XVI. LIPAEMIA AND MILK FAT SECRETION IN THE RUMINANT

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In a previous paper [Aylward & Blackwood, 1936], an attempt to determine the time and rate of fat digestion in the ruminant by blood sampling after meals of differing fat composition was reported. No alimentary lipaemia could however be demonstrated and subsequent inanition experiments having established the period of absorption of food materials from the rumen to be about 4–5 days, it was decided in the present work to use some special dietary fat which could be qualitatively demonstrated and quantitatively determined in the blood lipins and also, if present, in the milk fat.

The difficulties of such a procedure are many and too well known to merit repetition. One of the more recent contributions to such studies is that of Artom [1933, 1, 2], who made use of a fat saturated with iodine and sold as "Radiopol". Its iodine content was found by us to be 37 %, and it contained no free iodine. Artom found this fat to be tolerated by rabbits when given orally or as an intravenous injection, and while it may be argued that the results obtained by its administration are not necessarily physiological, the difficulties of other approaches to the problems of fat transport and milk fat secretion seemed to justify an investigation of the role of these iodized fats.

Accordingly experiments were devised to elucidate three main points: (1) the existence and nature of alimentary lipaemia in the bovine; (2) the rate at which fatty acids fed as glycerides enter the blood lipins and their relative distribution therein; (3) the existence of a relationship between the level of a particular fatty acid in the blood and its secretion in the milk.

EXPERIMENTAL

Iodized fat. Two proprietary preparations were obtained, "Radiopol" and "Lipidol". These exhibited the usual solubilities of triglycerides and appeared to be identical with normal fats except in their iodine content of some 40 %, their high specific gravity (1.4–1.5) and their high viscosity. Although both fats were liquid at room temperature, saturation was almost complete (i.v. 8–14).

Analytical methods

Blood. In general, an alcohol-ether extract of each blood sample [Bloor, 1928; Stewart & Hendry, 1935] was prepared and 1000 ml. aliquots were evaporated to dryness first at atmospheric pressure and finally *in vacuo*. The residues were left overnight in chloroform and then filtered from small traces of sediment, the resulting solutions being subsequently made up to 100 ml. from which suitable aliquots were taken for analyses.

Phosphatide. A suitable aliquot of each extract was evaporated, the residue dissolved in a little ether and the phosphatide precipitated with acetone and magnesium chloride. In the first three experiments in order to avoid loss of phosphatide in the acetone-soluble fraction only one precipitation accompanied by thorough washing with acetone was carried out, but in Exp. IV, to ensure complete removal of glycerides and cholesteryl esters from the phosphatide, three precipitations were made. In all cases the two fractions, phosphatide and non-phosphatide, were made up to known volumes for analyses.

Fatty acids. These were estimated by the method of Stoddart & Drury [1929].

Iodine. The halogen of halogenated fatty acids may be readily removed by the action of strong NaOH at high temperatures. To avoid loss by spurting or volatilization it was decided to use a Burgess-Parr sulphur bomb for the reaction. Accordingly a known aliquot of lipid extract was placed in the bomb, the solvent carefully evaporated, 1.4 g. NaOH added and the closed bomb heated strongly over a Bunsen flame for 7 min. After cooling, the contents were washed into a flask with water and analysed for iodine by the method of Kendall [1920], certain precautions noted by Harvey [1935] being incorporated. By the particular process of incineration used in the present work the lipid-I was completely converted into inorganic salt, and although small amounts of organic matter remained undestroyed, they did not interfere with the final end-point in the thiosulphate titration. The use of auxiliary oxidizing agents such as potassium nitrate and sodium peroxide was tested and it was found that by this means the organic matter was completely oxidized, but there was no other advantage in the present case either in accuracy or convenience.

Milk. A daily sample of the milk fat of each lactating animal was prepared in the form of an alcohol-ether extract in a similar way to that for blood. Suitable aliquots were taken for determination of lipid-I. Owing to the interference of lactose in the estimation it was found advisable to free the concentrated fat extract from sugar by careful washing with water.

Test experiments

(a) *Iodine in lipiodol.* Six determinations using *N*/200 thiosulphate gave values for the iodine content of lipiodol ranging from 38.4 to 39.0 with mean of 38.8%.

(b) *Stability of the iodized fat.* Owing to the small amounts of fat in blood and the large volumes of solvent required for their extraction, relatively long periods of heating are involved in reducing the extracts to suitable proportions. It was therefore essential to test the stability of the iodized fat under similar conditions of heating. Into a 50 ml. sample of blood added to alcohol-ether mixture in the usual way, 15 mg. of lipiodol were introduced and the whole refluxed for a few minutes, cooled and made up to 1 litre. The filtered extract was then concentrated. Analysis was carried out as already described and the lipid-I found in the blood agreed with the theoretical amount added to within 3%.

(c) *Extraction from milk.* Similar experiments to that described under (b) were also carried out using milk instead of blood. Again the accuracy of the estimation of lipid-I with the amounts of lipiodol used was found to be within 3%. Only when the iodine content was relatively small did the experimental error rise to any considerable extent and even then the methods were found to be reliable for the purpose of the present work.

Animal experiments

I. A non-lactating cow in the first month of gestation was given 450 g. of radiopol as a drench followed by a small quantity of linseed oil, and during the experiment 10 lb. of dry grass per day were given in two feeds. A blood sample, on which in each case detailed analyses were carried out, was taken from a subcutaneous vein immediately before the drench and at 8-hourly intervals thereafter. Results for the total fatty acids and total lipid-I may be read from Figs. 1 and 2 while the remaining values are summarized in Table I.

Fig. 1. The lipid-I content of the blood of two non-lactating cows after administration of iodized fat.

Fig. 2. Comparison between the amount of absorbed lipid-I in the blood and the total fatty acids.

The values for total fatty acids, lipid-P, free and combined cholesterol show no regular variation and are comparable with those found in animals under different conditions of fasting and of high fat feeding [Aylward & Blackwood, 1936]. The administration of iodized fat does not then result in any apparent

Table I. *The blood level of lipoid-P and cholesterol after a fatty meal together with the distribution of fatty acids and lipoid-I (mg./100 ml. blood)*

Time after drench hours	Total lipoid-P	Lipoid-I							
		Sterol		Fatty acids				% of total recovered in the fractionation*	% of total recovered which was present in the phosphatide fraction
		Free	As ester	Non-phosphatide	Phosphatide	Non-phosphatide	Phosphatide		
0	9.4	74.2	34.9	55	123	—	—	—	—
8	9.6	77.1	29.8	53.5	140	3.0	2.8	60.4	48.3
16	9.3	75.8	36.9	60	112	6.7	3.5	68.5	28.5
24	9.3	78.4	26.3	45	140	5.8	3.0	36.1	33.3
32	8.9	77.1	30.2	56	130	10.6	2.5	51.4	19.1
40	9.3	74.7	26.4	53	—	22.9	2.7	83.4	10.5
48	8.7	71.8	34.5	—	—	—	—	—	—
56	8.8	73.1	36.4	50	126	—	—	—	—
64	8.9	76.1	22.7	64	112	—	—	—	—
72	8.6	74.2	33.3	42	125	—	—	—	—
80	9.0	75.9	33.9	45	113	23.3	2.7	78.1	10.4
88	9.5	78.6	26.1	65	107	17.8	1.6	78.2	8.2
96	8.2	76.8	31.7	51	119.5	13.2	1.6	66.7	10.8
11 days	9.2	76.3	33.9	59.5	113	—	—	—	—

* The large losses in iodine which occurred during fractionation are discussed later.

quantitative change in the distribution of these fatty components of blood and so in subsequent experiments, where smaller doses of lipiodol were given, estimations of total fatty acids and of cholesterol were omitted.

As typical symptoms of iodism were present on the third day of the experiment, the dose of iodized fat was reduced in the three subsequent investigations.

II. A non-lactating cow in the third month of gestation was given 200 g. of lipiodol mixed with 200 g. of linseed oil and allowed free access to pasture. Blood samples were taken at intervals and analysed for total lipoid-I, the results appearing in Figs. 1 and 2. As in Exp. I (see Table I) considerable losses of iodine took place when the blood lipoids were separated into phosphatide and non-phosphatide fractions. For example, in samples taken 0.7, 1.5 and 2.0 days after the drench, the lipoid-I recovered after fractionation was only 84.4, 64.7 and 72.7 % of the total originally found, while the proportions present in the corresponding phosphatide fractions were 16.3, 14.4 and 12.3 %. These findings are discussed later.

III. A non-pregnant cow in the second month of its lactation was given 213 g. of lipiodol mixed with 250 g. of linseed oil and allowed free access to pasture. Records of the total daily milk production and of its specific gravity and fat content did not exhibit any variations to suggest that the milk yield (24–28 lb. per day) or the production of fat (370–436 g. per day) had been impaired by the experiment. The total lipoid-I in the blood and in the milk was estimated at intervals, the results being shown in Fig. 3.

The rise in the lipoid-I content of the blood is similar to that of Exp. II, but with the lactating animal the iodized fat is seen to pass quickly into the milk in amounts directly related to the lipoid-I content of the blood. As in the earlier experiments considerable losses of iodine occurred during the separation of the phosphatide and non-phosphatide fractions. In samples taken 1 and 2 days after the drench, the amounts of iodine recovered from the two fractions were 95.9 and 71.4 % of the lipin-I originally found, the proportions of the recovered total present in the phosphatide being 9.7 and 11.4 % respectively.

No symptoms of iodism having been detected in either of the last two subjects, it was decided to try the effect of a much larger dose of iodized fat on a second lactating cow.

Fig. 3.

Fig. 4.

Fig. 3. Amounts of lipid-I in the blood and milk after administration of 210 g. lipiodol (Exp. III).

Fig. 4. Amounts of total lipid-I in the blood and milk after administration of 293 g. lipiodol (Exp. IV). The distribution of the blood lipid-I between the phosphatide and non-phosphatide fractions is also shown.

IV. Daily records of yield (average 31 lb. per day), specific gravity, fat content and i.v. of the milk fat were made for the milk of a non-pregnant cow in the first month of its lactation. At the end of the second week the animal was given 293 g. of lipiodol and 300 g. linseed oil and allowed free access to pasture. Blood and milk samples were again taken at suitable intervals and the lipid-I determined. It will be seen from Fig. 4 that the results compare very closely with those of the previous experiment and that the amount of dietary fat present in the milk as measured by its lipid-I content is related to the amount present in the blood.

Known aliquots of the fat from seven samples of blood were then separated into phosphatide and non-phosphatide fractions by precipitating three times with acetone and magnesium chloride. The phosphorus content throughout demonstrated the efficiency of the separation, and iodine was estimated in both fractions. The proportion of the absorbed dietary fat which formed part of the phosphatide of the blood is of such importance that full details of the separation are given in Table II, the actual amounts of iodine in each fraction appearing in Fig. 4.

No significant changes in the daily yields of milk and milk fat having been noted throughout Exp. IV such data have been omitted, but one further detail of this particular section remains to be mentioned. In view of the fact that the

Table II. *The efficiency of the phosphatide fraction*

Time after drench days	Lipoid-P (mg./100 ml. blood)			% recovery in the fractionation	% of total lipoid-I recovered in the fractionation*
	Total	Phosphatide	Non- phosphatide		
0.34	9.8	9.3	0.6	101	85.0
1.0	9.4	8.4	0.3	93	95.1
1.35	9.7	8.7	0.5	95	80.3
2.0	9.85	8.6	0.3	90	85.4
3.0	9.9	9.4	0.4	99	94.4
4.0	9.0	8.7	0.5	102	77.5
6.0	9.4	9.4	0.3	103	117.0†

* The actual values for the iodine in each fraction may be read off from Fig. 4.

† In this case the error was high due mainly to the extremely small amount of iodine being measured in the phosphatide fraction.

presence of linseed oil in the diet of cows has been shown by Hilditch & Thompson [1936] to cause an increase in the proportion of oleic acid in the milk-fat, it was of interest to obtain information as to the rate at which this increase in oleic acid took place in order that it might be compared with that of the iodized fat. The i.v. of the milk fat was therefore determined daily. For the fortnight before the oil was given, it averaged 35.1 with extreme daily limits of 34.2-36.2. On successive days after the drench the i.v. was 37.5, 40.4, 39.9, 42.4, 42.2, 42.1, 42.1, 41.4, 40.6, etc., results which show that an increased secretion of oleic acid can be detected in 1 day after the drench and that it attains its maximum in 4 days. This observation confirms a similar finding by Maynard *et al.* [1936] and suggests that the ordinary action of linseed oil and also presumably its rate of absorption are not impaired by the presence of lipiodol, but that the increased secretion of oleic acid in the milk is much more intense and more prolonged than that of the iodized fat.

Phosphatide separation

It is apparent from all four experiments that loss of iodine tends to become quite considerable at some stage in the separation. The process of fractionation was therefore repeated on a slightly larger scale to determine, if possible, where the loss took place. Several alcohol-ether extracts remaining from Exp. I were bulked and the resulting lipoid-I content of the mixture was found to be equivalent to 23.85 mg. per 100 ml. of blood. A known volume of the mixture was evaporated to dryness and taken up in ether; a small residue remained and was filtered off. Using all precautions to avoid loss of iodine, the phosphatide fraction was separated. The iodine contents of the phosphatide and non-phosphatide fractions and of the ether-insoluble residue were equivalent to 3.9, 14.0 and 0.7 mg. per 100 ml. of blood respectively, making a total recovery of only 78%. No explanation has yet been found to account for this loss of 22%, a figure which seems extraordinarily high when due consideration is given to the stability of the iodized fat under the conditions used in these experiments.

DISCUSSION

Alimentary lipaemia. The absorption and digestion of fat by the ruminant have been shown to be not only very slow but also continuous processes lasting for several days with the result that the alimentary tides characteristic of the

non-ruminant are not observed [Maynard & McCay, 1929; Aylward & Blackwood, 1936]. The latter found that the reserves of the body depots together with the continuous nature of absorption were sufficient to maintain almost constant fat levels in the blood even during prolonged fasting before and after feeding. It is therefore of some significance to find that in all the experiments recorded here, it was possible to detect and follow the change in composition of the blood fatty acid in response to dietary stimulus. Fatty acids of the diet passed into the fat components of the blood enriching them in accordance with the composition of the dietary fat itself—the whole process following the course of a smooth lipaemia curve attaining a maximum in the case of the ruminant in 2 days and returning almost to normal by about the 5th day. If the assumption is made that the iodized fat in the blood still contained some 40% of iodine, reference to the figures shows that in 2 days conversion of the blood fatty acids into the iodized type had taken place to the extent of approximately 30% in Exp. I and 9% in the remaining three experiments.

The distribution of the iodized fatty acids. From Fig. 4, it may be seen that the amount of iodine combined in the phosphatide and in the non-phosphatide fractions followed the general trend of the curve for total lipoid-I, but that the amount in the phosphatide increased more slowly and to a much less marked degree. Thus, if it again be assumed that the iodized fat in the blood contained some 40% iodine, then at the maximum in Fig. 4, the amount of conversion of blood fatty acids into those of the diet must have been of the order of 1.5% for the phosphatides and 20% for the non-phosphatide fraction. Similarly in Exp. I, samples taken 80 hours after the drench suggest corresponding conversion figures of 3.5 and 78%. The general tendency is therefore for a high proportion of the absorbed fatty acids to enter the non-phosphatide fraction of the blood, and during the greater part of the lipaemia only some 10–16% of the total iodine present exists in the phosphatides. Thus it appears that paramount importance cannot be attached to the role of this particular fraction in the transport of fatty acids by the blood. It must, however, be borne in mind that these iodized fatty acids may not be entirely physiological in their action for it is possible that they are unsuitable for incorporation by the animal in the phosphatide molecule, so that this conclusion must be taken only in conjunction with confirmatory evidence from other sources.

In the experimental section it was noted that frequently quite considerable losses of iodine occurred during the separation of the phosphatide fraction. But although in some cases they were very high, these losses were not sufficient to reverse or even alter substantially the foregoing relative roles of phosphatide and non-phosphatide as fat transporters.

The secretion of milk-fat. It is evident from Figs. 3 and 4 that there is a close relationship between the amount of iodized fat in the blood and that secreted in the milk. It might have been expected that the arterio-venous technique of Blackwood & Stirling [1932] would reveal clearly the route by which this iodized fat gained access to the milk. When, however, the daily output of lipoid-I in the milk is considered and the rate of blood flow through the mammary gland is assessed at 6 l. per min., with animals and doses such as those of Exps. III and IV, an absolute diminution of 1% in the blood would more than account for the total output of iodine in the milk. Such a difference in the phosphatide and non-phosphatide fractions would be impossible to determine.

Circumstantial evidence is, however, not lacking. Blackwood [1934] showed conclusively that milk fat and milk phosphorus did not arise from blood phosphatides, results which, with others, were simultaneously confirmed by Lintzel

[1934] and 2 years later by Graham *et al.* [1936], from whose paper the above figure for the rate of blood flow is taken and who found a diminution of total fatty acids in the blood on passage through the mammary gland and little change in the phospholipin fraction.

By reviewing the evidence therefore the following position is reached.

(1) Direct evidence from arterial and mammary venous sampling has shown the non-intervention of phospholipins in the synthesis of milk fat [Blackwood, 1934; Lintzel, 1934; Graham *et al.* 1936]. (2) Evidence of the utilization of blood fatty acids by the mammary gland has been obtained [Lintzel, 1934; Graham *et al.* 1936]. (3) Special fatty acids have now been traced during their passage from the diet into the milk via the blood stream and the principal carrier of these fatty acids in the blood appears to be the non-phosphatide fraction. In its content of these special fatty acids this latter fraction seems to bear a much closer relationship to the milk than does the phosphatide fraction itself (Fig. 4).

SUMMARY

1. The rate of absorption of iodized fat has been followed. Its concentration in the blood is at a maximum after $1\frac{1}{2}$ –2 days and then falls gradually to a very small value within 5 days.

2. For the iodized fat administered in the experiments, the amount secreted in the milk appears to be directly related to the amount in the blood.

3. The proportion of the dietary fat carried in the blood as phosphatide is very much less in this case than that carried as non-phosphatide, from which it would appear probable that phosphatide is not the chief means of transport in the blood, nor is it the precursor of milk fat. This latter role appears to belong either to the glycerides or the cholesteryl esters or to both.

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