CXXVIII. BLOOD-FAT AND EXERCISE.

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IN the course of a series of investigations into the variations of the fasting blood-fat level on diets of low and high fat contents a very definite increase after exercise was noticed. This was accidentally discovered in samples withdrawn after a game of hockey, and was quite distinct from the original line of research, but so definite as to warrant a separate investigation.

EXPERIMENTAL.

The method of estimation of the blood-fat content followed in this paper is that of Stewart and White [1925] and has been found eminently satisfactory.

In spite of the lengthy series of manipulations involved it was found possible to estimate the fat in a solution of known concentration within 3 %, while duplicate samples of blood gave results differing by no more than 2 %.

In addition to the warnings given by Stewart and White, the author has found it necessary to redistil all the anhydrous spirit used with sodium hydroxide, as the slight acidity of the spirit supplied was sufficient to interfere with the accuracy of results. He has, furthermore, found it necessary to interpose a CO₂ trap between the siphon system and the capillary distributing the air current to the titrating fluids. This trap consists of a concentrated solution of sodium hydroxide through which the air bubbles, having passed through the siphon system. To rely entirely on a soda-lime tower guarding the inlet of the siphon system was unsatisfactory, and, moreover, was not effective when interchange of siphon bottles had to take place. By placing this trap beyond the siphon system, however, one can interchange the bottles without any fear of the air supply becoming contaminated with CO₂.

The blood-sugar was estimated by Benedict's method [1918]. The results are all high and in later investigations the method of Folin and Wu [1920] has been used. In a series of estimations comparing the results of estimation by both these methods, the Benedict technique has been observed to give results consistently 20 % above those by the Folin-Wu method.

The cholesterol content was determined by Bloor's modification [1915] of the Autenrieth-Funk process [1913].

The air samples for the determination of the respiratory quotient were collected by means of the Douglas Bag [1911] with subsequent analysis by means of Haldane's [1918] gas analysis apparatus.

The percentage of the corpuscles was estimated by the haematocrit.

RESULTS.

Preliminary experiments (Table I) showed that the fasting fat content remained remarkably constant for a given individual over a considerable period of time.

Table I.

Subject	Date	Fasting level mg. per 100 cc.	% variation
E. M. F.	Nov. 17, 1926	661	6.5
	Feb. 16, 1927	615	
I. H. M.	Dec. 4, 1926	535	3.0
	Feb. 28, 1927	520	
A. J.	Nov. 27, 1926	696	1.0
	Mar. 2, 1927	690	
W. M. A.	Dec. 3, 1926	612	3.0
	Feb. 14, 1927	627	
M. C. M.	Nov. 12, 1926	439	1.5
	Feb. 10, 1927	433	

The results of the effect of exercise are shown in Table II.

In each individual case the same amount of exercise has been performed (1) in the fasting state, (2) after the administration of 100 g. glucose by the mouth. In all cases specimen *A* is a basal fasting sample. In the experiments where glucose has been given the 100 g. are taken immediately after the collection of specimen *A*, and specimens *B* and *C* are collected at intervals of $\frac{1}{2}$ and 1 hour respectively, the patient resting in the interval. Specimen *D* is in all cases the sample immediately after exercise. Specimens *E* and *F* are collected at the times noted with the patient resting after exercise.

The subjects of the experiments were all healthy medical students.

Case E. M. F. Nov. 17, 1926.

Fig. 1. Showing effect of exercise in fasting individual.

Figs. 1 and 2 represent in graphic form each type of change.

Fig. 1 (case E. M. F.) shows the effect of exercise in the fasting individual. Cholesterol and R.Q. changes are recorded.

Table II.

Case	Specimen	Remarks	Time	Fat (mg. per 100 cc.)	Sugar (mg. per 100 cc.)	Cholesterol (mg. per 100 cc.)	% corpuscles in blood	R.Q.	Work done
(1)* E. M. F.	A	Fasting	9.35 a.m.	661.5	—	169.4	—	0.739	Running along definite route for 15 minutes
	D	After exercise	9.55 "	947.2	—	202.0	—	0.775	
	E	Resting	10.15 "	779.9	—	173.9	—	0.776	
	F	Resting	11.45 "	609.7	—	212.7	—	0.726	
(2)†	A	Fasting	10.10 a.m.	615.6	119.0	—	38	—	Running along same route for same time
	B	$\frac{1}{2}$ hour after sugar	10.40 "	627.5	154.0	—	—	—	
	C	1 hour after sugar	11.10 "	577.2	129.0	—	32	—	
	D	After exercise	11.30 "	571.2	285.0	—	52	—	
	E	Resting	12.5 p.m.	461.7	222.0	—	—	—	
(1)* I. H. M.	A	Fasting	10.30 a.m.	520.9	160.0	—	34	—	Bicycle ergometer. Work done = 28,632 kg. metres
	D	After exercise	11.0 "	701.5	270.0	—	38	—	
	E	Resting	11.5 "	734.0	116.0	—	57	—	
	F	Resting	11.30 "	686.7	163.0	—	46	—	
(2)†	A	Fasting	10.15 a.m.	535.7	—	200.0	—	—	Bicycle ergometer. Repetition of above exercise. Work done = 28,632 kg. metres
	B	$\frac{1}{2}$ hour after sugar	—	—	—	—	—	—	
	C	1 hour after sugar	11.15 "	515.0	—	215.0	—	—	
	D	After exercise	11.40 "	467.6	—	217.0	—	—	
	E	Resting	12.20 p.m.	518.0	—	208.0	—	—	
(1)* A. J.	A	Fasting	10.45 a.m.	695.6	138.0	—	40	—	Bicycle ergometer. Work done = 37,794 kg. metres
	D	After exercise	11.0 "	843.6	235.0	—	39.6	—	
	E	Resting	11.25 "	858.4	253.0	—	40	—	
	F	Resting	11.35 "	666.0	202.0	—	38	—	
(2)†	A	Fasting	10.10 a.m.	689.6	126.0	208.3	—	—	Bicycle ergometer. Repetition of above exercise. Work done = 37,794 kg. metres
	B	$\frac{1}{2}$ hour after sugar	—	—	—	—	—	—	
	C	1 hour after sugar	11.10 "	680.8	235.0	204.0	—	—	
	D	After exercise	11.25 "	710.4	135.0	204.0	—	—	
	E	Resting	12.15 "	666.0	154.0	212.7	—	—	
(1)* W. M. A.	A	Fasting	10.18 a.m.	627.5	160.0	—	—	—	Bicycle ergometer. Work done = 57,266 kg. metres
	D	After exercise	10.40 "	864.3	227.0	—	—	—	
	E	Resting	11.10 "	488.4	175.4	—	—	—	
	F	Resting	11.30 "	370.0	196.0	—	—	—	
(2)†	A	Fasting	9.50 a.m.	612.7	—	198.0	—	—	Bicycle ergometer. Repetition of above exercise. Work done = 57,266 kg. metres
	B	$\frac{1}{2}$ hour after sugar	—	—	—	—	—	—	
	C	1 hour after sugar	10.50 "	603.8	—	190.4	—	—	
	D	After exercise	11.20 "	580.6	—	202.0	—	—	
	E	Resting	—	—	—	—	—	—	
(1)* M. C. M.	A	Fasting	9.20 a.m.	439.5	—	210.4	—	0.763	Running along definite route for 15 minutes
	D	After exercise	9.35 "	597.9	—	197.2	—	0.798	
	E	Resting	10.5 "	630.4	—	206.9	—	0.787	
	F	Resting	10.40 "	598.8	—	200.0	—	—	
(2)†	A	Fasting	10.13 a.m.	433.6	120.0	—	53	—	Running along same route for same time
	B	$\frac{1}{2}$ hour after sugar	10.43 "	245.6	206.2	—	46	—	
	C	1 hour after sugar	11.13 "	553.5	176.9	—	54	—	
	D	After exercise	11.32 "	429.2	281.7	—	—	—	
	E	Resting	11.57 "	432.1	217.3	—	42	—	

* Without glucose.

† With glucose.

Fig. 2 (case M. C. M.) shows the effect of exercise after the ingestion of 100 g. of glucose. The relation of the sugar and fat curves during the first hour of glucose absorption (the subject resting during this period) is discussed later.

DISCUSSION.

The most striking feature of these experiments is the constant increase in the blood-fat content after exercise in the fasting individual, an increase which does not occur after the ingestion of glucose (Figs. 1 and 2).

Case M. C. M. Feb. 10, 1927.

Fig. 2. Showing effect of exercise after glucose (100 g).

The increase obtained is well beyond the margin of experimental error. Thus in case E. M. F. the blood-fat content is increased by 43 %, in I. H. M. by 35 %, in A. J. by 21 %, in W. M. A. by 37 %, and in M. C. M. by 36 %.

It may be argued that this increase is due entirely to the concentration of the blood consequent upon the severe exercise, but in cases E. M. F. and M. C. M., where this question was investigated, the increase in concentration of the blood corpuscles was found to be 4 % in the former and nil in the latter, while the increase in fat content amounted to 35 % and 21 % respectively. Similar findings have resulted from the investigation of this point in cases not yet published, so that one is in a position to state that the registered increase of blood-fat after exercise is greater than that which would result from simple concentration of the blood after exercise and is, in fact, a real and not an apparent increase.

Having established the fact of a definite increase in blood-fat after exercise, one or two other points require to be settled before discussing its significance.

In the first place Leathes and Raper [1925] do not regard the variations of blood-fat content in samples of blood taken "from any accessible vein" as being of any value whatsoever in determining the nature and mechanism of fat transport and utilisation. The significance of such data derived from examination of the blood is discussed in a paper by Lyon [1923] dealing with some factors which influence dosage. Secondly, before a variation in blood-fat content can be of any importance a constant must be established. If we have no constant the increase in blood-fat after exercise loses much of its significance, since such increase might be due to normal fluctuation of the blood-fat level.

The constancy of the blood-fat content in fasting individuals.

Most observers hitherto have found extreme variations in the blood-fat fasting content in successive examinations and White [1926] has mentioned the possible factors influencing the blood-fat level and "the difficulty of obtaining any definite correlation between them."

Terroine, on the other hand [1914], working with dogs, found that any one dog would show a similar amount of fat in the blood provided the conditions remained similar (the amount being subject to a variation of 15 % either way). He found considerable variation, however, in comparing the fat contents of different dogs under similar conditions. In other words, subject to a variation of 15 %, the individual fasting fat content remained steady under similar conditions. This latter view is borne out by the results of this series of experiments on men, save that here the percentage variation is much less. Thus it will be noted (Table I) that while the range of fasting blood-fat content in the five cases of normal students is from 439 mg. to 695 mg. per 100 cc., the fasting blood-fat level in the individual shows a definite tendency to remain constant, and that the increase of blood-fat after exercise is a real and not an apparent increase.

From Table II it is evident that a very definite increase in the blood-fat content occurs after exercise in the fasting individual. It is realised that the exercise involved in a patient moving from one room to another is sufficient to upset the estimations of basal metabolism and blood-pressure for an hour afterwards. It would appear, then, that the variations in fasting blood-fat content found hitherto by other observers may be accounted for by the fact that insufficient attention had been paid to the importance of the effect of exercise as a disturbing factor.

No basal sample was withdrawn in any of the present series of cases until the patient had rested on a couch for an hour.

The increase of the blood-fat content after exercise.

This increase 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 the 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.

This increase in blood-fat content after exercise has been remarked upon by Gage and Fish [1924], who determined the number of fat particles in the blood by microscopic dark-field, high-power investigation. While the method seems somewhat crude, these observers noted a definite increase in fat particles, or "chylomicrons" as they call them, after exercise, and they interpreted the results as meaning that in the ordinary activity of a fasting man the fat is poured out into the blood from the depôts only as it is needed and at the rate it is needed.

They found that it took some time for the fat content to resume normal proportions, and liken the effect of exercise to that of absorption after a fatty meal where the return of the fat to its previous level is slow, the fall being due to "metabolism or assimilation or both as in the curve of digestion."

While this point of the slow return of the blood-fat content to fasting level was not investigated in this series of experiments, the indications are not in support of this finding, for in no case was the examination maintained beyond 2 hours after the exercise, and yet in three of the five cases the blood-fat content had already returned to, or gone lower than, its previous fasting level.

The variations of the blood-fat content after exercise when 100 g. glucose had been taken prior to the exertion.

The increase in fat content does not take place if the patient has had glucose before the exercise, despite the fact that the amount and the severity of the exercise were the same in both the investigations with glucose and without it. In all cases the fat levels after exercise when glucose has been previously taken remain practically constant (Fig. 2).

This suggests that the working cells only call upon the fat when they have insufficient carbohydrate readily available.

The respiratory quotient in relation to exercise.

The existing views on this question are somewhat conflicting and not only do the interpretations of experimental data differ, but the actual results of similar experiments vary greatly.

Some observers [see Chauveau, 1896] maintain that carbohydrate alone furnishes the energy of muscular work and that fat can only be utilised by first undergoing conversion into carbohydrate.

Others [Zuntz, 1896; Loewy, 1908; Katzenstein, 1891] find no difference in R.Q. before and during muscular work, or [Benedict and Cathcart, 1914] maintain that this is true only for light work, and that during heavy muscular work the R.Q. is slightly raised.

Another theory [Atwater and Benedict, 1903] is that the working cells utilise fat and carbohydrate in the proportions in which they are presented to them, and that if fat is to be used in the production of muscular energy, then it is oxidised directly and does not undergo a preliminary conversion into sugar.

More recently Krogh and Lindhard [1920, 1, 2] suggest that during rest and during work the proportion of fat and carbohydrate catabolised is a function of the available supplies of these substances, and that carbohydrate is formed from fat and stored when the R.Q. is below 0.8, while a corresponding transformation of carbohydrate to fat takes place when the R.Q. is above 0.9.

Benedict and Cathcart [1914] find that the R.Q. rises during prolonged and heavy work, but when the work is finished the R.Q. falls below that observed previous to the performance of the work.

The investigations into this question in the present series of cases are not sufficiently extensive to warrant any sweeping deductions. In the two cases in which the R.Q. was followed up (cases E. M. F. and M. C. M.) it will be noticed (a) that the initial basal R.Q. is low, (b) that immediately after exercise the quotient tends to rise slightly, and (c) after an interval of rest the R.Q. has fallen again (in one case (Fig. 1) it has fallen below that observed previous to the performance of the exercise).

Taking into account the noted increase in blood-fat after exercise, one might interpret these changes as follows.

(i) That, on the basis of the observations of Krogh and Lindhard, in the initial fasting period the low R.Q. suggests that fat is being converted into carbohydrate and stored.

(ii) That during exercise the stored carbohydrate is utilised, thus tending to force the quotient up towards unity, which is the R.Q. for pure carbohydrate combustion. Coincidentally, more fat is poured out for conversion into sugar to maintain the supply of carbohydrate, so tending to lower the R.Q. The resultant slight increase in R.Q. noted immediately after exercise is the mean of these two effects.

(NOTE. The R.Q. was not taken during exercise, but immediately after it, when, it may be assumed, the physiological mechanism at work during exercise had not had time to readjust itself to resting conditions.)

(iii) After exercise, during the resting period, the depleted carbohydrate store is restored by the transformation of fat into carbohydrate with the consequent fall in R.Q. which such interconversion entails.

The relationship between the blood-sugar and blood-fat contents.

Although our knowledge at present remains insufficient for any very definite conclusions to be reached, there are certain indications that the blood-sugar and blood-fat contents are in some way related.

The evidence for such a relationship adduced in the present investigations may be briefly summarised as follows.

(1) In case M. C. M. it is noticed that after the taking of glucose (the individual resting), while the blood-sugar rises in the first half-hour and falls in the next half-hour, the blood-fat content does exactly the reverse. This has proved to be a fairly constant factor in many cases not yet published (Fig. 2).

(2) This apparent relationship in the resting individual between the blood-sugar and blood-fat, where, during absorption of sugar, the peak of the blood-sugar curve coincides with the trough of the blood-fat curve, is not maintained during exercise.

Here all the cases of exercise without glucose show an increase of blood-sugar side by side with an increase of blood-fat immediately after exercise, while in those cases where glucose was given before the exercise was started we have no increase in blood-fat but definite rise of blood-sugar in two cases

and a definite fall in another case. These changes are quite definite even when allowance of 10 % error is made for the colorimetric method of estimating the blood-sugar content.

The relation of cholesterol with the variations of blood-fat content.

Terroine [1914] found that the ratio of fatty acid to cholesterol was almost constant during the absorption of fat.

This was not substantiated by Bloor [1915], who found the variations of cholesterol during fat absorption to be irregular, small, and sometimes barely appreciable. Other observers [Hiller, Linder, Lundsgaard and Van Slyke, 1924] have confirmed Bloor's observation.

The present conception of the behaviour of cholesterol during the absorption of fat, stated briefly, is that up to a certain point the cholesterol tends to increase, but not beyond this level, depending, perhaps, on the amount available in the intestine from food or bile. The association of cholesterol with fat does not appear to be essential either to the process of absorption or of transfer from blood to tissues.

In the present series of cases where the cholesterol content was estimated it is necessary to modify the figures on account of the colorimetric method of estimation. This means an error either way of 10 %, and worked on this basis it will become evident that there is nothing conclusive in the variations—indeed, it may be deduced that the cholesterol content is unaffected, although it would be unwise to state definitely that no change occurs.

SUMMARY.

1. Figures are presented indicating a remarkable constancy of the fasting blood-fat content for the same individual.
2. A definite increase in the blood-fat content is noted as occurring in fasting individuals immediately after severe exercise.
3. There is no increase in blood-fat after exercise in the same individuals when they have received 100 g. glucose *per os* prior to the muscular exertion.
4. The variations of the R.Q. are discussed and the suggestion made that, taken in conjunction with the observed increase in blood-fat in fasting individuals after exercise, they might indicate that fat was being mobilised for its conversion into carbohydrate.
5. Indications of a relationship between the blood-fat and blood-sugar contents are noted.
6. The investigation of the cholesterol content of the blood seems to indicate that during the period of observation it remained unaffected by the exercise and, apparently, unrelated to the definite blood-fat variations.

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