very great. The calf shows an amount of milk "let-down" hormone per unit of body weight which is almost 10 times as great as that shown by the older cows. Work in progress may throw light on the factors influencing the amount of hormone in the dairy cow (Whittlestone *et al.*)(1).

All assays of milk "let-down" hormone were carried out on the lactating sow. The duration of the activity was used as a basis for comparison. It is interesting to note that despite their widely different sources all preparations appeared to exert the same action on the sow mammary gland. The changes in the tone of the gland as detected by the milker appeared to be the same regardless of the origin of the hormone. Similarly the rate at which the milk was ejected was not influenced by the source of the milk "let-down" substance. There is no reason to suspect that the active principle or principles from the posterior pituitary gland is different even in such widely separated creatures as the pig and the echidna.

It is worth noting that the pituitary gland of the fetus of one of the cows studied in another project. to be reported later, was examined for milk "let-down" activity and found to be inactive. This fetus, which was 12 weeks old, had not reached a stage of development at which milk "let-down" hormone was present. The dry weight of the whole posterior pituitary gland was 90 mg.

In addition to the mammalian pituitaries, the presence of a milk "let-down" factor was also demonstrated in the pituitary of a 260 lb marlin or sword fish. While this might appear surprising, it should be pointed out that both the oxytocic and vasopressor (antidiuretic) hormones cause the milk "let-down" phenomena (Whittlestone, 1952)(2). It is possible that the "let-down" effect observed might be due to the antidiuretic factor.

These data on the widespread presence of the factor influencing milk "let-down" may be compared with the assays of mammalian pituitaries for the presence of oxytocic and vasopressor factors by Geiling(3), who has shown that both hormones occur in whales, porpoise, armadillo, and even in chickens.

Summary. 1. A factor or factors which will stimulate the "let-down" of milk in the sow has been shown to be present in the posterior pituitary gland of representatives of the 3 divisions of mammals, the monotremes, the marsupials, and the placentals. 2. The pituitary of a marlin or sword fish also caused milk "let-down" in a sow.

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2. Whittlestone, W. G., *J. Endocrinology,* 1952, in press.

3. Geiling, E. M. K., P. R. Rockwood Lecture, College of Med. State Univ. of Iowa, 1940.

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Effect of Acetylcholine on Mammary Gland of the Lactating Sow. (19567)

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The role of the neurohypophysis as the source of the hormone controlling milk let-down has been clearly established by many workers since the first observations of Ott and Scott(1) on the effect of pituitary extracts on the mammary gland. Cross and Harris(2) have recently shown that the electrical stimulation of the pituitary stalk of the rabbit will result in the let-down of milk. There is evidence that both of the posterior pituitary hormones will cause milk let-down(3). Following milk let-down, it has been shown by Cross(4), and Peeters and Coussens(5) that antidiuresis occurs. Despite the clearly hormonal aspects of the control of milk let-down,

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TABLE I. Effect of Acetylcholine on Milk Let-Down.

Exp.	Treatment	Time from inj. to response, sec	Duration of milk let-down, sec	Milk yield, g	Remarks
I	.5 I.U. oxytocin	13.4	35.9	51.8	Sow in milk 1 wk.
	.2 g acetylcholine	13.9	40.3	12.7	Low milk yield.
	.5 I.U. oxytocin	13.9	30.2	61.4	
II	.2 g acetylcholine	—	—	—	No response.
	.5 I.U. oxytocin	15.9	47.1	172.9	Sow in milk 3 wk.
	.3 g acetylcholine	—	—	—	No response.
III	.5 I.U. oxytocin	15.7	45.8	71.8	Sow in milk 2 wk.
	.2 g acetylcholine	16.5	23.9	3.1	Low milk yield.
IV	.5 I.U. oxytocin	19.2	29.6	53.9	Sow in milk 2 wk.
	.2 g acetylcholine	16.9	21.3	48.4	
	.1 g " + .5 g prostigmine	15.2	19.3	52.6	
V	.5 I.U. oxytocin	15.4	49.6	68.3	Sow in milk 8 days.
	.2 mg Carbachol	15.6	42.6	12.4	Low in milk yield, sow sat down.
	.1 mg "	27	15	7.7	Abnormal delay after injection.

the possibility of an alternative neural pathway exists. That adrenalin is not the normal mediator is indicated by the research of Ely and Petersen(6), and Whittlestone(7) who showed the inhibiting role of this substance on the mammary gland. It is possible that the fibers supplying the posterior lobe are adrenergic. However, this action would be completely masked by the inhibiting action of adrenalin on the milk let-down process when this is used as a criterion of activity. Pickford (8) has shown that acetylcholine will evoke antidiuresis and the concentration of chloride in the urine of the dog. This suggests the possibility of the posterior pituitary being innervated by cholinergic fibers. Petersen(9) showed that acetylcholine will permit the removal of milk from the secreting tissue of a perfused mammary gland. This observation was complicated by the fact that no response was obtained from carbaminoylcholine.

In the light of our experience with the lactating sow as an experimental animal for the study of milk let-down(3) and in view of the results obtained by the injection of oxytocin and adrenalin in different orders(7), it was thought worthwhile to study the action of acetylcholine and its derivatives on the mammary gland of the intact animal. The present paper describes the results obtained from such preliminary experiments.

Material and methods. As in previous milk let-down experiments the standard of response has been taken as that evoked by 0.5 I.U. of oxytocin. A lactating sow was brought to a specially designed milking machine(10). Injection of the substance to be studied was made via an ear vein. The interval between injection and let-down and the duration of the latter as judged by the flow of milk obtained by hand-milking one of the front teats were recorded. The effects of a series of injections of 0.5 I.U. of oxytocin on the milk let-down time of the sow have been studied(3). Normally the first dose elicits a response of much greater duration than the second and subsequent injections and a slow decrease in duration of let-down follows with the third and subsequent injections. A minimum period of 5 minutes was allowed between injections in order to minimize interaction between consecutive doses. The amount of milk drawn by machine from the teats not hand milked was also recorded.

Experimental. In the first experiment it was observed that the injection of 0.2 g of acetylcholine into a lactating sow caused a milk let-down period of 40.3 seconds duration (Table I). However, the small amount of milk obtained compared with that obtained following the injection of oxytocin suggests the action of a substance with properties different from the latter. Since the time from injection to response is the same as that following the injection of oxytocin it is obvious that the immediate action of acetylcholine

was directly upon the mammary gland rather than on the pituitary. That it also stimulated the pituitary, however, is quite possible.

In a second sow neither 0.2 g nor 0.3 g of acetylcholine was effective. A third sow was stimulated to let down her milk but only a small amount of milk was obtained by the machine. A fourth sow was stimulated by 0.2 g of acetylcholine to give a normal let-down time and a normal amount of milk. When 0.1 g was injected together with 0.5 g of prostigmine there followed the expected augmentation of the action. This result was typical of a number of experiments which indicated the anticholine esterase action of prostigmine.

The derivative of choline, carbaminoylcholine (carbachol) at 0.2 mg and 0.1 mg levels gave graded responses. The milk yield may have been reduced due to the fact that the sow sat down during the milking process.

Discussion. These experiments clearly indicate that acetylcholine will cause milk let-down in the sow. From the fact that the time interval from injection of acetylcholine to milk let-down is essentially the same as that observed after oxytocin injection, it was concluded that it acted directly upon the mammary gland. Further research will be required to determine whether acetylcholine also stimulates the pituitary to release the let-down hormone which Pickford's results would indicate.

Two points of difference in the action of acetylcholine as compared to oxytocin were observed. In some animals the amount of milk removed was low in comparison to the duration of let-down time. The second difference was the variability in the response of some sows.

Prostigmine, which inhibits the enzyme choline-esterase, had the expected augmenting activity upon acetylcholine. The fact that small amounts of the closely related derivative carbaminoylcholine produced a let-down effect in the intact sow but not in the isolated udder of the cow(9) cannot be explained.

Summary. Acetylcholine was shown to cause the let-down of milk in the intact lactating sow. The time interval from injection to milk let-down was believed to indicate direct action upon the mammary gland, although action on the pituitary may also occur. The let-down effect was augmented by the anticholine-esterase action of prostigmine. Small amounts of carbaminoylcholine, a derivative, had let-down action similar to acetylcholine.

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