XCIV. THE ABSORPTION OF MILK PRE-CURSORS BY THE MAMMARY GLAND.

IV. ASPECTS OF THE PHOSPHORUS METABOLISM OF THE MAMMARY GLAND.

BY JANET HENDERSON BLACKWOOD¹
AND JOHN DEMPSTER STIRLING.

From The Hannah Dairy Research Institute, Kirkhill, Ayr.

(Received April 30th, 1932.)

THE mammary gland appears to have the power of effecting molecular transformations involving phosphorus. Thus, phosphoric acid must be linked up with the amino-acids hydroxyglutamic acid, aminohydroxybutyric acid and serine to give the phosphopeptone which Rimington [1927] has shown to be the phosphorus-containing group of caseinogen. Again, the secretion of milk involves the production of insoluble, colloidal calcium phosphate, while Kay [1925] has found that 15–25 % of the total phosphorus of milk is in the form of acid-soluble compounds hydrolysed by phosphatases. That phosphorus is concerned in the secretion of fat is suggested by Meigs, Blatherwick and Cary [1919], who claim that phosphatides are absorbed from the blood-stream by the mammary gland, that the amount of phosphorus so obtained is greater than can be accounted for by all the phosphorus in milk and that the excess phosphorus is returned to the blood-stream in the form of inorganic phosphorus. These suggestions are based on data obtained by the application of the Kaufmann-Magne technique, a procedure which, as we have already shown [Blackwood and Stirling, 1932, 2, 3; Blackwood, 1932], involves certain unjustifiable assumptions. In view of the recognition of the important part played by acid-soluble phosphorus compounds in metabolism it appeared desirable to investigate more fully the nature of the exchange of phosphorus between the blood-stream and the mammary gland. In the present investigation we have undertaken the examination of the organic and inorganic acid-soluble phosphorus contents of arterial, jugular, and mammary venous bloods with a view to establishing (1) if jugular blood may be taken to represent mammary arterial blood in so far as phosphorus is concerned, and (2) if non-lipoid phosphorus is utilised by the mammary gland.

¹ Carnegie Research Scholar.

EXPERIMENTAL.

Samples of blood were taken simultaneously from the radial artery and from the jugular and mammary veins of lactating and non-lactating animals, as described in Part I of this series [Blackwood and Stirling, 1932, 2].

The samples were analysed for:

Iron content. This was determined iodimetrically (Blackwood and Stirling, 1932, 1).

Corpuscular volume. The haematocrit technique was used.

Total acid-soluble phosphorus and inorganic phosphorus. The colorimetric method of Fiske and Subbarow [1925] was used; organic acid-soluble phosphorus being estimated by difference.

Table I summarises the results obtained for four lactating and four non-lactating cows. In Table II the differences implicit in Table I are expressed as

Table I.

No. of cow	Lactating or non-lactating	Iron content, mg. Fe/100 cc. blood			Total acid-sol. P, mg. P/100 cc. blood			
		Jugular	Arterial	Mammary	Jugular (corrected)	Arterial	Mammary	
1	Lactating	42.32	39.34	38.95	9.60	8.93	10.60	9.70
2	"	38.75	37.71	37.33	9.50	9.24	10.30	9.00
3	"	40.86	39.29	39.50	11.60	11.15	12.10	11.44
4	"	36.69	35.14	34.26	9.25	8.86	10.05	9.50
5	Non-lactating	39.31	36.28	36.81	9.10	8.40	9.70	9.60
6	"	34.74	—	32.94	9.00	8.53	8.80	9.20
7	"	34.23	32.56	33.46	9.90	9.41	11.00	10.60
8	"	37.28	34.60	34.89	10.00	9.28	9.80	10.40

No. of cow	Lactating or non-lactating	Inorganic P, mg. P/100 cc. blood				Organic acid-sol. P, mg. P/100 cc. blood			
		Jugular	Jugular (corr.)	Arterial	Mammary	Jugular	Jugular (corr.)	Arterial	Mammary
1	Lactating	5.16	4.80	5.20	4.92	4.44	4.13	5.40	4.78
2	"	5.26	5.12	5.48	5.34	4.24	4.13	4.82	3.66
3	"	5.72	5.50	5.86	5.76	5.88	5.65	6.24	5.68
4	"	4.44	4.25	4.72	4.70	4.81	4.64	5.33	4.80
5	Non-lactating	5.76	5.32	6.42	5.58	3.34	3.08	3.28	4.02
6	"	4.70	4.46	4.76	4.90	4.30	4.09	4.04	4.30
7	"	5.62	5.35	5.48	5.64	4.28	4.07	5.52	4.94
8	"	5.82	5.40	5.86	5.62	4.18	3.88	3.94	4.78

Table II.

No. of cow	Lactating or non-lactating	Total acid-soluble P			Inorganic P			Organic acid-soluble P		
		Jugular-Mammary	Arterial-Jugular	Arterial-Mammary	Jugular-Mammary	Arterial-Jugular	Arterial-Mammary	Jugular-Mammary	Arterial-Jugular	Arterial-Mammary
1	Lactating	- 7.30	15.80	8.5	-2.20	7.60	5.4	-12.03	23.52	11.48
2	"	2.32	10.29	12.62	-4.02	6.57	2.56	9.75	14.32	24.07
3	"	- 2.40	7.85	5.45	-4.44	6.14	1.71	- 0.48	9.45	8.96
4	"	- 6.40	11.9	5.5	-9.54	9.96	0.42	- 3.01	12.94	9.94
5	Non-lactating	-12.37	13.40	1.03	-4.05	17.14	13.08	-28.7	6.1	-22.56
6	"	- 7.61	3.07	-4.54	-9.25	6.30	-2.94	- 5.14	- 1.22	- 6.36
7	"	-10.8	14.4	3.6	-5.29	2.37	-2.92	-15.76	26.27	10.51
8	"	-11.43	5.31	-6.12	-3.75	7.85	4.10	-22.84	1.52	-21.32

percentages of the arterial values. Table III shows the partition of acid-soluble phosphorus compounds between the plasma and the corpuscles in the jugular blood of lactating and non-lactating cows. Table IV gives similar data for the mammary blood.

Table III. *Partition of acid-soluble phosphorus compounds between plasma and corpuscles in jugular blood.*

Corpuscular volume	Blood			Plasma			Corpuscles			Org. acid-sol. P of corpuscles Org. acid-sol. P of plasma
	Total acid-sol. P	Inorganic P	Org. acid-sol. P	Total acid-sol. P	Inorganic P	Org. acid-sol. P	Total acid-sol. P	Inorganic P	Org. acid-sol. P	
Lactating										
29.5	10.00	6.48	3.52	8.80	7.46	1.42	12.88	4.14	8.74	6.15
38.0	11.20	6.60	4.60	9.60	7.58	2.01	13.81	5.00	8.81	4.38
36.5	9.60	5.16	4.44	8.70	6.10	2.60	11.10	3.53	7.64	2.94
41.0	9.50	5.26	4.24	8.90	5.78	3.12	10.36	4.51	5.85	1.87
37.0	11.60	5.72	5.88	9.90	7.10	2.80	14.49	3.38	11.14	3.97
39.0	9.25	4.44	4.81	6.80	4.42	2.38	13.08	4.46	8.61	3.61
Mean	10.19	5.61	4.58	8.75	6.40	2.39	12.62	4.17	8.46	3.82
Non-lactating										
34.0	11.60	6.34	5.26	8.65	7.30	1.35	17.30	4.47	12.83	9.5
38.5	11.35	6.60	4.75	10.05	7.86	2.19	13.90	4.60	9.30	4.24
41.0	9.10	5.76	3.34	7.20	6.80	0.40	11.82	4.27	7.56	18.9
36.5	9.00	4.70	4.30	6.00	5.60	0.40	14.22	3.12	11.09	27.7
32.5	9.90	5.62	4.28	7.50	6.66	0.84	14.89	2.49	13.20	15.7
38.5	10.00	5.82	4.18	8.00	6.90	1.10	13.21	4.05	9.14	8.30
Mean	10.16	5.81	4.35	7.90	6.85	1.05	14.22	3.83	10.52	14.06

Table IV. *Partition of acid-soluble phosphorus compounds between plasma and corpuscles in mammary blood.*

Corpuscular volume	Blood			Plasma			Corpuscles			Org. acid-sol. P of corpuscles Org. acid-sol P of plasma
	Total acid-sol. P	Inor-ganic P	Org. acid-sol. P	Total acid-sol. P	Inor-ganic P	Org. acid-sol. P	Total acid-sol. P	Inor-ganic P	Org. acid-sol. P	
Lactating										
28.5	10.10	6.56	3.54	9.0	6.56	1.44	12.88	4.13	8.75	6.07
36.0	10.8	6.3	4.5	9.0	6.8	2.2	14.00	5.30	8.70	3.95
33.5	9.70	4.92	4.78	8.20	5.68	2.52	12.69	3.40	9.26	3.67
39.0	9.00	5.34	3.66	9.00	6.38	2.62	9.00	3.72	5.28	2.01
36.0	11.44	5.76	5.68	8.96	6.40	2.56	15.83	4.61	11.22	4.38
37.0	9.50	4.70	4.80	7.00	5.34	1.66	13.76	3.54	10.22	6.15
Mean	10.09	5.59	4.49	8.52	6.19	2.17	13.03	4.11	8.90	4.37
Non-lactating										
33.0	11.60	6.40	5.20	8.70	7.30	1.35	17.50	4.45	13.05	9.7
38.5	10.95	6.66	4.29	10.00	8.06	1.94	12.47	4.42	8.05	4.15
38.5	9.60	5.58	4.02	8.70	6.96	1.74	11.04	3.38	7.80	4.48
35.5	9.20	4.90	4.30	7.20	6.18	1.02	13.04	2.82	10.28	10.07
30.0	10.60	5.64	4.94	8.70	6.82	1.88	15.03	2.90	12.00	6.38
34.8	10.40	5.62	4.78	8.66	7.08	1.58	13.73	2.87	10.79	6.90
Mean	10.39	5.80	4.59	8.66	7.07	1.58	13.80	3.47	10.33	6.95

DISCUSSION.

Figures for the total acid-soluble phosphorus and inorganic phosphorus contents of cow's blood have been published by Hayden and Fish [1928] who give 5.70 mg. P per 100 cc. of blood and 9.29 mg. P per 100 cc. of blood as

inorganic and total acid-soluble phosphorus respectively. The mean values obtained by us for samples of jugular blood from twelve different cows are 5.71 mg. P per 100 cc. of blood as inorganic phosphorus and 10.18 mg. P per 100 cc. of blood as total acid-soluble phosphorus.

Discussing first the assumption of Meigs *et al.* [1919] that the phosphorus contents of arterial and jugular bloods are identical, reference to Tables I and II shows that this assumption is unjustified so far as acid-soluble phosphorus is concerned. In every case which we have examined we have found the acid-soluble phosphorus content of jugular blood to be lower than that of arterial blood. This results from a diminished content of both inorganic and organic acid-soluble phosphorus in jugular blood. It may therefore be assumed that, at the time when these blood samples were taken, both of these classes of phosphorus-containing compounds were being absorbed from the blood-stream by the tissues drained by the jugular vein. The qualification with regard to the time of observation is necessary because there cannot be continual retention of phosphorus, no matter how small the amount, by any tissue. The observations reported in this investigation were carried out on samples of blood taken 4 hours after milking and immediately after a meal. It is possible that, at such a period, the arterial blood is losing material to the tissues, a process which might be reversed at a later period in the day. In this connection Palmer *et al.* [1930] have reported large diurnal variations in the inorganic phosphorus content of the venous blood of dairy cattle. Such an observation is in accordance with a post-prandial period of absorption of phosphorus by the tissues followed by a period during which phosphorus is returned to the blood. Whether this interpretation be accepted or not, it is evident that the phosphorus content of jugular blood cannot be taken to represent that of arterial blood. If, then, any information on the phosphorus metabolism of the mammary gland is to be obtained from blood analysis it is apparent that analytical values for mammary blood must be compared with those for arterial blood.

It will be seen that the four lactating cows of Table I show smaller amounts of total acid-soluble phosphorus in mammary blood than in arterial blood. The difference appears to be due entirely to absorption of organic acid-soluble phosphorus compounds, since the amounts of inorganic phosphorus in the two bloods differ but slightly. For the four non-lactating animals both bloods have practically the same amounts of total acid-soluble phosphorus. With the exception of animal No. 5, for which an anomalous result was obtained, the inorganic phosphorus contents of both bloods are also equal. With the exception of one animal we have found that the organic acid-soluble phosphorus content of mammary blood is higher than that of arterial blood—an observation which was at first surprising. The work of Kay and Robison [1924], Martland and Robison [1926] and Lawaczek [1924] has indicated, however, that there is probably a continual interchange of phosphorus between the inorganic and organic phosphorus compounds in the blood. It may be that as the blood proceeds further from the heart the ratio, organic acid-soluble

phosphorus/inorganic phosphorus, is increased, without change in the total amount of acid-soluble phosphorus present. Such a process would explain our finding that the organic acid-soluble phosphorus content of mammary blood is higher than that of arterial blood in non-lactating cows. If such be the case, the figures shown for the amounts of organic acid-soluble phosphorus absorbed by the active mammary gland are too low. Meigs *et al.* [1919] have stated that the active mammary gland returns excess inorganic phosphorus to the blood-stream, basing their conclusion on the finding that the inorganic phosphorus content of mammary plasma is higher than that of jugular plasma. Figures for whole blood are likely to give more accurate information on this point. In every case examined we have indeed found that mammary blood contains more inorganic phosphorus than jugular blood. This fact cannot, however, be related to the state of activity of the mammary gland in any way for the result was obtained in lactating and non-lactating animals alike. Moreover, if the mammary gland absorbs more phosphorus from the blood-stream than is required for the secretion of milk—a point which has not yet been demonstrated—we should expect to find more inorganic phosphorus in mammary blood than in arterial blood. In no lactating animal was this the case and we believe that the difference between jugular and mammary bloods in this respect results from the fact that the mammary gland does not absorb the inorganic phosphorus compounds of the blood-stream as do the tissues drained by the jugular vein.

Our results lead us to suggest that the mammary gland utilises the organic acid-soluble phosphorus compounds of the blood-stream, and the question arises as to the function which they perform in the secreting cells. Kay [1925] has found that the active mammary gland of the goat contains an enzyme capable of hydrolysing such phosphorus-containing substances. The secretion of milk must to a certain extent resemble bone formation, for both processes involve the separation of insoluble (or colloidal) calcium phosphate from soluble phosphorus compounds. Robison and his co-workers [Kay and Robison, 1924; Martland and Robison, 1926] consider that the calcium phosphate of bone arises by enzymic hydrolysis of substances of the nature of hexosephosphates and we suggest that there is strong presumptive evidence for a similar process taking place in the mammary gland. (a) The gland can absorb acid-soluble phosphorus compounds from the blood-stream; (b) it contains an enzyme capable of splitting such compounds; and (c) it secretes insoluble (colloidal) calcium phosphate. The evidence is of the same nature as that adduced for the intervention of acid-soluble organic phosphorus compounds in bone formation.

One of the difficulties of accepting Robison's theory of bone formation is that most of the acid-soluble organic phosphorus of the blood is carried in the cells. It is of interest therefore to consider some data on the distribution of acid-soluble phosphorus compounds between the corpuscles and the plasma of cow's blood. In the first place it is to be noted that the total acid-soluble phosphorus content of cow's blood is only about one-third of that of human

blood. This is accounted for almost entirely by the extremely low acid-soluble organic phosphorus content of cow's blood. In human blood, the plasma is almost devoid of organic acid-soluble phosphorus. From Tables III and IV it will be seen that the plasma of lactating cows contains appreciable quantities of organic acid-soluble phosphorus—amounts which, indeed, constitute a considerable fraction of that in whole blood. We have found the organic acid-soluble phosphorus contents of the jugular blood of lactating and non-lactating animals to be almost equal. The partition of this phosphorus between cells and plasma appears, however, to differ in the two states. In non-lactating animals there is about fourteen times as much organic acid-soluble in the corpuscles as in the plasma; in lactating animals there is only about four times as much. This would appear to indicate that during lactation there is a greater demand for organic acid-soluble phosphorus than in the non-lactating condition. In the former state a large proportion is carried in the plasma—a medium of transport which would facilitate exchanges with the mammary gland. The ratio is not so high in the mammary blood of lactating animals (Table IV) although it is still higher than that in non-lactating animals. This is as one would expect if the active mammary gland absorbs organic acid-soluble phosphorus. It would appear therefore that the acid-soluble phosphorus compounds of blood play an important part in the secretion of milk.

SUMMARY.

The acid-soluble phosphorus content of the jugular blood of lactating and non-lactating cows is lower than that of arterial blood four hours after the ingestion of food. The lowering is shown both in organic and inorganic acid-soluble phosphorus.

The inorganic phosphorus content of mammary blood is higher than that of jugular blood and approximately equal to that of arterial blood in lactating and non-lactating cows. It is suggested that this is due to the uptake of inorganic phosphorus from the blood by the tissues drained by the jugular vein and to the absence of absorption of inorganic phosphorus by the mammary gland.

The organic acid-soluble phosphorus content of mammary venous blood is lower than that of arterial blood in lactating cows, but slightly higher in non-lactating cows. It is concluded that organic acid-soluble phosphorus compounds are utilised by the mammary gland during the secretion of milk. The suggestion is made that such compounds may be concerned in the secretion of calcium phosphate, thus bringing milk secretion into line with bone formation.

The partition of organic acid-soluble phosphorus between the corpuscles and the plasma is shown to be different for lactating and non-lactating animals. It is suggested that during lactation a greater proportion of organic acid-soluble phosphorus is carried in the plasma to meet the demand for such compounds during the secretion of milk.

We have to thank Dr N. C. Wright for his interest in this work, and Mr L. Jordan who obtained the blood samples.

One of us (J. H. B.) is indebted to the Carnegie Trust for a Research Scholarship during the tenure of which this work was carried out.

REFERENCES.

- Blackwood (1932). *Biochem. J.* **26**, 772.
—— and Stirling (1932, 1). *Biochem. J.* **26**, 353.
—— — (1932, 2). *Biochem. J.* **26**, 357.
—— — (1932, 3). *Biochem. J.* **26**, 362.
Fiske and Subbarow (1925). *J. Biol. Chem.* **66**, 375.
Hayden and Fish (1928). *Cornell Veterinarian* (April).
Kay (1925). *Biochem. J.* **19**, 433.
—— and Robison (1924). *Biochem. J.* **18**, 755.
Lawaczek (1924). *Biochem. Z.* **145**, 351.
Martland and Robison (1926). *Biochem. J.* **20**, 847.
Meigs, Blatherwick and Cary (1919). *J. Biol. Chem.* **37**, 1.
Palmer, Cunningham and Eckles (1930). *J. Dairy Sci.* **13**, 3, 174.
Rimington (1927). *Biochem. J.* **21**, 272.