SOME RECENT EXPERIMENTS ON THE PROBLEM OF MILK EJECTION

W. G. WHITTLESTON, Ruakura Animal Research Station, Hamilton.

In a previous paper read to this Conference (1) a series of problems connected with the milk let-down mechanism was discussed. The following paper presents some further data on some of these problems, and raises a number of questions in a more sharply defined form.

The Nature of the Milk Let-down Hormone.

The generally accepted view at present is that the milk let-down hormone is identical with the oxytocic principle of the posterior lobe of the pituitary gland. The milk ejecting properties of whole posterior lobe extracts have been known for some time (2), (3), but it was Ely and Petersen (4) who first suggested that oxytocin is the milk ejection hormone. This view was questioned by Turner and Cooper (5), who showed that, using a lactating rabbit as an assay animal, whole pituitary extract has more milk let-down activity than it should have for a given oxytocic activity. This suggests the presence of another hormone in addition to the pressor, oxytocic and anti-diuretic substances of the posterior lobe.

The experiments to be described were carried out to determine the relative milk ejecting activities of Pitocin and Pitressin. The former is a Parke Davis extract of the posterior lobe of the pituitary gland purified and separated so that the final product is essentially the oxytocic substance contaminated by not more than 5% of the pressor substance. Frequently the oxytocic preparation has as low a pressor contamination as 1% (6). Pitressin is the pressor preparation with an oxytocic content between 2-5%. A maximum contamination of 5% of one hormone by the other is guaranteed by the makers.

In the following experiments the sow was used as an assay animal.

Procedure.

A sow was selected and removed from her litter approximately 1½ hours before the first injection. Injections were given at 10 minute intervals into an ear vein. In all cases the dose was made up to 0.8ml. with normal saline. The milking machine teat cups were applied immediately after the injection and the total yield from all glands measured.

Experiment 1.

This experiment is a preliminary attempt to compare the activities of "Pitocin" and "Pitressin" by adjusting the doses to give a corresponding yield of milk. The following table summarises the experiment.

TABLE I.

Date	Solution	Dose in I.U.	Milk ejected (g.)
26/1/50	Pitressin	4	275
	Pitocin	2	262
	Pitressin	4	113
27/1/50	Pitressin	2	124
	Pitocin	1	121
	Pitressin	2	80
31/1/50	Pitocin	1	204
	Pitressin	2	86
	Pitocin	1	93

2/2/50	Pitressin	2	128
	Pitocin	0.5	86
	Pitressin	2	45
3/2/50	Pitocin	0.5	90
	Pitressin	2	59
	Pitocin	0.5	54
	(Sow: Phil)		

It would appear that the milk ejecting activity of Pitressin is of the order of 25% of that of Pitocin.

Experiment 2.

In this case the point at which a just measurable response was obtainable was determined for successive decreasing doses of the two extracts Pitocin and Pitressin. Table II gives the results.

TABLE II.

Date	Solution	Dose in I.U.	Milk ejected (g.)
31/1/50	Pitressin	0.5	131
	"	0.5	97
	"	0.5	112
2/2/50	Pitressin	0.25	50
	"	0.25	71
	"	0.25	58
3/2/50	Pitressin	0.125	59
	"	0.125	0
	"	0.125	0
7/2/50	Pitressin	0.2	94
	"	0.2	82
	"	0.2	89
7/2/50	Pitressin	0.15	35
	"	0.15	53
	"	0.15	22
	(Sow: Folly)		
7/2/50	Pitocin	0.25	203
	"	0.25	123
	"	0.25	173
9/2/50	Pitocin	0.125	45
	"	0.125	43
	"	0.125	56
14/2/50	Pitocin	0.1	33
	"	0.05	21
	"	0.05	22
	"	0.1	41
16/2/50	Pitocin	0.025	47
	"	0.025	12
	(Pitocin	0.025	
	(Pitressin	0.05	27
	(Sow: Phil)		
16/2/50	Pitressin	0.1	74
	(Pitocin	0.05	
	(Pitressin	0.05	76
	Pitocin	0.05	86
	(Sow: Folly)		

It is evident from the foregoing results that Pitressin is much more active than it should be if the milk let-down activity is attributed to the oxytocic contamination of the pressor preparation. One explanation of the results is that there is a separate milk ejection hormone or, to follow Sir Henry Dale's recent suggestion (7), there is a "galactergic" hormone.

The Rate of Destruction of the Hormone in the Sow's Blood.

Because of the absence of a milk cistern and definite teat sphincter milk trickles from a sow as soon as the let-down reflex is evoked. The duration can be determined by milking the teats by hand. The sharpness with which the flow ceases is a surprising feature of the process. Sections of a sow gland are shown in the accompanying figure (Fig. 1). The absence of large sinuses is obvious. In one experiment a dose of Pitocin which was 120 times the threshold dose (0.025 units) was reduced to an ineffective level in 1 minute 55 seconds, while 60 times the marginal dose took 1 minute 20 seconds. See also Table III.

TABLE III.

Dose	Solution	Milking Time (min.)
1.0	Pitocin	1.03
1.5	„	1.08
2.0	„	1.52
3.0	„	1.73

The roughly proportionate relationships between duration of milk flow, amount of milk and the dose injected suggest that the time during which the hormone injected (or secreted) is above marginal level in the blood controls the amount of milk released to a particular stimulus or following a particular injection. The snappy cut-off also suggests that in addition to a contraction of the myoepithelium around the alveoli there is a shortening of the ducts and thus an almost valve-like shutting off of the milk from the fine ductules when ejection ceases.

The Injection Time-lag.

The time interval from the point when the syringe is half empty until a response occurs has been measured for many experiments. Some of the results are set out in Table IV below.

TABLE IV.

Sow	Interval (secs.)	Solution	Dose units).
Phil	17	Pitressin	2
	19	Pitressin	1
	20	Pitressin	2
	17	Pitocin	1
	17	Pitressin	2
	20	Pitocin	1
	17	Pitressin	2
	17	Pitocin	0.5
	16	Pitressin	2
	19	Pitocin	0.5
	17	Pitressin	2
	16	Pitocin	0.5
	N = 15. Ra = 16-20.	Av. = 17.7.	
Ruth	17	Pituitrin	2
	20	Pitocin	1
	16	Pituitrin	2
	17	Pituitrin	1
	16	Pitressin	2
	N = 5. Ra = 16-20.	Av. = 17.2.	
Flirt	13	Pitocin	1
	15	„	1.5
	13	„	2
	11	„	3
	15	„	0.5
	16	„	0.5
	N = 6. Ra = 11-16.	Av. = 13.8.	

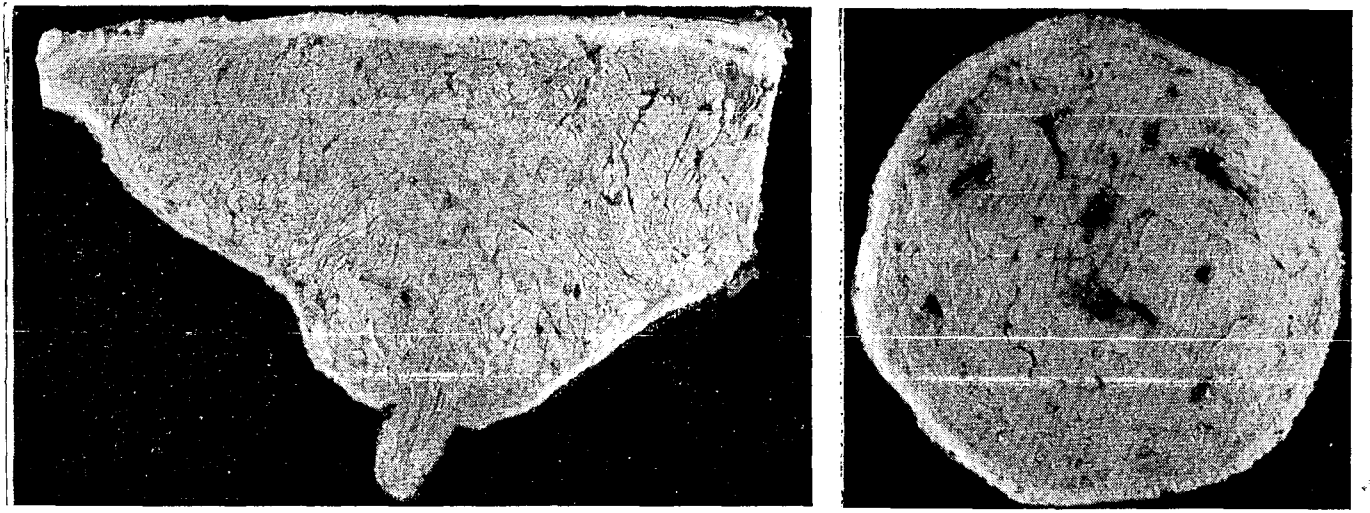


FIG. 1.—Sections of a Sow Mammary Gland.

(a) Vertical section of gland.

(b) Horizontal section of gland.

Folly	17	Pitressin	0.25
	19	"	0.25
	21	"	0.15
	23	"	0.15
	23	"	0.15
	24	"	0.1
	23	"	0.1

N = 7. Ra = 17-24. Av. = 21.4.

Considering such sources of error as the timing of the injection with a troublesome animal and the difficulty of detecting the first squirt from any of the dozen teats into the milking machine, the figures are surprisingly consistent. It is interesting to note a similar consistency amongst similar figures obtained by Peeters (8) for a number of cows injected via the jugular, the let-down response being measured by noting the resultant intramammary pressure change. These figures were as follows (seconds): 19, 22, 20, 19, 18.5, 19, 18, 18.5, 20, 20, 22, 21.5, 18.5. N = 13. Ra = 18-22. Av. = 19.7. For both the cow and the pig the rate of flow of the blood from the point of injection to the mammary gland is almost constant and the interval of about 20 seconds for the circulation from the jugular vein to the mammary gland in the cow is distinctly shorter than the figure of 45 seconds frequently given as the interval between the release of the pituitary hormone and its action at the mammary gland.

The Mechanism of Milk Ejection

The recent work of Richardson (9) has at last established the nature of the mechanism by which the milk is actually expelled from the alveoli. The basket-like networks of myoepithelial cells shown so clearly by his new staining method leave little doubt about the mechanism of the process. Whether or not the posterior pituitary hormone acts on these cells or whether some other mechanism is also involved, e.g. internal sphincters or a valve-like opening of the ductules (10) is not at present known. One point of considerable interest is the demonstration of the existence of contractile cells running along the ducts. The contraction of such fibres would explain the almost valve-like shut-off of the milk flow and the relatively low order of intramammary pressure fall subsequent to the dying away of the action of the milk ejection hormone. This point is referred to later.

The Effects of Inhibition on Milking Rate

The previously described experiments showed how milking rate could be reduced greatly by inhibiting a cow by electric shock. This observation immediately throws into question any experiments on the effects of mechanical factors on milking rate unless the internal letting down mechanism of the mammary gland is known not to be the limiting factor in controlling milking rate. Earlier experiments (11) have shown a surprising lack of dependence of milking rate on vacuum when measured over a number of cows. For example, a decrease in vacuum from 15in. to 10in. caused a decrease in milking rate of only 8% and this difference was not significant because of the high variance associated with this property. Some other factors appeared to be limiting milking rate. Another experiment, however, carried out during the earlier part of the season showed a significant difference in rate of milking between a high level and a low level milk pipe machine. The low level machine averaged 3.04lb. of milk per minute against 2.62lb. per minute for the high line plant. The low line plant had a vacuum at the teat cups between 2in. and 3in. of mercury higher than the orthodox plant when milk was flowing. Hence milking rate appeared to be related to vacuum in this experiment. It would appear that stage of lactation influenced the dependance of milking rate on the vacuum in the machine. The explanation offered in the earlier paper for these apparent contradictions was that "when the milk is

let down flow commences, the degree of crawling (of the teat cups) being roughly adjusted to the rate of flow into the milk cistern. This gives rise to a situation in which an increase or decrease in vacuum would not markedly affect the flow rate except in the case where the teat sphincter is the limiting factor.'' If, as seems likely, the sphincter is the limiting factor in the earlier months of the season when flow rates are high, then during that time milking rate would be dependent on external mechanical factors. As the rate of let-down internally decreases, due either to increased rate of destruction of the hormone or decrease in the amount released, the milk ejection curve will become more and more determined by the cow as in the case of the artificially inhibited animals. A study of the trends in ejection curve shape throughout a lactation supports this view. There remains, however, a question not discussed previously. In the case of the inhibited cow is the teat sphincter affected or is it inert?

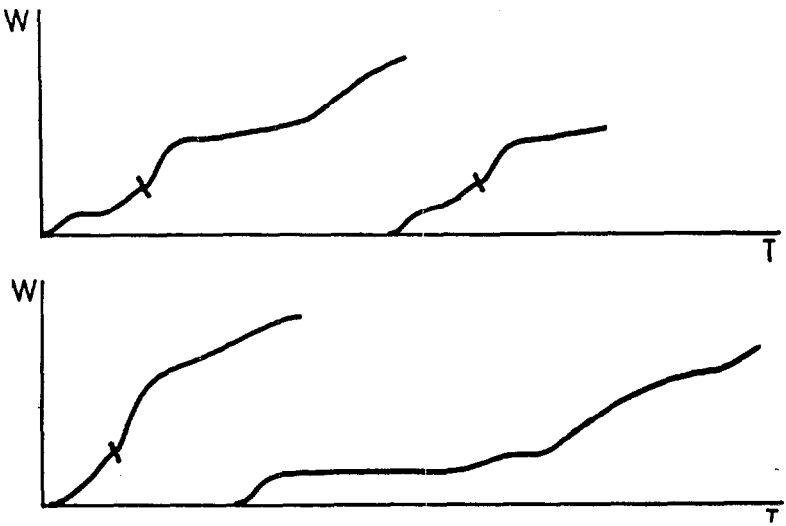


FIG. 2.

Milk ejection curves of cows inhibited by electric shock. The curves were interrupted for one minute at the points marked by the short lines. The lower right hand curve shows the initial rush of milk from an inhibited cow.

The accompanying figure (Fig. 2) shows the effect of removing the teat cups from an inhibited cow for one minute. On replacing them it can be seen that there is a sudden rush of milk followed by a resumption of the original slow rate. The same effect is observed when there is milk in the cistern of an inhibited cow. There is a sudden flow followed by a slow rate. The sphincter would appear to be inert even in the case of cows inhibited by electric shock.

The Suckling Stimulus and the Decline of Lactation.

Evidence has been produced (12) for the existence of a lactogenic hormone secreted by the anterior pituitary in response to the suckling stimulus. If this is correct an important question is raised by the demonstration of inhibition of the let-down reflex since as it is elicited by the same stimulus as the lactogenic hormone, it is possible that the inhibition process also works for both. Two cows were taken at a stage of lactation such that they were secreting half of their peak milk yield and were milked once a day. One of them, however,

in place of the omitted milking, was stimulated by washing and the use of the strip cup followed by withdrawing a pound of milk by the machine. The lactation curves are shown in the accompanying figure (Fig. 3). The results can only be regarded as a suggestion at this stage but it would appear that twice a day stimulation with once a day milking caused no change in the lactation curve compared with that of a control (twin) cow while the change to once a day milking only, caused an accelerated decline. If future experiments confirm this observation we have to answer the question, is the release of the lactogenic hormone involved in a conditioned reflex mechanism or does the posterior lobe hormone itself cause the release of the lactogenic hormone from the anterior lobe?

It is interesting to note that in the case of the cow stimulated at one milking but milked only to the extent of one pound, the ejection curves develop all the symptoms of an inhibited animal. Such an inhibition following the usual preliminaries to milking is to be expected if the removal of a small amount of milk can be treated as the non-reinforcement of the let-down reflex.

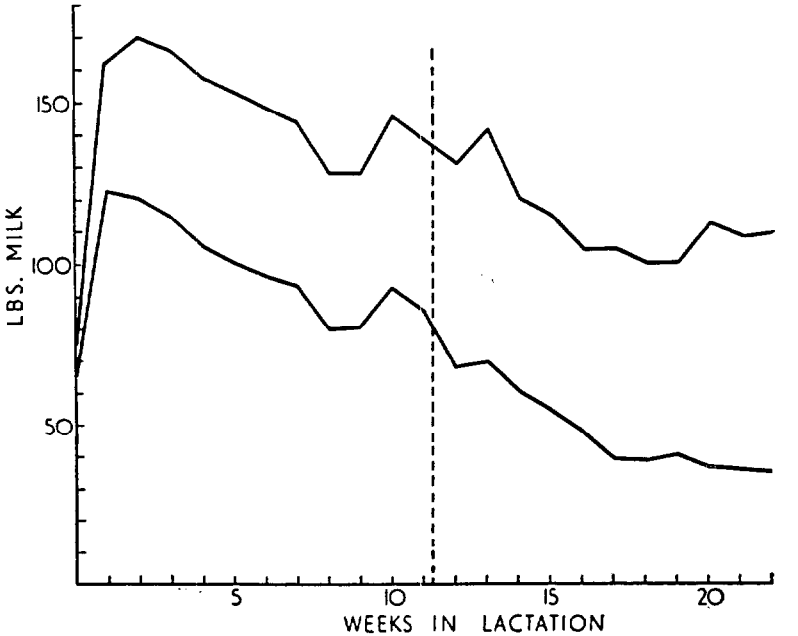
The Effect of "Double Milking" on the Milk Ejection Curve

A cow with a good ejection curve was subjected to the treatment of being half milked out (as judged by the previous corresponding milking) left for five minutes and then milking continued. After this treatment had continued for some three months, check normal milkings were started once a week. Figure 4 sets out the milk ejection curves from interrupted and control milkings. It is evident that this procedure had no tendency to produce inhibition or a double ejection curve. Whether the procedure had an effect on the cow's production or not is not clear from the lactation curve which is shown along with that of the cow's twin in Fig. 5. The results confirm what would be expected from the theory of conditioned reflexes, namely, that provided that the first milking is prolonged enough to act as a reinforcement for the preliminary stimuli, subsequent treatment will not influence the initial reflex. What happens to any second ejection reaction cannot readily be determined from the present experiment.

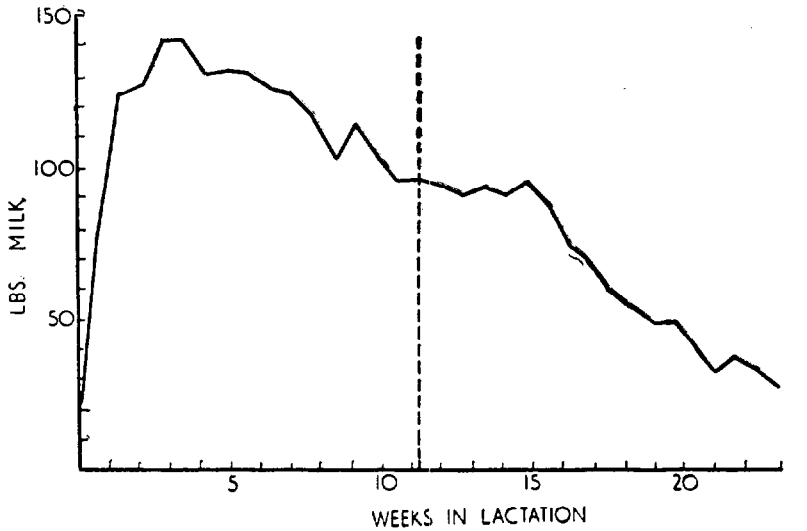
In the earlier paper read to this Society reference was made to the contradictory results obtained by different groups of American workers studying the effect of altering the time interval between stimulation and putting on the teat cups of the milking machine. The results were explained in terms of the multiple release of milk let-down hormone. In a similar way the development of strippy characteristics was explained in terms of a further release of hormone following the stripping stimulus. The obvious tendency on the part of some cows to require stripping during the latter part of the lactation can be explained in terms of (1) a decline in the effectiveness of the pituitary response to stimulation; (2) an increase in the rate of destruction of the hormone. In the previous discussion one point concerning the former factor was omitted, namely, the influence of the fullness of the udder on the effectiveness of the cow's response to the milking stimuli. It is well known from Pavlov's experiments with dogs that the efficiency of the salivary reflex is closely related to the hunger of the dog. An examination of the trends in the milk ejection curves of a group of cows indicates that as milk secretion fails, so the effectiveness of the first let-down decreases as judged by the amount of milk obtained when the cow is re-stimulated by stripping. There is great variation between cows but the effect is common and emphasises the need for a stimulating start to the milking process rather than vigorous machine stripping though in many cases the latter is needed as well.

These considerations raise a point about which there appears to be a considerable amount of confusion. Many years ago Tgetgel (13) published a paper in which he presented a graph showing the relation between intra-mammary pressure and time between two milkings (see

FIG. 3.



(a) Lower Curve. Lactation curve of cow milked once a day after the dotted line. The omitted milking was replaced by stimulation and the withdrawal of one pound of milk. Upper Curve. Lactation curve of twin cow milked normally.



(b) Lactation curve of cow switched to once a day milking only after the dotted line.

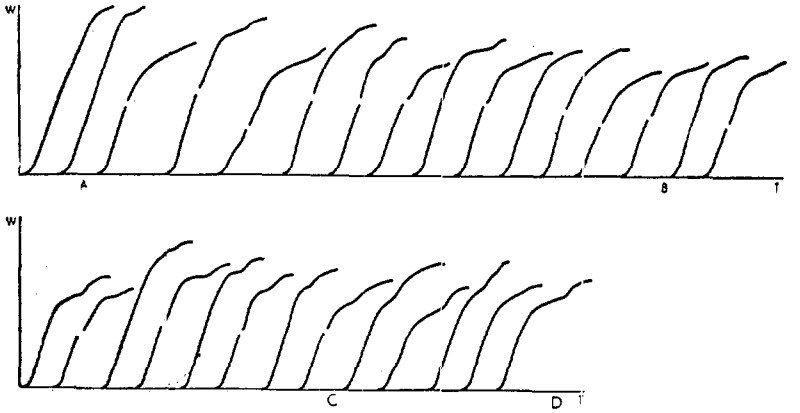


FIG. 4.

Milk ejection curves from cow milked twice by machine at each milking. The first two curves are samples of the pre-experiment curves; A-B, samples of double milked curves; B-C, alternate double milked samples and weekly curves milked normally; C-D, weekly samples during final normal milking period.

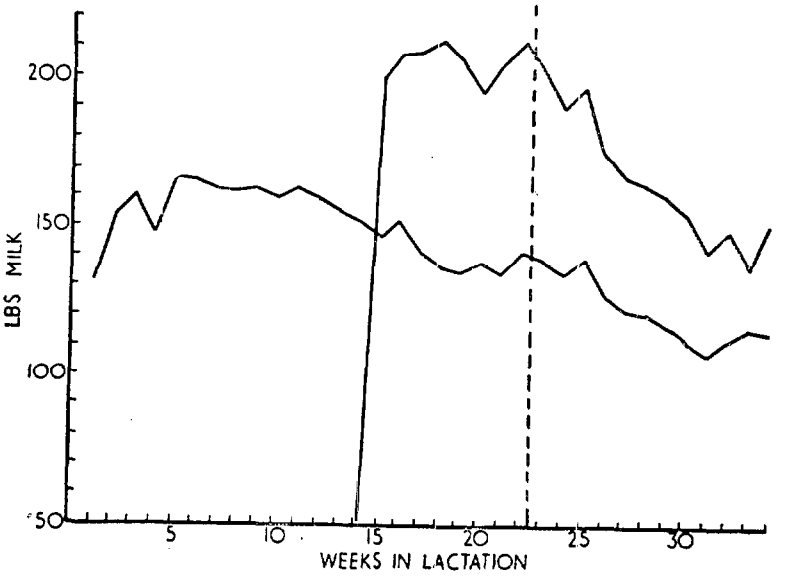


FIG. 5.

Lactation curve of double milked cow (upper) together with curve of her twin as control.

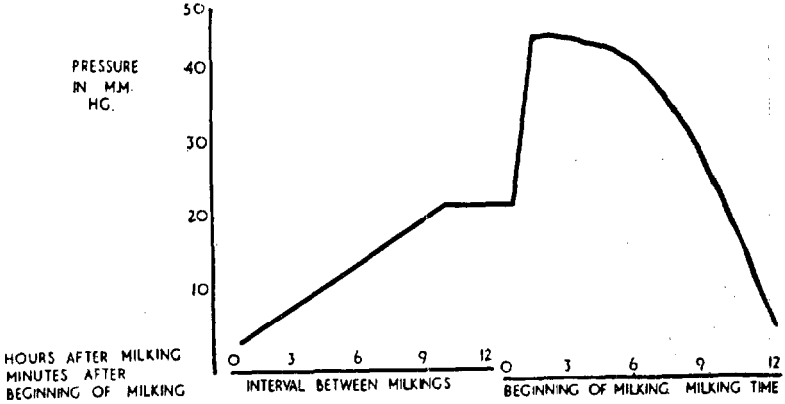


FIG. 6.
Curve showing intrammary pressure changes (after Tgetgel).

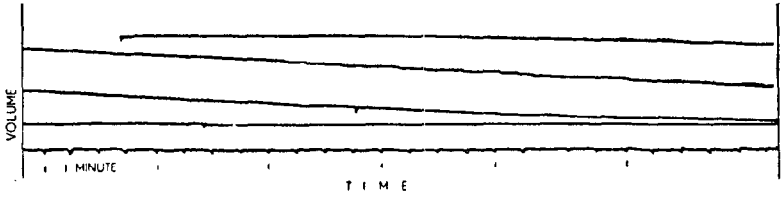


FIG. 7.
Tracing from kymograph drum showing volume/time relationships for flow of milk through a small orifice into a measuring cylinder. The rate of flow rises quickly, remains constant for a period and then falls quickly, fitting the Tgetgel graph for a milked out quarter.

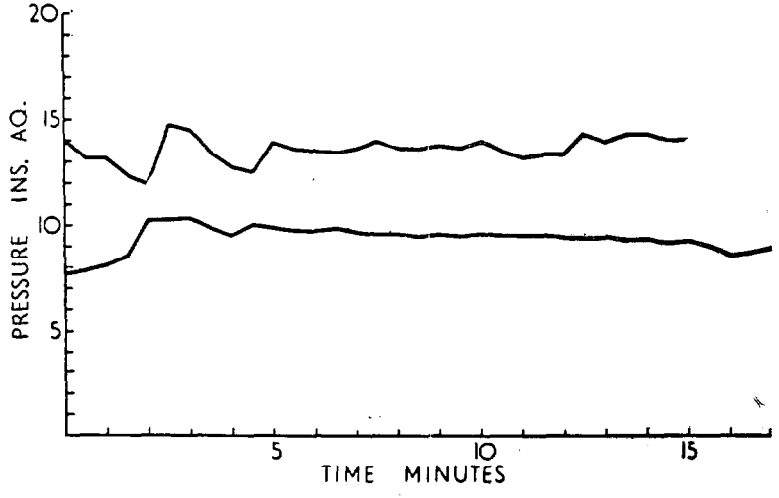


FIG. 8.
Pressure-time curves obtained for unmilked quarters with apparatus similar to that used by Tgetgel. This is quite different in nature from Fig. 6.

Fig. 6). The sudden rise following the milking stimulus is shown, followed by a rapid fall in pressure. So far we have been unable to obtain a copy of the original paper but it would appear from a later paper by the same author (14) that pressure measurement was done with a sphygmomanometer, leveling bulb and teat siphon. The part of the graph subsequent to stimulation would appear to represent the pressure change within a quarter **which is being milked out.** However, some authors seem to have treated this graph as obtaining for an unmilked quarter and deduced therefrom that unless a cow is milked within seven minutes most of her milk will have been withdrawn due to the relaxation of the alveoli. This, combined with the view that a cow can only release one lot of let-down hormone within an hour or so has resulted in some odd practical recommendations being made to the farmer. Using a volume recording instrument and a simple siphon inserted into the teat to permit flow into the recorder, the graph shown in Fig. 7 was drawn. It is quite apparent that the flow/time relationships shown fit Tgetgel's curve if it represents pressure changes within a quarter **being milked out.** Further, by using the more sensitive version of Tgetgel's apparatus, the pressure-time curves of Fig. 8 were obtained. While this system of measurement will not give a precise indication of actual intramammary pressure it does repeat the conditions of the original measurement and does show that the dramatic pressure drop which has been depicted in popular articles bears little relation to the facts. The changes in pressure seen in the figure are due to a further let-down occurring and as can be seen effects due to the let-down mechanism going out of action are certainly not a rapid change in pressure seven minutes from the application of the stimulus. Mr. Phillips will discuss other aspects of these changes in intramammary pressure. His experiments supply additional evidence for the multiple let-down theory advanced in the earlier paper and also support the view that milk ejection involves both contraction of the alveoli and the opening of the ductules. Work with both the sow and the cow indicates that the milk ejection mechanism involves both a forcing of the milk from the alveoli and a decrease in the resistance of the ductules, due presumably to the shortening tendency caused by the contraction of the longtiudinal myoepithelial cells shown by Richardson. Should this prove to be a correct interpretation its importance in the problem of treating mammary infection will be at once apparent. Infusing a drug into the udder while the ductules are open as it were to the action of injected posterior lobe hormone should greatly increase the effectiveness of such treatment.

Acknowledgments:

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Discussion on Mr. Whittleston's Paper

Prof. CAMPBELL: What was the level of production of the cows that were milked once a day?

Mr. WHITTLESTON: The trial was done when the yield had fallen to half the maximum figure, in order to avoid pressure effects.

Mr. GERRING: What is the difference in time lag after stimulation and after injection Might not 45 seconds be a more accurate figure?

Mr. WHITTLESTON: Peterson's figure of 45 seconds for the flow from the jugular vein to the udder was on a circulation time basis. Our figures and those of Peeters were 20-25 seconds. The stimulus may be the washing or the application of the machines.

Mr. BISHOP: Advantage may be taken of the duct opening effect of extracts of the pituitary, to increase the effectiveness of udder infusion treatments for mastitis. In a preliminary trial carried out at Ruakura by Mr. Whittleston and myself 450 ml. of a solution of proflavine was injected into one quarter at a pressure of 70 mm. of mercury. At this pressure no more fluid would flow. The injection of pitocin into the jugular, however, resulted in a further 200 ml. being taken up by the gland and presumably this passed into the smaller ducts and perhaps the alveoli.

In the past, fluids have been injected into the quarter which was then massaged in order to distribute the material through the udder tissue. Pituitary injections may achieve this more effectively. We have, however, made no experiments so far on quarters with acute mastitis, and so we can give no idea as to whether the injection of pituitary extract will help to overcome the "inflammatory barrier" which presents a very real obstacle to successful infusion therapy. This remains to be done.

Mr. MacFARLANE: My interest in the bovine mammary gland stems from five years' work on bovine mastitis. This involved careful microscopical examination of many thousands of pieces of mammary tissue and gave rise to several preliminary physiological experiments. The main point on which I wish to join issue with Mr. Whittleston is the interpretation of myoepithelial function particularly in the intralobular ductules and the suggestion of a valve-like shutting off of milk.

While working at the Royal (Dick) Veterinary College in 1942 my colleagues and I demonstrated two important structural features. Firstly, myoepithelium was clearly demonstrated as existing around all acini and intralobular ductules, both those directly draining the acini and the larger collecting ductules. We never managed to demonstrate their distribution as beautifully as has since been done by Richardson. The second point was the occurrence of elastic tissue. This is found surrounding all duct and ductule structures but never around the acini.

My own interpretation of the let-down phenomenon, based on my own experience and the additional data provided to-day, would differ somewhat from Mr. Whittleston's. Essentially I visualise the mammary gland as consisting of a large acinar volume having the main function of milk production and storage. This is separated from the smaller more or less inert reservoir in the milk cistern. The elements separating the two main reservoirs consist of ductules and ducts becoming larger progressively from the acini to the gland cistern. At the acinar end the acinar ductule is minute and drains a group of acini. Further it is surrounded throughout its length as are all intralobular ductules, by other acini. The elastic tissue surrounding all intralobular ductules would, I should think, tend to maintain the ductules in a collapsed state, thereby creating a distinct barrier between the acini and the larger ductules. It is not suggested that the lumen of these ductules is impassable therefore, but simply that the capillary resistance is suffi-

ciently high to ensure that only a trickle of milk escapes from the secreting structures. Immediately after milking the position would probably be that the acini contain a small quantity of milk and the acinar epithelium is rapidly secreting. The milk accumulates in the acini due to the resistance offered by the ductules closed by elastic tension, and I presume that as the acinar structures become full this capillary resistance is reinforced by the compression pressure of widely distended acini. The nearer the cow approaches to the next milking the greater such pressure must be. It would thus appear that the let-down stimulus has to overcome a mechanical system of this kind.

Mr. Whittleston suggests a shortening of the ductules causing a dilatation of the lumen thereby overcoming the elastic resistance and compression pressure exerted on the ductules and as a consequence capillary resistance is sufficiently reduced to permit the pressure difference which is developed between the acini and the gland cistern to become effective thereby discharging milk towards the cistern.

I am at a loss to understand how, in the structural set-up I have described, any distinct shortening of myoepithelium can occur. All structures are widely distended and shortening would be dependent on major milk displacements which cannot occur until milk has been removed from the cistern. I would agree, however, that a potential towards shortening has been created. It is not possible for a contraction of peri-acinar myoepithelium by increasing intra-acinar pressure and rendering the wall of the acinus more rigid and so relieving the acinar compression pressure on the ductules to overcome the remaining capillary resistance and so permit flow to the cistern. Another possible alternative hypothesis might be that a tonic contraction of myoepithelium longitudinally distributed along the ductules may convert them into semi-rigid tubes, thereby combating compression pressure and at the same time by creating a distinct lumen, permitting the flow of milk from the acini to the cistern. It also appears to me that the cessation of the let-down, presumably on relaxation of the myoepithelium, would automatically permit elastic tissue virtually to close the lumina of the ductules. Depending on the rate of the relaxation this could be sufficiently snappy to simulate valve action.

In a very extensive examination of the bovine mammary gland, no structures which could be called valves or sphincters were noted in the ductule system. An explanation such as this not requiring any new and undescribed structures has many advantages.

Milking rates and ejection curves used in studying them require critical consideration. In the bovine mammary gland the ejection curve must always be complicated by the reservoir function of the gland cistern, and in any critical study of the activity of the let-down process the buffering effect of the gland cistern must be eliminated. This of course does not occur in the sow where no significant cistern is found.

Mr. Whittleston consistently uses the term "conditioned reflex" with regard to the let-down of milk excretion upon direct stimulus. As far as I remember Pavlov designated as "unconditioned" inborne visceral and somatic reflexes including postural, feeding and sexual reflexes etc. in which internal and external stimuli produce predictable and fixed reaction which are not dependent on surrounding conditions or only slightly so. I feel that the let-down of milk is, in the vast majority of cases an unconditioned reflex in the bovine in this sense at the same time agreeing that in some cases cattle may become conditioned to dairy shed routine. Conditioned let-down reflexes are common in the human female. Based on the supposition that let-down in the cow is a true Pavlovian conditioned reflex, Mr. Whittleston suggests that possibly the trends of milk ejection curves may reflect the functional capacity of the mammary gland in the same way that hunger effect the efficiency of the hunger reflex in the dog. I would suggest that the efficiency of the let-down is possibly more related to the efficiency of myoepithelial

contraction, possibly based on a degree of stretch of the muscle on the initiation of let-down or to the concentration or persistence of the pituitary principle in the blood stream.

Mr. Whittleston has discussed the question of whether in the cow the teat sphincter is an active factor in the removal of milk. So far as I can see the measurements made on the sphincter have not been made with a view to demonstrating any variability in tonus. Structurally there is definitely a muscular sphincter and it seems reasonable to assume that variations in muscle tonus do occur. Control mechanisms are at present unknown and unless some means of measuring the variability is evolved it seems likely that we will remain in the dark.

Mr. WHITTLESTON: I am very grateful to Mr. MacFarlane for raising a number of very interesting points. There is first the question of the nature of the valve-like mechanism within the udder. Mr. MacFarlane's suggestion that contraction of myoepithelium along the ductules results in an increase in rigidity and so the creation of a distinct lumen in place of the flattened cross-section caused by pressure from neighbouring acini is a very attractive one. It is a more satisfactory explanation than one involving an actual shortening of the ducts and fits the facts just as well. It also explains the "notch" in the pressure/volume curves obtained by Peeters when he pumped known amounts of liquid into an udder and measured the resultant pressure. He found a flat portion in his graphs at about 20 cm. of water pressure. This he interpreted as being due to the opening of valves between the great and small ducts. Mr. MacFarlane's suggestion fits these observations admirably.

The second question, whether a normal let-down is a conditioned reflex or not, is one which could be debated at length because of the lack of precision in the distinction between a natural and a conditioned stimulus. The difference between the hot moist mouth of a vigorous calf and the crude action of certain metal topped teat cups is much greater than the difference between the taste stimulus and the smell of food, and yet the latter is by definition a conditional stimulus. If let-down is not usually a conditional reflex, it is difficult to explain the large number of cows which let down their milk to the sound of the machine, and even more difficult to understand why quite small extraneous stimuli will inhibit the let-down reflex. Once let-down has occurred quite acute stimuli, such as electric shocks, will not influence the milking process, thus showing that if adrenalin is the normal inhibiting agent, it apparently cannot act on the mammary mechanism after the pituitary hormone has acted. This appears from American work on the effect of adrenalin to be unlikely. However, the problem needs more experimental work before the argument can be finally decided.

The final point about the sphincter is simply that the decline in milking rate observed in earlier experiments to follow the effect of electric shock could have been partly explained by the involvement of the sphincter. The experiments just described show that the sphincter is not the main factor. From the nature of the experiment small differences in sphincter tone would not have been measurable.