LXXXI. FAT METABOLISM IN MUSCULAR EXERCISE.

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A RISE in the blood-fat during muscular exercise was first described by Patterson [1927], whose experiments left no doubt as to the reality of the phenomenon. His suggestion that the rise in blood-fat is a response to the demand of the working muscles for fat agrees with the observation of Lafon [1913] that the blood leaving a working muscle contains less fat than the blood entering it. The phenomenon of an alteration in the lipid content of the blood during muscular exercise appears to be of importance not only from the point of view of blood chemistry, but also on account of the controversy which has arisen as to whether muscle is capable of utilising fat as a source of energy. The experiments described in this paper have been undertaken to extend the work of Patterson by elucidating the source and the nature of the extra fat which appears in the blood, the cause of its appearance, the chemistry of muscle with respect to the use of fat, and the relationship between the rate of utilisation of fat and its concentration in the blood.

EXPERIMENTAL.

Preparation of the subject. The subjects, with one or two exceptions noted later, were healthy young men, used to taking moderate exercise, and were on a normal mixed diet. At the time of the experiment they had fasted for 13–15 hours. They had either spent the night in the laboratory, or had rested there in bed since early morning. In either case, a state as nearly basal as possible was assured at the commencement of the experiment. For those experiments involving measurements of the gaseous exchange, this condition was obviously essential, since it provided the only possible base line from which to measure the excess metabolism during the exercise. Further, preliminary experiments showed that any previous activity on the part of the subject considerably modified the effect of a given amount of muscular work on the level of the blood-fat. These precautions were not observed by Patterson in all of his experiments, which doubtless explains some of the quantitative discrepancies between his results and those about to be described.

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Mode of exercise. The subjects were exercised on a bicycle ergometer at a steady rate. To most of the subjects cycling was an unaccustomed form of exercise, and it was found impossible for them to work for long at a greater rate than about 1100 kg. m. per minute. Short periods of work such as were employed by Patterson in his running experiments were not feasible, since amount rather than rate of work seemed to be the important factor. To avoid a training effect the same subject was not used for more than one experiment except after the lapse of a considerable period.

Experiments involving measurement of respiratory exchange. After the subject had reached basal conditions, and had emptied his bladder, his expired air was collected in a 200 litre spirometer for a period of 25 minutes. A sample of blood was drawn and the bladder was again emptied. The work was then begun, and at intervals during its progress the expired air was collected for a period of 1 minute, the subject being connected to the spirometer for this purpose without interruption of the steady state of work. A number of preliminary experiments satisfied us that extrapolation from the results of these separate collections gave nearly as accurate a figure for the gaseous exchange over the whole period as the much more cumbersome and uncomfortable procedure of collecting all the expired air throughout the exercise. The oxygen consumption reached a steady value about 5 minutes from the commencement of exercise, and it was therefore necessary only to confirm this in each case by taking two samples during the latter part of the exercise with, in addition, one sample at the second or third minute of exercise. Special care was needed at the end of the exercise on account of the sudden alteration in the respiratory exchange at the beginning of recovery. On the completion of the work, the subject immediately lay down and was once more connected to the spirometer. A second sample of blood was then taken. Collection of the expired air was continued until the subject had again reached a basal condition, as shown by pulse and respiration rate, and minute volume of expired air. At the end of this period—usually an hour was allowed—the subject again emptied his bladder and a third sample of blood was procured.

Experiments not involving measurement of respiratory exchange. In two series of experiments it was not feasible to measure the respiratory exchange. The first of these was undertaken in order to determine the effect of a short period of work, following immediately after apparent recovery from one of sufficient magnitude to produce a rise in the blood-fat. In one or two of these the respiratory exchange was determined over the first part of the experiment, but it was found that the discomfort of the mask, worn over such a long period of time, precluded the proper return of the subject to basal conditions after the second period of exercise. In these experiments blood was drawn before and after each period of exercise. The second series had as its object the determination of changes in the blood during the progress of exercise, and blood samples were withdrawn, not only before and after, but at intervals during the performance of work. This procedure necessitated

interruption of the exercise, and, though the withdrawal of samples was performed as expeditiously as possible, the interruption rendered fallacious any measurement of the respiratory exchange with the means at our disposal.

Chemical methods. The respiratory exchange was calculated in the usual way by analysis of samples removed from the spirometer.

The urine was analysed for total nitrogen and urea-nitrogen in order to determine whether any of the extra metabolism during exercise and recovery was due to protein breakdown. It may be said at once that no increase in the rate of nitrogen excretion was found during the experiments (Table I).

Table I.

Subject	Nitrogen excreted during exercise and recovery	Nitrogen excreted during same time under basal conditions
	g.	g.
R.G.	1.23	1.36
D.M.D.	0.80	0.80
C.P.S.	0.79	1.03
J.B.R.	1.28	1.05
D.S.	1.29	1.25
R.E.I.	1.08	1.18
De V.	0.75	0.84
W.McL.	0.35	0.49

Usually, in fact, the urine secreted during the period of exercise and recovery contained rather less nitrogen than that for the corresponding basal period. This slight decrease, no doubt, is to be attributed to an increased elimination of nitrogen by the skin.

In the various blood samples, total fat, soap, total cholesterol, lipid phosphorus, the iodine number of the fatty acids, and the carbon dioxide-combining power were estimated.

The method used to estimate the total fat in blood was a modification of that of Stewart and White [1925]. This method has been criticised by Stoddart and Drury [1929]. In the work reported here no difficulty was found in obtaining duplicate analyses agreeing to within 5 % by using the technique of Stewart and White, and several other workers in this laboratory have also used this method with good results. It has long been realised, however, that this method of analysis estimates the phosphoric acid from the phospholipins as well as the fatty acids obtained from the fats and phospholipins by hydrolysis. To overcome this difficulty, a modification of the original technique was devised, involving filtration—the same device as was introduced by Stoddart and Drury [1929]. As before, the total lipoids were extracted from blood with alcohol-ether mixture, and were saponified by evaporation almost to dryness with 5 cc. of *N*/10 sodium hydroxide. Whereas in the original method, this alkali was exactly neutralised by adding 5 cc. of *N*/10 hydrochloric acid, a slight excess of acid was now added, with thorough shaking to aid liberation of the free fatty acids; the mixture was warmed to produce coagulation of the fine particles, and filtered through a fat-free filter-paper. The precipitated fatty acids were washed three times with 1 cc. of 5 % sodium chloride. The precipitate was then dissolved in alcohol and the solution was made up to 10 cc. 1 cc. of this solution was titrated with *N*/10 sodium hydroxide, using the Rehberg burette. From the mean of three titrations, the blood-fat was calculated as tripalmitin.

The use of this modification gave results which were consistently lower than those given by the original Stewart and White method. If the phosphoric acid of the phospholipins of the blood were titrating as a monobasic acid, the difference in the results given by the two methods would

be accounted for. Table II shows the results of analyses of bloods in which fat was estimated by both methods, and the lipid phosphorus was also determined. It shows how closely the difference in the fat content as determined by the two methods agrees with the fatty acid which would correspond to the lipid phosphorus if it were titrating as a monobasic acid.

Table II.

Blood sample	Fat (mg./100 cc.)		Lipid phosphorus (mg./100 cc.)	
	Original method	Modified method	Observed	Calculated
1	654, 657	501, 521	15.0	16.0
2	659, 679	444, 454	20.1	24.0
3	426, 429	331, 338	12.3	10.4
4	461, 491	320, 350	16.4	16.0
5	659, 660	446, 442	22.5	24.0

The method of Stewart and White [1929] was used for the estimation of soaps, that of Myers and Wardell [1918] for cholesterol, that of McClure and Huntsinger [1928] for iodine values, that of Van Slyke and Neill [1924] for carbon dioxide-combining power, and that of Briggs [1924] for lipid phosphorus.

RESULTS AND DISCUSSION.

The utilisation of fat by muscle. The use of the R.Q. for determining the nature of the fuel used during muscular exercise demands as a primary condition that the body shall be in exactly the same condition at the beginning and end of the measured period except for the loss of fuel. The probability is that this condition, except perhaps in very mild exercise of short duration, is never entirely fulfilled. Not only must the oxygen consumption and the R.Q. have returned to their original levels—which involves the inclusion of a recovery period—but so must the lactic acid, carbon dioxide content and combining power of the blood. Even so, the conditions before and after exercise may not be strictly comparable, since even moderate exercise involves the excretion of lactic acid, and it is not known how this excretion affects the urinary content of bicarbonate ions or the ammonia/urea ratio. Such factors as these, however, though they may introduce considerable errors in experiments of short duration where the gas volumes dealt with are small, cannot have a very great effect on the longer experiments reported here. The particular ones mentioned above, in fact, would tend to make the observed R.Q. higher than the actual one. Hence though they, and others of the same kind, may afford at any rate a partial explanation of the very high respiratory quotients observed by Best, Furasawa and Ridout [1928], in violent exercise of very short duration, they do not invalidate the deductions drawn here.

In Fig. 1 the amount of work performed by a number of subjects is plotted against the R.Q. for exercise and recovery at the end of which the oxygen consumption, R.Q., and carbon dioxide-combining power of the blood had returned to normal. The figures used in constructing this curve were, of course, those for the extra metabolism due to the exercise, the basal metabolism having been deducted from the gross figures.

In three cases the R.Q. for small amounts of work was very much lower

than was expected, a result which indicates, if the conclusions drawn later are justified, a much earlier usage of fat. In these cases there appears to be either a relative inability to utilise glycogen in the normal way or a very low store of glycogen, and it is significant that all three are cases of diabetes mellitus. Since the evidence in the literature [Lusk, 1928] indicates that in

Fig. 1. Relationship between the amount of work and the R.Q. for exercise and recovery.

• Normal.

○ Diabetic.

this condition the muscle-glycogen is maintained at the usual level long after the remaining stores are depleted, and since the cases reported here were under control, it is reasonable to conclude that the fault lay in the utilisation of carbohydrate rather than in its lack. It is significant that glycosuria was not produced by the exercise.

With small amounts of work (4000–5000 kg. m.), the excess R.Q. was at or very near 1.0 (in two cases values appreciably above this were obtained), but with increasing amounts of work, the R.Q. for exercise and recovery steadily fell. In spite of the fact that twelve individuals are represented in the curve, the proportionality between the amount of work performed and the R.Q. is definitely marked, and goes far to support the substantial truth of the figures. It is perhaps advisable to reiterate here that the rate at which the different subjects performed the work was nearly constant, the tension on the ergometer bicycle and the rate of pedalling being kept as nearly constant as possible. The conclusion seems justified that, although the muscles prefer carbohydrate as a fuel, they are fairly rapidly forced to fall back on some other

fuel of lower R.Q. In no case did the results of urine analysis indicate any usage of protein.

There remains fat, which, therefore, one is forced to suppose is used by the muscles as a secondary source of energy. The alternative hypothesis that extra fat is used in the basal processes, thereby liberating carbohydrate for use in the working muscles, is disproved by a consideration of the amounts involved. Thus in one experiment, with R.Q. 0.96 for exercise and recovery, the relevant data are as follows. This experiment has been chosen for illustrative purposes since, with the small amount of fat utilisation involved, it offers a less convincing example than those experiments with lower R.Q.

R.Q. for exercise and recovery	0.96
Oxygen consumption for exercise and recovery	24.17 litres
Duration of exercise and recovery	85 minutes
Calculated fat used during exercise and recovery	3.31 g.
Heat equivalent of fat used	31.35 cal.
Basal oxygen consumption for 85 minutes	26.28 litres
Basal R.Q.	0.77
Nitrogen excretion during 85 minutes	1.097 g.
Carbohydrate used in basal metabolism during 85 minutes	3.55 g.
Heat equivalent of carbohydrate	14.86 cal.

The amounts of fat and carbohydrate are calculated from the tables given by Lusk [1928]. It is evident that the energy available from the whole of the carbohydrate used for basal metabolism during the period of exercise and recovery would not, if entirely diverted to the working muscles, obviate the necessity of their calling upon fat.

The R.Q., though it allows the deduction that fat is used as a fuel by the working muscles, gives no information as to whether the fat is used directly or only by conversion to carbohydrate.

The controversy as to which of these alternative routes is followed by the fat used in muscular exercise hinges largely on the problem of the relative efficiency of fat and carbohydrate.

It is rightly argued that if fat is utilised by direct oxidation, muscular efficiency should be unaltered when it replaces carbohydrate; on the other hand, utilisation only of carbohydrate derived from fat necessarily implies a loss of energy, and therefore a decreased efficiency. Moreover, the loss of efficiency should be the same for every individual. In considering the magnitude of such a hypothetical loss of efficiency, one is faced with the difficulty that the conversion of fat to carbohydrate in the animal body has never been conclusively proved, and that those workers whose experiments have seemed to demonstrate the possibility of such a conversion have thrown no light on the mechanism. The smallest wastage of energy, as has often been pointed out, would occur in the unlikely event of conversion of the whole of the carbon of the fat molecule into carbohydrate, and even then some 25 % of the total available energy would be lost.

The classical work of Krogh and Lindhard [1920], in which the maximum

loss of efficiency was found to be only 11 %, is often cited as disproving the fat to carbohydrate conversion hypothesis. The original authors, however, are more cautious, and merely state that their experiments cannot be used as proof that fat is necessarily converted to carbohydrate before being utilised by working muscle.

The change over from carbohydrate to fat as muscular fuel is, of course, not sudden and complete. Even when fat has become the preponderating source of energy, carbohydrate is still being used though in diminishing amount. This is shown in Fig. 2, in which the amounts of fat and carbohydrate used for various amounts of work (and recovery) are plotted against the amounts of external work performed. The figures for the amounts of fat and carbohydrate used are calculated in the usual way from the observed excess oxygen consumption and R.Q. It is evident from the curves that as the amount of work increases the amount of fat used increases, but so also does the amount of carbohydrate. Even with R.Q. 0.80, carbohydrate is still being used; the R.Q. is not simply the mean result of the early exclusive use of carbohydrate and the later exclusive use of fat.

Fig. 2. Relationship between the amount of external work and the amounts of fat and carbohydrate oxidised.

The R.Q. for a complete period of work and recovery can never be that of fat alone—except possibly in the case of a complete diabetic, and then only on the assumption that the diabetic cannot oxidise carbohydrate or lactic acid. Hence the loss of efficiency to be expected is always less, and, with R.Q. 0.80

and over, very much less than the calculated 25 %. Thus, in one of the experiments made by the present authors, the R.Q. and oxygen consumption for work and recovery showed the utilisation of 56.8 g. of carbohydrate and 21.56 g. of fat, with R.Q. 0.86. A simple calculation shows the expected loss of efficiency to be only 11.5 %.

In the experiments of the present authors no loss of efficiency was observed when work was performed at the expense of fat (Table III). It is admitted that the experiments were not specially designed for the purpose of showing such change in efficiency, and that in no case was a series of experiments undertaken upon the same individual with varying amounts of work and at varying R.Q. Nevertheless, since the conversion of fat into carbohydrate involves a definite wastage of energy irrespective of individuals, it seems fair to suppose that any such wastage would be shown in the average results from a large number of subjects. Since the subjects were not trained cyclists, the fact that only one experiment was performed upon each was an actual advantage, for it eliminated any fallacy due to training. The numbers reported here are not, it is true, large, but, as they show no suggestion of a wastage of energy with the lower R.Q., and as the distribution in each series is nearly the same, it has been thought worth while to report them (Table III).

Table III.

R.Q.	...	...	1.0	0.95-0.99	0.90-0.94	0.85-0.89	0.80-0.84
Lowest			4.76	4.23	4.32	4.11	4.06
Highest			5.71	5.15	5.38	5.65	5.70
Mean (no. of exps)			5.12 (6)	4.82 (4)	4.86 (6)	4.78 (7)	4.60 (6)

They differ radically, of course, from those of Krogh and Lindhard [1920], and it seems possible that the difference may lie in the fact that all the subjects dealt with here were on an ordinary mixed diet, while Krogh and Lindhard used high and low carbohydrate diets to obtain the variations in R.Q. Moreover, the rate of work in the experiments reported here was much greater than in those of Krogh and Lindhard.

A wastage of energy in the utilisation of fat should become apparent not only in the calculated efficiency, but also in another way. The wastage, if fat is converted to carbohydrate, is due to some such process as loss of hydrogen which is oxidised to water. Thus, in the most favourable conversion,

The utilisation by the muscles of 1 g. of glycogen derived from fat demands, according to this equation, the consumption of 1.099 litres of oxygen instead of the 0.828 litre required by the glycogen itself—an oxygen wastage of 31.5 %. On the other hand, the direct utilisation of fat involves no loss of oxygen, and the curve showing oxygen consumption per calorie equivalent of work done is simply that of the “calorific value” of oxygen calculated by Zuntz and Schumburg [1901]. These two curves for the consumption of oxygen

per calorie equivalent of work are shown in Fig. 3. In these curves allowance is made for the simultaneous use of carbohydrate, the relative amounts of the two fuels being calculated on the basis of Fig. 1. The experimentally determined values are also shown in Fig. 3, and, though there is a certain amount of scattering due to the employment of many different individuals, it is evident that by far the closer approximation is given by the curve corresponding to direct utilisation of fat.

In the curve, as in the table of efficiencies, are included the results from the three diabetic subjects, and it is interesting to note that in spite of their

Fig. 3. Relationship between the amount of work and the oxygen consumption for work and recovery. The continuous line represents the volume of oxygen expected if there is no wastage consequent upon the utilisation of fat. The dotted line represents the oxygen consumption calculated on the basis of a 30 % wastage for the conversion of fat to carbohydrate.

• Normal.

○ Diabetic.

abnormally low R.Q., indicating the utilisation of an abnormally high proportion of fat, they show no deviation from the normal efficiency.

Though no finality is, of course, claimed for these results, they tend very

definitely to oppose the view of the necessary conversion of fat to carbohydrate prior to its utilisation by the working muscles, and to support the view that fat and glycogen are alternative fuels although the latter appears to be used preferentially.

The theoretical need for a conversion of fat to carbohydrate has, indeed, been greatly weakened by the recent work of Eggleton and Eggleton [1927] on creatinephosphoric acid and of Lundsgaard [1930] on muscle poisoned with iodoacetic acid, which appears to show that the breakdown of glycogen to lactic acid can no longer be regarded as an essential in muscular contraction. Even if the glycogen-lactic acid cycle be considered essential, it may well be that fat, by its direct oxidation, is capable of sparing lactic acid, leaving more for resynthesis to glycogen and so conserving the supplies of carbohydrate.

It is perhaps worth pointing out, though with diffidence in view of modern ideas as to the function of insulin, that if it be admitted that diabetes mellitus involves a failure to oxidise carbohydrate, calculations from cases of nearly complete diabetes show that such diabetics do not convert fat to carbohydrate. Such a conclusion follows, *e.g.* from the data in the case reported by Geyelin and Du Bois [1916], in which the observed respiratory exchange and heat production can only be explained in this way. The diabetic can oxidise fat as far as acetoacetic acid, and it is therefore a fair conclusion that any conversion of fat to carbohydrate is possible only after this stage, *e.g.* by oxidation to acetaldehyde followed by aldol condensation. Such a conversion would involve a wastage, not of 25 %, but of 80 % of the energy of fat, and would render quite impossible of accomplishment the amounts of work actually observed.

The variation in blood-fat in muscular exercise. The results of blood analysis (Table IV) show that the majority of normal subjects do experience some increase in the blood-fat, when, under fasting conditions, they perform work in excess of approximately 8000 to 10,000 kg. m. This figure is only approximate, since the exact point at which the rise in the blood-fat occurs depends to a considerable extent on the rate at which the work is performed. Table IV gives typical results of blood analyses. The experiments on normal subjects (*A*) show that the rise in blood-fat does not occur in every case (*e.g.* C.P.S., D.S.); when it does occur, it is transient, and has disappeared at the end of the recovery period.

Patterson [1926] observed that diabetic subjects almost invariably showed a fall in the blood-fat, even with the relatively small amounts of work they were able to perform. All his diabetic subjects, however, had initially an abnormally high blood-fat, a state of affairs which is usually present in this disease. The experiments summarised in Table IV *B* show that this fall in the blood-fat produced by exercise is characteristic, not of diabetes mellitus, but of the incidental high blood-fat level. It occurred in subjects whose fasting blood-fat was abnormally high, but who were apparently healthy in all other respects. Moreover, in two diabetic cases (Table IV *C*), with the basal blood-

Table IV.

	Subject	Work kg. m.	Blood sample	Fat mg./100 cc.	Cholesterol mg./100 cc.	Lipoid P mg./100 cc.	Soap mg./100 cc.
A	R.G.	5,500	<i>a</i>	492	134	—	40.0
			<i>b</i>	433	139	—	37.9
			<i>c</i>	481	128	—	41.2
			<i>d</i>	579	133	—	41.2
	C.P.S.	14,966	<i>a</i>	689	136	21.7	27.9
			<i>b</i>	693	136	21.9	17.6
			<i>c</i>	522	137	21.6	24.6
			<i>d</i>	562	137	20.0	27.2
	D.S.	18,564	<i>a</i>	478	143	20.7	—
			<i>b</i>	453	144	22.3	—
			<i>c</i>	450	125	23.1	—
			<i>d</i>	481	127	20.3	—
	R.E.I.	19,123	<i>a</i>	607	118	13.3	30.7
			<i>b</i>	791	114	12.1	30.2
			<i>d</i>	583	118	11.6	31.2
	R.G.	28,050	<i>a</i>	480	145	—	30.7
			<i>b</i>	588	144	—	45.7
			<i>d</i>	434	146	—	39.8
	R.E.I.	33,000	<i>a</i>	511	118	18.8	23.9
			<i>b</i>	674	118	16.5	25.2
			<i>c</i>	467	—	19.0	25.0
			<i>d</i>	425	—	15.0	23.9
	De V.	37,150	<i>a</i>	376	120	15.3	23.6
			<i>b</i>	511	142	15.2	24.6
			<i>c</i>	464	127	17.2	24.1
			<i>d</i>	460	122	17.5	24.5
B	D.M.D.	4,300	<i>a</i>	853	208	21.7	28.2
			<i>b</i>	685	209	21.9	26.7
			<i>c</i>	522	208	21.8	22.6
			<i>d</i>	562	207	21.7	27.2
	J.B.R.	14,000	<i>a</i>	1103	139	15.5	29.7
			<i>b</i>	831	141	18.3	35.7
			<i>c</i>	694	140	15.1	42.7
			<i>d</i>	899	139	14.5	42.7
C	J.McC.	8,600	<i>a</i>	770	—	—	—
			<i>b</i>	825	—	—	—
	W.McL.	12,300	<i>a</i>	690	—	—	—
			<i>b</i>	812	—	—	—
D	F.P.C.	22,250	<i>a</i>	310	119	15.9	36.0
			<i>b</i>	660	121	15.5	34.7
			<i>c</i>	510	121	—	35.7
			<i>d</i>	410	120	15.6	35.2

a = Before work.*b* = After work.*c* = After partial recovery.*d* = After complete recovery.

fat not much above the normal level, exercise produced a small but definite increase as easily as in normal subjects. Nor, probably, is it mere coincidence that in one case the converse appeared to hold, and that a healthy subject with an abnormally low fasting blood-fat level showed an abnormally great increase after a moderate amount of work (Table IV *D*). These results lead to the conclusion that the blood-fat content tends to reach the normal level before it shows the increase usually given by healthy individuals. This preliminary change takes place under the influence of moderate work, definitely

below the amount required to produce a change in the blood-fat content of subjects whose basal blood-fat is within the normal range.

Those experiments in which the subject performed a second period of work after recovery from the first (Table V) demonstrate that a rise in the blood-fat is rather more easily obtained during the second period than during the first. This point is of importance as emphasising the necessity of obtaining basal conditions before starting the exercise if comparable results are to be obtained from a series of experiments on different individuals. It also tends to support the contention that the increase in blood-fat during exercise is in some way connected with the utilisation of fat by the muscles. There can be no doubt that at the commencement of the second period of exercise the body stores of glycogen have been somewhat depleted, and hence the muscles—if they can use fat—must call for fat earlier, though only slightly so, than in the first period.

Table V.

Subject	Blood sample	Fat mg./100 cc.	Cholesterol mg./100 cc.	Lipoid phosphorus mg./100 cc.
R.G.	Basal	611	145	14.6
	After 1st work (28,000 kg. m.)	719	144	14.2
	After recovery	565	146	14.6
	After 2nd work (11,000 kg. m.)	619	147	14.3
R.E.I.	Basal	607	118	13.3
	After 1st work (19,000 kg. m.)	791	114	13.1
	After recovery	583	118	11.6
	After 2nd work (9000 kg. m.)	647	118	13.7

The results so far described confirm the statement that muscular exercise is accompanied by an increase in the blood-fat, though they show that this is not an invariable result. It was desired, however, to find in what constituents of the total blood-fat the increase occurred. The analyses (Tables IV and VI) showed that the increase lay solely in the triglyceride fraction, which, according to Channon and Collinson [1929], forms a very small part of the total blood-lipoids under resting conditions. While the total fatty acids increased, the lipoid phosphorus and the cholesterol showed either no change or only small and inconstant variations. The free fatty acid (soap) content of the blood remained unaltered, and though there was possibly an increase in the amount of esterified cholesterol, any such increase was quite incapable of accounting for the observed increase in the total fatty acid. Figures in support

Table VI.

Subject	Total cholesterol mg./100 cc.	Fat required to esterify completely mg./100 cc.	Rise in fat mg./100 cc.
R.E.I.	118	13.7	163
FF.P.C.	121	14.0	350
De V.	142	16.5	135
R.G.	144	16.7	108
R.E.I.	114	13.2	184
R.M.L.	136	15.8	92
R.G.	129	15.0	220

of this statement are given in Table VI, which is based on the ratio of esterified to unesterified cholesterol given by Channon and Collinson [1929].

Finally, since Patterson's figures were obtained by the use of the original Stewart and White method, it is important to note that exactly similar results are obtained—as in the experiments here described—by use of the modified method which estimates only fatty acids and does not include phosphoric acid. Otherwise the apparent increase in the blood-fat during exercise might conceivably have been regarded as due merely to an altered state of dissociation of the phospholipins.

The iodine value of the blood-fat before and after exercise was determined in a number of cases (Table VII), and although it was found to be very variable for different subjects, it altered only very little in the same subject as a result of exercise, and in no case did it show a rise. This suggests very strongly that the source of the fat poured into the blood stream in response to stimulation by exercise is the adipose tissue and not the liver.

Table VII.

Subject	Work	Fat mg./100 cc.	Iodine value	Subject	Work	Fat mg./100 cc.	Iodine value
T.F.	Basal	426	63	E.B.H.	Basal	364	26
	11,550 kg. m.	421	65		13,250 kg. m.	374	24
	16,885	661	52		20,500	485	14
	24,650	448	32		26,650	387	26
E.G.	Basal	660	84	A.T.B.	Basal	444	22
	12,750 kg. m.	690	65		10,250 kg. m.	454	27
	18,965	865	62		16,250	454	34
	28,000	757	78		23,050	444	36

The variation of the blood-fat during muscular exercise. Those experiments in which blood-fat estimations were made during the performance of work showed a curious result which ultimately shed light on the marked individual variations and apparently haphazard responses obtained in previous experiments. It appeared that, instead of the blood-fat steadily increasing during exercise, it first rose and then fell. This result was obtained in a considerable number of individuals, and typical cases are shown in Fig. 4. When the results were plotted together as a composite curve, it appeared that actually there was a rise in the blood-fat, followed by a fall and then by a second rise (Fig. 5, dotted line). Moreover, the results obtained from nearly all the previous experiments could be fitted to this curve. Certain exceptions were noted, as when the rate of work differed markedly from that usually employed, or when the subject had an abnormal resting blood-fat.

The shape of the composite curve had been disguised in the later individual experiments shown in Fig. 4 by the fact that only three points were obtained. To test whether the exceptions to this composite curve were really due to the one observed variation in experimental technique—the rate of work—a second series was undertaken in which the subjects worked at a rate of 1100 kg. m. per minute instead of the 900 kg. m. per minute which had

Fig. 4. The blood-fat and carbon dioxide-combining power during exercise. Individual cases. (Ordinates as for Fig. 5.)

Fig. 5. Composite curves showing changes in the blood-fat concentration and the blood carbon dioxide-combining power during exercise. The dotted line represents changes at 900 kg. m. per minute, the continuous line at 1100 kg. m. per minute.

previously been the rule. The results of blood-fat determination from this series fell on a second composite curve (the continuous line in Fig. 5) of exactly the same shape as the first, but showing the changes in blood-fat concentration to take place after a smaller amount of work than previously. These variations in the blood-fat during work were entirely unexpected, and could not be correlated with a steadily increasing consumption of fat by the working muscles. Yet experiment had shown that such a steadily increasing consumption did indeed exist (Fig. 2).

It became necessary, therefore, to discard the simple suggestion that the increase in blood-fat during exercise was a direct response to a demand by the muscles for an alternative fuel consequent upon the utilisation of glycogen. Attention was thus directed to the discovery of some stimulus which could act as a connecting link between the two phenomena. The production of lactic acid had suggested itself as a possible causative factor in the rise in blood-fat, which, at that time, was all that had been observed. For ease of measurement the carbon dioxide-combining power of the blood had been determined in all samples. The results of these analyses showed at first somewhat irregular decreases in the carbon dioxide-combining power of the blood following exercise, but it was only when blood samples were analysed during the course of exercise that any real relationship between the blood-fat and the carbon dioxide-combining power was observed. It then transpired that the carbon dioxide-combining power, instead of falling steadily with increasing amounts of work to the acidosis level, followed a course which was roughly the reciprocal of that followed by the blood-fat (see Fig. 4). A composite curve of all the results obtained, just as in the case of the blood-fats, was sinuous, showing a fall followed by a partial recovery and only then a steady progression to the acidosis level.

The second series of experiments in which blood samples were drawn during the progress of exercise showed a composite curve of exactly the same character as the first. Again, however, as in the case of the blood-fat concentration, the fall occurred at an earlier period of the work. Moreover, in both series, the fall in the carbon dioxide-combining power preceded the rise in blood-fat, and its partial recovery preceded the fall in blood-fat. Whether this time relation between the curve of carbon dioxide-combining power and that of the blood-fat concentration has any real significance, it is too early to say definitely. It is none the less interesting to speculate whether it indicates a causative relationship between the two phenomena.

SUMMARY.

1. When normal healthy men performed muscular work on an ergometer bicycle at rates varying from about 800 to 1200 kg. m. per minute, the blood-fat usually rose after about 8000 kg. m. of work had been done.

2. The increase in the blood-fat concentration appeared earlier with greater rates of work.

3. After recovery from a first period of work, a second period produced a rise in the blood-fat more easily than usual.

4. A high fasting blood-fat in normal or diabetic subjects led to a preliminary fall when work was performed; an abnormally low blood-fat was increased by relatively small amounts of work.

5. This alteration in the blood-fat was confined to the glyceride fraction, and the extra fat was probably derived from the adipose tissue.

6. Continuance of work led to a return of the blood-fat towards normal (at about 18,000 to 21,000 kg. m.), and later to a second rise.

7. The carbon dioxide-combining power of the blood during muscular work of this type followed a course which was roughly the reciprocal of the blood-fat, but the changes in the carbon dioxide-combining power preceded those in the blood-fat.

8. The R.Q. for exercise and recovery was unity for amounts of work up to about 5000 kg. m., and thereafter fell steadily with increasing amounts of work.

9. Analysis of the R.Q., oxygen consumption, and nitrogen excretion showed that protein was not used for work, that carbohydrate continued to be used throughout, though in decreasing amounts, and that fat was used in increasing amounts.

10. There was no relationship between the utilisation of fat and the changes in the blood-fat concentration.

11. No diminution in efficiency was observed at the lower R.Q., and the figures did not suggest any conversion of fat to carbohydrate.

12. Diabetic subjects, though performing work at a lower R.Q. than normals, showed no less efficiency, and no wastage of oxygen.

13. A modification of the Stewart and White [1925] method for the estimation of blood-fat is described.

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