CLXXIV. BLOOD-PHOSPHORUS IN FAT ABSORPTION.

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THE view held that the phosphatides are concerned in the transport of fat for utilisation or deposition in the tissues seems to have found rather a general acceptance. To a great extent this view is experimentally based on the observation that the phosphatide content of blood is very often increased on occasions when an increased fat transport with the blood is presumably taking place, *e.g.* after fat ingestion. This phenomenon has been studied particularly by Bloor [1915, 1916], but has also been observed by many other investigators, usually in experiments on dogs¹.

According to Bloor [1916] most of the increase of the phosphatides takes place in the blood-corpuscles, in which this author found at the same time an augmented content of glycerides. On the strength of these observations Bloor advanced the interesting and much cited hypothesis that the fatty acids absorbed from the intestine are converted into phosphatides in the corpuscles and mainly as such are removed from the blood. Noticeably little additional work has, however, been done in order to test the correctness of this hypothesis. Knudson [1917] confirmed the observation of phosphatide increment in the corpuscles after fat ingestion. On the other hand Bloor [1920] himself in a later paper mentions that "further unreported work in this laboratory has not, however, entirely borne out these findings, since certain dogs have been found, in which the increase of lipid phosphorus took place in the plasma and not in the corpuscles." Further Iwatsuru [1926] found no such increase (in experiments with rabbits). Under these conditions I have thought it desirable to submit the question of the alimentary phosphatide increase in the corpuscles and in plasma to renewed investigation. For a further trial of the hypothesis in question I have, concurrently with the determination of lipid P, also determined the non-lipid P of plasma and corpuscles. This was done with the idea that if the phosphatides added to the blood during fat absorption are formed in the blood itself, either in corpuscles or in plasma,

¹ It is noteworthy that no alimentary increase of blood phosphatides has been found in man [Hiller, Linder, Lundsgaard and Van Slyke, 1924; McClure and Huntzinger, 1928; Page, Pasternack and Burt, 1930]. This discrepancy is not unlikely to be explained by the fact that the human subjects tested in this respect have received, in spite of the ingestion of about 100 g. of fat, a much smaller quantity of fat in relation to their body weight than the dogs.

it might reasonably be expected that the non-lipoid P of the blood should show a simultaneous decrease.

Methods.

The investigations were performed on dogs which were starved for 24 hours before the test-meal. Between the experiments the dogs were fed on a mixed diet, for the main part consisting of meat. Blood (about 15 cc. each time) was drawn from a cutaneous vein on the hind leg just before the fat ingestion and 2, 4, 8, 12 and generally also 24 hours afterwards. The test meal consisted of olive oil in an amount of about 5 cc. per kg. body weight (usually 100–120 cc. in all) and of 100–200 g. of lean meat. The oil was given by stomach tube or simply by pouring it directly into the pouch produced in the mouth of the dog, by pulling the lips away from the jaws on one side. In Exps. 5, 6 and 7 the animals were given 100 g. of glucose in water (+ 0.25 g. of tannic acid) 18 and 12 hours before the fat meal (*i.e.* 200 g. of glucose in all). In Exp. 6 no meat was given. The blood was transferred to a flask containing solid sodium oxalate in a quantity of about 0.2 % of the blood taken. After the oxalate had been mixed up with the blood, this was centrifuged for 30 minutes at a rate of 2700 r.p.m. and the plasma cautiously drawn off. The leucocyte layer and the uppermost layer of the red corpuscles were removed and the rest of the erythrocytes taken for analysis.

For the phosphatide determination 2 cc. of plasma were dropped slowly into 40 cc. of a mixture of 3 parts of alcohol and 1 part of ether which was kept rapidly rotating. The mixture was heated to boiling in a water-bath and thereafter cooled to room temperature. The entire fluid was then filtered into a 100 cc. Erlenmeyer flask, two washings with 10 cc. of the alcohol-ether mixture being employed. The filtrates were evaporated to dryness on a water-bath, the residue incinerated with sulphuric and nitric acids and the phosphorus determined titrimetrically according to the modified Neumann method, described by Widmark and Vahlquist [1931].

To a weighed quantity (about 1.5 g.) of corpuscles was added an approximately equal amount of water and the mixture again weighed. The fluid was then shaken vigorously and allowed to stand for a few minutes. The haemolysed corpuscles were poured drop by drop into the alcohol-ether mixture and the amount poured in was determined by weighing before and after. For the rest the determination was performed in the same way as with the plasma.

In plasma the so-called acid-soluble P is practically identical with the non-lipoid-P. The acid-soluble P was determined as follows. 4 cc. (in some cases only 2 cc.) of plasma were added drop by drop to an Erlenmeyer flask containing ten times the amount of saturated picric acid in 0.25 % hydrochloric acid. After standing for 1 hour the mixture was filtered and an aliquot part (usually 30 cc. out of 44 cc.) was evaporated, incinerated and the phosphorus determined according to the Widmark-Vahlquist method.

In the corpuscles the non-lipoid P was determined as the difference

between total and lipid P. For the determination of the total P of the corpuscles quantities of about 0.75 g. were weighed, thereafter incinerated and analysed for P as above.

The experimental confirmation of the accuracy of the methods used for the determination of lipid P and acid-soluble P of plasma was given by Widmark and Vahlquist [1931]. For the quantities of plasma used in the present work the error does not as a rule exceed ± 0.05 mg./100 cc. With regard to the determination in the corpuscles the accuracy becomes somewhat less. According to our experience the error of the determination of lipid P and of total P in the corpuscles seldom exceeds ± 0.2 mg./100 cc. and ± 1 mg./100 cc. respectively.

Table I. *Experiments with fat ingestion.*

Exp. No. Date Exp. animal	Hours after fat ingestion	Plasma		Corpuscles		Turbidity of plasma
		Phos- phatide P mg./100 cc.	Acid- soluble P mg./100 cc.	Phos- phatide P mg./100 cc.	Non- lipid P mg./100 cc.	
I	0	—	—	14.2	48.6	0
20. viii. 1930	2	16.1	5.3	14.3	51.2	++
Dog 1. ♂	4	17.4	5.1	14.9	49.2	+++
	8	16.8	5.1	14.2	49.9	+(+)
	12	16.9	4.6	14.1	50.0	+
	24	14.2	3.1	14.3	47.1	0
II	0	15.3	3.9	16.6	53.6	0
27. viii. 1930	2	16.1	4.7	16.2	54.1	++(+)
Dog 1. ♂	4	17.4	5.4	17.0	50.8	+++
	8	16.6	4.7	16.8	51.8	++
	12	15.6	5.4	16.3	54.1	+
	24	14.5	3.3	16.5	51.7	0
III	0	14.7	4.5	16.7	48.8	0
3. x. 1930	2	14.4	5.0	16.5	51.0	(+)
Dog 2. ♂	4	16.9	6.2	16.0	48.0	+
	8	15.8	6.1	17.0	48.3	+(+)
	12	15.0	5.1	16.4	50.1	0
IV	0	12.3	3.3	14.6	—	0
26. x. 1930	2	12.0	3.3	13.8	—	++
Dog 3. ♀	4	14.4	4.0	15.0	—	+++
	8	13.3	3.3	14.5	—	+
	12	11.9	4.1	14.4	—	(+)
	24	11.6	3.7	14.2	—	0
V	0	12.2	3.6	15.5	51.0	0
12. x. 1930	2	12.8	3.4	15.2	49.0	++
Dog 1. ♂	4	14.4	3.8	15.3	50.8	+++
	8	15.4	3.8	14.6	49.4	+++
	12	15.5	4.4	14.7	52.7	++
	24	14.8	3.4	14.7	52.2	(+)
VI	0	9.7	3.7	14.4	—	0
17. xii. 1930	2	10.0	4.0	14.3	—	+(+)
Dog 2. ♂	4	11.1	—	14.5	—	+(+)
	8	11.4	3.7	14.1	—	(+)
	12	11.2	4.6	14.4	—	0
VII	0	9.7	4.3	14.7	—	0
20. xii. 1930	2	9.8	4.9	—	—	+
Dog 2. ♂	4	11.5	4.6	14.2	—	++(+)
	8	10.3	4.7	14.5	—	(+)
	12	9.9	4.9	14.4	—	0
	24	9.9	4.4	14.0	—	0

+ cloudy, ++ slightly milky, +++ milky

Experimental results.

The analytical data are comprised in Tables I and II. In all experiments with fat ingestion (Table I) there is a clear increase of the plasma phosphatides, varying between 14 and 26 % of the fasting values. In five of the experiments the maximum is reached after 4 hours, in one experiment after 8 hours, and in another experiment after 12 hours. In the last two cases the animals were given glucose before the fat meal. On the contrary the phosphatides of the corpuscles show only small and quite irregular changes. As to the acid-soluble P increases were found in all the experiments (in No. 1, the fasting value was lost), in some cases small indeed, but certainly exceeding the experimental error. In Exps. 2 and 3 the rise and fall are regular with a maximum 4 hours after fat ingestion. In the rest of the experiments the changes are more irregular but in no instance is there a significant fall below the fasting values. The level of the non-lipoid P of the corpuscles is not moved definitely in any direction. Small variations occur but in quite an irregular way. In all experiments the plasma became markedly lipaemic, the maximal milkiness appearing as a rule after 4 hours. In the three control experiments (XI–XIII, Table II) in which lean meat was given but no oil the phosphatides of the plasma remained constant but the acid-soluble P of the plasma showed a rise of about the same magnitude as in the experiments with fat ingestion. In the three control experiments (VIII–X, Table II), with no food supply the plasma phosphatides as well as the acid-soluble P were unchanged.

Table II. *Control experiments.*

Exp. No. Date Exp. animal	Hours	Plasma		Corpuscles Phosphatide P mg./100 cc.
		Phosphatide P mg./100 cc.	Acid-soluble P mg./100 cc.	
VIII	0	11.7	4.5	—
24. i. 1931. Dog 1. ♂	4	11.6	4.3	—
IX	0	11.4	3.4	—
15. ii. 1931. Dog 1. ♂	4	11.3	3.5	—
X	0	9.9	3.5	—
15. ii. 1931. Dog 2. ♂	4	10.0	3.6	—
XI	0	15.5	3.5	14.1
14. viii. 1930. Dog 3. ♀	5	15.2	5.2	13.6
XII	0	11.5	3.7	—
24. i. 1931. Dog 2. ♂	4	11.5	4.4	—
XIII	0	11.7	4.2	—
30. i. 1931. Dog 1. ♂	4	11.7	5.0	—

DISCUSSION.

My experiments were arranged in much the same way as Bloor's experiments in which this author found a marked phosphatide increase in the corpuscles. Among the obvious differences entailed in the experiments, however, none can to my mind be presumably held responsible for the marked discrepancy found in the results. My dogs (with one exception) were given

some meat together with the oil, whereas Bloor's animals had oil only. In Exp. 6 in which I gave no meat the outcome is, however, quite the same as in the rest. I made my determinations directly on the corpuscles, whereas Bloor obtained his corpuscle values as differences between whole blood and plasma, guided by haematocrit determinations. Bloor's corpuscle values, therefore, contrary to mine, include the leucocyte phosphatides. Yet, not even a considerable digestive leucocytosis, in spite of the relative richness of the leucocytes in phosphatides [Boyd, 1931] could produce the difference. The facts that I drew the blood from a vein of a hind leg, whereas Bloor used blood from the jugular vein and that different analytical methods were employed cannot reasonably explain the different results. There are certain reasons for believing that the diet during the period preceding the experiments might have been of some influence. According to Wendt [1928] and to Rony and Ching [1930] the fatty acid increase in plasma is much higher in animals which have fasted for some days previous to the fat meal, than in animals fed in the usual way. Bang [1918], Rony and Ching [1930] and others have found that ingestion of carbohydrates together with fat tends to repress the alimentary lipaemia. On the other hand, our experiments in which glucose was given before the fat meal show practically the same results with regard to phosphatides and non-lipoid P as the rest. In two of the glucose experiments the phosphatide increase in plasma appears somewhat protracted, but in the third the course of the phosphatide level was quite the same as in the experiments without glucose. Bloor does not mention anything about the diet used between the experiments. My animals received, as stated above, a mixed diet consisting mainly of meat. It remains to state that at present it is impossible to explain why a fat meal should be followed by a phosphatide increase in the corpuscles in some dogs and not in others.

After having found that in haemorrhagic lipaemia the phosphatide increase in the corpuscles is inconsiderable as compared with that in plasma, Bloor suggests that the absence of the rise in the corpuscles here and in certain cases of alimentary lipaemia may be due to the rapid passage of lecithin from the corpuscles to the plasma. Yet if this assumption were right, the new formation of lecithin in the corpuscles would be expected to cause a decrease of the non-lipoid P of the corpuscles and eventually of the plasma, especially when the new formation of lecithin had been going on for some time, that is towards the end of fat absorption. In our experiments, however, the non-lipoid P shows in no instance the least tendency to fall. On the contrary, in the plasma the non-lipoid P shows some increase. (This increase could possibly be ascribed to the meat given, as it was only in the control experiments with meat that a similar rise was found. As in Exp. 6, where only oil was given and no meat, an increase was also found, the fat may be at least partly responsible for the rise in the rest of the experiments.)

My results are thus clearly against the view of a phosphatide formation within the corpuscles during fat absorption. They cannot, however, be said

definitely to disprove it. To settle this question qualitative investigations of the corpuscle phosphatides during fat absorption will no doubt also be necessary.

SUMMARY.

1. In experiments on dogs the phosphatides of plasma showed after fat ingestion a regular increase, whereas the phosphatides of the corpuscles remained practically unchanged.

2. The non-lipoid P of plasma showed a small increase during fat absorption, the non-lipoid P of the corpuscles being unaltered.

3. The results argue against the assumption that the fat absorbed from the intestine is transformed into phosphatides of the red corpuscles of the blood.

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REFERENCES.

- Bang (1918). *Biochem. Z.* **91**, 111.
Bloor (1915). *J. Biol. Chem.* **23**, 317.
—— (1916). *J. Biol. Chem.* **24**, 447.
—— (1920). *J. Biol. Chem.* **45**, 180.
Boyd (1931). *J. Biol. Chem.* **91**, 1.
Hiller, Linder, Lundsgaard and Van Slyke (1924). *J. Exp. Med.* **39**, 931.
Iwatsuru (1926). *Pflüger's Arch.* **214**, 295.
Knudson (1917). *J. Biol. Chem.* **32**, 337.
McClure and Huntzinger (1928). *J. Biol. Chem.* **76**, 1.
Page, Pasternack and Burt (1930). *Biochem. Z.* **223**, 445.
Rony and Ching (1930). *Endocrinology*, **14**, 355.
Wendt (1928). *Klin. Woch.* **7** (II), 2183.
Widmark and Vahlquist (1931). *Biochem. Z.* **230**, 245.