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SUCKLING ANTIDIURESIS IN RABBITS

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The neurohormonal concept of 'let-down' of milk (Ely & Petersen, 1941) postulates that stimulation of the teats in suckling or milking evokes a reflex release of oxytocic hormone from the posterior pituitary gland. On reaching the mammary gland this hormone produces a contraction of the effector elements around the alveoli and fine ducts, which forces the milk down into the lactiferous sinuses where it is readily removed from the glands by the sucklings or the milking machine. Although much circumstantial evidence supports this view (Folley, 1947), the ability of the suckling stimulus to excite the neurohypophysis has not been demonstrated. The object of the present experiments was to see whether suckling resulted in an inhibition of a water diuresis which could be attributed to release of antidiuretic hormone from the neurohypophysis.

In this paper the term 'suckling' will be used only in reference to the activity of the young.

METHODS

Nine crossbred rabbits of body weight 2·5–3·7 kg. were studied over thirteen lactations. Diet consisted of compound pellets (diet 18, Associated London Flour Millers Ltd.) and fresh greenstuff when in season. Water was available at all times. Observations were made between the seventh and thirtieth days after parturition, and during this period the does were confined apart from their litters and were returned to the nests once daily for suckling. Provided the litters had access to water they maintained normal growth and vigour on this regime. Observation windows in the darkened nursing cages permitted note to be taken of the behaviour of the doe and her litter, and of the duration of actual suckling—a time determined by the doe herself. Litters were weighed immediately before and after suckling to obtain an estimate of the quantity of milk withdrawn.

Diureses were effected by a hydrating dose of 50 ml./kg. body weight tepid water by stomach tube followed 90 min. later by a second dose of 40 ml./kg. body weight. Thereafter the rabbits were confined in diuresis cages from which they were only removed for collection of urine by manual expression every 15 min. and for suckling or one of the control procedures. Any urine passed between collections was added to the appropriate sample. The urine volumes were measured and the chloride concentration estimated by the Whitehorn method.

Injections of posterior pituitary extracts were made in the marginal ear vein. The extracts were given in normal saline in a volume of 0·5–1·0 ml. Assays were performed by interposing an anti-

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diuretic response to suckling between two graded doses of posterior pituitary extract. As far as possible all three observations were made on the same day. The extracts used were the products of the Parke Davis Company: 'Pituitrin', undifferentiated posterior pituitary extract containing the oxytocic and vasopressor fractions in equal proportions; 'Pitressin', containing the purified vasopressor fraction with about 5% contamination with oxytocin, and 'Pitocin', containing the purified oxytocic fraction with about 5% contamination with vasopressin.

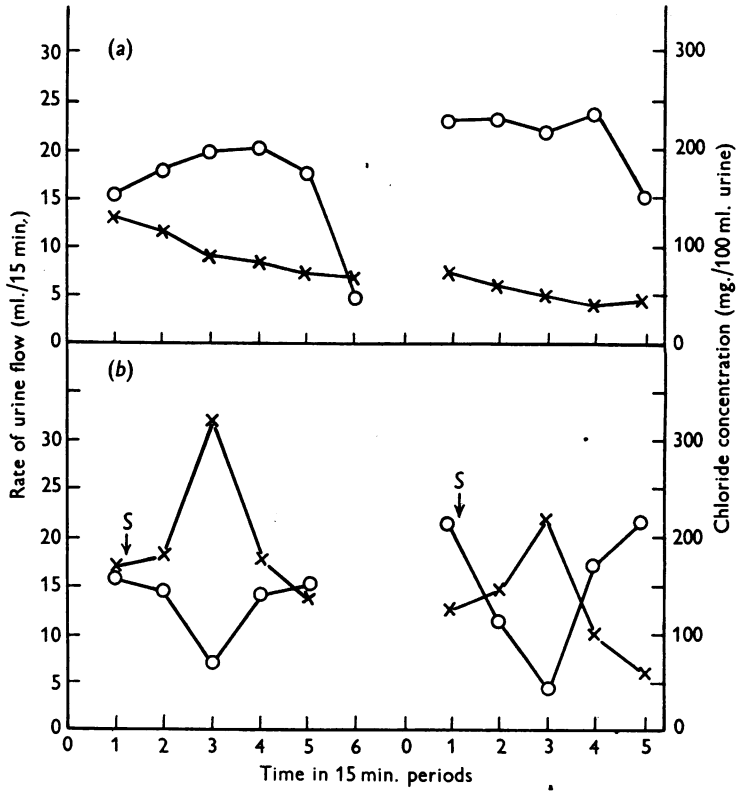

Fig. 1. (*a*) Water diuresis curves from rabbits nos. 3 and 6. (*b*) Antidiuretic responses from same rabbits. *S*=suckling; O——O =rate of urine flow; ×——× =chloride concentration in urine. These antidiuretic responses are typical of those observed to follow suckling.

RESULTS

Normal course of water diuresis

In most cases the urinary excretion rate rose to between 10 and 20 ml./15 min. half an hour after the second dose of water, and maintained a steady level for an hour or more before subsiding (Fig. 1*a*). No significant difference was observed in the diuretic responses of lactating and non-lactating rabbits. Incidental laboratory noise or presence of strangers did not affect the course of a water diuresis, and rehydration involving gentle restraint and passage of the stomach tube rarely caused observable inhibition of urine flow. The urinary

chloride typically followed the course indicated in Fig. 1*a*, namely, a gradual fall in concentration and absolute excretion rate.

Effect of suckling on water diuresis

In forty-nine out of fifty-five experiments in which suckling was allowed at the height of a water diuresis an antidiuretic effect was observed (see Fig. 1*b*). Maximum oliguria was present in most cases at the second urine collection following suckling, and these samples showed a chloride concentration of as much as four times the pre-suckling level. Chloruresis occurred in eight experiments in three rabbits. Five of the experiments in which no antidiuresis resulted were with one rabbit (no. 3) in its first lactation. Chloruresis was, nevertheless, observed in three of these cases.

The antidiuretic responses to suckling were similar to those following intravenous injections of small doses of posterior pituitary extract (see below). Assays in four animals showed the suckling antidiuretic response to be greater than that produced by 0·4 mU. but less than that produced by 1·0 mU. 'Pituitrin' (Fig. 2). These responses were typical of those obtained in the forty-nine positive experiments.

The magnitude of the antidiuretic response to suckling bore no obvious relation to the size of the litter (four to seven young), the duration of suckling ($2\frac{1}{4}$–7 min.), or the quantity of milk withdrawn (30–216 g.).

Experiments were carried out in an attempt to find the minimal requirements of an effective suckling stimulus. The first method adopted was to paint the nipples (rabbit no. 6) with collodion in order to prevent egress of milk without unduly interfering with mammary stimulation during nursing. On the first two occasions no milk escaped despite vigorous suckling attempts by the litter. No antidiuresis occurred. On the third occasion the collodion seals broke allowing the young to obtain 86 g. milk in $2\frac{3}{4}$ min. A typical antidiuretic response was obtained in this case. In the fourth test the doe frustrated attempts to suckle by her litter and no diminution of urine flow was observed. In the next lactation of the same animal an alternative method was tried, namely normal suckling for 1 min. only. It was hoped that appreciable milk loss would not have occurred in this short interval, although 'let-down' might occur. However, 43 g. milk was withdrawn by the litter, and suckling antidiuresis was again exhibited. Curtailing the suckling period to $\frac{1}{2}$ min. in two successive tests prevented milk loss and no antidiuresis ensued. Likewise, in three further experiments (rabbits nos. 1 and 3), normal suckling for 1 min. without escape of milk did not result in an antidiuresis, although longer suckling with milk loss produced antidiuretic responses later the same day. Finally, in three experiments (rabbits nos. 6 and 15) in which a single young rabbit was allowed to suckle for a period of 5–15 min., a transient diminution of urine flow unaccompanied

by increased chloride concentration resulted, although in one case 25 g. of milk was removed.

Effect of posterior pituitary extracts on water diuresis

The effect of posterior pituitary extracts on a water diuresis was examined in sixty-two experiments in eleven rabbits.

The responses to 'Pituitrin' and 'Pitressin' in doses up to 10 mU. were strictly comparable. Maximum inhibition of urine flow had usually occurred

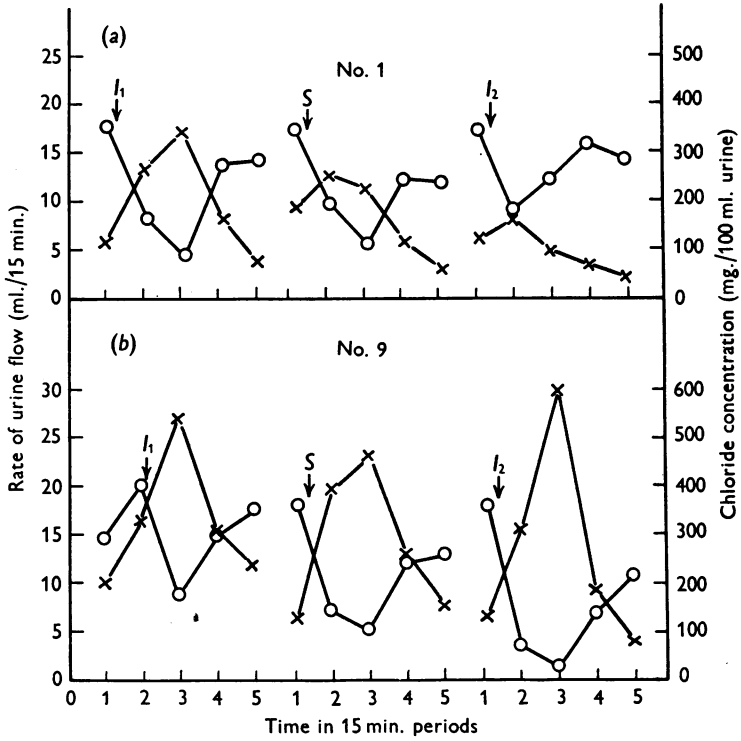

Fig. 2. Assay of suckling antidiuresis. (*a*) Rabbit no. 1: I_1, injection of 1·0 mU. 'Pituitrin'; S=suckling; I_2, injection of 0·4 mU. 'Pituitrin'. (*b*) Rabbit no. 9: I_1, injection of 0·4 mU. 'Pituitrin'; S=suckling; I_2, injection of 1·0 mU. 'Pituitrin'. O——O =rate of urine flow; ×——× =chloride concentration in urine.

by half an hour, and only with doses of 0·5 mU. or less was recovery of urine flow sometimes apparent at this time. With the larger doses recovery had begun by the third or fourth urine collection after injection (Fig. 2). The chloride concentration of the oliguric samples increased to as much as ten times the pre-injection level. Chloruresis was observed in twelve cases. One rabbit (no. 3) failed to give antidiuretic responses to 0·5–1·0 mU. 'Pituitrin' on four occasions in her first lactation, but this refractoriness was not present in two subsequent lactations.

Doses of 1·0–2·0 mU. 'Pitocin' were not found to produce an antidiuretic effect, although chloruresis was observed. 100 mU. 'Pitocin' was injected in one experiment and an antidiuretic response was obtained. This was probably attributable to the small impurity of 'Pitressin'.

Effect of emotional stimuli on water diuresis

The possibility that emotional factors might contribute to the production of the antidiuretic response to suckling was examined by subjecting diuretic rabbits to: (1) intravenous injection of 1·0–10·0 μg. adrenaline in 1·0 ml. normal saline; (2) the noise of a klaxon horn; (3) subcutaneous faradic shocks; (4) coitus; (5) confinement with the litter without the occurrence of suckling. With the exception of faradic shocks of sufficient duration and intensity to provoke resentment, these procedures failed to elicit changes in urine flow comparable with those following suckling or injection of 'Pituitrin'. Faradic shocks produced antidiuretic responses similar to those resulting from 0·5–8·0 mU. 'Pituitrin' and some of these closely resembled suckling antidiuretic responses in magnitude, time course and concomitant increase in urinary chloride concentration.

DISCUSSION

It is clear from the data presented in this paper that the stimulus of normal suckling results in an inhibition of a water diuresis in rabbits. These findings are in accord with recent work in the cow (Peeters & Coussens, 1950) in which up to 40% inhibition of urine flow lasting $\frac{1}{2}$–1 hr. followed milking in eleven out of fourteen experiments in two diuretic animals. Maximal bovine responses were matched by injection of 10 mU. posterior pituitary extract.

The essential components of an adequate suckling stimulus remain in doubt. It would seem unlikely that emotional factors are involved (Rydin & Verney, 1938), since all the emotional stimuli tested except subcutaneous faradism failed to give a comparable response. It is possible, however, that a specific type of emotional pattern is concerned. The removal of milk from the glands would appear to be a more important factor, since this occurred in all the cases in which suckling resulted in an antidiuretic response, while in the seven experiments in which escape of milk was prevented no antidiuretic effect was observed. This might suggest that the circulating blood is concentrated by the withdrawal of fluid from the mammary glands, and that in consequence an osmotic stimulus to antidiuretic hormone release might be implicated. Opposing this idea, however, is the fact that the milk withdrawn is actually present in the glands at the start of suckling (Gaines & Sanmann, 1927; Gowen & Tobey, 1927; Petersen, Palmer & Eckles, 1929). It is difficult to see how removal of this preformed milk could affect the composition of the circulating blood. It seems more likely that the distinctive pattern of sensory impulses from the

mammae occasioned by the outward passage of milk through the teats is necessary for stimulating release of the antidiuretic hormone.

The pituitary origin of a suckling antidiuresis is supported by the similarity in magnitude, time course and urinary chloride changes of antidiuretic responses to small doses of 'Pituitrin' or 'Pitressin'. Further, preliminary work (Cross & Harris, unpublished) has shown that suckling antidiuresis may be abolished by electrolytic lesions in the region of the supraoptico-hypophysial tract.

Most workers agree that it is the oxytocic factor that is chiefly concerned in the 'let-down' process (Ely & Petersen, 1941; Linzell, 1950; Petersen, 1942; Whittleston, 1950), although the vasopressor fraction has some activity (Cross & Harris, unpublished; Petersen, 1942; Turner & Cooper, 1941; Whittleston, 1950) and the possibility that unfractionated extracts may contain a still more powerful ejection principle is not ruled out (Turner & Cooper, 1941). The experimental results reported in this paper would suggest that the antidiuretic hormone is not responsible for the 'let-down' of milk, for a dose of 0·5–1·0 mU. 'Pitressin' has no ejection effect. In the rabbit 50–200 mU. posterior pituitary extract is probably necessary to produce 'let-down' (Cross & Harris, 1951). In the cow the corresponding figure is in the region of 1000 mU. (Peeters, 1950). That is to say, the ratio of antidiuretic effect to milk ejection effect resulting from the suckling stimulus is of the order of 1 : 100 in both species. This large ratio is further strong evidence against both phenomena being due to a release of antidiuretic hormone, but does not exclude the possibility that the antidiuresis is a side-effect of a large release of the oxytocic factor which causes the 'let-down'. This, however, is made unlikely by the occasional occurrence of 'let-down' without antidiuresis, and in any case there is no evidence for an antidiuretic action of oxytocin. On these grounds it seems likely that the antidiuretic and milk ejection responses are mediated by separate hormones released independently from the neurohypophysis. In conformity with this idea is the evidence suggesting that removal of milk from the mammary glands may be necessary for the appearance of an antidiuretic response to suckling, whereas 'let-down', which normally occurs within 30–60 sec. of the onset of nursing, precedes withdrawal of milk. It is possible, therefore, that while stimulation of the teats alone is sufficient to evoke release of the milk ejection hormone, passage of milk out of the glands is required to excite the additional release of antidiuretic hormone. As cows can be trained to 'let-down' in response to massage of the teats with a warm udder cloth before actual milking is commenced, it would be interesting to see if such a procedure resulted in an antidiuresis.

SUMMARY

1. In forty-nine out of fifty-five experiments an inhibition of urine flow was observed in diuretic rabbits following suckling.

2. The antidiuresis lasted ½–1 hr., maximal oliguria usually occurring half an hour after suckling.

3. Oliguric urine samples showed increased chloride concentration. Chloruresis occurred in eight of the fifty-five experiments.

4. The antidiuretic responses to suckling closely resembled those from intravenous injection of 'Pituitrin' or 'Pitressin' in doses of 0·4–1·0 mU.

5. The effect of various emotional stimuli on a water diuresis was investigated. Only subcutaneous faradic shocks resulted in antidiuretic responses resembling those from suckling or 'Pituitrin'.

6. In seven experiments suckling in the absence of withdrawal of milk did not evoke an antidiuretic response.

7. The significance of these findings in relation to the neurohormonal theory of 'let-down' of milk is briefly discussed. The results are consistent with the view that suckling stimulates the neurohypophysis.

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