THE SECRETION OF MILK*

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OUR knowledge of the cytology of milk secretion —what structures in the large cells lining the alveoli of the mammary gland are concerned with the formation of the various milk constituents—is meagre. There is no doubt that all the milk constituents are secreted by the same type of cell; there are not separate cells secreting fat, others secreting casein, others secreting salts and so on. It is now usually agreed also that the milk-secreting cells are fairly permanent structures; that is, that milk does not originate from the breakdown and dissolution of complete cells which are renewed again and again from the basement membrane. This would entail far more mitosis and repair in cell structure than can be seen in the actual functioning gland, and would probably entail the presence in milk of fairly large amounts of substances derived from the cell nuclei, such as nucleoproteins or breakdown products of these proteins which are, in fact, only present in traces in milk. What appears to be the case is that apart from occasional breakdown and repair, the alveolar cells maintain their nuclei and general integrity throughout a large part of a lactation period.

The day's work of one of these cubical cells entails the following cycle of operations. It begins as a rather short squat cell with the nucleus in the middle. Granules or globules, some of which stain with fat-soluble dyes, then begin to appear in the part of the cell nearest the alveolar space. The cell begins to increase in length and size, the nucleus remaining fairly close to the basement membrane. The secretory products soon fill the whole of one end of the tall, distended cell. These products, and possibly a small part of the cytoplasm of the cell itself, are now extruded into the lumen of the alveolus as milk, following which discharge the cell returns to its original squat shape. This whole process is repeated several times until the alveolar spaces become distended with secreted milk and the whole of the rest of the storage space in the gland is also occupied, whereupon further secretion ceases. During twenty-four hours in an actively lactating cow there may be up to four or five or even more cellular cycles of operation. Milk secretion proceeds continuously throughout the major part of the twenty-four hours, possibly interrupted during the actual milking process.

Though the cells are tiny compared with, say, the size of an *Amœba*, there is plenty of room inside each one for a very complicated structure, a part of which has been revealed by ordinary microscope and histological methods. The development of the electron microscope gives an increased hope of further accurate knowledge of the lay-out of these tiny factories, but it will probably be a long time before their detailed structure, the mode of action of the enzymic systems which are known to exist in these cells, and the associated biochemical changes, many of which have already been observed, and which I now propose to discuss, have been completely co-ordinated.

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The Biochemistry of Milk Synthesis

It is clear that the basic materials from which milk is made, as well as those needed for producing the energy for milk secretion, must be brought to the mammary gland by the circulating blood. The main problems can be stated in the following general terms: (1) What are the chemical materials in this blood which are used for (*a*) the manufacture by the milk-secreting cells of the principal milk constituents, (*b*) as a source of energy for the work done by the active gland? (2) How is this manufacture actually brought about in these cells? In the cow, two mammary arteries, one of which serves the front and rear quarters on each side of the udder, provide the whole of the incoming blood. The arteries rapidly sub-divide to form arterioles and capillaries in close touch with the alveolar cells. A sample of blood taken from any part of the arterial system will contain all the materials either for milk manufacture or to meet the energy needs of the udder.

Three constituents of milk (Table 1), at least, are not present as such in the circulating blood, namely, milk fat, casein and lactose. Two at least of these, namely, lactose and casein, are produced nowhere in Nature but in the cells of the mammary gland.

TABLE 1. RELATION BETWEEN THE CONSTITUENTS OF COWS' BLOOD AND THOSE OF COWS' MILK.

(Quantities shown in mgm. per 100 ml.).

Constituent	Blood plasma	Milk	Ratio (approx.) blood : milk
Casein	Nil	2800–3000	—
Lactalbumin	Nil	350–450	—
Globulin	1200–2000	50–150	1 : 0·1
Lactose	Nil	4600–4900	Blood glucose : milk lactose
Glucose	45–60	Traces	as 1 : 80
Fat (as fatty acids)	150–300	3000–4000	1 : 15
Ca (total)	10–12	120–140	1 : 12
K	16–20	120–180	1 : 9
Cl	270–300	90–120	1 : 0·4
Inorganic P	4–6	60–80	1 : 15
Total acid-soluble P	4·2–6·5	65–90	1 : 15
Lipin P	5–7	4–12	1 : 1·5
Urea	30–40	30–40	?1 : 1

Other constituents of milk are present either in much larger or much smaller concentrations than in blood. A very few, such as urea, which is freely diffusable through most of the tissues of the body, are present in equal concentration in both blood and milk. Whereas the inorganic salts of milk must clearly come from the inorganic salts of the blood plasma (for example, calcium, potassium and chlorine) it is by no means necessary that the organic constituents of the milk are derived from the more or less obvious precursors in the blood, for example, milk sugar from blood sugar.

As regards venous drainage, the system of blood vessels is more complicated. There are actually three routes by which venous blood leaves the gland, the main one being the subcutaneous abdominal vein which goes forward from the gland just below the skin (the so-called milk vein) and enters the abdomen through a small hole—the 'milk well'—in the abdominal wall. There are considerable anastomoses between the various veins, so that a sample of blood taken from the 'milk vein' gives a fair picture of the composition of the blood leaving the gland.

By comparing, therefore, the composition of the arterial blood reaching the secreting gland with that of the venous blood leaving it, one should, it would seem, be able to determine what blood constituents are being used by the gland.

The veins are not, however, the only channel through which circulating fluid leaves the mammary gland. There is also, as in most other active tissues of the body, a well-developed lymph drainage system, as extensive as the venous system. The lymphatic system drains the tissue spaces, whence the fluid which originates from the circulating blood is slowly but continuously removed, passed through lymph nodes (there is a particularly large lymph node, up to some 10 cm. long, on the top of the udder on each side) and by a long lymphatic channel furnished, like the venous system, with valves, and returned to the circulating blood through the so-called thoracic duct, which pours a slow stream of lymph into the venous blood of the anterior vena cava, just before the blood reaches the heart. The rate of lymph flow from the mammary gland is undoubtedly very much less than that of the venous blood flow, but in what quantitative relationship the two stand is at present a matter of guesswork. Nevertheless, any balance sheet which might otherwise be drawn up over any given period of secretion, say twenty-four hours, as between the quantity and constituents of blood entering the gland on one side, and the blood plus milk leaving the gland on the other, is complicated by this small but quantitatively unassessed expenditure from the arterial blood which does not appear either in the venous blood or in the milk itself.

The first whole-hearted attempts to discover the nature of the milk precursors gave misleading results. The particular method used was to analyse samples, taken simultaneously, of mammary venous blood and of jugular venous blood. Satisfactory methods for obtaining true arterial blood had not then been worked out, and it was assumed that the easily obtainable jugular blood, draining the head, which was considered to be a relatively inactive part of the organism, in the cow at any rate, would be fairly close in composition to true arterial blood.

This was clearly shown, by later workers at the Hannah Research Institute, not to be the case, so that one at least of the main conclusions drawn from the first experiments, that butter fat in milk was mainly derived from the phosphorized fats of the blood, was incorrect.

Most of the recent work, that is, since about 1932, has been carried out by more reliable methods. Methods of arterial sampling without disturbance of the cow have been developed in various research centres; for example, sampling from the internal iliac artery, which can be easily approached through the rectum of the animal, or principally in goats, from a portion of the carotid artery which has in a previous minor operation been exteriorized in a loop of skin. It may be said at once that the method of obtaining the blood samples is more than half the battle. It was clearly demonstrated at the National Institute for Research in Dairying, at Shinfield, in 1934, that disturbance of the animal during sampling must be avoided, otherwise analysis of the blood samples was largely waste of time. It is not at all easy to take an arterial and venous blood sample practically simultaneously without some disturbance, though individual animals vary very greatly in their perturbability.

To get over this difficulty, two other methods have been used fairly recently: one is the use of an anæsthetic, nembutal, which removes nervous excitement and appears not to interfere with the secretory process; the other is by, so to speak, removal of the animal altogether (a method first used, tentatively, by Foá some thirty-five years ago), namely, by excising the mammary gland and perfusing the latter, with as little delay as possible, either with oxygenated defibrinated blood or with oxygenated whole blood prevented from clotting with a suitable anticoagulant such as sodium citrate or heparin.

None of these methods is perfect, but each has its advantages and disadvantages. The perfusion technique very recently developed by Petersen, Shaw and Visscher in Minnesota is one requiring much experimental skill. It is said that the gland can be in action within seven or eight minutes of the slaughter of the cow. By this method various blood pressures can be used, various materials can be added to the arterial blood, and blood sampling, both 'arterial' and venous (a single venous exit is arranged), is easy, while milk can be collected without too much difficulty. The isolated gland cannot be used too long as it is, of course, steadily deteriorating, and again the question arises as to how far the interference with lymphatic drainage affects the findings.

The nembutal anæsthesia method, also developed in the United States during the War, is said to have no effect on blood sugar or on the rate of milk secretion in goats, or on the energy requirements of the mammary gland.

An entirely different method for the study of mammary gland metabolism is the incubation, in well-oxygenated salt solutions, of fresh slices of mammary tissue with various possible precursors of the milk constituents.

Still another method is by the use of radioactive elements such as radioactive phosphorus or calcium, or of 'heavy' elements such as deuterium ('heavy hydrogen') or 'heavy' nitrogen, which, suitably combined, can be given to a lactating animal by mouth or intravenously, and followed through into the milk by appropriate physical means. This very promising method has so far been used only in a preliminary way, though Hevesy and Aten in Holland, in 1938, made a few useful observations using radioactive phosphorus.

Chemical Changes from Arterial to Venous Blood

Blood sugar. These changes are the largest in actual percentage; there is a fall varying from 10 to 25 or even 30 per cent of the total arterial blood sugar.

Blood fat. There is a fall of about 3–5 per cent in the neutral fat of the blood in passing through the gland.

Lactic acid. The position here is not yet clear. Some earlier experimenters claimed that fairly large amounts of blood lactic acid disappear while going through the mammary gland. More recent work shows little change with normal, unanæsthetized animals, but there appears to be a slight uptake by anæsthetized animals in the first twenty minutes of anæsthesia, but not, it seems, later. It is an open question whether lactic acid is or is not one of the blood constituents normally used by the secreting cells.

β-*Hydroxybutyric acid.* This substance, which occurs in normal cow's blood in appreciable quantities, and in animals in 'ketosis' in much larger amounts, is taken up by the mammary gland in quantities which appear to vary with the concentration in the blood.

Globulin. Apparently an appreciable quantity of plasma globulin, but no albumin, leaves the blood in traversing the mammary gland. The globulin which is mainly involved is a glycoprotein, the molecule of which contains some 9 per cent of a sugar complex containing galactose, mannose and glucosamine. There is some evidence that plasma albumin is actually greater in mammary venous blood than in arterial blood.

Amino-acids. Small quantities of uncombined amino-acids appear to leave the arterial blood, and there is a suggestion that slightly more urea nitrogen is present in mammary venous blood than arterial blood.

Inorganic phosphorus. There is a drop of about 5–7 per cent in the amount of inorganic phosphorus in blood plasma in passing through the gland.

Calcium. A fall takes place of about 2·5 per cent in the amount of calcium in the arterial blood.

Since the milk contains relatively large quantities of end-products, of which several of the materials just mentioned must be the precursors, it is obvious that, since there is such a small quantity of precursor removed from unit volume of blood, the rate of blood-flow through the gland must be very high (see below).

Mammary Gland Slices

Grant, working in Great Britain, found some years ago that lactose was formed when mammary gland slices were incubated in oxygenated physiological salt solutions containing glucose, but not, apparently, if fructose, mannose, galactose or various sugar-phosphoric acid esters were used. Some of these findings were later called into question, but very recent work of Knodt and Petersen has confirmed that lactose is formed when mammary slices are incubated with glucose, and also with glucose plus lactic acid, maltose and glycogen. It is not perhaps surprising that the last two should give positive results, since both will presumably be hydrolysed to glucose by enzymes present in mammary gland tissue. β-Hydroxybutyric acid disappeared when incubated with mammary slices, the rate depending on the amount of the acid present.

Perfusion of Excised Mammary Gland

It has been shown that tissue glycogen is increased by perfusion of the excised mammary gland with blood containing added quantities of glucose, but increased blood glucose caused no significant change in the amount of lactose secreted in a six-hour period. It is very likely that, as in other tissues, glycogen can be synthesized from various normal blood constituents by the mammary cells, and later hydrolysed again to form glucose and, through glucose, lactose. Normal mammary tissue contains about 0·2 per cent of glycogen, according to American findings.

Further sidelights on the biochemistry of milk secretion are thrown by the study of the composition of milk when the secreting tissue is infected by *Streptococcus agalactiæ*, which may persist in the gland for long periods without too seriously inhibiting the flow of milk.

In the infection known as streptococcal mastitis, the secreting cells are physiologically abnormal in that instead of true milk, a fluid is secreted in which the characteristic milk constituents lactose, casein and fat and the less characteristic but very important constituent vitamin A are diminished. There is an increase in globulin and a large increase in chloride. Davies believed that mastitis milk could be regarded as ordinary milk containing larger or smaller proportions of what he called "an isotonic diluent" approximating in composition to blood plasma, with its relatively high proportion of globulin and sodium chloride. A varying proportion of udder tissue is, in effect, in a catarrhal condition and forms an ineffective barrier against this leakage. This view has recently received some support from the finding that while in the blood plasma of a cow receiving green fodder carotene is high, in normal milk the carotene content is only 3 per cent of that in blood, but in mastitis milk (even say from one infected quarter of a cow the other three quarters of which may be still normal) the carotene may be as high as 20 per cent or even more of the blood level. Vitamin A, on the other hand, is lower in blood plasma than in milk—it is not yet certain whether the udder cells concentrate the vitamin A of the plasma or actually synthesize it from blood carotene—but in mastitis milk it is, like the fat, lower than in normal milk.

Any connected account of the processes by which milk is made in the udder must provide an explanation of the interesting carotene-vitamin A-butter fat relationships in milk. Both carotene and vitamin A are present in milk in solution in the fat globules; but it has been shown quite recently that whereas the vitamin A-butter fat ratio is more or less independent of the size of the fat globule in milk, the carotene-fat ratio in the fat globule increases as the size of the globule diminishes, that is, as its relative surface area increases. This suggests that fat and vitamin A are synthesized, or perhaps one had better say assembled, by the same mechanism in the secreting cell, whereas the closely related (and also fat-soluble) carotene finds its way into milk by a different process associated in some unknown way with the *surface* of the fat globules.

All the water-soluble vitamins investigated—vitamin C, riboflavin and vitamin B_1—are considerably more concentrated in milk than in blood. Vitamin C is concentrated some eight times, riboflavin perhaps about four times, and total vitamin B_1 two or three times. All three are depressed in amount in mastitis milk, as would perhaps be expected since a function of the normal secretory cell is to take them up from the blood and concentrate them, a function which partially breaks down in the infected cell.

Energy Changes in Milk Secretion

Despite the rapid flow of blood through the mammary gland, a considerable proportion of the oxygen reaching the gland from the arterial blood is used up for combustion of one or more oxidizable substances in the tissue. Quantitatively, in some Shinfield experiments in which we were satisfied that the cows were undisturbed, the amount of oxygen used by the secreting gland was 4–5 volumes for each 100 volumes of blood, while at the same time 5–7 volumes of carbon dioxide were given out to the venous blood. The so-called respiratory quotient—volumes of carbon dioxide given out divided by volumes of oxygen used—was invariably higher than 1 in all experiments where the cows were undisturbed.

Similar findings have been made in at least two laboratories abroad, with both goats and cows; that is, in the undisturbed, normal animal, a mammary gland respiratory quotient of well above 1, usually between 1·1 and 1·3, is found.

If these findings are to be interpreted along the usual lines, it would mean that the secreting cells were synthesizing fat from carbohydrate. The American workers, who found that β-hydroxybutyric acid was being taken up from the blood by the gland, suggested that it was being partially oxidized and partially transformed into the short-chain fatty acids which occur specifically in milk fat. But it is known that the amount of blood *fat* taken up by the mammary gland is sufficient to account for all the milk fat secreted, so that this suggestion requires further evidence before it can be accepted.

Until we are fairly certain what substances are actually serving the gland as sources of energy and what blood precursors are transformed into milk constituents, exact knowledge of the energy changes in the gland will not be forthcoming, and it is probably idle to make any thermodynamic speculations at present.

Rate of Blood Flow through the Secreting Udder

One of the earliest estimates of the number of volumes of blood required, on the average, to produce one volume of milk, was made by the Shinfield workers in 1935, by comparing the fall in fatty acid, inorganic phosphorus and sugar between arterial and mammary venous blood. It was considered that probably between 400 and 500 volumes of blood must circulate through the udder to produce one volume of milk. This figure was little more than a guided guess, and it was pointed out at the time that implicit in this conclusion was the view that the lymphatic drainage of the udder could be regarded for the purpose of calculation as negligible—a likely assumption, but little more than an assumption. Afterwards, by use of a 'stromuhr' or volume-meter actually inserted in the circulation, Graham found that the blood flow was only about half our estimate. Still later findings of Shaw and others, who have used a rather different method of assessment, namely, by determining the amount of total calcium and total phosphorus taken, on the average, out of unit volume of blood by the udder, and the amount of the same materials in the milk secreted by the same animal during twenty-four hours, point again towards our original estimate of 400–500 volumes. Their average figure is given as 494 volumes of blood for each volume of milk—an average finding, of course, the lowest figure being 331 volumes for total phosphorus in one experiment and the highest 650 in one experiment for total calcium.

The results summarized in the accompanying table are probably not very far from the truth, though the experimental errors in the second column are quite appreciable, and also the efficiency of uptake of any blood precursor by the mammary cells will, like the efficiency of any other physiological process, undoubtedly vary from one cow to another and in the same cow at different stages in the lactation period and even at different times on the same day. It may nevertheless be concluded that, with a reasonably good cow at the peak of her lactation, giving say four gallons of milk a day, approximately nine tons of blood, say eighteen times her own weight, will pass through the udder each day.

TABLE 2. MAMMARY GLAND BALANCE. SUMMARY.

(From Shaw, Powell and Knodt, 1942.)

Blood precursor	Gland utilization per litre of blood (gm.)	Suggested end product				Ratio of blood volume to milk volume
		Milk substance	24 hr. production (gm.)	Blood required (litres)	Milk produced (litres)	
Calcium	0·0021	Calcium	22·52	10,931	19·40	563 : 1
Phosphorus	0·0021	Phosphorus	17·61	8,246	,,	425 : 1
Calcium +phosphorus	0·0042	Calcium +phosphorus	40·13	9,588	,,	494 : 1
Neutral fat	0·0684	Fat	707·5	10,317	,,	532 : 1
Glucose	0·1122	Lactose	985·5	9,070	,,	468 : 1

Precursors of Milk Constituents

To sum up, our present knowledge of the precursors of the main milk constituents is as follows:

Milk fat. This is almost certainly derived from the neutral fat of the blood. The short-chain fatty acids in milk fat are probably derived, as Hilditch first suggested, from the breakdown of some of the long-chain fatty acids in the blood fat, though the recent findings of Barcroft and his co-workers that the ruminant is able to take up short-chain fatty acids direct from the rumen and that these may circulate in the blood has to be borne in mind.

Lactose. This is almost certainly derived from the glucose of the circulating blood, though the latter sugar may pass, in part in any event, through intermediate stages such as lactic acid or even glycogen in the gland cells before being transformed into the lactose. Part of the lactose may come from the sugar-containing globulin which is taken up by the active gland cells from the blood.

Casein and lactalbumin. There is little doubt that amino-acids in the circulating blood contribute at least a little toward the synthesis of one or both of these proteins by the gland cells. Doubtless a major portion is contributed by the blood globulin which is now known to be taken up by the secreting tissue. Using radioactive phosphorus, it has been shown that the inorganic phosphate of the circulating blood provides the phosphorus organically combined in the casein molecule. We know less about protein synthesis by the gland than about fat and carbohydrate synthesis.

Energy. We know next to nothing as to what carbon compound is burnt in the mammary gland to provide the energy needed for synthesis of the milk constituents. It may be glucose, lactic acid, the sugar-containing moiety of the blood globulins just mentioned, or β-hydroxybutyric acid, or a part of the blood fat, or a combination of two or more of these.

In this connexion it seems more than likely that the secreting cells may be capable, like other tissues of the body, of using different materials for energy production or for milk production at different times and under different nutritional conditions. Although the secreting tissue of the mammary gland appears histologically to be simpler than that of, say, the kidney, it is clear from what has just been said that the activities of these large mammary cells are very complex. Their internal structural pattern and enzymic equipment are at present almost unknown.