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A CONTRIBUTION TO THE PHYSIOLOGY OF LACTATION

A DISSERTATION

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WALTER LEE GAINES**

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A CONTRIBUTION TO THE PHYSIOLOGY OF LACTATION

W. L. GAINES

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FOREWORD

The development and functioning of the mammary gland in the female mammal is closely associated with, and dependent upon the reproductive function.¹ The normal course of activity of the gland is somewhat as follows.

Embryonic—	The first sign of the mammary organs, the milk line, appears at an early stage in embryonic life, and growth is continued until the gland reaches a considerable development at
↓	
Birth—	At birth a milky fluid may be expressed from the gland in either sex, indicating a secretory activity, but this activity soon ceases and the gland remains quiescent until
↓	
Puberty—	At puberty under the influence of the ovaries growth is resumed and carried to a greater or less development, varying with the individual and the occurrence of
↓	
Pregnancy—	Pregnancy induces a great hypertrophy of the gland, and it reaches a high state of development, accompanied by the accumulation of colostrum, by the time of
↓	
Delivery—	At this time milk secretion proceeds actively and if the milk is removed at short intervals, by nursing or artificially, secretion continues for a period of days, months, or years. The rate of secretion, however, after a time gradually decreases to zero. This decrease is favored by a succeeding
↓	
Pregnancy—	Further growth of the gland ensues but not as marked as in the preceding gestation, and at
↓	
Delivery—	milk secretion is again actively resumed. Whence, the pregnancy-delivery cycle is repeated.

¹ The mammary gland may, in turn, influence the reproductive and other body functions, although such influence is, apparently, not so fundamental.

This paper presents some data and discussion based on an investigation of certain phases of the above outlined course of activity of the mammary gland carried out at the Hull Physiological Laboratory of the University of Chicago. I wish to acknowledge especially the assistance and suggestions of Dr. Carlson throughout the work. I am also indebted to Dr. Mathews and Dr. Koch, among others, for various suggestions.

EXPERIMENTAL METHODS AND RESULTS

Prenatal development. The milk lines may be distinguished as early as the 4 mm. stage in the human embryo, according to Strahl (1) Knoepfelmacher (2) states that in children born prematurely there is no appearance of milk in the mammary glands. In a post mortem examination of two kids, the mother of which died about two weeks before term, I found mammary tissue plainly developed but no appearance of milk in the transected gland. Chemical examination for the presence of sugar was not made and this test might have shown the presence of milk in amount too small to be distinguished by simple cutting of the gland. The udder of the mother, a multipara, was at the time undergoing a rapid hypertrophy as evidenced by increase in size. (Data on this point will be found in table 1, Goat No. 4). A part of the increase in size was due to the accumulation of colostrum, which exuded freely from the cut ducts of the gland after death.

The influence of pregnancy on the growth of the mammary gland. The growth of the mammary gland during gestation is a very striking phenomenon. This development is in preparation for its function of milk secretion following delivery. Ribbert (3) transplanted the mammary gland of a guinea pig into the skin back of the ear. Five months later (the gestation period is about two months) living young were born, whereupon the transplanted gland functioned promptly. Ribbert states that hypertrophy was not sufficiently marked to make the gland apparent beneath the skin, that the nipple did not develop, and there was, therefore, no outlet for the secretion, but that milk was present in the gland. Marshall (4) cites the case of the Bohemian pygopagous twins, Rosa-Josepha, one of whom became pregnant. The breasts of both underwent development during pregnancy and functioned following delivery. Lane-Claypon and Starling (5) produced a development of the mammary glands in a virgin rabbit closely resembling that occurring during pregnancy by means of injecting extracts of rabbit foetus.

On the other hand, Lombroso and Bolaffio (6) using parabiotic methods with rats found no evidence that the pregnancy of one of a pair of females produced any effect on the mammary glands of the other one of the pair. Knoepfelmacher (2) injected blood serum from a pregnant goat into a non-lactating goat. The amounts used were 55, 33, and 90 cc. taken respectively 6 days, 2 days, preceding delivery, and 1 day following delivery. The results were negative.

I have injected defibrinated blood from a pregnant non-lactating, multiparous goat into a non-pregnant, non-lactating, multiparous goat with negative results with reference to increase in the volume of the mammary glands or milk secretion. The volume of the udder was determined by pressing a suitable vessel containing warm water up

TABLE 1

Showing the effect on the volume of the udder of a non-pregnant goat from the intravenous and intraperitoneal injection of defibrinated blood from a pregnant goat

GOAT NO. 4—DONOR (PREGNANT, NON-LACTATING, MULTIPAROUS)		GOAT NO. 3—RECIPIENT (NON-PREGNANT, NON-LACTATING, MULTIPAROUS)		
Date	Volume of udder	Volume of udder	Blood injected	Method of injection
1915	cc.	cc.	cc.	
1-21	935	170	90	Intraperitoneal
1-23	935	165	115	Intraperitoneal
1-25	950	175	100	Intraperitoneal
1-27	980	175	75	Intraperitoneal
1-30	1005	160	15	Intravenous
2-3	1060	170	85	Intraperitoneal
2-6	1090	170	35	Intraperitoneal

around the udder and firmly against the body wall. The amount of water displaced (determined by the difference between the amount left in the vessel and its capacity) was taken to represent the volume of the glands. The method is not exact but is sufficiently accurate to show any marked changes in volume. The data are given in table 1. No appearance of milk resulted during the period of injections or following.

The figures in table 1 show that the udder of the donor was increasing steadily in size but the amount of blood injected failed to affect the size of the udder of the recipient. The latter at a previous time during a similar stage of pregnancy as represented by the donor in the present instance had fully as large an udder as the donor. The figures, then

serve to show the enormous difference in size of the udder with the stage of pregnancy.

The influence of pregnancy on milk secretion. Lane-Claypon and Starling (5) advance the theory in connection with the work referred

TABLE 2

Daily milk and fat yield of goat No. 2, showing the effect of intravenous injections of defibrinated blood from a pregnant goat, No. 3; from her two days old kid; and again from No. 3 six days after delivery. No. 2 said to have dropped her kids about April 12. No. 3 kidded August 8, 1914

DATE	MILK	FAT	FAT	REMARKS
1914	cc.	per cent	gms.	
7-24	823	5.1	41.9	
7-25	796	6.4	51.0	
7-26	730	6.4	46.8	
7-27	645	5.4	35.0	
7-28	720	6.4	46.3	} Injected intravenously 175 cc. defibrinated blood from No. 3 (10 days before term)
7-29	463	6.9	31.9	
7-30	708	6.8	48.2	
7-31	674	6.2	41.7	
8-1	783	6.8	53.7	} Injected intravenously 300 cc. defibrinated blood from No. 3 (7 days before term)
8-2	552	7.0	38.6	
8-3	783	7.0	54.8	
8-4	847	7.8	66.1	} Injected intravenously 175 cc. defibrinated blood from No. 3 (3 days before term). Caused some general depression, weather hot, flies troublesome
8-5	913	7.5	68.4	
8-6	349	4.9	17.1	
8-7	633	8.0	50.8	
8-8	832	6.2	51.6	
8-9	752	5.5	41.1	
8-10	665	5.9	39.3	} Injected intravenously 100 cc. defibrinated blood from male kid of No. 3. Age of kid 2 days
8-11	538	6.6	35.4	
8-12	806	6.8	54.7	
8-13	873	6.0	52.4	} Injected intravenously 100 cc. defibrinated blood from No. 3 (6 days after delivery)
8-14	853	6.5	55.0	
8-15	765	7.0	53.7	
8-16	880	6.3	55.4	
8-17	823	7.3	59.6	
8-18	848	6.2	52.3	

to above that the developing foetus passes a substance into the maternal circulation which favors the growth of the mammary gland but inhibits its secretory activity. The removal of this inhibitor, then, at delivery allows the gland to function in its milk secretory capacity.

Bearing on this point I have made the following series of injections

of defibrinated blood into a lactating goat: 1, from a pregnant goat near term; 2, from a kid of this same goat 2 days after birth; 3, from the same goat 6 days after delivery. Data of this test are given in table 2.

The figures indicate an inhibitory action from each of the transfusions. The milk and fat data as given for each day are the sum of two milkings made throughout at approximately 12-hour intervals. In every case the inhibitory influence was most marked at the first milking following the transfusion. Except in the case of the transfusion made August 5 there was no apparent systemic disturbance

TABLE 3

Daily milk and fat yield of goat No. 6, showing the effect of intraperitoneal injection of a water extract of dried, fat-free cow's placenta; also, defibrinated blood from goat No. 5. No. 6 said to have kidded March 12, 1915. No. 5 dropped kids April 17, 1915, and at the time of transfusion, April 24, was milking around 2000 cc. per day

DATE	MILK	FAT	FAT	REMARKS
	cc.	per cent	gms.	
1915				
4-18	475	6.8	32.3	
4-19	488	7.3	35.5	
4-20	460	6.4	29.3	{ Injected intraperitoneally a water extract of 5 gms. of dried, fat-free cow's placenta
4-21	168	9.4	15.8	
4-22	238	8.5	20.4	
4-23	365	6.7	24.4	{ Injected intraperitoneally 200 cc. defibrinated blood from No. 5 (fresh 7 days and giving about 2000 cc. daily)
4-24	315	5.9	18.5	
4-25	182	8.6	15.7	
4-26	147	7.1	10.5	
4-27	111	9.4	10.4	
4-28	183	7.8	14.3	
4-29	154	8.3	12.8	

in the condition of the recipient. Leaving this case out of consideration the inhibitory action seems to be equally marked for either the blood of the pregnant goat or that of her two-day old kid.

The transfusion made 6 days after delivery had only a very slight inhibitory action as seen in the yield of milk and was not at all reflected in the yield of fat. Results in another trial with two other goats were similar to the above except in the transfusion following delivery (see table 3).

Effect of placental extract on milk secretion. Niklas (7) found that an extract of placenta caused milk production in virgins and mothers.

Lederer and Pribram (8) found greatly increased secretion of milk upon intravenous injection of placental extract.

Table 3 presents the data of a test of the effect of an extract of cow's placenta on milk secretion in the goat. The fresh placental tissue was ground, dried, extracted with gasoline and ether to rid of fat, and the residue extracted with water as needed. The figures show a distinct inhibition, quite similar in nature to that just observed from the blood of a pregnant goat or new-born kid (see table 2).

Relation of the blood to milk secretion. The activity of the mammary gland with reference to milk secretion varies enormously. A large part of the time it may be at zero, but immediately following a normal pregnancy and delivery it is at a high point. To test for the presence of a substance in the circulating blood stream which accelerates the secretory function of the mammary gland during the period of its highest activity following delivery, transfusions were made from a fresh, heavy milking goat to one giving a low yield. No increase in secretion was obtained by this means. In table 2 no change, or a very slight decrease was shown. In table 3 there is shown a very marked decrease. In this latter case the donor was milking heavily at the rate of about 2000 cc. daily, including the milk taken by two nursing kids. The recipient although giving a relatively low yield, about 300 cc. daily, had been fresh only about 6 weeks and would seem to have offered a favorable subject for positive results. No general disturbance was apparent to account for the decrease noted.

The effect of mammary gland extract on milk secretion. Gavin (9) fed mammary gland preparations to cows without affecting the yield or quality of the milk. The mammary gland of the same cow whose placenta was used in the data shown in table, 3, was prepared in the same way as given for the placenta. Results from the injection of water extracts of this material are given in table 4.

The figures appear to show a temporary inhibition followed by an increase to something above the previous yield. The net result of the several injections seems to be a considerable increase, but it may be that this should not be attributed to the injected material. Reference to table 3 shows that goat No. 6 was, a few days before, giving more than the yields recorded in table 4, and the apparent increase may be nothing more than recovery following the blood transfusion of April 24. However, the immediate inhibition, similar to that noted for the placental extract, seems to be unequivocal.

The action of pituitrin on the mammary gland. Since Ott and Scott (10) first noted that pituitrin had a galactagogue action this material has received considerable attention in its relation to milk secretion. All reports of investigators on the immediate action of pituitrin confirm the observation of Ott and Scott, viz., that its injection into the circulation causes an immediate flow of milk in a lactating animal. MacKenzie (11) working with cats in anesthesia and observing the flow of milk from the cut surface of the gland found pituitrin the most active of the several animal extracts he studied. Gavin (9) working with

TABLE 4

Daily milk and fat yield of goat No. 6, showing the effect of intraperitoneal injection of a water extract of dried, fat-freed cow's mammary gland (pregnant). No. 6 said to have dropped kids March 12, 1915

DATE	MILK	FAT	FAT	REMARKS
1915	cc.	per cent	gms.	
5-3	113	8.3	9.4	
5-4	85	6.7	5.7	
5-5	90	7.7	6.9	
5-6	119	7.6	9.0	} Injected intraperitoneally water extract of 5 gms. cow's mammary gland
5-7	75	6.5	4.9	
5-8	135	6.2	8.4	
5-9	143	6.6	9.5	
5-10	176	6.4	11.2	} Injected intraperitoneally water extract of 1 gm. cow's mammary gland
5-11	157	7.1	11.1	
5-12	190	6.9	13.1	} Injected intraperitoneally water extract of 4 gms. cow's mammary gland
5-13	134	7.8	10.4	
5-14	189	7.4	13.9	
5-15	220	7.5	16.4	} Injected intraperitoneally water extract of 5 gms. cow's mammary gland
5-16	177	7.7	13.7	
5-17	194	7.3	14.3	

cows yielding 15-35 pounds of milk daily found no effect on quantity or quality of milk as the average result of 3-5 days treatment with pituitrin. In some cases, he reports, there was a distension of teat and cistern with milk immediately following injection. Hill and Simpson (12) report similar results. Hammond (13) has studied in some detail the effect of pituitrin on the composition of milk secreted under its influence. He finds that the fat content is greatly increased and that the protein, sugar and ash content remain very constant. This refers to the milk secured immediately after the injection of pituitrin and is not in conflict with Gavin's results which referred to longer periods;

for the yield of milk and its fat content are later depressed. Hammond concludes from his data that the action of pituitrin is not muscular. Hill and Simpson (14) confirm Hammond's results as to the composition of milk obtained after the use of pituitrin except they did not find the depression in fat content later, as noted by Hammond.

Heaney (15) found in the human a contraction of the breast upon injection of pituitrin followed by a return to initial volume, and attributes the milk secretory action of pituitrin to its muscular effect.

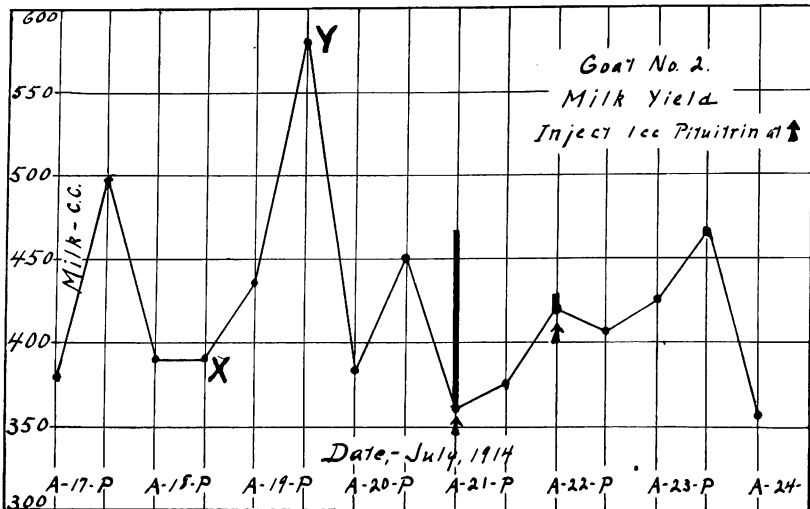


Fig. 1. Showing fluctuation in yield of milk by a goat milked at regular 12-hour intervals and the dependence of the flow of milk produced by the injection of pituitrin upon this fluctuation. The heavy vertical lines represent the yield of milk obtained upon intravenous injection of 1 cc. of pituitrin immediately after the regular milking. It is inferred, for example, that pituitrin would have caused a large further flow at X; while at Y little or no extra milk would have been obtained. A = morning milking; P = evening milking.

As to the quantity of milk and fat yielded as a result of the use of pituitrin (Parke & Davis preparation was used throughout the work here reported on pituitrin) based on the average of two days and not the immediate yield, my data show no effect either way. My results, based on three goats, confirm those of Hammond who found a decrease in fat content of the milk for a day or two following the use of pituitrin. I have found no marked or consistent variations in the sugar or protein content of the milk.

The flow of milk from an active mammary gland, which has not been drained for a few hours, upon injection of pituitrin is a very striking reaction. In the goat I have never failed to secure some flow of milk from its use even immediately after milking "dry." The amount obtained, however, varies considerably.

Figure 1 illustrates this point. The goat was milked regularly at 12-hour intervals but the yield is seen to fluctuate widely. The intravenous injection of pituitrin immediately following a regular milking produces a further flow varying in amount inversely with the yield secured by the hand milking which preceded it. That is, injection of pituitrin following a relatively high yield of milk produces a small flow; and following a relatively low yield it produces a large flow. A second, third or fourth dose produces only a very slight flow, 2-4 cc. in the goat.

I have followed the yield of milk in the nursing dog very satisfactorily by keeping the pups separated from the mother and allowing them to nurse at 8-hour intervals. The pups were balanced on a smooth-working balance, with shot, then allowed to nurse, and again balanced with the standard weights. The increase in weight represents approximately the yield of milk by the mother.

Under the above conditions, after the pups have nursed fully (6 or 7 minutes), I have never succeeded in producing any further flow of milk by the intravenous injection of pituitrin. But if the mother be placed under ether at the regular nursing hour and the pups then allowed to nurse the yield of milk is greatly depressed, although the pups do their part of the act in the usual vigorous manner. Often, in fact usually, the yield is so low as not to be detectable by the method used (that is, it appears, well under 5 gms., considering errors in loss of saliva, etc.). If, now, pituitrin be injected intravenously, with the mother still under ether, the pups immediately secure the normal yield, or the balance of it which they had failed to get in the first nursing, and this, in rather less time than is required in nursing under normal conditions. A second dose following the first in 10 minutes, produces no further flow.

Figure 2 shows an illustration of the effect of pituitrin under normal conditions and also with the mother under ether, but in the latter case some milk yielded to the pups nevertheless. Ordinarily the ether curve would hold to the base line until the pituitrin was administered.

While pituitrin causes no further flow of milk in the dog after nursing, this does not seem to hold true for the goat. Data from two

experiments are given in table 5 which show that after the kid had nursed and secured all the milk it could, injection of pituitrin still caused a further marked flow. The high fat content (15.8 to 19.6 per cent) of the milk thus obtained is also remarkable.

Bearing on the nature of the mechanism involved in the flow of milk produced by pituitrin under certain conditions, the following test was carried out. A cannula was inserted in the teat of a goat and connected with a chloroform manometer recording on the revolving drum of a kymograph. The gland was then inflated with air through a T-

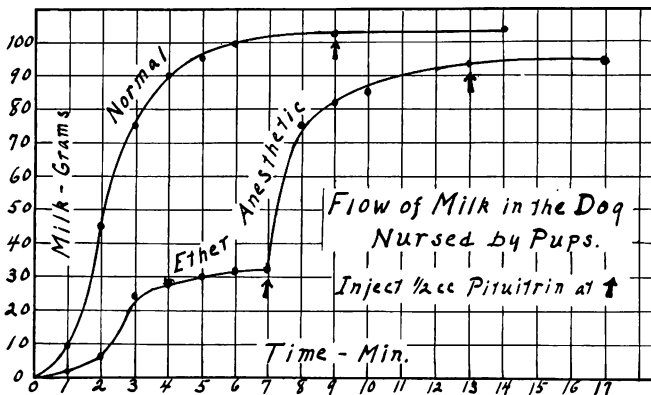


Fig. 2. Showing failure of pituitrin to cause a flow of milk following normal nursing in the dog; and, its restoral of the flow normal to the stimulus of nursing when the normal flow is inhibited by anesthesia; and, the failure of a second dose to cause any further flow. Also, showing under the stimulus of normal nursing the latent period in the flow of milk during the first minute, followed by the very rapid flow for the next two minutes, and then the gradual decline to zero (cf. figs. 4-8).

connection to a pressure of 8-10 cm. chloroform. The device may be made quite sensitive in recording any change in pressure within the gland. With the apparatus properly adjusted pituitrin was injected intravenously. The effect varies with the stage of activity of the gland. In the non-pregnant, non-lactating goat there is no apparent effect. This is true, also, in the pregnant, non-lactating goat up to a time close to term. A short time before term the result, following a latent period of 15-20 seconds, is a slight increase in pressure with a return to the initial. In the freely lactating goat, after a latent period

of 15-20 seconds, there is an abrupt rise in pressure followed by a gradual and nearly uniform decline to the initial pressure. The decline occupies about 5 minutes. A series of 3 curves, taken from the same goat before and after delivery, is given in figure 3.

A second dose of pituitrin in the freely lactating goat gives a curve similar to the first dose, except that it is much reduced in the extent of the rise. In the case of a goat milking from one gland and dry in the other, each gland being separately connected as above, pituitrin

TABLE 5

Showing the effect in the goat of nursing following milking "dry" by hand; and of pituitrin following nursing. After milking by hand, kid nursed left gland and at the same time right gland was milked by hand. Following the nursing 1 cc. pituitrin was injected intravenously and both glands milked by hand. Goat No. 5, kidded April 17, 1915

EXPLANATION	RIGHT GLAND			LEFT GLAND			BOTH GLANDS		
	Milk	Fat	Fat	Milk	Fat	Fat	Milk	Fat	Fat
	cc.	per cent	gms.	cc.	per cent	gms.	cc.	per cent	gms.
4-22-15									
Milked "dry" by hand.....							358	3.5	12.5
Nursed by kid.....				153			343		
Milked by hand.....	190	9.8	18.6						
Injected pituitrin—milked by hand...	70	15.8	11.1	98	17.1	16.8	168	16.6	27.9
5-2-15									
Milked "dry" by hand.....							315	4.7	14.8
Nursed by kid.....				175			330		
Milked by hand.....	155	13.3	20.6						
Injected pituitrin—milked by hand...	45	19.1	8.6	40	19.6	7.8	85	19.3	16.4

produced the typical curve in each gland, that is, the abrupt rise and slow decline in the active gland, and no change in the non-lactating gland.

While the above test indicates a contraction of the ducts and alveoli of the gland with a subsequent relaxation, it does not definitely localize the seat of the contraction. That is, the effect might be produced by the activity of the muscles of the large ducts. Indirect evidence on the point is afforded by the following test.

A lactating guinea pig was separated from her nurslings for two hours to allow the accumulation of milk in the glands. The animal was then anesthetised and a mammary gland dissected entirely away from the body with as little injury to the gland as possible. The end of the nipple was cut off to allow free egress to any milk. A few drops of pituitrin were now injected into the gland substance, whereupon there was a copious flow of milk from the cut nipple. A control in-

jection of salt solution gave no such effect.

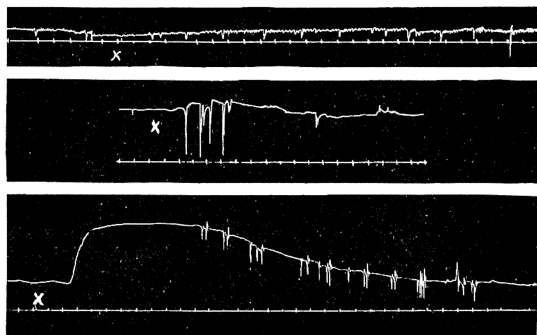


Fig. 3. Showing the effect of pituitrin on the pressure of an air-inflated mammary gland of a goat. The teat was connected with a cannula and tube to a chloroform manometer and the gland inflated to a pressure of 8-10 cm. At X $\frac{3}{4}$ cc. of pituitrin was injected intravenously. The time line marks 10-second intervals. The upper tracing was taken 37 days before delivery; the middle tracing, 17 days before delivery; the lower tracing, 12 days after delivery (the daily milk yield at that time being around 1800 cc.). The shallow waves, seen especially in the upper tracing, are due to respiratory movements. The deep, sharp irregularities are due to bleating and struggling of the animal.

A similar gland preparation was cut across with a razor, exposing a cut surface as remote as possible from the large ducts and vessels. A drop of pituitrin was placed on the cut surface and in a few seconds many minute white dots appeared beneath the pituitrin and slowly swelled to tiny milky rivulets streaming beautifully through the clear liquid. Similar preparations from the cat and dog gave like results.

✓*The reflex involved in milking and nursing.* According to Flower and Lydekker (16) the mother whale ejects the milk from her mammary glands into the mouth of the young without the active sucking on the part of the young which is generally common to mammals. Schäfer (17) states that the discharge of milk during the act of sucking or milking is in part the result of direct mechanical pressure upon the milk reservoirs of the larger ducts; and partly due to a contraction of the plain muscular tissue which accompanies these ducts, and which appears to

be set in action by mechanical stimulation of the nipple. He adds that the flow is probably aided by the swelling of the gland due to a reflex dilatation of its arterioles.

The curve of normal nursing in the dog given in figure 2 shows roughly the rate at which the pups secured milk during the process of nursing. The data on which the curve is based were obtained by working the pups in two relays of three pups each. Group I was allowed to nurse for 1 minute, then removed and Group II given way for 1 minute, Group I being meanwhile weighed. Places were then again exchanged and so on until they had obtained all the milk possible.

The normal curve shows that the pups could secure only a small amount of milk the first minute, but during the second and third minutes there was a relatively large amount of milk available to their

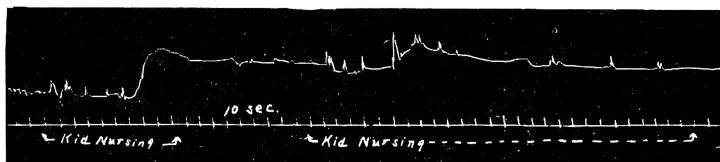


Fig. 4. Showing the pressure changes produced in one gland under the influence of kid nursing the other gland. Goat No. 5, fresh 10 days, milked 3 hours previously. Left gland connected with chloroform manometer (no inflation of gland). Kid nursing right gland. The sharp irregularities in the curve are due to bunting of the kid. Time line marks 10-second intervals and represents 7 cm. pressure (cf. figs. 5, 6, 7, 8).

efforts. After the third minute the amount available declines and at the end of 6 minutes the total supply appears to be exhausted. The very marked changes in the amount of milk secured with the progress of nursing are not attributable to any differences in the efforts of the pups themselves to secure their food.

In table 5 it is shown that, after milking the mother goat as completely as possible by hand, if a kid be allowed to nurse one gland he not only obtains a considerable quantity of milk but there is also a practically equal flow in the other gland which may now be readily obtained by hand milking. The full significance of this reaction with reference to the operation of a reflex contraction and ejection of milk from the gland is perhaps better given by the records of the following tests.

I. Change in pressure. One gland was connected by a cannula and tube to a chloroform manometer recording on a kymograph. A kid

was then allowed to nurse the other gland. An example of the effect is given in figure 4. The pressure registered upon insertion of the cannula was about 9 cm. During the period when the kid was nursing the curve shows a latent period of 60 seconds with no change in pressure aside from the purely mechanical effect of the impatient bunting of the kid. At the end of this latent period there appears a marked rise in pressure. That, coincident in time with this rise in pressure, the kid was obtaining a satisfactory supply of milk, was plainly evident from the cessation of his bunting (shown, as well, in the smoothness of the curve), his rapid guzzling, and the rounding out of his little paunch. Two minutes later a second kid was allowed to nurse and a rise in pressure occurred; again only after a latent period of about 1 minute.

II. Flow of milk. A cannula was inserted in one teat and the milk flowing through it led by a short tube to one limb of a U-tube of large bore. The U-tube was held in a vertical position and its other limb was provided with a float and rod recording on a kymograph after the usual fashion of a manometer. Enough water was put in the U-tube to allow the float to ride freely, so that the addition of any further liquid would be properly registered. The connection of the tube from the teat with the U-tube was open so that the liquid in both limbs was constantly under atmospheric pressure only. The outlet for the milk remained constantly about 10 cm. below the level of the teat. With the apparatus adjusted a kid was allowed to nurse the free teat. Examples of the results are given in figures 5-7.

In the experiment represented by figure 5 the right gland was connected as described above and the left gland milked by hand. After the hand milking was completed a kid was allowed to nurse the left teat. During the first 35 seconds of the kid's nursing the curve shows a flow amounting to a few drops only from the right gland. At the end of a 35-second latent period it shows a rush of milk reaching a maximum in 20 seconds and being practically complete in 80 seconds. The form of this curve is strikingly similar to that of the curve of flow in normal nursing of the dog shown in figure 2.

The high fat content (13.3 per cent) of the milk obtained under the stimulus of the nursing of the kid is noteworthy and significant. The amount of fat contained in the second portion was 50 per cent greater than that obtained under the stimulus of the first milking operation.

In the trial shown in figure 6 preliminary hand milking was not used. Fifteen cc. of milk flowed upon insertion of the cannula in the course of 3 minutes. Upon nursing of the opposite gland by the kid there is

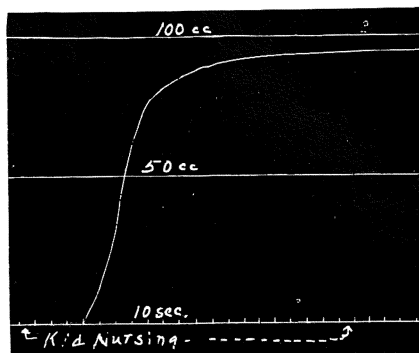


Fig. 5. Showing flow of milk through cannula from one gland under the stimulus of kid nursing the other gland. Goat No. 5, fresh 24 days, milked 11 hours previously. Cannula inserted in right teat and led to instrument recording the flow of milk. 84 cc. of milk flowed immediately on insertion of cannula. Left gland then milked as dry as possible by hand, yielding 136 cc. 45 seconds after the commencement of hand milking a further flow started from the right gland, yielding in 2 min. 68 cc. (results to this point not shown in the figure). Kid then nursed left gland causing a rapid flow from the right gland, after a latent period of 35 seconds as shown (cf. figs. 2, 4, 6, 7, 8).

	Milk, cc.	Fat, per cent	Fat, gms.
I { Left gland, hand milked.....	136	5.6	7.6
I { Right gland, cannula flow.....	152	5.3	8.1
II { Left gland, kid nursed.....	90		
II { Right gland, cannula flow.....	95	13.3	12.6

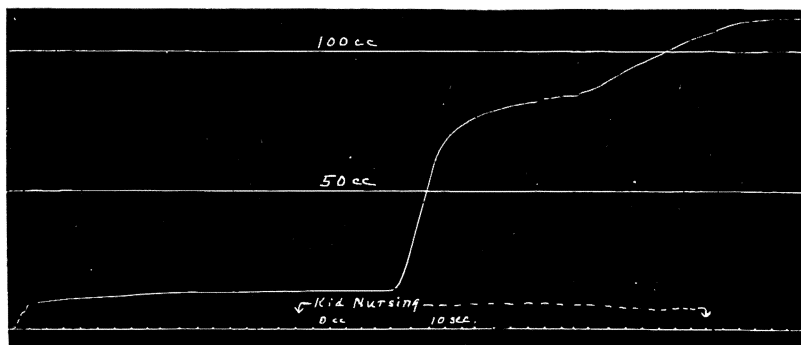


Fig. 6. Showing same as figure 5. Goat No. 5, fresh 11 days, milked 3½ hours previously. Left gland connected with flow recorder, kid nursed right gland (no hand milking)¹. Initial flow on insertion of cannula, 15 cc. Flow under stimulus of kid nursing other gland, after latent period of 60 seconds, 100 cc. Total flow 115 cc., 7.1 per cent fat (cf. figs. 2, 4, 5, 7, 8).

shown a latent period of 60 seconds and then a rapid flow from the unnursed gland. A second marked increase in flow is shown to occur 2 minutes following the first ejection. The curve shows, in comparison with figure 5, first, a longer latent period, and second, the occurrence of a second ejection of milk. In some cases the curve takes the form of 4 or 5 distinct steps.

The conditions of the test shown in figure 7 are practically identical with those of figure 6. The curve is given as showing another type of reaction, viz., a protracted and more or less uniform flow of milk (although a tendency to steps in the curve is shown here, also) under the stimulus of the kid's nursing. That the kid was also experiencing delay in the speed with which he was served his meal is evidenced by the length of time he continued to nurse.

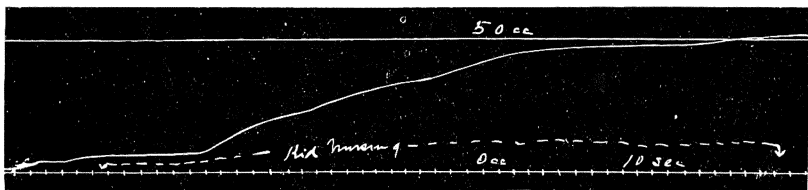


Fig. 7. Showing same as figure 5. Goat No. 5, fresh 16 days, milked 4 hours previously. Left gland connected with flow recorder. Kid nursed right gland (no hand milking). Initial flow on insertion of cannula, 7 cc. Flow under stimulus of kid nursing other gland, after latent period of 60 seconds, 44 cc. (cf. figs. 2, 4, 5, 6, 8).

Figure 8 brings out two points: (1) The curve of milk flow with the simple insertion of a cannula and without any other manipulation may take a distinct step form, as shown in the left hand part of the figure. (2) Hand milking of one gland produces a flow in the opposite gland, similar to the effect noted for the kid's nursing. (Other trials gave higher and steeper curves, very like that of figure 6.) The latent period is distinct, being 55 seconds in the present instance.

The question arises as to the nature of the factor or factors involved that cause the further ejection of milk upon nursing of the kid following hand milking, and which it seems to be impossible to call into play by usual methods of milking. I have tried various methods of electrical stimulation without success. I have imitated the nursing action

of the kid as actively as I could with my own mouth for several minutes at a time without result in the way of a further milk flow. The presence of the kid, nosing about the udder but not allowed to take a teat into his mouth, while using the greatest possible persuasiveness in the art of dry-hand milking was not sufficient. Neither did it suffice to add to the last mentioned recourse conditions of moisture and temperature by immersing the teat in water at 38–40°C. Only by allowing the kid to nose about the udder, while applying my own mouth to a teat in

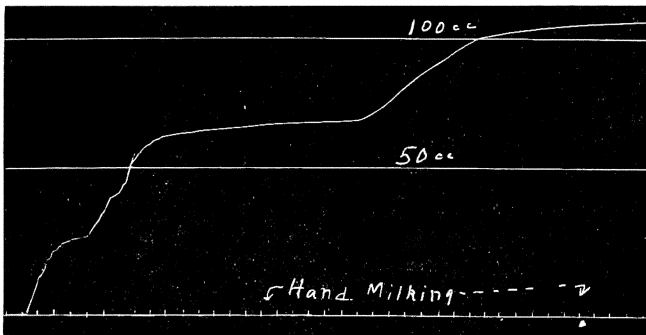


Fig. 8. Showing flow of milk through cannula from one gland under stimulus of hand milking of other gland. Goat No. 5, fresh 23 days, milked 12 hours previously. Left gland connected with flow recorder. Initial flow on insertion of cannula, 27 cc., followed by second flow of 44 cc. Flow under influence of hand milking other gland, after latent period of 55 seconds, 33 cc. Total flow 104 cc. Kid then nursed right gland obtaining 140 cc. and causing a further flow from left gland of 124 cc. (not shown in the figure), (cf. figs. 2, 4, 5, 6, 7).

	Milk cc.	Fat per cent	Fat gms.
I { Right gland, hand milked.....	136	3.6	4.9
I { Left gland, cannula flow.....	104	3.3	3.4
II { Right gland, kid nursed.....	140		
II { Left gland, cannula flow.....	124	9.3	11.5

imitation of his sucking, have I been able to bring about the desired reaction without the actual nursing of the kid. The following protocol illustrates.

Goat No. 5. 5/16/15, 7.15-7.45 p.m.

	Milk cc.	Fat per cent	Fat gms.
Milked by hand in usual manner (kid not around).....	285	7.9	22.5
Sucked teat with mouth, then milked by hand.....	00	00	00
Kid introduced and attempting to nurse but not allowed to take teat in mouth, then milked by hand.....	00	00	00
Sucked teat with mouth (no milk drawn from teat), with kid present but prevented from nursing, then milked by hand.....	160	17.3	27.7
Kid nursed vigorously and secured.....	3		

The second flow of milk induced by the treatment outlined is not quite as great as in the data given in connection with figures 5 and 8, and table 5, but the rise in fat content is greater, and, in this respect, is much the same as the effect noted from the use of pituitrin given in table 5.

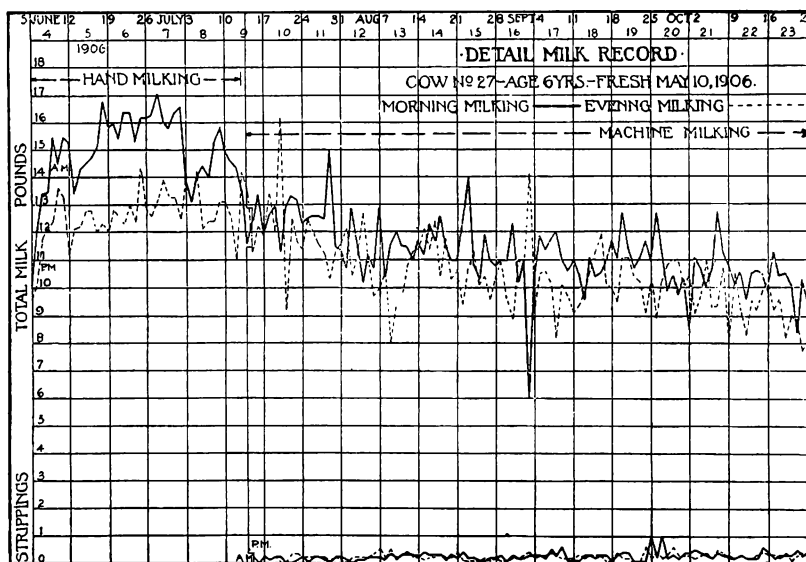


Fig. 9. Showing an illustration of the failure of a milking machine, followed by hand stripping, to secure a uniform degree of removal of the milk from the udder in the cow. Figure from M. S. thesis of the writer, University of Illinois, 1910.

While hand milking does not remove all the milk from the udder that it may be possible to obtain by the use of some stronger stimulus, it usually secures a fairly uniform degree of removal. Thus, in figure

9 showing the yield of milk by a cow after a 13-hour period (morning milking) and after an 11-hour period (evening milking) it is seen that, with hand milking, the curve for the longer period is always above the curve for the shorter period and the two do not cross. However, when machine milking commences, even though it was followed by hand stripping, the character of the curves changes and they cross and intercross repeatedly. This means that the stimulus provided by the machine work did not produce a uniform result. The total yield over a period of some months, it appears, need not be decreased by this condition.

Change in volume of the udder in milking. That the udder of the goat and cow decreases in volume during the process of milking is a matter of common observation. It has been stated [see Marshall (4) p. 557] that the udder of a cow could not contain the quantity of milk which can sometimes be obtained from it at one milking. No quantitative data are given to back up the statement. In making measurements of the change in volume of the udder of the goat by removal of the milk I have found in some cases a decrease equal to the amount of milk secured, and in other cases a decrease less than the volume of milk drawn. An average of four determinations shows a yield of 339 cc. of milk and a decrease in volume of udder of 323 cc. The method of volume determination, as previously noted, was not exact. The results of one trial were as follows:

Goat No. 5. 5/5/15, a.m.

	cc.	cc.
Volume of udder.....	1540	
Milked out.....	105	
Kids nursed.....	255	
Total milk.....		360
Volume of udder.....	1170	
Decrease in volume of udder by milking.....		370
Injected salt solution at 25 cm. water pressure.....		480
Volume of udder.....	1635	
Increase in volume.....		465
Milked out (1.1 per cent fat).....	185	
Milked out one hour later (2.5 per cent fat).....	82	
Kids nursed.....	265	
Total fluid recovered (1½ hours).....		532

The pressure of injection used was probably somewhat greater than that at which the milk existed in the udder. It is noteworthy, however, that in this case the decrease in volume of the udder by milk-

ing and nursing was equal to the volume of milk removed. Also, the internal capacity of the udder after milking and nursing was shown to be considerably greater than the volume of milk yielded.²

Change in fat content of milk with progress of milking. Data previously given serve to emphasize the well known fact that as milking progresses the fat content of the milk rises greatly. The effect may be duplicated to a certain extent by allowing milk to pass through a quantitative filter paper. The results of such a test are given in table 6. The goat was first turned on her back and the milk cisterns gently massaged to destroy the results of any tendency for the milk to cream in the cisterns. 25 cc. was then drawn, designated as fore-milk; then the main portion,

TABLE 6

Showing the fat content and size of fat globules in fore-milk and stripping milk in comparison with similar data for the main portion of the milking, mid-milk, and two fractions of a 50 cc. portion of the mid-milk separated by allowing to pass through a filter paper until 20 cc. had filtered

SAMPLE	FAT	AVERAGE DIAMETER OF FAT GLOBULES
	per cent.	μ
Fore-milk, 25 cc.....	4.8	2.2
Strippings, 40 cc.....	8.3	2.8
Mid-milk, 245 cc.....	6.0	2.5
Filtrate, 20 cc.....	5.3	2.3
Non-filtrate, 30 cc.....	6.6	2.7
Filter control.....	6.0	
Creaming control.....	6.0	

245 cc., designated as mid-milk; and finally the last portions, amounting to 40 cc. and designated strippings.

A 50 cc. portion of the mid-milk was placed in a filter and 20 cc. allowed to filter through, requiring about 20 minutes. The portion remaining unfiltered was transferred with the paper to a beaker, the paper gently washed through the milk and removed. This portion is designated non-filtrate. As controls another 50 cc. portion of the mid-milk was treated the same as the above except the unfiltered part

² A characteristic reaction of the goats I have worked with is for them to start chewing the cud when milking begins and to continue during the process of milking. Upon the injection of the salt solution in this trial the goat started again to chew her cud and continued as if she were being milked. That is, the passive distension of the gland produced the same reflex, with reference to this particular, as is usually produced during its active contraction during the milking act.

with the paper was added to the filtrate and the paper, after light washing through the milk, removed. This is designated filter control. Still another 50 cc. portion of the mid-milk was allowed to stand in a beaker during the foregoing process and a sample drawn from the surface. This is designated creaming control.

From table 6 it appears that the filter paper retards the passage of the fat globules more than it does the rest of the milk. The fat content and size of the fat globules in the filtrate are decreased. A certain parallelism appears between this effect and that shown in the natural separation of fore-milk and strippings in the removal of milk from the mammary glands. The effect of treating cow's milk (where the fat globules are larger than in goat's milk) as above, is even more marked on the fat content. Thus, in one case a sample of cow's milk containing 7.1 per cent fat showed in the first portion filtered 0.7 per cent fat.

DISCUSSION

It seems highly probable that the remarkable growth of the mammary gland during pregnancy is excited by the presence in the blood of a specific hormone, speaking in terms of current point of view. The evidence of such natural experiments as that of the pygopagous twins, the transplantation experiments of Ribbert, and the foetal extract experiments of Lane-Clayton and Starling, is readily interpreted in such a way. On the other hand it is clear that the hypothetical hormone is not present in large or potent quantities in the blood of the pregnant animal, or else that the mammary gland of the pregnant female is for some reason more susceptible to its influence than the gland of the non-pregnant female; for the transfusion of considerable quantities of blood does not produce positive results. The negative results of Lombroso and Bolaffio with rats united in parabiosis would seem to overthrow the hormone hypothesis. But the authors state that a starved member of such a pair suffered death as quickly as a normal starved control, even though its parabiotic mate was given full feed. That is, there was, apparently, no interchange of circulating food substances. From this it might be well contended that passage of a possible hormone in the blood of the pregnant individual of a pair to the blood of the other, non-pregnant member, was not possible. The fact that lactation may occur in the virgin does not argue against a hormone of pregnancy being *normally* the main factor in the peculiar increased growth activity of the mammary gland during pregnancy. From a teleological point of view

such a hormone, produced by the foetus (or foetal membranes), would be simply an organ for its own post-natal welfare.

While a development of the mammary gland similar to that occurring in pregnancy is not readily produced by the introduction of blood from a pregnant animal into a non-pregnant female, yet such treatment of a lactating animal immediately and temporarily depresses milk secretion. Either inhibition of milk secretion is due to a different hormone than the one causing growth of the gland in pregnancy, or the gland is more sensitive to its influence in the lactating stage. The Lane-Clayton and Starling theory that milk secretion occurs as a result of the removal of this hormone inhibitor, with the removal of the foetus, serves to explain lactation following normal delivery and abortion. The preliminary preparation of the gland, however, seems to be the essential factor for its secretory activity, since lactation ceases normally after a time even though pregnancy does not recur, and, therefore, without the inhibition of any foetal hormone. Further, while lactation is initiated or increased by premature delivery it is not as large in amount as it is following a normal delivery.³

The failure of blood transfusion from the actively lactating goat to a feebly lactating one to accelerate the secretion of milk in the latter indicates that lactation is not due to the presence of some particular substance (hormone) in the blood. The fact that one gland may cease lactation before the other also argues against such a factor. The response of the lactating gland to pituitrin is not dependent on the presence of anything in the nature of an activator in the blood; for its non-lactating mate, supplied with the same blood, does not respond. This supports, indirectly, the contention that lactation is not determined by the presence of any exciting substance in the blood stream.

The form of the curves showing the effect of pituitrin on an active, air-inflated mammary gland shows a muscular response. The relatively rapid rise of pressure as expressive of a contraction of the milk passages,

³ If this point of view is correct it would seem possible that the foetus, by influencing the development and preparation of the gland preceding delivery, might affect the yield of milk by the mother following delivery. Then, the sire of the foetus, by means of the foetus, might influence the milk yield of the mother. In dairy theory and practice, as far as I know, the bull to which a cow is bred is not recognized as a factor in the yield of milk by the cow following the birth of the calf by that mating. Probably the maximum yield of milk is fixed by the genetic endowment of the milk secreting cells of the gland and influences of pregnancy and delivery serve purely a qualitative purpose in initiating activity and have no quantitative effect. The point, however, is worthy of investigation.

followed by the slow decline as expressive of a relaxation is characteristic of the response of smooth muscle. The failure of a second dose to duplicate the effect of the first is not a thing unusual to the action of drugs. Indeed, with pituitrin itself while a first dose causes a rise of blood pressure due to constriction of peripheral vessels, a second dose may not cause a rise of pressure. That the pressure changes in the gland upon injection of pituitrin are not due to changes of blood pressure seems certain from the fact that no change is produced in the non-lactating gland in a late stage of pregnancy, although the gland is at that time highly vascular. That it is not due to a secretory action of the cells is shown by the rapidity of the rise and the return to the initial pressure. A rise of pressure through a secretory action could occur only by the absorption of water and its passage into the ducts. If this occurred it is extremely unlikely that resorption would commence at once and be carried to a point that would exactly restore the initial pressure. A muscular explanation seems the only one to fit the data. The difference in sensitiveness of the contractile elements of the gland to pituitrin according to the stage of activity finds an analogue in the response of uterine muscle to adrenalin, where the gravid uterus is strongly contracted and the virgin uterus practically unaffected.

The outflow of milk caused by pituitrin is well explained by a muscular action. This seems the more likely as a correct explanation when it is remembered that the act of nursing causes an active increase of pressure in the gland closely resembling the effect of pituitrin. The flow of milk produced by pituitrin when simply placed on the cut surface of the excised gland containing milk shows that its effect is independent of the circulation (except, of course, to bring it in contact with the gland when injected), and gives, also, pretty conclusive evidence that it causes constriction of the very small milk passages of the gland.

Hammond bases his conclusion that pituitrin stimulates the secretory activity of the cells, and has no muscular action on the gland, on the yield of milk obtained by hand milking during a test period of a few hours. Rather positive evidence of a muscular action has been shown above. It must also be clear, from the data given, that any attempt to measure the secretory activity of the gland cells by the amount of milk that may be obtained by hand milking in a test extending over a few hours, is subject to a large error because of variations in the completeness of removal of the milk from the gland. Milk secretion, in the sense of the formation of the milk constituents, is one thing; the ejection of the milk from the gland after it is formed is quite another

thing. The one is probably continuous; the other, certainly discontinuous. And inasmuch as no one has shown any material increase in the milk obtained over a period of days by the use of pituitrin any true secretory action of the drug must be held unproven.

The data given on the flow of milk during milking and nursing show very plainly that there is a reflex constriction of the gland (very similar to that produced by pituitrin) involved; that the removal of the milk from the gland is dependent upon the operation of this reflex; and that the reflex is conditioned. The stimulus which, naturally, excites the reflex must be found in the friction and warmth of the sucking action of the young acting on the cutaneous sense organs of the teat; with, possibly, a further source in the passive dilatation of the sphincter muscles of the nipple by the passage of the milk.

If the mother dog be placed on her back the pups experience no difficulty in getting the full yield of milk, although it must be secured directly against the force of gravity. But if the mother be placed under ether, in a normal position, the pups are unable to secure any milk. Evidently some connection in the reflex arc is broken by the anesthesia, and the indication would seem to be that the central nervous system is involved. The failure of vigorous and continued nursing to secure milk under this condition indicates that the normal removal of the milk is not passive with respect to the activity of the gland. Pituitrin restores the flow, presumably by acting directly on the contractile elements of the gland and producing the same sort of contraction that is ordinarily produced by the nursing reflex.

An artificial imitation of the stimulus afforded by the nursing kid which must have duplicated the natural stimulus very closely, failed to excite the nursing reflex in the absence of the kid; but with the kid present the reflex was called into effective operation by the artificial stimulus as quickly and thoroughly as by the nursing of the kid himself. This may be interpreted to mean that conduction in the reflex arc is dependent upon the psychic condition of the mother.

When the recently delivered cow or mare has been separated from her young some time and her udder is distended with milk, she shows, when brought to her calf or colt, marked symptoms of maternal concern, and accompanying this there is often a spurting of milk from the nipples. Apparently, with the gland under this extreme tension, the psychic state produced by the recovery of the young induces a contraction of the gland and an ejection of milk. But in the case of the goat above, with the udder previously drained of milk by hand milking,

recovery of the young, although causing plainly an altered psychic state of the mother, was not sufficient to cause a further flow of milk. But this psychic state in combination with the mechanical and thermal stimuli of nursing permits a reflex more powerful than that excited by the preceding hand milking and calls into play, as it were, a certain reserve strength in the contractile elements of the gland which results in a large flow of milk. In the dog this seems to be the limit of contractile power of the gland, but in the goat pituitrin causes a still further contraction as seen in the ejection of milk. (The statement is based on trials with one goat only.) It is of interest to note the somewhat analogous action of adrenalin on the vascular system. The drug produces a stronger constriction of the blood vessels than can be produced by any reflex nervous excitation; but direct stimulation of the motor nerve to a vessel may cause an equal constriction.

It is difficult to reconcile the seeming dependence of proper nursing or milking on a central nervous mechanism with the results of Eckhard (18) who found no effect on yield of milk in the goat by section of the nerves to the mammary gland; or, with those of Goltz and Ewald (19) who found that removal of the spinal cord in the dog did not interfere with the nursing of her pups.

The determinations of volume change in the udder with milking here reported are hardly extensive or varied enough to be absolute, but so far as they go the indication is that practically the entire quantity of milk obtained at any one time is present as such in the udder at the beginning of milking. Certainly, the capacity of the ducts and alveoli of the udder in the goat is as great as the milk yield at one time. There is, therefore, no *a priori* reason why the milk should not be regarded as being accumulated as such in the udder during the interval between milkings, and the milking operation regarded as a more or less effectual method of stimulating a reflex which causes a contraction of the gland musculature resulting in the ejection of a greater or less part of the milk therein present, according to the effectiveness with which the reflex is excited. It has been claimed that in the heaviest milking cows it is impossible for the udder to contain the quantity of milk that may be secured from it at one time, but that is a matter for determination rather than speculation.

Histologists, as a rule, have been unable to demonstrate any contractile elements in the alveoli of the mammary gland. Positive physiological evidence, however, should outweigh negative histological evidence. Benda (20) has demonstrated contractile elements in the

basement membrane of the alveoli to his satisfaction. The muscular mechanism of the mammary gland seems to be a very important factor in its functioning. It is, indeed, not inconceivable that the activation of the muscular mechanism of the gland may be an important factor in the onset of lactation. Thus, colostrum is secreted by the cells and fills the ducts during pregnancy long before the contractile mechanism is sensitive to pituitrin or to the stimulus of milking. More extensive data than are here available are needed for definiteness, but, at least, it is clear that the contraction of the gland under the influence of pituitrin runs more closely parallel to the appearance of an actual outflow of milk than does the formation of milk (colostrum) by the secreting cells.

The high fat content of strippings milk as compared with fore milk can not be accounted for by a creaming process in the udder for the same phenomenon occurs in the human (Engel (21)). How far the effect noted by simple passing of milk through a filter paper is comparable to the mechanism that causes the result in the mammary gland, is not altogether clear. It is, however, suggestive that the natural process may be partly one of physical retardation of the fat globules in the rapid passage of the milk through the small ducts which must occur in milking. It is generally regarded that the fat of milk is formed within the secreting cells and the globules then extruded through the ruptured end of the cell. It would seem quite possible, also, that under the strain and stress imposed upon the cells by the reflex constriction produced by the milking act this rupture and escape of fat globules from their free ends may be greatly increased and thus cause the higher fat content of the later portions of milk.

SUMMARY AND CONCLUSIONS

Transfusion of blood from a pregnant goat into a lactating one temporarily inhibits milk secretion. Placental and mammary gland extracts from a pregnant cow have an effect similar to the blood. There is some indication of a subsequent accelerating action in the case of the gland extract. Transfusion of blood from a fresh, heavy-milking goat into a low milking one fails to accelerate milk secretion.

Pituitrin has a muscular action on the active mammary gland causing a constriction of the milk ducts and alveoli with a consequent expression of milk. This action holds, also, on the excised gland in the absence of any circulation. The flow of milk produced by pituitrin

is dependent on the amount of milk present in the gland. There is no evidence of any true secretory action. The non-lactating gland, up to a late stage of pregnancy, is not sensitive to pituitrin.

Nursing, milking, and the insertion of a cannula in the teat, excite a reflex contraction of the gland musculature and expression of milk. There is a latent period of 35 to 65 seconds. Milking is a stronger excitant than the cannula; nursing is stronger than milking; [and the direct action of pituitrin (in some cases) is stronger than nursing]. Removal of milk from the gland is dependent upon this reflex, and it may be completely inhibited by anesthesia. The adequate stimulus for the receptor in the reflex arc is the thermal and mechanical effects of nursing; but the strength of the excitation thus aroused is profoundly modified by the psychic state of the mother—especially striking are anesthesia which greatly weakens it, and recovery of the young after separation which greatly strengthens it.

The internal capacity of the mammary gland of the goat is greater than the volume of milk drawn at one time, and the udder shrinks in volume during milking to nearly the same extent as the volume of milk drawn. Practically all the milk drawn is present as such in the gland at the beginning of milking, and is actively ejected by a reflex contraction of the gland musculature under the stimulus of milking.

Passing milk through a filter paper separates it into fractions of different fat content, somewhat similar to fore-milk and strippings. A physical filtration may account, at least in part, for the rise in fat content of the milk with progress in milking.

REFERENCES

- (1) STRAHL: *Verhandl. d. Anat. Gesellsch. zu Kiel*, 1898, 236.
- (2) KNOEFFELMACHER: *Jahrb. f. Kinderheilk.*, 1902, lvi, 791.
- (3) RIBBERT: *Arch. f. Entwicklungsmech.*, 1898, vii, 688.
- (4) MARSHALL: *Physiology of Reproduction*, London, 1910, 585.
- (5) LANE-CLAYPON AND STARLING: *Proc. R. Soc.*, 1906, lxxvii, B, 505.
- (6) LOMBROSO AND BOLAFFIO: *Arch. Ital. d. Biol.*, 1910, liii, 447.
- (7) NIKLAS: *Monatsschr. f. Geburtsh. u. Ggn.*, 1913, xxxviii, 60 E. H.
- (8) LEDERER AND PRIBRAM: *Pflüger's Arch.*, 1910, cxxxiv, 531.
- (9) GAVIN: *Quart. Jour. Exp. Physiol.*, 1913, vi, 13.
- (10) OTT AND SCOTT: *Proc. Soc. Exp. Biol. and Med.*, 1910, viii, 48.
- (11) MACKENZIE: *Quart. Journ. Exp. Physiol.*, 1911, iv, 305.
- (12) HILL AND SIMPSON: *Proc. Soc. Exp. Biol. and Med.*, 1914, xi, 1.
- (13) HAMMOND: *Quart. Journ. Exp. Physiol.*, 1913, vi, 311.
- (14) HILL AND SIMPSON: *Quart. Journ. Exp. Physiol.*, 1914, viii, 103.
- (15) HEANEY: *Surg. Gyn. and Obst.*, 1913, xvii, 103.

- (16) FLOWER AND LYDEKKER: An Introduction to the Study of Mammals, London, 1891, 228.
- (17) SCHAEFER: Text b. Physiol., Edinburg and London, 1898, i, 668.
- (18) ECKHARD: Beitr. zu Anat. u. Physiol., 1858, i, 3.
- (19) GOLTZ AND EWALD: Pflüger's Arch., 1896, lxiii, 362.
- (20) BENDA: Dermatolog. Zeitschr., 1894, i, 94.
- (21) ENGEL: In Sommerfeld's Handbuch der Milchkunde, Weisbaden, 1909.