INTRODUCTION.

The chemical changes which fat undergoes in the living organism are largely obscure. It is true that the oxidation of fatty acids by successive stages, such involving the removal of two carbon atoms, has been established by the ingenious experiments of KRÖN and of BIRD, but it is generally conceded that this is by no means the only way in which they are catabolised. Many other suggestions have been made, such as the introduction of double bonds into the carbon chain, oxidation at many points of the molecule simultaneously, or of carbohydrate into sugar. Nevertheless, none of these has been proved by evidence sufficient to justify our knowledge of fat catabolism incomplete.

31st March, 1930.

Yet the importance of fat in the animal body can hardly be over-estimated. Even in normal it is one of the main sources of energy both for basal metabolism and for external work; in pathological/
pathological conditions, it may become almost the only one. Its imperfect breakdown in such conditions as diabetes, may become a menace to life itself.

A study of fat metabolism then, needs no apology. The subject is one, not only of theoretical interest to the bio-chemist or the physiologist, but it is one of vital practical importance to the clinician.

THE CHEMICAL MECHANISM OF MUSCLE ACTIVITY.

Although HERMAN discovered in 1879 that muscle can work perfectly well in the absence of air, or when oxidation has been stopped by the addition of cyanide, it was not until 1907, that the real foundation of our present knowledge of the chemistry of muscle action was laid by FLETCHER & HOPKINS at Cambridge. They showed that when muscle is made to work anaerobically, lactic acid is produced, and steadily accumulated up to a maximum. When this maximum is reached, the accumulation of lactic acid, and the working of the muscle stop simultaneously. If oxygen be admitted at this stage, the accumulated lactic acid begins to disappear, and the muscle regains its power of contracting. Further, there is little or no accumulation of lactic acid when muscle is made to work in the presence of oxygen. FLETCHER & HOPKINS concluded, that during muscle activity there is formed a precursor of lactic acid which is transformed into lactic acid, and then synthesised from it again. These changes were supposed to be merely a lubricating system.
the real energy coming from the oxidation of some other substance. Although these conclusions are now known not to be altogether tenable, yet the experiments leading to them are the pioneers and have led to our present knowledge of muscle action.

It was next shown by some more accurate experiments, that the maximum amount of anaerobic work which can be obtained from an isolated muscle is not constant, but depends upon the state of nutrition of the muscle. Again, if the lactic acid produced by this working muscle, is allowed in some way to escape, a greater amount of work can be obtained.

These discoveries led to the conclusion that the precursor of lactic acid in muscle contraction, is glycogen. The total amount of lactic acid which can be obtained from a working muscle either by allowing the lactic acid to drain away as it is formed, or by suspending the muscle in alkaline solution, buffered with phosphate (Meyerhof), so that the muscle continues to contract, depends upon the stores of glycogen originally present in the muscle.

In 1912, A. V. HILL commenced his work on muscle action. His first approach to the subject was along lines quite different from those already studied. The method of attack which he adopted, was an examination of the heat changes involved in all phases of muscle action. When a muscle contracts in the absence of oxygen, part of the glycogen which is present in the muscle is converted into lactic acid, and it is this lactic acid which
by its action on the muscle fibres, brings about the actual contraction. The change from glycogen into lactic acid involves the production of heat, and HILL, by measuring the heat produced in this phase of the muscle action, showed that it corresponded to the heat evolved in the change from glycogen to lactic acid. Further, in order to allow the muscle to regain its original position of rest, MEYERHOF showed that it is necessary for the free lactic acid to be removed. He suggested that the protein of the muscle acts as a buffer and neutralises the lactic acid as it is formed. Certainly the amounts of bicarbonate and phosphates in muscle are quite insufficient for this purpose. Again by measurement of the heat evolved, HILL showed that this was a possible mechanism.

In the presence of oxygen, the reverse of all these processes takes place at the same time, so that the muscle is recovering its store of glycogen, and is able to continue to contract. When the muscle attains a steady state of work, both (a) the direct breakdown of glycogen to lactic acid and the neutralisation of lactic acid by protein, and (b) the reverse resynthesis of glycogen takes place.

It is at once obvious that in order to reconvert the neutralised lactic acid to free acid, and to synthesis this to glycogen, energy must be supplied. This energy is obtained by the combustion of part of the carbohydrate of the muscle, either in/
in the form of glycogen or lactic acid. It is at this point that oxygen takes part in muscle action. HILL showed that approximately for every five molecules of glycogen resynthesised, one molecule is burned, and part of the energy thus evolved is used to recover the lactic acid and to synthesise it to glycogen. Since the recovery process is, therefore, not quite perfect, it follows that the store of glycogen in the muscle becomes less and less.

The most recent work shows that in its essentials the view of HILL and MEYERHOF probably presents the correct view of the chemical changes during muscular contraction and recovery. There is little doubt that the breakdown of glycogen to lactic acid and its resynthesis are a necessary part of the mechanism. Criticism has been directed mainly against the suggestions to explain the neutralisation of lactic acid, and the discovery of such substances as phosphocreatin together with the work of PARNAS on ammonia formation in muscle, make it obvious that in HILL and MEYERHOF'S hypothesis, we have not yet a complete story of muscle contraction.

Although these results have been obtained from a study of isolated muscle, they apply also to muscle action in the intact animal. Thus, the fact that during muscular exercise the amount of lactic acid in the blood is increased shows that this substance is being produced by the working muscles, whence it is in part removed by the blood. Further, under certain conditions which will be more fully dealt with later, it is found that muscular exercise/
exercise involves the using of oxygen and the production of an equal volume of carbon dioxide. This condition demands the consumption of carbohydrate and of carbohydrate alone.

It has long been known, however, that the intact animal does not always always perform muscular work entirely at the expense of carbohydrate. Thus ZUNTZ showed that fat could be used, and considered it to be as efficient a fuel as carbohydrate. Many workers have obtained similar results, but finer methods of experimentation have led to the discovery that fat, though readily available as a fuel, is slightly less efficient than carbohydrate (KROGH & LINDHARD). The conditions under which fat is utilised by muscle have been discovered by HILL & LUPTON, and their collaborators. These workers found that the performance of relatively small amounts of work involved the combustion of carbohydrate only; but that as the amount of work increased, the respiratory quotient fell further and further from unity, showing that fat was being used in increasing amounts. Protein whose oxidation would have produced a similar effect, was excluded, since the exercise did not increase the nitrogen excretion.

A step further in the elucidation of fat utilisation in muscular exercise was made in 1927, when PATTERSON was able to correlate the utilisation of fat with changes occurring in the fat content of the blood. The discovery of this relationship was quite accidental, made during a very close study of the blood fat level and its variations, in human beings. He found that the fat content/
content of a sample of blood withdrawn after a strenuous exercise was much higher than normal. This rise was so definite that PATTERSON investigated the phenomenon further. A series of the results of some of his experiments was published, in which he showed that there was a rise in blood fat with prolonged muscular exercise, and that the blood fat returns to its normal level after a recovery period of about an hour. By observing the concentration of the blood corpuscles before and after exercise, he showed that this rise is not merely apparent and due to concentration of the blood. PATTERSON did not put forward a detailed explanation of his discovery, his experiments not being sufficiently full nor controlled. He did, however, suggest that the increase in the blood fat may be due to either of two factors. It may be a definite response to a demand for more fat on the part of the working cells, or it may be due to a non-removal of fat from the blood stream, the working cells either refusing it or being unable to utilise it. The first hypothesis appears to be the more logical.

ORIGINAL EXPERIMENTAL WORK ON FAT METABOLISM IN EXERCISE.

With a view to confirming PATTERSON'S results, to finding a relationship between them and the already known facts about muscular exercise, to elucidating the mechanism of fat metabolism during exercise, and to applying the results to the problem of fat/
fat metabolism in pathological conditions, STEWART, DUNLOP & GADDIE undertook a series of investigations which are outlined in the following pages.

In order to correlate the changes in the composition of the blood with the utilisation of fat in muscular exercise, it was necessary to follow the complete metabolism during the period of exercise and recovery. The fact that in the intact animal lactic acid may be removed from the working muscle by the bloodstream, introduces a factor which complicates the determination of the respiratory quotient during, and immediately after exercise. The introduction of lactic acid into the blood tends to alter the hydrogen ion concentration by abstracting sodium proteinate, sodium bicarbonate, etc., and the balance can only be restored by increased elimination of carbon dioxide via the lungs. There is thus, during exercise a fictitiously high production of carbon dioxide. This tends to make the observed respiratory quotient during exercise higher than that actually due to the metabolic changes taking place, and a second factor operates in the same direction. A knowledge of the oxygen requirement of the exercise undergone is of value as furnishing a measure of its vigour. When the exercise is mild, the oxygen intake may be able to balance the oxygen requirement, and the requirement may then be ascertained by measuring the oxygen intake during a steady state of exercise. When the exercise is severe, the oxygen requirements of the muscles/
muscles cannot be met by the oxygen taken into the body, through the respiratory-circulatory system, and the steady state of exercise is never reached. The oxygen intake may remain constant at its maximum value, but the lactic acid continues to accumulate in muscle and blood. In HILL'S words: "In such a state, the muscle has to 'go into debt' for oxygen, to obtain its energy on the 'security' of a concentration of lactic acid which it will require future oxygen 'income' to eliminate."

The only way to ascertain the oxygen requirement during severe exercise, is to measure both the oxygen intake and the oxygen debt, or in other words, to ascertain the oxygen used both during exercise and in complete recovery therefrom. It is these factors which render difficult, the question of utilizing respiratory data, in order to ascertain the metabolic changes occurring in the body due to exercise.

When the body is at rest, the respiratory quotient, i.e. the ratio of the carbon dioxide produced by the body to the oxygen used, gives valuable information as to the type of substance undergoing combustion in order to provide the energy necessary for the basal requirements of the body. It is well known that the respiratory quotient due to the combustion of carbohydrate alone is unity, and of fat alone is .707, while that of protein is intermediate between the two. From a knowledge of these figures, and from certain data given in Lusk's tables, it is possible to calculate the actual weights of each class of substance/
substance metabolised during a known period. The metabolism of protein over a certain interval of time can be ascertained directly by measuring the nitrogen output over that period of time. By calculating the oxygen requirement and the carbon dioxide production due to the ascertained amount of protein, and subtracting these values from the total oxygen consumption and carbon dioxide production, the values due to the combustion of carbohydrate and fat are ascertained. These latter values give the respiratory quotient due to the carbohydrate and fat used, and it is an easy matter to calculate the relative amount of the two metabolites. The last stage in the calculation is to obtain the actual grains of carbohydrate and fat used, from the oxygen consumption.

It is now obvious that in studying exercise in the intact animal, viz: the human animal, that measurement of the respiratory exchange would give very valuable information as to the source of the energy necessary for the exercise. This can be done, but there are many pitfalls which must be avoided in its accomplishment.

At no instant during either exercise or recovery from exercise, does the respiratory quotient give any indication of the metabolic changes going on in the body at that instant. Although the respiratory quotient is high during exercise, owing to a greater proportion of carbohydrates being used than normally, it has been pointed out that various other factors are present/
present which make its value still higher and give entirely fallacious information as to metabolism.

After cessation of exercise, according to HILL, the respiratory quotient continues to rise, at first owing to the carbon dioxide elimination diminishing more slowly than the oxygen intake. So long as over-breathing continues, so does the high carbon dioxide elimination. As the lactic acid formed in exercise is removed in recovery, it is necessary for the body to retain carbon dioxide to replace that which was previously eliminated, otherwise the tissues would become excessively alkaline. Consequently, in the stage of recovery, there is a retention of carbon dioxide and a very low value of the respiratory quotient.

During recovery too, oxidation of the accumulated lactic acid is taking place. In other words, the muscles are paying the oxygen debt incurred during exercise. This also tends to the production of a low observed respiratory quotient. Considering, however, the whole process of exercise and recovery, it is obvious that the factors which tend to raise unduly the respiratory quotient during exercise, are cancelled by those which tend to lower it excessively during recovery. Hence the total oxygen consumption and carbon dioxide production over the two periods combined, represent accurately the amount of metabolism due to the exercise. The respiratory quotient, however, represents only an average, and does not give any information as to the sequence of events in relation to time. It allows deductions to be drawn as/
as to the relative amounts of the various substances metabolised but not as to the time at which the metabolism of a given substance began or ended.

In the experiments of STEWART, DUNLOP & GADDIE, the subject either stayed overnight in the laboratory or came in very early in the morning and lay down in bed immediately, avoiding all except the very minimum of exercise. All precautions were taken to ensure that the subject was perfectly basal, no food having been taken for at least fourteen hours previously. Before commencement of the experiment, the subject emptied his bladder, and was given a drink of water, the intention being to follow the nitrogen excretion until complete recovery from exercise. In order to ascertain the basal oxygen requirement and carbon-dioxide production, the subject breathed into a large spirometer for a period of twenty-five minutes. The spirometer used was more convenient than the usual Douglas' bag, in that its capacity was much greater, and it offered no resistance to the breathing of the subject. At the end of this basal period a sample of blood was withdrawn and urine again passed. The subject now performed a definite amount of work upon a bicycle ergometer. This constituted an improvement upon the first of PATTERSON'S experiments, where his subjects were made to run over a certain distance in a given time. The ergometer allowed the exact amount of work to be calculated. During the exercise, the subject breathed into the spirometer for periods of one minute at a time, at suitable parts of the exercise. One minute sample was taken/
taken at the third minute of exercise, a second in the middle of the exercise, and a third just before the end of the exercise. When the oxygen consumption and carbon dioxide production during these minutes of exercise were obtained by analyses of the respiratory exchange, the points were found to be on a perfectly definite curve. Thus by interpolation, the total oxygen requirement and carbon dioxide production for the whole period of exercise was found. At the end of exercise, a second blood sample was immediately taken and urine again passed. As quickly as possible the subject was again made to breathe into the spirometer until he had returned to, and remained at basal conditions for at least five minutes. In calculating the respiratory exchange during the period of recovery, allowance was made for the short period intervening between completion of exercise and the beginning of the collection of the expired air.

These experiments, therefore, gave two series of data —
(a) respiratory data, and
(b) data from blood analyses.

The nature of the problem was to find an explanation of the results obtained from each set of data, and to find the connection, if any, between the two.

Consider, firstly, the respiratory data. Since the total oxygen consumption and carbon dioxide requirement, over the whole period of exercise and recovery are known, by subtracting the oxygen consumption and carbon dioxide production due/
due to basal function, the respiratory exchange due entirely to exercise and recovery may be obtained. It is quite obvious that this procedure entirely avoids errors due to oxygen debt and increased carbon dioxide elimination, since these are quite corrected before the subject has resumed the basal condition.

Under basal conditions, with normal subjects, values of respiratory quotient varying from 0.71 to 0.85 were obtained, with the maximum number in the neighbourhood of 0.80. The respiratory quotient due to exercise and recovery, was found to depend entirely upon the amount of work performed. The results of HILL & LUPTON were thus confirmed with the added result, that the second source of energy besides carbohydrate was shown to be fat. When the amount of work performed was small the respiratory quotient was found to be unity, confirming that small amounts of work are performed at the expense of carbohydrate. When, however, the amount of work performed was increased, the respiratory quotient was found to fall below unity, showing that fat was being utilised as well as carbohydrate. That the fall in respiratory quotient is not caused by increased protein metabolism, was shown by the nitrogen excretion. It was found that the nitrogen excretion remained practically constant. Instead of rising during exercise, it rather tended to fall. This fall is accounted for by the loss of nitrogen in sweat. Therefore the fall in respiratory quotient for exercise and recovery is due to increased utilisation of fat.
In these experiments the amount of work performed was varied between wide limits, and it was shown that the respiratory quotient continued to fall as the amount of work increased. The change due to the utilisation of fat instead of carbohydrate in exercise is not sudden, but a very gradual process. With small amounts of exercise, no fat is used at all, and the respiratory quotient is I. As the work gradually increases in amount, the respiratory quotient gradually falls. Many different values were obtained, down to 0.83, and the respiratory quotient tended to be inversely proportional to the amount of work performed.

The accompanying graphs will help to make these results clearer. By means of the calculation previously outlined for basal metabolism, the actual amounts in grams, of carbohydrate and fat used by each subject in order to perform a known amount of work, can be obtained.

In Graph I., the actual litres of oxygen used and carbon dioxide produced are plotted against the amount of work performed. It will be seen at once, that fair correlation is obtained between the volumes of gas and the amount of work, the slight deviations being due to individual variations in efficiency. The corresponding points for each experiment lie in the same vertical line. The ideal curve relating oxygen consumption and the amount of work should, theoretically, be almost a straight line, and the observed points fulfil this condition fairly satisfactorily/
Diagram II.

Relation between R.Q. and amount of fat used.
- Normal: 
- Obesity: 
- Diabetes: [Diagram with points and symbols]
satisfactorily. The points representing carbon dioxide production on the other hand, lie on a curve which diverges further and further from the oxygen curve, showing how the respiratory quotient falls as the amount of work performed increases. The points plotted on this diagram, and on succeeding diagrams, are the results of all the experiments performed on normal people, without exception. They show very clearly that the change over to fat instead of carbohydrate as the source of energy for muscular work, is a gradual change, and not a sudden one.

The two points at the top of the diagram which are so far from fitting the curves, are of special interest. They are the results of two experiments upon subjects who were highly trained athletes, the other experiments being upon subjects who led normal lives without playing strenuous games. These results show that athletes are able to perform more work than normal individuals, before they begin to call upon fat to any great extent. It might be suggested here that 'training' consists partly in storing glycogen to a greater extent than normally. This view has already been put forward by LUSK, who has evidence in support of it, from the results of experiments on animals. This point, however, is one which might bear further investigation.

DIAGRAM II., shows the relation between the respiratory quotient and the actual amounts of fat used in performing work.
Diagram III

Relation between R.Q. and alteration in blood fat.

- Normal
- High initial blood fat
- Obesity
- Diabetes
This, at first sight, may seem as though something had been plotted against itself, since the amount of fat used is calculated from the respiratory quotient. This is not absolutely so, however, because another factor, viz: the total oxygen requirement for exercise and recovery is also used. The points on this graph show better agreement with the smooth curve.

A point of great interest might conveniently be mentioned here, but be more fully dealt with later. It will be seen from Diagram II., that a number of points lie on a quite distinct curve from the others. These points were obtained from experiments on abnormal people, comprising diabetics and obese persons. The chief point to be noted here is that the case of obesity behaves in the same way as the cases of diabetics. As a matter of fact, one of the points on the 'normal curve' belong to the same case of obesity, who, however, was at the time of this experiment, receiving thyroid treatment, which made her behave in an entirely normal manner. The clinical significance of these results will be discussed later. The text used in the construction of these results are summarized in Table I.

Turning now to the second series of data obtained from these experiments, the blood fat picture will be discussed. In the first experiments, no rise in the blood fat was found after exercise. This was in complete disagreement with the results of Patterson's experiments, so much so, that in one or two instances even a slight fall in the fat content was noted. On closer examination/
examination of the conditions of the experiments it was found, that the subjects were only performing a small fraction of the work done by PATTERSON'S subjects. The former were performing up to 20,000 kilogram metres without any appreciable change in blood fat, while the latter had run at full speed for fifteen minutes, which is very severe exercise indeed. It was found impossible to get subjects to perform large amounts of work on the bicycle ergometer in the short time of fifteen minutes. Partly because this form of exercise is an unaccustomed one, it was found that the working muscles very quickly became stiff, showing that the recovery mechanism was unable to cope with the large quantities of lactic acid being formed. In order to obtain large amounts of work on the bicycle ergometer, it was found necessary to lengthen the time taken to do exercise. By decreasing the break tension of the bicycle, it was found that the subjects were able to keep on working at the reduced rate for a very long time, and thus perform great amounts of work. With these large amounts of work thus obtained, increases in blood fat similar to PATTERSON'S were found.

The results are best followed by a consideration of Diagrams III and VI., when it will be at once apparent that the blood fat changes follow very closely the respiratory data. Diagram III shows the relationship between the R.Q. and the alteration in blood fat, while Diagram IV. shows the relationship between/
Diagram IV

% Alteration in Blood Fat.

Relation between alteration in blood fat and amount of fat actually used.

- Normal
- High initial blood fat.
- Obesity
- Diabetes

Grams Fat used
between the amount of fat actually used, and the alteration in blood fat. When the basal blood fat content is about normal level, small amounts of work produce no change. Even when fat is used, the proportion of fat to carbohydrate oxidised must reach a certain level before the ordinary available blood content is unable to supply the demand of the muscles for fat, and the fat depôts are stimulated to pour out fat into the blood stream. Once, however, the point is reached when the fat depôts are stimulated, it appears that the rise in blood fat above the normal level bears a close relationship to the ratio of fat to carbohydrate oxidised to provide the energy necessary for exercise.

It will also be seen from these diagrams, that there are several points which are 'abnormal'. A number of points are shown which were obtained from experiments on subjects whose normal fat blood levels were high. The reason for this high fasting blood fat level is not known, the subjects being perfectly normal in other respects. It has been suggested that they are examples of incipient diabetes. These subjects, even when performing small amounts of work show a fall in blood fat. Again, in one experiment the subject had an abnormally low resting blood fat level, and with a comparatively small amount of work, (circa 19,000 Kg.metres), showed a very considerable rise, out of all proportion to the amount of work performed. These cases lead to the conclusion that the blood fat content tends to reach the normal level before it shows the increase given/
### TABLE I.

<table>
<thead>
<tr>
<th>SUBJECT</th>
<th>BLOOD</th>
<th>FAT</th>
<th>SOAP</th>
<th>CHOLESTEROL</th>
<th>LIPOID</th>
<th>PHOSPHORUS</th>
<th>WORK</th>
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<tr>
<td>R.E.I.(1)</td>
<td>a.</td>
<td>680 mgs. 0/o</td>
<td>23.9 mgs. 0/o</td>
<td>118 mgs. 0/o</td>
<td>18.8 mgs. 0/o</td>
<td>33,000</td>
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<td></td>
<td>b.</td>
<td>824</td>
<td>25.2</td>
<td>118</td>
<td>16.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.</td>
<td>560</td>
<td>23.9</td>
<td>118</td>
<td>15.0</td>
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<tr>
<td>F.P.C.</td>
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<td>36.0</td>
<td>119</td>
<td>15.9</td>
<td></td>
<td></td>
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<tr>
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<td>800</td>
<td>34.7</td>
<td>121</td>
<td>15.5</td>
<td></td>
<td>22,25</td>
</tr>
<tr>
<td></td>
<td>c.</td>
<td>550</td>
<td>35.2</td>
<td>120</td>
<td>15.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D.V.</td>
<td>a.</td>
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<td>23.6</td>
<td>120</td>
<td>15.3</td>
<td></td>
<td></td>
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<td>b.</td>
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<td>24.6</td>
<td>142</td>
<td>15.2</td>
<td></td>
<td>37,15</td>
</tr>
<tr>
<td></td>
<td>c.</td>
<td>616</td>
<td>24.5</td>
<td>122</td>
<td>17.5</td>
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<td>32.9</td>
<td>145</td>
<td>11.6</td>
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<td></td>
</tr>
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<td>45.7</td>
<td>144</td>
<td>17.2</td>
<td></td>
<td>28,00</td>
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<td></td>
<td>c.</td>
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<td>156</td>
<td>19.6</td>
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<td></td>
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<td>812</td>
<td>38.2</td>
<td>160</td>
<td>20.4</td>
<td></td>
<td>12,000</td>
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<td></td>
<td>c.</td>
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<td>166</td>
<td>20.0</td>
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<td>262</td>
<td>20.8</td>
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<tr>
<td>(Diabetic)</td>
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<td>263</td>
<td>22.1</td>
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<td>8,600</td>
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<td>c.</td>
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<td>19.1</td>
<td>260</td>
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<tr>
<td>R.M.M.R.</td>
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<td>43.9</td>
<td>138</td>
<td>16.1</td>
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<td>b.</td>
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<td>44.1</td>
<td>136</td>
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<tr>
<td></td>
<td>c.</td>
<td>654</td>
<td>43.6</td>
<td>136</td>
<td>16.3</td>
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Blood (a) Basal Sample.  
(b) After Exercise.  
(c) After Recovery.
given by normal individuals. This preliminary change takes place under the influence of moderate amounts of work, definitely below the amount of work required to produce a change in the blood fat content of normal people.

Further abnormal points are given by cases of diabetes experimented on, and by the cases of obesity untreated by thyroid.

Briefly these results show that when the blood fat level is normal, and when the proportion of fat being used for exercise is below a certain value, the amount of fat circulating in the blood stream is sufficient to supply the needs of the muscles. When, however, the muscles begin to call for more fat, than can normally be supplied by the blood stream, the depots are stimulated to greater activity. The results of this increased activity is made apparent by the increase in the blood fat content. On the other hand, when the resting blood fat level is higher than normal the muscles first use up this excess of fat before the depots are stimulated. Also when the resting blood fat level is lower than normal, the blood stream is unable to supply the muscles even when their demand for fat is small, and consequently the fat depots are called upon much sooner than in absolutely normal subjects.

A question which at once arises is - what is the chemical nature of the increased blood fat? The study of blood chemistry undertaken in these experiments shows conclusively that the rise is in the tryglyceride fraction of the blood fat content. No marked variations were observed in the lecithin fraction, the cholesteryl ester fraction, nor in the soap fraction. TABLE I. summarises...
summarises these results of the blood chemistry study.

An investigation concerning the nature of the triglyceride fraction is now being undertaken, with a view, first of all, to finding the degree of unsaturation. This might be expected to give information as to the source of increase in blood fat. If the fat is being withdrawn from the liver, an increase in the degree of unsaturation is to be expected since the liver fat is highly unsaturated. On the other hand, the fat in the adipose tissues consists principally of a mixture of oleic and stearic acids, so that it is relatively less unsaturated than liver fat, and contains about the same amount of unsaturated fatty acid as does blood fat. Mixture of fat from adipose tissue with the blood fat would, therefore have little or no effect on the iodine number which is the measure of the degree of unsaturation.

Another line of investigation being entered upon, is the question of the nature of the stimulus to the fat depots, calling upon them to mobilise fat and cause it to circulate in the bloodstream to be carried to the sites of muscular activity. When muscles perform work, lactic acid is produced from glycogen. When the amount of work is small, this lactic acid is confined to the working muscles, and it does not accumulate to any great extent. When, however, the amount of work performed increases, lactic acid accumulates in the working muscles and is washed out into the bloodstream, and is there neutralised by sodium proteinate, and bicarbonate etc., producing a depletion of the alkali reserve of the blood. That is to say, that when the glycogen stores in muscle are/
are practically normal, no acidosis is produced, but when the glycogen stores are depleted by the production of large amounts of lactic acid, acidosis is produced. Further, it is the depletion of the stores of glycogen in muscles which causes them to use more fat as a source of energy, and the more the glycogen stores are depleted, the more do muscles use fat. Therefore, since the same condition leads to a depletion of the alkali reserve of the blood as leads to an increase in the blood fat content, it is an obvious suggestion to make, that the stimulus to the fat depôts is lowering of the alkali reserve.

Preliminary investigation in this direction suggests, that as work is performed at the expense of carbohydrate, a tendency in the direction of acidosis occurs. When this acidosis reaches a certain value, the fat depôts appear to be stimulated and the blood fat content increases. When this stimulation has occurred, the carbon dioxide combining power returns towards its basal value. It is well known that after the performance of a large amount of work, a definite acidosis is produced. Unfortunately in the experiments so far performed in this connection, difficulty has been experienced in obtaining this final acidosis; but further experiments are at present being undertaken with this end in view.

The present stage of the investigations suggest that the stimulus to the fat depôts, to transfer fat into the blood, is brought about by a fall in alkali reserve. When the fat depôts have been stimulated, a temporary recovery of the alkali reserve takes place, before the onset of the acidosis commonly found after prolonged exercise.
THE MODE OF UTILISATION OF FAT.

An interpretation of the experimental results obtained in these studies of muscular exercise is based upon the assumption that the diabetic organism has lost part or all of its power of oxidising glucose. This basic assumption it is necessary to examine, since it has been suggested by MACLEOD and others that in diabetes, the power to oxidise glucose is unaffected, but that the disease consists essentially in an over-production of carbohydrate from protein and fat.

If the power of oxidising glucose is retained by the diabetic animal, it seems necessary to postulate, not merely over-production of carbohydrate, but inability to synthesise glycogen, and indeed inability to prevent hydrolysis of the stored glycogen. Otherwise it is impossible to explain the observed fact, that the diabetic, far from increasing its glycogen store as a result of the increased supply of carbohydrate - as a normal animal would do - progressively loses glycogen, rapidly from the liver, and more slowly from the muscles.

That there is over production is obvious, but that this over-production exists, even in the 'complete' diabetic, along with a continued ability to oxidise glucose, is by no means obvious. In phlorhizinised dogs, LUSK and others have shown repeatedly that the oxygen consumption and respiratory quotient are exactly what would be expected if no glucose were being oxidised, and if carbohydrate were being synthesised - and excreted - to the maximum.
maximum possible amount from protein, and not at all from fat. Similar agreement is obtained from a consideration of cases of 'complete' diabetes in human subjects. This can hardly be mere coincidence. If we accept MACLEOD'S suggestion that carbohydrate is being oxidised (R.Q. = 1.0), and simultaneously fat is being converted to carbohydrate (R.Q. about 0.23), certainly we may arrive at the same resultant respiratory quotient, as would be produced by the direct oxidation of the fat, but we are faced with the curious absurdity that the diabetic organism can oxidise whatever amount of carbohydrate is produced from fat, but excretes whatever carbohydrate is directly ingested and whatever is obtained by synthesis from protein!

The excretion of ketone bodies, too, is difficult to account for on the hypothesis that in diabetes, carbohydrate oxidation proceeds as in the normal. Each molecule of fatty acid can give one molecule of hydroxybutyric acid, and at least two molecules of glucose if, as is apparently assumed, the remaining carbon is completely utilised in glucose synthesis. The respiratory quotient shows that this sugar - or its equivalent from other sources - must then be oxidised, and if so, the hydroxybutyric acid should also be oxidised, since it has been abundantly demonstrated that one molecule of glucose suffices for the combustion of two molecules of 'ketone bodies'.

It is fair to conclude that these considerations uphold the view that in diabetes there is failure to oxidise sugar, and that/
that being so the evidence for and against the conversion of fat into sugar will now be examined. This has recently been done by LUSK, who concludes that the evidence is totally against any such conversion. In the 'complete' diabetic, totally unable to oxidise glucose, the excretion of sugar is never greater than can be accounted for by the conversion of protein to carbohydrate, and in 'incomplete' diabetics it is invariably less than this. Moreover calculation shows that the observed heat production, respiratory quotient sugar, nitrogen and hydroxybutyric acid excretion cannot be accounted for on the supposition that fat is converted to carbohydrate, but can be accounted for on the supposition that protein is converted to carbohydrate, while fat is oxidised in the usual way, as far as hydroxybutyric acid.

Another suggestive piece of evidence leading to the same conclusion is, that alteration of the protein content of the diet leads to a parallel alteration in the sugar excretion, whereas no connection whatever is observed between the fat content of the diet and the excretion of sugar.

On the basis of these and many other similar facts, it may be accepted that the diabetic does not convert fat to carbohydrate, and from this bearing in mind the circumstances of the disease, it is a legitimate conclusion that he cannot do so. This, however, affords no proof of a corresponding inability in the normal subject. Yet the only known difference between the fat metabolism of the diabetic, and that of the normal/
normal lies in the power of the latter to oxidise hydroxybutyric acid, beyond which the diabetic cannot go. The normal, with a plethora of fat, produces hydroxybutyric acid, which, as such evidence shows, lies on the normal line of fatty acid oxidation, and it is a fair conclusion that up to that point, the diabetic exhibits no abnormality of fat metabolism - save, perhaps, in quantity, for he undoubtedly uses fat in relatively large amounts. This leads, then, to the important conclusion, that if the normal organism can convert fat into carbohydrate at all, he can only do so from hydroxybutyric acid. In other words, of a fat molecule containing some fifty-three atoms of carbon, only twelve, or fifteen, if one includes the glycerol, can be available for glucose synthesis. But if hydroxybutyric acid goes to sugar, the probable course is: first oxidation to acetoacetic acid, thence (by splitting at B-carbon atom) acetaldehyde which can undergo aldol condensation. Hence of fifty-three carbon atoms in the fat molecule, nine may appear as carbohydrate.

If it be granted that the cycle glycogen to lactic acid is essential for muscular contraction (and of this there is little doubt) and that when glycogen alone is being burnt, the resynthesis of glycogen takes place at the expense of part of the lactic acid - which is oxidised to carbon dioxide and water, it is clear that fat can be utilised in muscular contraction only in one of these two ways.

I. It may be oxidised completely in place of lactic acid or II. It may be converted into glycogen to replace that lost by combustion of lactic acid.
If the latter alternative is the one actually employed, only the glycerol molecule and two carbon atoms from each fatty acid molecule can be utilised for glycogen synthesis.

Which of these two routes is actually taken by the fat cannot be decided from a consideration of the respiratory quotient. In each case the fat is completely burned to carbon dioxide and water: in the first case directly, and in the second case, with intermediate formation of carbohydrate which is itself oxidised.

The two processes, however, differ very considerably in efficiency. Combustion of a gram of fat by the first method will allow the performance of work equivalent to 9.46 Cals. — assuming, for the moment an efficiency for the human body of 100 per cent; by the second method, a gram of fat will allow of the performance of only 1.22 cals. of work. Since it is supposed that the glycogen-lactic acid cycle is essential to muscular contraction. In other words, 87 per cent of the energy of the fat is wasted.

In actual practice no such wastage is found. Thus in the early experiment of GUNTZ and of HEINEMANN, the average result showed no appreciable difference in the mechanical efficiency of the body whether working on a fat diet or on a carbohydrate diet. ANDERSON & LUSK observed that with a respiratory quotient of 0.92, work was done at the expense of 5 per cent less/
less energy than with a respiratory quotient of 0.78; conversion of fat (which is supplying 73.7 per cent of the heat when the respiratory quotient is 0.78) to carbohydrate by the method under consideration, would involve a loss, not of 5 per cent of efficiency, but of approximately 60 per cent.

KROGH & LINDHARD state that:

"In the three best series of experiments the net expenditure of energy per calorie of technical work, varies from about 4.6 Calories when fat alone is catabolised (respiratory quotient = 0.71) to about 4.1 calories when carbohydrate alone is catabolised (Respiratory quotient = 1.0). The waste of energy from fat is 0.5 calorie or 11 per cent of the heat of combustion of the fat."

They consider that when the respiratory quotient is below 0.8 carbohydrate may be formed from fat and provisionally stored, but that their experiments do not prove the necessity for the conversion of fat to carbohydrate prior to its utilisation for muscular work.

The existing evidence, then, is against the existence of any such waste of energy as would be involved in the conversion of fat to carbohydrate by the method just suggested. In so far, therefore, as it may be taken to support the use of fat as a sperer of lactic acid, it indicates that fat is rather less efficient than lactic acid as a source of energy for glycogen synthesis.

Pointing/
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<th>Litres $O_2$</th>
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<th>Gm. of Fat used.</th>
<th>Cals. of Work used.</th>
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D. = Diabetic

Ob. = Obese Subject on Thyroid.

Ob.2 = Obese Subject off Thyroid.
Pointing in the same direction is the finding, in severe diabetes, that muscular exercise involves no oxidation of carbohydrate (GRAFE & SALOMON), that the muscles, nevertheless hold tenaciously to glycogen (RINGE) and that in partial diabotes at any rate, exercise involves the breakdown of glycogen to lactic acid just as in normal cases (HIMWIEH, LOEBEL & BARR).

In the experiments of STEWART, DUNLOP & GADDIE, which have been described, sufficient data is furnished to enable calculation to be made of the amount of external work which could possibly be accomplished, (1) when fat is oxidised directly and spares lactic acid, and (2) when fat is first converted to glycogen, and the glycogen alone is used.

The results of these calculations in a considerable number of cases, show without exception that the observed and calculated amounts of work agree closely when fat is taken to be directly oxidised, but that the observed amount of work could not possibly have been done at the expense of glycogen derived from fat. These results are summarised in TABLE II. It will be noted that when the amount of work performed is small, the difference between the two is not marked, but with greater amounts of work, owing to the increased utilisation of fat, the difference becomes very considerable.
It remains now to discuss the results of application of the methods used in these experiments to abnormal subjects. The first abnormal condition studied was that of diabetes. As is to be expected from their incapacity to oxidise glucose, diabetics utilise fat as the source of energy, from the very start of muscular exercise. Not being complete diabetics, these subjects were able to metabolise a certain amount of carbohydrate, and did so to their maximum ability, yet in all the cases studied, the disease was severe enough to give abnormal results. The blood picture given by diabetic subjects was similar to that given by normal subjects, with the important exception, that the increase in blood fat content is caused by very small amounts of work. Most diabetics are incapable of performing more than 10,000 kilogram metres of work, this being a generous limit, yet even with 6,000 kilogram metres, increases in the blood fat content were found. It is a well known fact that in diabetes, the blood fat content is usually high, although there are many exceptions to this rule. Just as is the case with normal subjects whose resting blood fat content is abnormally high, the first effect of exercise on diabetics with a high blood fat is to produce a decrease. This decrease would undoubtedly be followed by an increased blood fat, if it were possible for the subjects to perform enough work, but, owing to their state of health, those subjects studied, were unable to perform/
perform more than a small amount, so that this secondary effect was not obtained.

The respiratory quotient for exercise and recovery obtained with diabetic subjects performing small amounts of work, were in the neighbourhood of 0.30, and even lower, when, with the same amounts of work, normal subjects give a respiratory quotient of unity.

In GRAPH II., which shows the relationship between the respiratory quotient and the actual amounts of fat used, the points given by diabetic subjects show at once their very abnormal behaviour. The more severe the case of diabetes, the more does the corresponding point on this diagram deviate to the right of the curve on which lie the normal points. This means that, while with normal subjects, on account of the large quantity of carbohydrate being oxidised, it is necessary to burn large amounts of fat, in order to lower the respiratory quotient for exercise and recovery, much below unity, with diabetics, even small amounts of fat used lower the respiratory quotient considerably, because very little carbohydrate can be oxidised. In other words, small amounts of work produce a low respiratory quotient for exercise and recovery in diabetes.

Such an experiment may thus be used to give an idea of the severity of the disease. Preliminary treatment of a diabetic with insulin and glucose, enables him to perform exercise in an entirely normal manner.
A second point of great interest, is the effect of exercise on obese subjects. A preliminary short survey of the known facts of obesity will facilitate the interpretation of the results obtained from the experiments. Obesity may be roughly divided into two classes, Exogenous Obesity and Endogenous Obesity. The division is somewhat artificial, because it is seldom that one meets with a case of purely exogenous obesity.

Exogenous obesity may be due to a variety of causes, such as to simple over-eating, to a wrongly balanced diet to a lack of exercise, and to the effect, though small, of wearing too many clothes. It is difficult to ascribe this type of obesity to any one of these causes alone, all probably contributing a share. Furthermore, the obesity is often due immediately to the repercussion of the exogenous factor, which is merely the ultimate cause, on the delicate balance of the internal secretions. Probably the effect of a wrongly balanced diet is greatest. When a person, usually a woman, persists over a long period in eating a large excess of carbohydrate, two things may happen:

Firstly, the carbohydrate metabolic mechanism becomes strained, and a sudden bordering upon diabetes results. It is well known that many fat women show traces of glycosuria.

Secondly, there is an increased tendency for carbohydrate to be connected to fat, and stored thus in the adipose tissue. Endogenous obesity is characterised by a laying down of fat all over the body, the limbs being affected as well as the trunk.

A case of pure endogenous obesity gives a history of none of these factors which are conducive to the exogenous type, but is due to/
to disturbance of function of one or other of the endocrine glands, of which the thyroid pituitary body, and ovary are, in this connection, the most important.

A very typical case of exogenous obesity, Miss M., was made the subject of experiment. This was a woman, who, for many years had eaten large quantities of white bread, scones and sweet things generally. She had taken little exercise, and wore many clothes. In her case there was no evidence that endocrine disturbance was the primary cause of obesity. Her periods were normal and regular, her basal metabolic rate was normal or slightly increased, and the distribution of the fat was quite atypical of hypopituitrism. With exercise, she showed behaviour typical of a diabetic. With a small amount of work, 11,000 kilogram metres, her respiratory quotient for exercise and recovery was 0.75, showing how quickly she utilised fat as a source of energy. Her blood fat content also increased, with this small amount of exercise. This close resemblance between the behaviour with exercise, of exogenous obesity and diabetic may be due to either or both of two causes. Either it may be due to the derangement of the carbohydrate oxidative mechanism, or to an increased availability of fat. These points will be further discussed later.

Endogenous obesity was studied in the case of John H. who suffered from hypopituitrism. This is a condition found in both men and women, and there is no definite age period. The
fat is laid down in definite parts, while the ankles and wrists remain thin, fat is laid down over the hips, abdomen and chest, giving in men the typically rounded feminine contours, which were observed in this case, associated with retrogression of the sexual organs. In this condition, too, there is an increased sugar tolerance, it being almost impossible to produce glycosuria. The following report was obtained from an X-ray examination of John H.:

"The sella is abnormally small, and the anterior and posterior clinoid bones meet so as partially to enclose the pituitary fossa. The appearances suggest a deficiency of the pituitary".

He responded to exercise in an opposite manner to the exogenously obese person Miss M. He showed an entirely normal ability to utilise carbohydrate as the source of energy for muscular work. With 6000 kilogram metres, his respiratory quotient for exercise and recovery was only slightly below unity, which is what one would expect to obtain from a normal subject who had spent a long time in bed, as had this subject. It will be very interesting indeed to see if, when able to perform larger amounts of exercise, he will continue to utilise sugar to a normal, or to a slightly greater than normal extent.

The point of interest in this study, is the entirely different response to exercise given by exogenous and endogenous types of obesity. So marked was the difference, that this method might well be used to distinguish between the two types, often a matter/
matter of considerable difficulty to the clinician.

A further interesting subject for speculation was found in the course of the series of experiments upon the exogenously obese subject, Miss M., a part of whose treatment consisted in the administration of thyroid extract. So tolerant was she to this drug, that doses up to 18 grains a day could be administered without anxiety as to her cardiac condition. It was found that although the administration of thyroid produced no alteration in her basal respiratory quotient which remained as before at the low figure of 0.68, it yet had a profound effect on her response to exercise. As has already been said, her respiratory quotient for exercise and recovery, when without thyroid, was 0.75 for a small amount of work, yet when fully under the influence of the drug, as shown by an increased loss of weight, her respiratory quotient, for practically the same amount of work, was 0.96. This striking though somewhat paradoxical result would suggest that the effect of thyroid therapy in exogenously obese subjects, is to cause them to perform work in the same way as it is performed by a normal person, though the precise manner in which such a result is obtained, is still a subject of mystery, and will require future research to elucidate. It also suggests another field of investigation, namely, the effect of thyroid administration on the metabolism during exercise, of myxoedematous persons.

This study, then, incomplete though it is, has undoubtedly/
undoubtedly elucidated a number of problems interesting alike to the biochemist and the physician. The difficult diagnosis of exogenous as distinct from endogenous obesity, bids fair to become a comparatively easy matter, if the preliminary results which have been discussed are confirmed by further work. Of great interest, too, particularly in view of the common coincidence of obesity and glycosuria, is the response to exercise in exogenous obesity. The relationship between the alkali reserve and the fat content of the blood, raises questions of interest to the chemist, who must consider the effect of altered ion equilibrium on the stability of a fat emulsion, and to the clinician, who, as in cases of severe untreated diabetes, is frequently confronted with co-incident acidosis and lypaemia.

The action of thyroid in stimulating carbohydrate metabolism in obesity to follow the normal course, affords a scientific rationale of its therapeutic action in this condition which has hitherto been largely a matter of empiricism, its administration in myxedema having a directly opposite though equally beneficial effect.

Though apparently without any direct clinical application, the conclusion that fat is used as such in muscular exercise, and is not previously converted into carbohydrate - a conclusion which follows inevitably from the view that the diabetic suffers from an incapacity to oxidise glucose - provides an answer to a question which has been debated by many biochemists and/
and physiologists for many years.

The problems outlined in the introduction to this essay, have not yet been completely solved, yet it is perhaps not going too far to say, that the experimental work which has been discussed, throws light on some of them, and suggests lines along which further research may lead to their ultimate solution. If this be so, the labour involved has not been in vain.