LXXXVIII. THE ORIGIN OF MILK FAT, AND ITS RELATION TO THE METABOLISM OF PHOSPHORUS.

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AN outstanding feature of the variation appearing in the yield of milk from individual cows for successive days, as shown by the work of Crowther [1905], Hammond and Hawk [1917], and Gowen [1919], is the want of proportion between the weight of the fat and that of the other constituents of milk. Sheehy [1919] has demonstrated that the fat varies from day to day independently of the solids not fat and water.

To explain this phenomenon it is assumed that

(1) Fat is not derived from the same source as the other constituents of milk, or that

(2) It has the same origin but is secreted in quantity independent of the other components of milk through the peculiarity of mammary gland metabolism.

An experiment to elucidate this matter was conducted at the Albert Agricultural College, Dublin. It was sought to compare the secretion of the mammary gland when supplied with blood rich in absorbed fat with that produced when carbohydrate was assimilated. A good milking cow, No. 71, in moderate condition, receiving the usual daily ration of the herd, *i.e.* roots 80 lbs., concentrates 5 lbs. (fed in three equal portions at 6 a.m., 11 a.m., and 3 p.m.) and hay given *ad lib.*, was milked five times a day—at 6 a.m., 9 a.m., noon, 3 p.m., and 6 p.m., for seven days. She was fasted for the last eighteen hours of the seventh day, and on the following morning—6 a.m.—was given a feed consisting of

Mangels	...	...	...	36 lbs.
Hay	...	...	...	3 „
Oil (linseed)	...	...	...	1 pint

and got nothing except water for the remainder of the day. She was milked as during the previous week, and the weight of milk and the percentage and weight of fat determined as before. The yield at 9 a.m., 12 noon, 3 p.m., and 6 p.m., of the eighth day was taken as representing the manufactured product of the mammary gland when supplied with blood containing a large quantity

Table I

Day	Food	6 a.m.			9 a.m.			Noon			3 p.m.			6 p.m.			Total daily milk (lbs.)	Total daily fat (lbs.)
		Milk (lbs.)	Fat %	Fat (total lbs.)	Milk (lbs.)	Fat %	Fat (total lbs.)	Milk (lbs.)	Fat %	Fat (total lbs.)	Milk (lbs.)	Fat %	Fat (total lbs.)	Milk (lbs.)	Fat %	Fat (total lbs.)		
1	Normal	19½	3.6	0.693	1	7.6	0.076	3½	3.4	0.119	8	4.6	0.368	3	4.3	0.129	34½	1.385
2	"	14	3.2	0.448	½	6.9	0.052	4½	3.8	0.181	3½	4.8	0.168	3	4.5	0.135	26	0.984
3	"	14	3.1	0.434	1½	4.5	0.056	4½	4.3	0.194	4	4.3	0.172	3½	4.9	0.172	27½	1.028
4	"	15½	3.1	0.473	2	4.7	0.094	3½	3.9	0.146	4½	4.5	0.191	2½	5.4	0.149	28	1.053
5	"	16½	3.1	0.512	2½	3.7	0.102	3½	3.9	0.127	4½	5.9	0.266	2½	4.7	0.118	29½	1.125
6	"	7½*	2.3	0.173	7½	3.4	0.264	4½	5.7	0.256	3½	5.6	0.210	2½	4.5	0.101	25½	1.004
7	Fast from noon	8*	2.8	0.224	3½	5.4	0.203	3½	4.6	0.150	3½	4.9	0.184	3	3.2	0.096	21½	0.857
8	Fat	12½	2.8	0.350	4½	4.8	0.204	2½	5.4	0.122	3	4.6	0.138	½	3.6	0.018	22½	0.832
9	Normal	10	1.9	0.190	7½	4.5	0.349	2½	4.9	0.135	3	4.2	0.126	4½	3.5	0.149	27½	0.949
10	Fast from noon	10	2.6	0.280	5½	4.7	0.270	3½	5.3	0.199	2½	5.4	0.135	3½	4.4	0.143	25½	1.007
11	Molasses	15½	2.7	0.412	3	5.1	0.153	3½	5.3	0.172	3	4.8	0.144	2½	4.1	0.103	27	0.984
12	Normal	11	3.2	0.352	3	4.6	0.138	2½	4.4	0.121	3	5.1	0.153	2½	3.9	0.098	22½	0.862

* There was no apparent reason for small yields on these two occasions. Such abnormal yields did not appear in the repeated experiments.

of absorbed fat. The cow got her usual feed on the ninth day and up to and including her noon meal on the tenth day. At 6 a.m. on the eleventh day, after an eighteen hours' fast, she was given the following feed:

Mangels	...	...	...	36 lbs.
Hay	...	...	...	3 „
Molasses	...	...	...	5 „

The milking, five times a day, was continued up to the twelfth day.

The results obtained are tabulated in Table I and plotted in Fig. 1. The graphs on Fig. 1 enable a comparison to be made between the milk-fat produced from blood containing much neutral fat in suspension and that from blood not so enriched. The heavy lines join the milk-fat yields, percentage and total, given at 9 a.m., noon, 3 p.m., and 6 p.m., on the eighth and eleventh days when the possible effects of the fat and carbohydrate fed would be respectively shown. The character of the graphs for these two days is similar, showing that the absorption of fat from the intestine affects the percentage and the total quantity of fat in the milk produced in the same way as the absorption of equidynamical proportions of carbohydrate. If the production of milk-fat were to depend on the proportion of neutral fat floating in the blood the percentage fat in the milk yielded on the eighth day should be higher than that of the eleventh day unless it is assumed that all the carbohydrate absorbed on the eleventh day is directly converted into neutral fat and delivered into the blood as quickly as fat is poured into it from the thoracic duct on the eighth day, an assumption which is in contradiction to our knowledge of general metabolism. But even the adoption of this premise would not explain why, in a repeated experiment, when double the dose of fat was given on the eighth day there was no higher percentage of fat in the milk than on the days when the cow was normally fed or fasted from noon, in which case the fat fed on the eighth day was greater than the fat equivalent of the ration of the other days. Presumably the immediate source of milk-fat is some other chemical compound of the blood, and for the production of this compound fat and sugar can replace one another. Evidence from another experiment conducted by the writer, not yet published, shows that fat and carbohydrate can replace one another for the production of milk-fat over protracted periods.

The experiment was repeated on two other cows, Nos. 68 and 70. On the eighth day at 6 a.m., after an eighteen hours' fast, they were fed as follows:

Cow No. 68.

Mangels	...	...	36 lbs.
Hay	...	...	3 „
Linseed oil	...	...	1 pint

Cow No. 70.

Mangels	...	...	36 lbs.
Hay	...	...	3 „
Molasses	...	...	5 „

The yields were recorded as before and on the eleventh day at 6 a.m., after a similar fast, they were again fed the special diets this time as follows:

Cow No. 68.

Mangels	...	...	36 lbs.
Hay	...	...	3 "
Molasses	...	...	5 "

Cow No. 70.

Mangels	...	...	36 lbs.
Hay	...	...	3 "
Linseed oil	...	...	1 pint

The relative effects of carbohydrate and fat could thus be compared for one cow on different days and for two cows on the same day. The results from these two cattle led to the same conclusion as those from No. 71. The experiment was repeated with cows Nos. 68 and 70 with margarine instead of oil and this gave a similar result. Lest the amount of fat fed in these instances was insufficient the experiment was again repeated this time on cows 52 and 76; the procedure was similar to that with 68 and 70 except that the quantities of fat and carbohydrate given with the special feeds on the eighth and eleventh days were increased. On the eighth day the cows were fed as follows:

Cow No. 52.

6 a.m.	{ Mangels	...	25 lbs.
	{ Hay	...	2 "
	{ Oil	...	1 pint
9 a.m.	{ Mangels	...	15 lbs.
	{ Hay	...	1 lb.
	{ Margarine	...	1 "

Cow No. 76.

6 a.m.	{ Mangels	...	25 lbs.
	{ Hay	...	2 "
	{ Molasses	...	5 "
9 a.m.	{ Mangels	...	15 "
	{ Hay	...	1 lb.
	{ Starch	...	2½ lbs.

and on the eleventh day the feeds were reversed, *i.e.* 76 got the ration previously fed to 52 and *vice versa*. The results led to the same conclusion as those from the previous experiments.

In the case of sugar it has been shown that the glucose of the blood [Röhmman, 1919] diffuses into the mammary gland cells where it is converted into the less diffusible lactose of milk. It is highly probable that, in like manner, the non-diffusible fat of milk is made from some diffusible fatty compound. The phosphatides of the blood are soluble compounds containing complex groupings of fatty acids, carbohydrate, phosphorus and nitrogen. In fact, Meigs, Blatherwick and Cary [1919, 1920] have shown that the phosphatide content of the blood of the mammary vein in a lactating cow is less than that of the jugular vein, and conclude that the mammary gland utilises some of the phosphatides of the blood as it passes through it.

Since the phosphatides contain definite proportions of phosphorus, nitrogen and fatty acid groupings in their constitution, their metabolism in the mammary gland should give rise to constant proportions of phosphorus and fat in milk. It must be remembered, however, that the inorganic phosphorus is diffusible whilst fat and some of the nitrogen compounds of milk are not, and it is conceivable that part of the phosphorus might diffuse back into the blood leaving fat and other non-diffusible compounds imprisoned in the gland. Possibly some of the phosphorus liberated is utilised in the synthesis of caseinogen, but in any case if all the inorganic phosphorus derived from the disintegration of phosphatides in the gland cells were secreted in the milk

there should be some definite relationship between the content of either the total or inorganic phosphorus and the content of fat or caseinogen.

To investigate this matter the following percentage determinations were made in milk from individual cows over successive days: fat, caseinogen, total and inorganic phosphoric acid. Table II gives a few of the results:

Table II.

		% fat	% caseinogen (Van Slyke)	% total phosphoric acid	% phosphoric acid in caseinogen (calculated from Richmond's formula for caseinogen)	% inorganic phosphoric acid
Cow 15	{ May 4	2.7	2.88	0.208	0.108	0.100
	„ 5	3.1	2.90	0.218	0.109	0.109
Cow 135	{ „ 4*	2.0	2.16	0.214	0.081	0.133
	„ 5*	8.0	2.76	0.215	0.104	0.111
Cow 34	{ „ 4	3.2	2.59	0.236	0.097	0.139
	„ 5	5.2	2.53	0.225	0.095	0.130
Cow 76	{ „ 4	2.8	2.93	0.194	0.110	0.084
	„ 5	2.8	2.55	0.162	0.096	0.066
Cow 73	{ „ 8	3.4	—	—	—	—
	„ 9	3.4	—	—	—	—
	„ 10	3.4	—	0.217	—	—
	„ 11	3.3	—	0.212	—	—
	„ 12	3.4	—	—	—	—
	„ 13	3.5	2.53	0.216	0.095	0.121
	„ 14	3.3	—	0.210	—	—
	„ 15	3.1	2.41	0.214	0.090	0.124
	„ 16	3.5	—	—	—	—
Cow 52	{ „ 8	3.8	—	—	—	—
	„ 9	3.5	—	—	—	—
	„ 10	3.2	2.83	0.177	0.106	0.071
	„ 11	—	—	—	—	—
	„ 12	3.8	—	0.175	—	—
	„ 13	3.4	—	0.197	—	—
	„ 14	3.5	—	—	—	—
	„ 15	3.8	2.44	0.187	0.092	0.095
	„ 16	3.4	—	—	—	—

* Selected because of marked difference in the percentage of fat on two consecutive days.

There certainly is no constant relationship between the percentage of phosphorus secreted in milk and the quantity of either fat or caseinogen. An increased secretion of fat or caseinogen or both is not always accompanied by an increased amount of phosphorus, the secretion of which appears to be influenced by some other conditions. Since a large increase in the fat secreted is not accompanied by a corresponding increase in the phosphorus, evidently some of the inorganic phosphorus must be reabsorbed from the mammary gland into the blood, and possibly variation in the permeability of the gland cells regulates the secretion of inorganic phosphorus in the milk.

The two experiments quoted lead to the following conclusion regarding the metabolism of the mammary gland: soluble phosphatides diffuse from the circulating blood into the gland cells, are disintegrated there to produce phosphorus, fat, and other products. The non-diffusible products remain in the gland and are afterwards secreted as milk with part of the soluble phosphorus, some of which meanwhile diffuses back into the blood. This view is supported by the work of Meigs, Blatherwick and Cary [1919, 1920] who on comparing the blood of the mammary vein with that of the jugular found that the inorganic phosphorus content of the former was greater than that of the latter. Evidently the mammary gland had elaborated part of the phosphatide of the blood and had returned to the mammary vein inorganic phosphorus.

SUMMARY.

1. For the manufacture of milk-fat, carbohydrate and fat can replace one another in the food. Presumably the immediate source of milk-fat is a diffusible compound to be found in the blood, and there is evidence that the precursor is phosphatide.

2. The phosphatides diffuse from the blood into the mammary gland cells, are there elaborated, and phosphorus, fat and other substances are produced; the non-diffusible products, fat, etc., are all secreted into the lactiferous tubules; the diffusible inorganic phosphorus is partly secreted into the milk and partly diffused back into the blood.

REFERENCES.

- Crowther (1905). *J. Agric. Sc.* **1**, 149.
Gowen (1919). *J. Agric. Res.* **16**, 79.
Hammond and Hawk (1917). *J. Agric. Sc.* **8**, 139.
Meigs, Blatherwick and Cary (1919). *J. Biol. Chem.* **37**, 1.
— (1920). *J. Biol. Chem.* **40**, 469.
Röhmman (1919). *Biochem. Zeitsch.* **93**, 237.
Sheehy (1919). *Sc. Proc. Roy. Dub. Soc.* **15**, 574 (N.S.), No. 41.