eisentrant, A.M., Madison, L.L. and Siperstein, M.D. (1965). Nature, 205, 804.

Wenke, M., Muhlbachova, E. and Hynie, S. (1962). Proceedings of the 5th National Congress of the Czechoslovak Physiological Society, Prague, p. 82.

Wertheimer, H.E. (1965). in Handbook of Physiology, Section 5. American Physiological Society, Washington D.C.

Wertheimer, H.E. and Shapiro, B. (1948). Physiol. Rev. 28, 451.

White /

White, J.E. and Engel, F.L. (1958). Proc. Soc. exp. Biol. N.Y., 99, 375.

Wilgram, G.F., Campbell, J., Lewis, L. and Patterson, J. (1959). Diabetes, 8, 205.

Wilhelmi, A.E. (1960). Ciba Found. Coll. Endocrine, 13, 25.

Young, F.G. (1945). Biochem. J., 39, 515.

Young, F.G. (1953). Recent Prog. Hormone Res., 8, 471.

Zuntz (1911). in Oppenheimer's Handbuch Biochemie, 4, 826.

Zuntz and Schumburg (1901). Physiologie des Marsches, Berlin.