CXXXIII. THE ABSORPTION OF MILK PRECURSORS BY THE MAMMARY GLAND.

V. PHYSICO-CHEMICAL ASPECTS OF MILK SECRETION.

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THE tendency to regard secretion as an osmotic phenomenon has, as yet, found very little expression in the particular case of the secretion of milk. Beyond the fact, reported by Van der Laan [1915] that milk is isotonic with blood, no data on the osmotic relationships of the mammary gland appear to have been established. Taylor and Husband [1922], it is true, have suggested that the flow of milk through the mammary gland is the result of osmosis induced by the elaboration of lactose. Osmosis could, however, only be induced by this means if the lactose were derived from a larger molecule such as the glycoprotein suggested by Hammond [1913]. At the present time there is no evidence for the existence of such a substance in the mammary gland, and it seems more reasonable to assume that a large part of the blood-glucose, shown by us to be absorbed by the gland [Blackwood and Stirling, 1932, 1], is transformed directly into lactose. The synthesis of lactose would therefore involve a decrease and not an increase in osmotic pressure as suggested by Taylor and Husband. This fact demonstrates that definite knowledge of the nature of milk precursors is a pre-requisite for the study of the physico-chemical basis of milk secretion.

In previous papers of this series we have discussed the nature of milk precursors. In the present communication an attempt will be made to consider their absorption and subsequent fate from a physico-chemical standpoint. In such a treatment it is logical to consider first the transference of material from the blood-stream to the gland cells.

The nature of the transudate from the blood-stream to the gland cells.

The first stage in milk secretion must be the passage of a solution of milk precursors from the blood-stream to the secreting cells of the mammary gland. This might be brought about in one of two ways. (1) The intervening cell membrane might be freely permeable to all blood-plasma constituents which

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are in true solution, in which case the transudate would consist of blood-plasma *minus* its colloids. (2) The cell membrane might be endowed with the property of selective absorption and thus be capable of adjusting the composition of the transudate to the requirements of the secreting cells.

The first hypothesis would imply a filtration through the cell membrane analogous to that which has been demonstrated for the glomerulus of the kidney, since the glomerular filtrate has been shown [Richards and Plant, 1917, 1921] to be identical with blood-plasma *minus* its colloids. There are, however, strong reasons for rejecting the hypothesis that a filtration of this type takes place between the blood-plasma and the cells of the mammary gland. This may be concluded from a consideration of the following experimental data.

In Table I A are given the results of analysis of blood from six lactating cows and in Table I B the analysis of milk from six cows (not the same animals).

Table I.

A. *Blood.*

Amounts mg. per 100 cc. blood			Ratios		
Glucose	NH ₂ -N	NaCl	Glucose	NH ₂ -N	NaCl
44	6.92	491	6.36	1	70.96
43	6.57	497	6.55	1	75.65
36	7.16	459	5.03	1	64.10
42	7.65	500	5.49	1	65.36
43	8.46	475	5.14	1	56.82
49	7.15	466	6.85	1	65.18

B. *Milk.*

Amounts g. per 100 cc. milk			Ratios		
Lactose	Protein-N	NaCl	Lactose	Protein-N	NaCl
4.87	0.51	0.17	9.55	1	0.33
4.84	0.55	0.17	8.80	1	0.31
5.98	0.38	0.13	15.73	1	0.34
4.88	0.36	0.16	13.55	1	0.44
4.63	0.55	0.15	8.42	1	0.27
4.88	0.45	0.15	10.90	1	0.32

Blood-glucose was determined by Hiller, Linder and Van Slyke's [1925] modification of the Hagedorn-Jensen method—true sugar being found in this way and not total reducing power. Blood-amino-nitrogen was determined by the Van Slyke manometric method [1929] and chloride by the thiocyanate procedure [Whitehorn, 1921].

Lactose was determined in the milk by the Shaffer-Hartmann iodimetric method [1920], nitrogen by the Kjeldahl method, and chloride by thiocyanate titration.

It will be seen that the ratios for blood, amino-nitrogen : sugar : sodium chloride are approximately 1 : 6 : 65, while the ratios for milk are approximately 1 : 11 : 0.35. It is evident therefore that milk cannot be considered

merely as a concentrated (and transformed) transudate of blood-plasma *minus* colloids, and that, in order to arrive at the typical ratios found in milk, it would be necessary to assume that certain of the constituents of the transudate are reabsorbed into the blood-stream. Such a process is considered to take place in the kidney [Cushny, 1926]. In the latter case, however, re-absorption of water and of certain solutes takes place during the passage of the glomerular filtrate along the renal tubules. This implies the existence of two complementary cell structures, one for filtration and one for re-absorption. The histological picture of the mammary gland presented by Martin [1919] shows, however, that the blood capillaries have direct access to the secreting epithelial cells and that no complementary structures akin to the renal glomeruli and tubules are present. The mammary gland is therefore not provided with any apparent means of securing specific re-absorption of part of the transudate.

That this criticism is a serious one may be shown from the following quantitative data. The sugar content of the blood of the lactating cow may be taken as roughly 40 mg. per 100 cc. The experimental animals used by us secrete per 12 hours about 10 litres of milk containing 450 g. of lactose. In order to furnish this amount of lactose there would have to be filtered from the blood in 12 hours $\frac{450,000 \times 100}{40 \times 1000} = 1125$ litres of transudate. Thus, on the filtration hypothesis, of this 1125 litres of transudate 10 litres would appear as milk, while the remaining 1115 litres would be re-absorbed into the blood-stream. This hardly appears to be a likely process.

In our view the nature of the transudation is best explained by the second hypothesis, namely, the selective absorption of milk precursors at the membranes separating the secreting cells from the blood supply. In this connection it might be observed that the existence of a renal threshold value for such a blood solute as glucose is explained by preferential absorption of this substance by the cells of the tubules. Further, the substances which are most easily absorbed by the mammary gland (glucose, amino-acids) are precisely those for which renal threshold values exist. We suggest therefore that the membranes separating the cells of the mammary gland from the blood-stream have similar properties to those separating the blood-stream from the renal tubules. Such a similarity would account for the free passage of amino-acids and glucose into the mammary gland and for the comparative impermeability to inorganic salts such as sodium chloride. The postulated preferential absorption would not appear so complex if it could be shown that the amino-acids and sugar were absorbed into the mammary gland in the same ratio as that in which they occurred in the blood. Blackwood [1932] has, in fact, provided positive evidence on this point. Further support for this suggested resemblance of transudation in the mammary gland to tubular resorption in the kidney is to be found in the behaviour of phloridzin. Phloridzin has the property of paralysing the re-absorptive power of the renal tubules for glucose. We should therefore expect that administration of phloridzin to the lactating animal would be followed by diminution in lactose secretion.

This has been noted by Paton and Cathcart [1911] and others.

We suggest therefore that the first stage in milk secretion is the separation from the blood of a transudate which contains the organic milk precursors (glucose and amino-acids) in the proportions in which they occur in the blood, and the inorganic milk constituents, for example sodium chloride, in much smaller relative concentration.

We may mention in this connection our previous finding [Blackwood and Stirling, 1932, 2] that inorganic phosphate is not absorbed by the mammary gland. Since, as has been pointed out above, the supposition that the transudate contains glucose in the same concentration as in blood does not appear likely, it follows that the transudate must be more concentrated with respect to glucose and amino-acids than is blood.

It is appreciated that this view of the transudation from the blood-stream to the mammary gland is attended by the difficulties common to all attempts to account for the passage of fluids across physiological membranes. For example, the theory first suggested by the work of Tamman [1896] and Starling [1899] to account for the filtration from the blood-stream to the glomerulus of the kidney, and later developed by Govaerts [1923] and Schade and Claussen [1924] as a general theory of transudation across cell membranes considers the effective filtration pressure to be the hydrostatic pressure of the blood less the osmotic pressure of the plasma-colloids (oncotic pressure). If the membranes of the mammary gland are specifically permeable towards blood-solutes, then those blood-solutes towards which they are relatively impermeable will exert a high partial osmotic pressure in opposition to transudation. If we accept 150 mm. mercury as the typical arterial pressure and 35 mm. mercury as the typical oncotic pressure of blood, we obtain 115 mm. mercury as the effective filtration pressure. According to our observations, the partial osmotic pressure of sodium chloride in cow's blood is about 1.7 atmosphere or 1300 mm. mercury. It is difficult to see how transudation can be effected against such an oncotic pressure for sodium chloride. For the present, it must suffice to conclude that, in the mechanism of filtration across physiological membranes, considerations exist regarding the nature of which it is not at present justifiable to speculate.

Intracellular osmotic changes in the mammary gland.

Turning now to consider the nature of the osmotic changes produced in the transudate in the gland cells, it is of primary importance to recognise that, no matter what be the osmotic pressure of the transudate from the blood-stream to the gland, the onset of synthetic activity within the secreting cells will be accompanied by a lowering of osmotic pressure. The formation of one molecule of lactose from two molecules of glucose and the elaboration of osmotically inactive colloidal protein from osmotically active amino-acids are reactions involving lowering of osmotic pressure. The formation of insoluble colloidal calcium phosphate would have a similar tendency, but the

direction of the osmotic change involved in fat secretion is more difficult to judge. The precursors of milk-fat are probably of the nature of phosphatides and as these exist in the plasma in the colloidal state they will contribute but slightly to the osmotic pressure of the blood. If they are absorbed into the gland cells in a colloidal state, and if the reactions by which fat is formed take place out of true solution the secretion of fat will involve very little alteration in tonicity within the secreting cells. It is known that the fat content of milk is readily affected by external factors which have no influence on the concentrations of lactose and protein in the milk secreted. The synthesis of these latter constituents involves osmotic changes and their secretion will be controlled by osmotic relationships. The variability of the fat content of milk seems, accordingly, to indicate that fat secretion is unconcerned with the osmotic conditions in the mammary gland. It seems feasible therefore to consider that by far the largest part of any osmotic change taking place during milk secretion is to be accounted for by the synthesis of protein and carbohydrate.

Since milk is isotonic with blood, we have to consider how this lowering of osmotic pressure due to synthesis is related to the osmotic pressure finally attained during secretion—namely the osmotic pressure of milk. It will be apparent that the osmotic pressure attained at the end of synthesis will be a function of the amounts of glucose and amino-acids. It seems possible therefore to obtain data upon which to consider the osmotic relationships of the mammary gland by determining the freezing-point, lactose and protein contents of the milk secreted. In order to obtain such data we have collected and weighed the daily secretion of milk from six animals. The freezing-point of the milk has been determined by the Hortvet cryoscopic method [Hortvet, 1921] together with the amounts of water (by evaporation), lactose [Shaffer and Hartmann, 1920] and protein (Kjeldahl) secreted in 12 hours. These are shown in Table II. With the data available we propose to consider the nature

Table II.

Cow No. ...	1	2	3	4	5	6
Total yield of milk (g.)	8164	8164	9979	9298	10433	10866
Water (<i>P</i>) (g.)	7202	7129	8734	8174	9138	9429
Lactose (<i>a</i>) (g.)	389	387	586	443	471	519
Protein (<i>b</i>) (g.)	260	281	240	211	355	303
Depression of freezing-point (Δ)	0.531°	0.545°	0.544°	0.546°	0.545°	0.543°

of the osmotic changes involved in a hypothetical process the reverse of synthesis—namely the transformation of lactose back into glucose and protein into amino-acids. By this means it is possible to calculate the theoretical composition of the transudate from which the milk is derived.

One gram molecule (342 g.) of lactose dissolved in 1000 g. of water will have an osmotic pressure of 22.4 atmospheres at N.T.P. This is represented by a depression of the freezing-point of 1.86°. If the lactose is now transformed

into glucose there will be an increase in the depression of the freezing-point of approximately 1.86° . The increase for a g. lactose in P g. water will be:

$$1.86 \times \frac{1000}{P} \times \frac{a}{342} = \frac{5.44 a}{P}.$$

A similar expression may be developed for the transformation of protein into amino-acids. In column 1 of Table III are given the amounts of the

Table III.

	Yield from 100 g. caseinogen g.	Molecular weight	Yield from 100 g. caseinogen g.-mol.
Glycine	0.4	75.06	0.00533
Alanine	1.8	89.07	0.02020
Valine	7.9	117.10	0.06745
Leucine	9.7	131.11	0.07399
Aspartic acid	4.1	133.07	0.03080
Glutamic acid	21.8	147.08	0.14820
Hydroxyglutamic acid	10.5	163.08	0.06437
Serine	0.5	105.07	0.00476
Proline	8.0	115.08	0.06950
Hydroxyproline	0.2	131.08	0.00153
Phenylalanine	3.9	165.10	0.02417
Tyrosine	6.5	181.10	0.03589
Cystine	0.3	240.26	0.00125
Arginine	5.2	174.15	0.02985
Histidine	2.6	155.10	0.01670
Lysine	7.6	146.13	0.05200
Tryptophan	2.2	204.12	0.00108
Total	93.2	—	0.64707

various amino-acids to be obtained by hydrolysis of 100 g. of caseinogen. The figures have been taken from Mitchell and Hamilton [1929] and while they do not represent a quantitative recovery they may be taken as an approximation. From these have been calculated the g.-molecular yield of each amino-acid (column 2). It will be seen that the hydrolysis of 100 g. of caseinogen will increase the osmotically active concentration of any solution by approximately 0.647 g.-mol. For b g. of caseinogen dissolved in P g. of water the increase in depression of the freezing-point will therefore be

$$1.86 \times \frac{1000}{P} \times \frac{0.647 b}{100} = \frac{12.02 b}{P}.$$

This expression may be used to give an approximate idea of the change in osmotic pressure caused by the hydrolysis of milk-protein.

If we have a solution of depression of the freezing-point Δ , and containing P g. of water, a g. of lactose and b g. of protein, the depression of the freezing-point (Δ_1) after hydrolysis of the lactose and protein will be given by the formula

$$\Delta_1 = \Delta + \frac{5.44 a}{P} + \frac{12.02 b}{P}.$$

This value Δ_1 has been calculated for each of the six milks the analyses of which are shown in Table II. The values for Δ_1 are given in column 6 of Table IV. We have now to consider the relationship of these calculated values to osmotic phenomena within the mammary gland during secretion.

Table IV.

Cow No.	<i>P</i>	<i>Q</i>	<i>a</i>	<i>b</i>	Δ	Δ_1	Δ_2	<i>W</i> Cals.
1	7202	17080	389	260	0.531°	1.259°	0.244°	-0.650
2	7129	17190	387	310	0.545°	1.314°	0.225°	-0.688
3	8734	19910	586	349	0.544°	1.240°	0.239°	-0.722
4	8174	17240	443	307	0.546°	1.152°	0.259°	-0.541
5	9138	21670	471	355	0.545°	1.292°	0.230°	-0.844
6	9429	19600	519	303	0.543°	1.129°	0.213°	-0.592

It will be appreciated that, at the end of the hypothetical hydrolysis considered above, the milk constituents are in the form in which they were obtained from the blood. In other words, by a process the reverse of that which must actually take place in the mammary gland, we have regained the transudate stage. The depression of the freezing-point (Δ_1), calculated for each milk, will be that which the transudate from the blood-stream must have in order that the osmotic pressure reached after synthesis has taken place may be that of milk. We may conclude that in order that the secretion at the end of the synthetic stage may be isotonic with blood the transudate must have an osmotic pressure of about three times that of blood. If the value is greater than this, then at the end of synthesis, water will have to be absorbed from the blood to give a product isotonic with blood; if less, then, before the milk can become isotonic with the blood, water will have to be returned to the blood. It has been suggested by Hammond [1913] that an osmotic flow of milk through the mammary gland might be produced by the breakdown of a glycoprotein precursor into lactose and milk-protein. If such a process were to take place it is certain that synthesis of glycoprotein must first take place with consequent lowering of osmotic pressure. A flow of water could result from hydrolysis of a glycoprotein only if the osmotic pressure reached at the end of the synthetic reactions were equal to or greater than that of blood. As has been noted above, this requires the separation of a transudate of osmotic pressure much higher than that of blood. When we remember the many mechanisms utilised by the living organism for the preservation of intracellular osmotic equilibrium, the separation of an intracellular fluid from the blood of higher osmotic pressure than the blood itself seems a very unlikely process.

In view of the isotonicity of blood and milk it appears to us a more profitable hypothesis to consider that at no point in mammary tissue does the osmotic pressure rise above that of blood. On this view the first stage in milk secretion is the charging of the gland cells with a transudate isotonic with blood. Synthesis will then commence and, in order to preserve osmotic equilibrium, water will be returned to the blood-stream. When synthesis is complete, the cells will be filled with milk isotonic with blood. On this view it is impossible to account for the discharge of milk into the alveoli by an osmotic effect. When, however, we remember the difficulties which have beset attempts to explain the continuous flow of other secretions by osmosis, it

seems advisable to ascribe this phenomenon, so far as milk secretion is concerned, to agencies not yet elucidated. It is then possible to test the likelihood of this suggested mechanism of milk secretion in the light of experimental data presented in this paper.

For example it is possible to calculate the amount of transudate required according to the hypothesis for the secretion of a known amount of milk. For this purpose we shall assume that in the gland cells the return of water to the blood takes place after completion of synthesis, whereas in practice the two processes will take place simultaneously. We have previously seen that hypothetical hydrolysis of the protein and lactose in milk gives a fluid of higher osmotic pressure than milk. Since the transudate is considered to be isotonic with blood, the amount of water which must be separated from transudate during milk secretion is identical with that which must be added to the hypothetical fluid to bring the osmotic pressure to the same value as that of the blood.

Let P = g. of water secreted in milk in time t .

Q = g. of water in transudate in time t .

Δ = Depression of freezing-point of the blood.

Δ_1 = Depression of freezing-point of the milk obtained after hydrolysis of protein and lactose.

Then

$$\frac{Q}{P} = \frac{\Delta_1}{\Delta}, \quad Q = \frac{P\Delta_1}{\Delta}.$$

The values of Q for the six milks examined have been calculated and are given in column 2 of Table IV. It will be seen that, on the theory suggested above to describe the various phases of milk secretion, approximately 2/3 of the water obtained by the mammary gland in the form of the transudate will be returned to the blood during synthesis, the remaining third appearing in the milk. While the figures can only be taken as rough approximations, it cannot be said that the theory imposes on the mammary gland the necessity of dealing with excessive amounts of fluid. The theory does of course require that cell membranes shall have a permeability from the blood towards the gland different from the permeability in the opposite direction. This is an assumption which has to be made in describing many biological phenomena.

The energy requirements of the mammary gland.

In a previous paper [Blackwood and Stirling, 1932, 1] we have pointed out the error implicit in the assumption that all the glucose absorbed by the mammary gland is utilised for lactose synthesis. We have suggested that part may be utilised in providing energy and it seems desirable to consider the amount of energy which would be expended by the mammary gland according to the theory of milk secretion suggested in this paper.

Synthetic reactions are considered to be almost thermoneutral, but it is

as well to have some idea of the energy required for the daily synthetic requirements of the mammary gland.

From the above equation it will be seen that the heat of formation of 1 g.-mol. of lactose from 2 g.-mols. glucose is about 5 Cals. The experimental animals used by us secreted on an average about 1.5 g.-mols. of lactose per 12 hours, as shown in Table II. This will require the expenditure of about 7.5 Cals. which could be provided by the combustion of about 2 g. of glucose. The other synthetic reactions of the mammary gland will also involve the completion of secondary bonds (carbon to oxygen and nitrogen) and it is not to be expected that the energy required for the synthesis of protein and fat will be much in excess of that required for the synthesis of lactose. It is to be concluded therefore that the energy required for synthetic reactions by the mammary gland is so small as to be negligible.

It has been pointed out that the assumption of a transudate isotonic with blood requires the return of water to the blood-stream during synthesis for the preservation of osmotic equilibrium. For convenience of treatment we may consider synthesis to go to completion without separation of water and to be followed by return of water to the blood in amount sufficient to bring the osmotic pressure again to that of the blood. We should thus have a lowering of osmotic pressure due to synthesis and the lowest value obtained would be represented by a new depression of the freezing-point (Δ_2) given by the formula

$$\Delta_2 = \Delta - \frac{5.44 a}{Q} - \frac{12.03 b}{Q},$$

where a , b , Q and Δ have the same meanings as before. Values of Δ_2 have been calculated for the six milks examined and are given in Table IV.

It will be seen that, according to the view expressed in this paper, the secretion of milk involves, in 12 hours, the concentration of a fluid containing Q g. of water and of depression of the freezing-point Δ_2 to milk containing P g. of water and of depression of the freezing-point Δ . Since the process of concentration is considered to take place by passage of water from a solution of low osmotic pressure to one of higher osmotic pressure separated by a semi-permeable membrane it will not entail the expenditure of energy by the mammary gland. This fact is best illustrated by treating the matter formally according to the methods for evaluating osmotic work.

If p_1 = osmotic pressure of the solution to be concentrated,

p_2 = osmotic pressure of the blood,

V_1 = initial volume of solution,

V_2 = final volume of solution,

and W = work done in effecting concentration.

Then

$$dW = (p_1 - p_2) dv$$

$$W = \int_{v_2}^{v_1} (p_1 - p_2) dv = \int_{v_2}^{v_1} p_1 dv - \int_{v_2}^{v_1} p_2 dv.$$

Since p_2 remains constant throughout the process we may write

$$W = \int_{v_2}^{v_1} nRT dv - p_2 \int_{v_2}^{v_1} dv,$$

$$W = nRT \log_e \frac{V_1}{V_2} - p_2 (V_1 - V_2).$$

Instead of V_1 and V_2 we may write Q and P since these are the amounts of water (in g.) in the transudate and milk respectively. p_2 , the osmotic pressure of the blood, is the same as that of milk and at body temperature (37°) is equal to $\frac{22.4 \times 310 \Delta}{1.86 \times 273}$ atmospheres = 13.7Δ atmospheres. $n = \frac{\Delta P}{1.86}$.

$$\begin{aligned} W &= \frac{\Delta PRT}{1.86 \times 1000} \log_e \frac{Q}{P} - \frac{13.7 \Delta}{1000} (Q - P) \text{ litre atmospheres} \\ &= \frac{2.303 \times 0.082 \times 310}{1860} \Delta P \log_{10} \frac{Q}{P} - \frac{13.7 \Delta}{1000} (Q - P) \text{ litre atmospheres} \\ &= 0.03148 \Delta P \log \frac{Q}{P} - 0.0137 \Delta (Q - P) \text{ litre atmospheres} \\ &= 0.000762 \Delta P \log \frac{Q}{P} - 0.000332 \Delta (Q - P) \text{ Cals.} \end{aligned}$$

This expression has been evaluated for each of the milks for which analytical data are available, the values of W so calculated being given in Table IV, column 8. In each case a negative value is obtained. In this fact we have a mathematical expression of the statement made above that the theory of the secretion of milk presented does not require the expenditure of any energy other than that required for synthesis.

It should be noted that when an energy balance is struck according to the equation:

Total energy requirements = energy for synthesis + energy for concentration, the negative values obtained for the last factor are insufficient to give the total energy factor the negative sign. The conceptions are therefore in harmony with the idea of secretion requiring the expenditure of a small amount of work by the gland cells.

SUMMARY.

The absorption of milk precursors by the mammary gland and the mechanism of milk secretion are considered from the physico-chemical standpoint.

The nature of the transudation from the blood-stream to the mammary gland may best be explained by the hypothesis of selective absorption of milk precursors at the membranes separating the secreting cells from the blood supply.

It is considered that during milk secretion a transudate is separated from the blood having the same osmotic pressure as blood, and that during synthesis water is returned to the blood to maintain the tonicity of the cell fluid. At

the end of synthesis the gland cells will therefore be filled with milk isotonic with blood.

Quantitative aspects of this theory of the mechanism of milk secretion are considered in the light of analytical data for the composition and yield of milk from six cows.

It is shown that this theory of milk secretion does not require the expenditure of any energy by the gland cells other than that necessary for the synthesis of milk constituents.

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