XLVI. FORMATION OF PHOSPHATIDES IN LIVER PERFUSION EXPERIMENTS

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ABOUT 2 hr. after a meal containing fat, the fat content of the blood begins to rise. This alimentary lipaemia is followed by lecithinaemia [Reicher, 1911; Bloor, 1915], the phosphatide content of the blood increasing more or less parallel with the fat content. A maximum is reached after about 4 hr. and after 8 hr. the fat and phosphatide contents of the blood are almost at the initial level. As to the origin of the phosphatides responsible for alimentary lipaemia the following possibilities exist:

- (1) the phosphatides are synthesized in the intestinal mucosa and resorbed into the blood;
- (2) they are synthesized in the blood;
- (3) they are mobilized under the influx of lipaemic blood from the liver or other organs and possibly wholly or partly formed in the latter during the influx.

To obtain further information on the above problem oil, together with labelled (radioactive) sodium phosphate, were administered to a dog [Hevesy & Lundsgaard, 1937]. If the increase in the phosphatide content of the blood which amounted to 15% after 4 hr. was due to phosphatides taken up from the intestine, the phosphatides extracted from blood should have shown a marked radioactivity. The latter was however much smaller than to be expected on this assumption. It follows that while phosphatides are synthesized in the intestinal mucosa [Artom *et al.* 1937; Sinclair & Smith, 1937] and some do enter the circulation from the bowels [Himmerich, 1934; Süllmann & Wilbrandt, 1934; Freeman & Joy, 1935] the bulk of the phosphatides which are responsible for the alimentary lipaemia must originate from outside the intestinal tract. We next tested the possibility that the additional phosphatides are formed in the lipaemic blood [Hahn & Hevesy, 1938]. A few ml. of dog blood were shaken with labelled sodium phosphate under the usual precautions for 4-5 hr. The phosphatides extracted after the experiment were only slightly radioactive, the labelled phosphatides formed amounting to only about 0.1% of the total amount present. No difference was found in the behaviour of normal and lipaemic bloods.

Formation of labelled phosphatides in perfusion experiments

Through the great kindness of Prof. Lundsgaard and Dr Blixenerone, who carried out perfusion experiments on isolated livers, we were enabled to test the formation of labelled phosphatides in the blood of cats circulating through an isolated liver and also in the liver tissue. To 120-160 ml. of cat blood diluted to about twice its volume with physiological NaCl solution a minute amount of active sodium phosphate was added. The blood was then defibrinated and allowed to circulate through an isolated liver for 2.5 hr. The labelled inorganic P present in the blood was determined at the start and at the end of the experiment and also the labelled phosphatide P of blood and liver at the end of the experiment.

The ratio of the specific activity (activity per mg. P) of the blood phosphatide P to that of the blood inorganic P is seen from Table I. In interpreting the figures of the table we should recall that if all phosphatide molecules present are newly formed the specific activities of the inorganic P and phosphatide P should be equal. The figure of 0.85×10^{-3} for the ratio quoted, for example, shows that the new formation of phosphatide molecules within the experiment amounts to only 0.085%. In the course of the experiment inactive inorganic P of the liver and also a part of the P present in the organic P compounds of the liver exchange with the active plasma inorganic P and lower the specific activity of the latter. The specific activities of the plasma inorganic P being thus different at the start and at the end of the experiment we have calculated the ratio (seen in Table I) for the beginning of the experiment (col. 1), for the end (col. 2), and also an average value (col. 3).

Table I

	$\frac{\text{Specific activity of blood phosphatide P}}{\text{Specific activity of blood inorganic P}}$		
	1	2	3 (average value)
Normal blood (av. of 3 expts.)	0.5×10^{-3}	1.2×10^{-3}	0.85×10^{-3}
Lipaemic blood (av. of 2 expts.)	1.6×10^{-3}	3.4×10^{-3}	2.5×10^{-3}

The figures of Table I for normal blood hardly differ from the figures obtained in the experiments *in vitro* (average value 0.8×10^{-3}). While, however, in the experiments *in vitro* no definite difference was found in the formation of phosphatides in normal and lipaemic bloods, in the perfusion experiment about three times as many newly formed phosphatide molecules were found to be present in the lipaemic blood as in the normal. This result is supported by figures obtained when investigating the labelled phosphatides extracted from the livers used in the perfusion experiments. Here also (see Table II) a greater part of the phosphatide present became labelled when lipaemic blood was used.

Table II

Liver	Phosphatide P per g. fresh tissue mg.	Total P per g. fresh tissue mg.	$\frac{\text{Spec. activity of phosphatide P} \times 100}{\text{Spec. activity of inorganic P}}$
Perfusion with normal blood			
1	0.96	2.6	1.25
2	1.13	2.9	1.53
3	0.88	2.0	1.78
Perfusion with lipaemic blood			
4	0.75	2.4	2.75
5	0.69	2.2	2.59
6	1.32	3.7	2.79

The livers used were taken from fasting cats, except in Exp. 3. In Exp. 6 the specific activities of the ester P of the liver and the protein P (remaining P after extraction with ether-alcohol and trichloroacetic acid) were determined as well. The relative figures obtained were: specific activity of the inorganic P, 1; of the ester P, 0.218; of the protein P, 0.068. The radioactive P atoms can only enter into the phosphatide molecules by a synthetic process. If the radioactivity of 1 mg. organic P of the liver were equal to that of 1 mg. inorganic P, all organic P atoms would have been replaced. If the organic P were not radioactive at all, none of the organic molecules could be newly formed. If all the phosphatide molecules present in the liver after the perfusion experiment had been newly

formed the value of the ratio in the last column would be 1. From the figures it follows that 1.5% of all phosphatide molecules present in the experiment with normal blood and 2.7% in that with lipaemic blood are formed in the course of the experiment.

During the perfusion experiment some molecules may have decomposed thus introducing an uncertainty into all conclusions based on the determination of the amount of phosphatides present. Our conclusions are not influenced however by this source of error, since they are not based on determinations of the phosphatide content before and after perfusion but on the ratio of labelled and non-labelled phosphatide molecules present in the liver. We may, therefore, conclude from the results described above that lipaemic blood is more effective in the formation of phosphatides in the liver than is normal blood. This result suggests that one of the main reasons for alimentary lipaemia is that during the influx of lipaemic blood phosphatide formation in the liver is increased and phosphatides are discharged into the circulation [cf. Aylward *et al.* 1935]. As the lipaemic blood is changed into normal blood the excess phosphatides are taken up by the liver and other organs, until the "normal" phosphatide content of the blood is reached. The chance that the influx of lipaemic blood will remove a labelled phosphatide molecule from the liver will be a small one in view of the great preponderance of non-labelled molecules and therefore we should not expect to find the phosphatides which pass into the blood during alimentary lipaemia to be labelled to any great extent. We mentioned on p. 342 an experiment in which labelled sodium phosphate and olive oil were administered to a dog. The animal's blood showed, as expected, an increased phosphatide content, the latter being, however, only slightly labelled. This result is just what would be expected in the light of the above conclusions.

SUMMARY

In experiments *in vitro* the amount of labelled phosphatide obtained in shaking blood with radioactive sodium phosphate is the same whether normal or lipaemic blood is used.

In perfusion experiments the lipaemic blood is found to contain more labelled and thus newly formed phosphatide than the normal blood. The same result applies also to the phosphatides extracted from the liver in the perfusion experiment.

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