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THE FLOW AND COMPOSITION OF MAMMARY GLAND LYMPH

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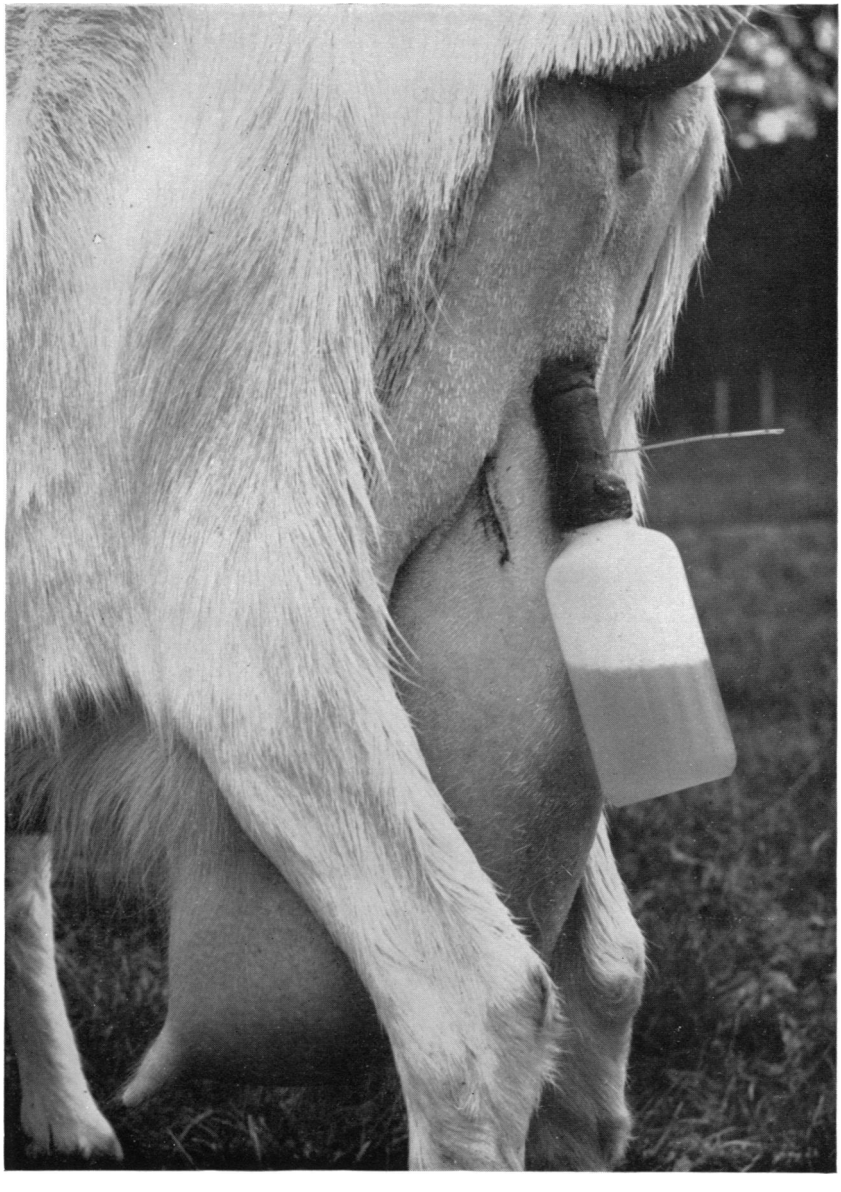
There are no published figures for the rate of lymph flow from the mammary glands, although a number of authors have stated that it should be considered in the study of the uptake of milk precursors from the blood by the mammary glands. In recent years Belgian workers have suggested the study of mammary lymph composition as a means of investigating mammary gland metabolism and have published some detailed analyses of the lymph taken from the udders of dead cows (Heyndrickx & Peeters, 1958*a*, *b*, 1960; Heyndrickx, 1959). Mammary gland lymph has been collected on a number of occasions in this laboratory over a period of 10 years, chiefly from goats during studies of the transfer of metabolites from blood to milk (Linzell, 1960) and during a study of the perfused goat's udder (Cargill-Thompson, Drury, Hardwick, Linzell & Tucker, 1958; Hardwick, Linzell & Price, 1960). The results are presented now because sufficient data have accumulated to suggest that mammary lymph flow does reflect the metabolic activity of the tissue and also to sound a note of caution, because the composition of the lymph collected from conscious goats differs in some important respects from that obtained from dead cows. In addition, it seems worth recording that large quantities of lymph can be obtained for long periods with minimal disturbance from goats' udders, because this type of preparation may be of use to workers interested in lymph.

METHODS

The lymphatic vessels from the two mammary glands forming the udder in the goat and sheep pass to the superficial inguinal lymphatic glands on each side, from whence a single lymphatic trunk passes into the abdomen through the inguinal canal, parallel and caudal to the external pudic blood vessels. This efferent duct was cannulated with polythene tubing (1–2 mm outside diameter; 20–30 cm long) a few centimetres from the lymph gland (Fig. 1). In some animals there are 2 or 3 efferent lymph trunks; in such cases one was cannulated and the others ligated.

For continuous collection in conscious goats a polythene bag or bottle (100–250 ml.) was fixed to the animal. In early experiments epidural anaesthesia was used (5–8 ml. lignocaine 2% in the lumbosacral space), but this suffered from the disadvantage that some animals staggered about as the anaesthetic wore off and had to be temporarily supported in a sling to prevent the cannula from being dislodged. Later, satisfactory anaesthesia was obtained



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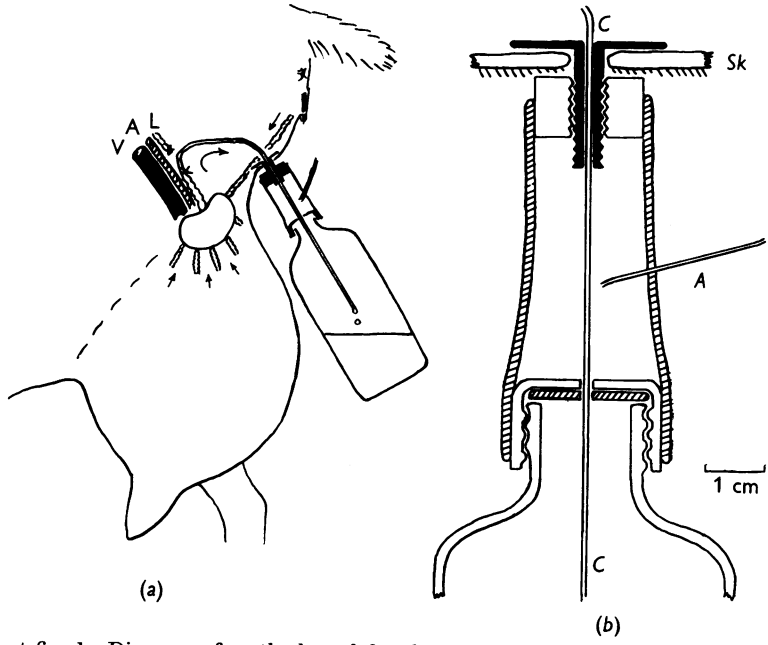
by blocking the udder nerves paravertebrally (Linzell, 1959); in this case there was no paralysis of the hind quarters. A tranquillizing dose of chlorpromazine hydrochloride, 1·70 mg/kg was given ½–1 hr before the operation. The lymph cannula was anchored to the lateral suspensory ligament of the udder when this was stitched up after cannulation, and the distal end was led into the collecting bottle via a stainless-steel cannula, inserted through the skin 5–7 cm from the vulva, with a flexible rubber tube joining it to the cap of the bottle (Text-fig. 1 and Pl. 1). The animal thus had complete freedom and the collecting bottle could be changed without disturbing it or the cannula. The narrow space between the lymph cannula and the skin cannula quickly filled with wound fluid, which clotted and effectively sealed the space so that uncontaminated lymph was obtained. Some of the goats



Text-fig. 1. Diagram of method used for the continuous collection of mammary lymph in conscious goats. (*a*) Position of lymph duct (L) and cannula, external pudic artery (A) and vein (V). (*b*) Scale diagram of flexible rubber connector used to join the polythene collecting bottle to the animal. *C*, Lymph cannula; *Sk*, skin; *A*, air vent. Plastic, white; stainless steel, black; rubber, cross-hatched.

used for continuous collection were also used for mammary blood-flow measurements and had a mammary vein and a carotid artery exteriorized, so that blood samples could also be obtained without disturbance during lymph collection.

Lymph and plasma were analysed by the following methods: Protein, as total N, by a micro-Kjeldahl method; Na and K by flame photometry (EEL Photometer); Ca and Mg, Wilson (1955); Cl, Van Slyke & Hiller (1947); HCO_3, Van Slyke & Sendroy (1928); volatile fatty acids (VFA), Annison (1954); glucose, Nelson (1944) and Somogyi (1952); lactate, Barker & Britton (1957); inorganic P, Berenblum & Chain (1938); amino acids, Hamilton & Van Slyke (1943).

EXPLANATION OF PLATE

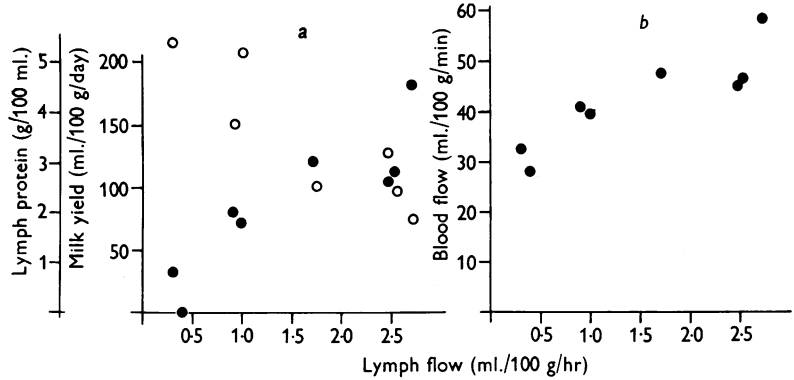
Photograph of the position of the lymph-collecting bottle in a conscious goat.

RESULTS

Mammary lymph flow in conscious goats

Collections were made in 8 Saanen goats for periods varying from 8 hr to 17 days. The volume of lymph collected in non-pregnant animals ranged from 150 to 840 ml./day and the most comprehensive observations are summarized in Table 1.

It must be pointed out that the lymph duct cannulated drains the whole inguinal region including the mammary gland, skin, the teat and the vulva, so that it has to be decided to what extent the lymph obtained represents mammary-gland lymph. It was not at first realized that an appreciable proportion of the lymph from the vulva drains through the superficial inguinal lymph gland at the base of the udder (Text-fig. 1). This was



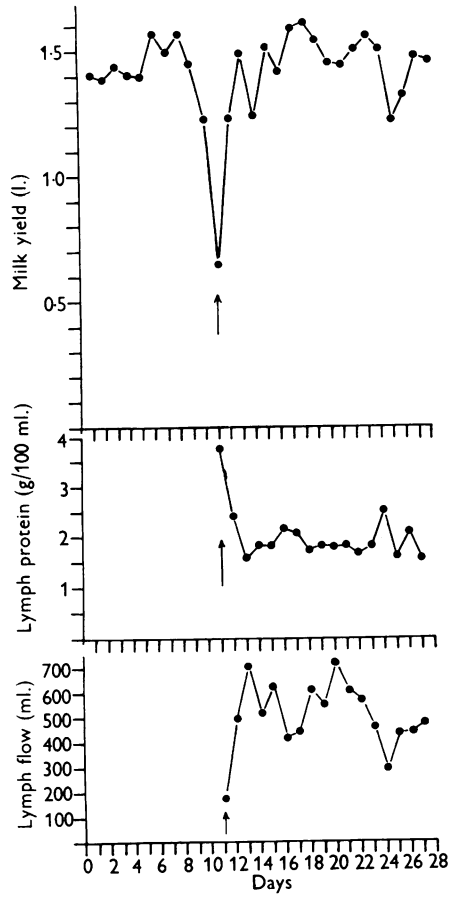
Text-fig. 2. (*a*) Relation of lymph flow (●) to milk yield/100 g tissue and to lymph protein (○). (*b*) Relation of lymph flow to blood flow/100 g tissue. Blood-flow figures from Linzell (1960).

suspected when it was observed that, whereas the lymph flow in dry non-pregnant goats was less than in non-pregnant lactating animals (Text-fig. 2), in pregnancy the flow per gram of tissue remained equal to the highest flows, irrespective of the milk yield, and was often 10–30 % higher. The highest flows recorded (800–1000 ml./day, or 3 ml./100 g/hr) were obtained in one animal in the 3 days before parturition, when the whole perineum was oedematous. In this animal red blood cells appeared in the lymph during parturition and the lymph unexpectedly clotted in the cannula 7 hr later. Next day, following the injection of Evans Blue dye (4 %) around the vulva, 3–4 small lymphatics containing the dye were seen to be running from the vulva towards the superficial inguinal lymph gland in company with the perineal blood vessels. Similar vessels were demonstrated by the same method in other goats, although they were smaller in non-pregnant animals (Text-fig. 1).

TABLE 1. Summary of observations (mean ± S.E. of mean) made during collection of lymph from one mammary gland in each of 4 conscious lactating Saanen goats, together with the analysis of plasma from lactating goats in the same herd. Lymph analyses refer to 24-hr samples. The numbers of animals used for plasma analysis are shown in brackets; mean of 1– 40 estimates for each animal. A—arterial; V—mammary venous

Animal	Jill	Pam	Bessie	Gracie	Plasma
Milk yield (ml./day)	1,207 ± 23	626 ± 13	901 ± 34	1,445 ± 31	—
Weight of mammary gland (g)	1,000	600	800	800	—
Length of lymph collection (days)	7	15	7	17	—
Lymph flow (ml./day)	415 ± 14	357 ± 12	488 ± 14	520 ± 16	—
Lymph flow (ml./100 g/hr)	1·72	2·47	2·54	2·71	—
Na (m-equiv/l.)	150·3 ± 1·1	150 ± 0·9	151 ± 1·8	148 ± 1·2	153 ± 1·3 (13)
K (m-equiv/l.)	4·8 ± 0·16	4·47 ± 0·05	4·66 ± 0·15	4·46 ± 0·08	4·23 ± 0·08 (13)
Ca (m-equiv/l.)	2·61 ± 0·2	3·09 ± 0·15	2·71 ± 0·2	2·68 ± 0·21	4·38 ± 0·21 (10)
Mg (m-equiv/l.)	2·33 ± 0·21	2·06 ± 0·07	1·88 ± 0·07	2·02 ± 0·09	2·29 ± 0·33 (10)
Cl (m-equiv/l.)	118·6 ± 1·1	114·5 ± 0·95	120·4 ± 0·8	112·9 ± 0·71	109·25 ± 0·8 (12)
HCO_3 (m-equiv/l.)	23·6 ± 0·7	27·6 ± 0·37	25·0 ± 0·9	26·0 ± 0·65	A 24·1 ± 0·5 / V 26·1 ± 0·6 (10)
Inorganic P (m-equiv/l.)	2·13 ± 0·17	3·10 ± 0·07	3·25 ± 0·17	1·9 ± 0·04	3·50 ± 0·22 (7)
Lactate (m-equiv/l.)	0·68 ± 0·12	0·78 ± 0·10	0·44 ± 0·22	0·35 ± 0·04	0·42 ± 0·05 (9)
Volatile fatty acids (m-equiv/l.)	0·91 ± 0·09	0·82 ± 0·05	0·68 ± 0·09	0·68 ± 0·05	A 1·43 ± 0·1 / V 0·36 ± 0·08 (6)
Protein (g/100 ml.)	2·47 ± 0·035	3·18 ± 0·14	2·4 ± 0·15	1·88 ± 0·07	7·55 ± 0·2 (9)
Glucose (mg/100 ml.)	59 ± 2·7	82 ± 3·8	82 ± 4·0	53 ± 0·75	A 61 ± 1·7 / V 40 ± 1·0 (13)
Lymphocytes (cells/mm³)	17,500 ± 4·900	7,000 ± 650	2,930 ± 700	4,350 ± 1,100	—
α-amino N (mg/100 ml.)	—	4·09 ± 0·35 (4)	4·42 ± 0·5 (3)	3·12 ± 0·4 (8)	A 5·0 ± 0·31 / V 3·39 ± 0·34 (6)
pH (38° C)	—	7·342 ± 0·015	—	—	A 7·40 ± 0·015 / V 7·35 ± 0·015 (17)

Thus the figures recorded in pregnant animals are unreliable as estimates of mammary lymph flow, but the error in lactating non-pregnant goats is probably negligible. During lactation the skin and teat form only 20% of the total weight of the udder, which again is at least 40 times the weight



Text-fig. 3. Daily variations in milk yield, lymph flow and lymph protein from right mammary gland of goat Gracie. Cannulation on day 11, shown by arrows; the lymphatics from the vulva were resected in this animal on day 12. The fall in milk yield before cannulation occurred when the goat was moved from the farm to the animal house. It was associated with a lower rate of lymph flow and a higher lymph protein.

of the vulva at this stage because goats are anoestrous for the first 5–6 months of lactation and the vagina and vulva are small. In one goat (Gracie, Table 1 and Text-fig. 3), after the lymph duct was cannulated, 2 ml. Evans Blue (4%) solution was injected beside the vulva and the dye appeared in the cannula within 45 min. However, only about 36% of the

injected dye was recovered in the lymph, and when the perineal blood vessels and lymphatics were completely resected 26 hr later there was no significant change in the lymph flow.

Although there are blood vessels crossing the mid line between the two halves of the udder, there are no conspicuous lymphatics. The lymph flows were not significantly different in the two goats (Jill and Bessie, Table 1) in which the two glands of the udder had been previously permanently separated by a plastic skin operation.

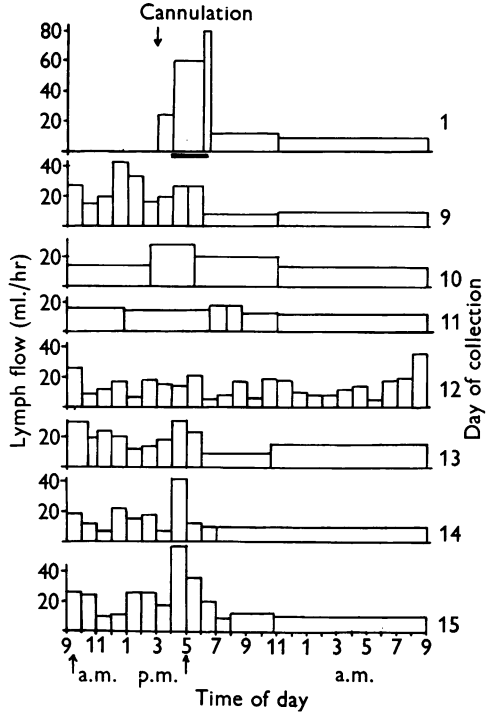
The daily loss of large quantities of lymph from these goats did not produce any noticeable ill effects. Adequate minerals were provided in the diet for milk production and pregnancy and water and salt licks were continuously available. There was no fall in plasma Na or Cl even after continuous collection for 17 days and no significant fall in milk yield in lactating animals. The losses of water, Na, Cl and protein were not great in relation to the weight of the animals (about 70 kg) and were usually less than the daily loss in the milk.

It will be seen from Table 1 and Text-fig. 2 that the average daily lymph flow varied with the daily milk yield. A similar relationship was also seen in individual animals from day to day and the clearest case is shown in Text-fig. 3. Since it has been found already that blood flow is related to milk yield (Linzell, 1960), as might be expected, lymph flow also varied with the average blood flow (Text-fig. 2).

The daily variations in lymph flow were not great, the standard deviation being about 10 % of the mean. In one goat the flow increased 50 % on the second day after cannulation when the operation wound was unusually oedematous. Hourly variations in flow were much greater (up to 300 %). Hourly collections for 33 hr in the goat Pam (Text-fig. 4) showed a standard deviation of 46 % of the mean. Although the rate of flow was sometimes highest at about the time the goats were milked and fed (9.30 a.m. and 5.0 p.m.) and was usually lowest in periods when the animals were mostly lying down (as at night and during the week-ends), at other times variations appeared to be random (Text-fig. 4). A marked increase in lymph flow (300–500 %) was produced when the animals' hind quarters were suspended in a sling during the recovery from epidural anaesthesia, presumably due to a rise in pressure in the abdominal veins produced in this way (Text-fig. 4). In one goat lymph flow was measured during pregnancy at the end of lactation and milking was stopped on the second day of lymph collection. There was no change in lymph flow either when the udder became distended with milk 6 days later or when this was relieved by milking out.

Lymph composition. A general analysis of the lymph collected daily is given in Table 1, together with the average composition of plasma from lactating Saanen goats of the same herd, collected over several years in

the course of other work. Variations in the composition of lymph collected hourly, which was investigated only in the case of Ca, Mg, P and glucose, were of the same order as the daily variations. However, variations in glucose content as high as 30 % of the mean were encountered in successive 5 min samples.



Text-fig. 4. Record of hourly lymph flow from right mammary gland on 8 days in the goat Pam. The animal was suspended in a sling to recover from the epidural spinal anaesthetic on day 1 as signalled by black bar and the lymph flow rose. The goat was milked each day at 9.30 a.m. and 5 p.m., shown by arrows.

As has been reported for lymph from some other organs (Yoffey & Courtice, 1956) the protein concentration tended to be higher at the lower rates of flow (Text-figs. 2, 3). As a result, the daily loss of protein in the lymph for the goats in Table 1, which were all in full lactation, was fairly constant (10·2, 11·3, 11·7 and 9·8 g/day). Electrophoretic analyses, by Dr A. E. Pierce of this Institute, of serum and lymph proteins collected simultaneously from the goat Jill (Table 1) showed no significant difference between arterial and mammary venous serum proteins, which consisted of 44·5 % albumin, 25·7 % α-globulin, 6·8 % β-globulin, and 23 % γ-globulin. By contrast, the lymph protein consisted of 55·2 % albumin, 21·8 % α-globulin, 7·1 % β-globulin and 15·9 % γ-globulin. There was also

probably fibrinogen in the lymph, because a fibrin-like material came down when heparinized lymph was thawed out after storage at $-10°$ C.

The differences between lymph and plasma shown in Table 1 are similar to those reported for other organs. Since the samples were not collected at the same time from the same goats the differences have not been tested for significance. However, in addition to these analyses, samples of arterial and mammary venous plasma (4 samples of each, collected every 2 hr and pooled) were compared with the lymph produced simultaneously (during 8 hr) in the goat Bessie. The analyses were very similar to the figures in Table 1; lymph Cl, K and HCO_3 were all 5% higher than the venous plasma values, Ca and Mg 18·5 and 19% lower and Na and inorganic P not significantly different. Lymph glucose and VFA, which are actively taken up from the blood by the lactating mammary gland and were identical to the average figures for this animal in Table 1, were midway between arterial and venous plasma levels. In other goats the lymph concentrations of glucose and VFA were nearer the venous plasma figures.

Lymph flow in anaesthetized animals

Shorter collections ($\frac{1}{2}$–4 hr) were also made in 15 goats, 2 sheep and a dog under general anaesthesia (cyclopropane in goats and sheep, and pentobarbitone in the dog) mostly during the course of other experiments involving dissection around the mammary glands, which often reduces both the blood flow and rate of milk secretion (Linzell, 1960 and unpublished). Although the milk yields of the goats before the experiments were in the same range as those in Table 1, the rates of lymph flow during the experiments were much less than in the conscious animals (0·1–1·25 ml./100 g/hr) and the average protein concentration was significantly higher (4·41 ± 0·35 g/100 ml.). A smaller number of analyses showed raised levels of Ca (2·2–4·5 m-equiv/l.), Mg (3·3 and 3·8 m-equiv/l.), inorganic P (3–7·4 m-equiv/l.) and glucose (36–156 mg/100 ml.). In two experiments lymph K and lactate were measured at the start of perfusion of the isolated mammary gland of the goat (time between removal from the anaesthetized animal and starting the perfusion 5 and 10 min). K was initially raised to 5 m-equiv/l. and lactate to 0·9 and 1·2 m-equiv/l., but these quickly fell once perfusion had started to 4·6 m-equiv/l. for K and 0·14 m-equiv/l. for lactate. Lymph flows from one mammary gland of the anaesthetized sheep were 1 and 2 ml./100 g/hr and in the dog about 4 ml./100 g/hr.

DISCUSSION

Mammary lymph has been collected in this laboratory for two reasons. First, to assess the variable rates of lymph flow encountered in perfused goats' udders (Cargill-Thompson *et al.* 1958; Hardwick *et al.* 1960) and

secondly to determine the proportion of potential milk precursors that are lost from the mammary glands in the lymph in conscious animals. Having measured the uptake quantitatively by simultaneous measurement of blood flow and arteriovenous differences (Linzell, 1960) it seemed necessary to know the quantities removed as lymph, since several workers have previously pointed out that this is an unmeasured source of error in work of this sort.

In answer to the first problem it seems that the rate of lymph flow reflects the general metabolic activity of the tissue, running roughly parallel to blood flow and to the milk yield of each gram of tissue, and is thus one means of investigating the condition of glands being perfused. As to the second problem, it is clear that only a small proportion of milk precursors removed from the plasma return to the circulation via the lymph. In many studies this factor may be ignored because the quantities in lymph are small in relation to the errors in measuring the mean uptake from the plasma (because of the high blood flow the arteriovenous differences are often small). It has been calculated from the present data and those from Linzell (1960) that only 2% of the Ca removed from the plasma appears in the lymph and about 0·5% of the glucose.

One of the unexpected features of this work was the high rate of lymph flow from the mammary gland, which in conscious lactating animals is about 10 times the average flow from the thoracic duct in other animals (Yoffey & Courtice, 1956) and about equal to the flow of lymph from the liver in unanaesthetized dogs (Ritchie, Grindlay & Bollman, 1959) and rats (Friedman, Byers & Omoto, 1956). Unlike that from the liver, however, the protein content at the higher rates of flow is low and is inversely related to the flow. Mammary lymph flows spontaneously not only in conscious resting animals, but in anaesthetized animals and in perfused mammary glands. The rate of flow, which has been found to be related to the rates of milk secretion and blood flow per unit volume of tissue at different stages of lactation, is ordinarily, like the milk yield and blood flow (Linzell, 1960) fairly constant in conscious animals. The greater variation encountered from hour to hour than from day to day may merely reflect the greater scatter of observations to be expected when a randomly varying process is sampled more frequently. The fact that the rate of lymph flow was not clearly related to milking (when the udder is massaged) or to the state of fullness with milk, conditions under which venous pressure variations might be expected, was surprising. However, it may be said that this is testimony to the remarkable adaptation of the udder of domesticated milking ruminants, that can hold and release the large quantities of milk that accumulates between milkings (up to 4 l. in the goat) whilst maintaining a constant over-all rate of metabolism.

The high rates of lymph flow during pregnancy were also surprising, and, as has already been pointed out, the amount of lymph coming from the vulva must partly account for this increase. Nevertheless, the mammary gland during pregnancy in these goats was considerably larger than all the perineal tissues and it seems likely that there was an increase in mammary lymph flow as well. However, there is the possibility that this could be due to a raised venous pressure associated with pregnancy. It has been demonstrated that a raised abdominal pressure, as produced by supporting the animal in an abdominal sling, greatly increased the lymph flow. One of the main veins draining the udder passes into the abdomen, through the inguinal canal, where it would be subjected to raised average abdominal pressure and also could occasionally be mechanically compressed by the foetus.

In general composition mammary lymph is similar to that from other organs. According to the classification of Yoffey & Courtice (1956) the lymph collected in this work is intermediate (i.e. lymph that has passed through one lymph gland only), as distinct from peripheral and central lymph. The numbers of lymphocytes are in agreement with the numbers in intermediate lymph from other regions. As would be expected according to the generally accepted theory of lymph formation, mammary lymph is lower than the plasma in Na plus K, Ca and sometimes Mg, but higher in Cl and slightly higher in HCO_3. In addition there is significantly less protein, but a greater proportion of those of smaller molecular weight (e.g. albumin). In conscious animals during lactation the concentration of those substances (glucose, VFA, amino acids) actively utilized by the mammary tissue lies between arterial and venous levels, but in non-lactating or anaesthetized animals, when the uptake is reduced, the levels approach arterial values as is the case with non-electrolytes in lymph generally.

The lymph flow during acute experiments under general anaesthesia in goats was lower than in conscious animals and there were in addition significant changes in lymph composition. These changes may well be due to the reduced blood flow following dissection around the mammary glands in these experiments, but general anaesthesia itself may lower the mammary blood flow and a lower lymph flow was also encountered in one experiment where only lymph cannulation was done with general anaesthesia.

It must be pointed out that there are some significant differences between the composition of the lymph collected in this work from conscious goats and that taken from dead cows by Heyndrickx & Peeters (1958*a*, *b*, 1960) and Heyndrickx (1959). There is some evidence to suggest that many of the differences may be due to post-mortem changes. The Belgian workers themselves point out that Na and K were unexpectedly higher in the

lymph collected after death than in plasma taken before slaughter and stress that their lymph had an extremely high level of lactate (about 10 m-equiv/l.). Comparison of the present figures with their published findings shows the following additional differences. In the goat lymph HCO_3 is slightly *higher* than in the plasma, and lymph inorganic P, Mg and amino acids are slightly *lower* than in plasma. Some of these changes occurred in lymph collected during acute experiments under general anaesthesia when the rate of lymph flow was also reduced, possibly associated with a reduced blood flow and relative anoxia. However, the most striking differences are that the K and lactate in dead cow udder lymph are 2 and 12 times higher than in that from conscious goats. Both these changes would be expected in anoxia and indeed were seen to a lesser degree when goats' udders (cooled during dissection) were removed for perfusion, under epidural anaesthesia. This suggests that the recent findings of Heyndrickx & Peeters (1960), that the lymph content of lactose, citrate and α-oxoglutarate are higher than in plasma, may also be due to post-mortem anoxic changes.

Although it may be that a knowledge of the variations in the quantities of metabolites in mammary lymph at different stages of lactation will provide clues to the type of metabolism going on at the time, it would seem necessary to interpret with caution the results of analyses of lymph taken from dead or anaesthetized animals.

SUMMARY

1. A method is described for the continuous collection of mammary gland lymph in unrestrained conscious goats.

2. Lymph was collected for up to 17 days at the rate of 150–840 ml./day from one mammary gland without disturbing the animal.

3. Mammary lymph flows spontaneously at a rate, which varies with the rate of milk secretion and the mean blood flow, from 0·3 to 2·71 ml./100 g/hr. Daily variations in flow were 10% (S.D.) of the mean but the hourly variations averaged 46%. The protein concentration varies inversely with the rate of flow.

4. The composition of the lymph and its relationship to plasma are similar to those of lymph from other organs.

5. Lymph flow was reduced in experiments under general anaesthesia and the composition altered. This is discussed in relation to the high K and lactate recorded by Heyndrickx & Peeters (1958*a*) in the udder lymph from dead cows.

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