MILK-EJECTION RESPONSES FOLLOWING ADMINISTRATION OF “TAP” STIMULI AND POSTERIOR PITUITARY EXTRACTS

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Since the original work of Ely and Petersen (1941), various views (Andersson, 1951; Cross, 1953, 1954, 1955; Cross and Harris, 1953; Whittlestone, 1952, 1954 a, b,c; see review: Petersen, 1948; Folley 1952; Folley and Malpress, 1948; Macaulay, 1950) have been accumulated as to the mechanism of milk “let-down” (or ejection, in the terminology since suggested by Cowie, Folley, Cross, Harris, Jacobsohn, and Richardson, 1951; see also Yokoyama, 1954). These evidences put forward the hypothesis that the mechanical stimulation of the teats of the lactating animal had induced the reflex release of oxytocic hormone from the posterior lobe of pituitary gland. Oxytocic hormone thus secreted, provoked a contraction of the myoepithelial cells of mammary gland and caused milk ejection response (Richardson, 1949; Linzell, 1952).

On the other hand, there were some observations which tended to indicate that the mechanical stimulation of the teat was also important for the secretion of prolactin (Selye, 1934; Hooker and Williams, 1940; Meites and Turner, 1942) and the maintenance of milk secretion (galactopoiesis: see review, Cowie and Folley, 1955; Folley and Malpress, 1948). Prolactin and the other galactopoietic hormone accepted to be originated from the anterior pituitary gland (Cowie and Folley, 1955; Folley, 1952; Folley and Malpress, 1948).

Involvement of the hypothalamus in the control of the release of these hormones, i.e. oxytocic and galactopoietic hormones, were well known but distinct relationship between the release of these hormones had not been studied.

It is of great interest to study the mechanism of the release of these hormones, which was caused by suckling stimulus, under the light of the hypothalamic control. But prior to the investigation of this mechanism, following experiments were performed in order to determine the type of milk ejection responses to posterior pituitary extracts and to “tap” stimuli, and the effect of adrenaline on these responses.

MATERIAL AND METHODS

Lactating rabbits of mixed strains, weighing 2.0~4.0 kg, were used and maintained with green food and wheat bran. The experiments were carried out during the period of 4~20 days of lactation.

The animals were anaesthetized with ethanol-urethane. Metal needle cannula was cannulated
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into a single teat duct of one mammary gland. Five to 7 ml of physiological saline at 37°C was introduced through the cannula for the convenience of “tap” stimuli. The cannula was connected to tambour and ejection response was recorded kymographically. These methods were similar to those of Cross and Harris (1953) and Cross (1954).

Whole posterior pituitary extracts (Pituitan; Dainippon Zoki Laboratory, Osaka, Japan.) and adrenaline (Bosmin; Daiichi Seiyaku Co. Ltd., Japan.) were injected into the marginal ear vein. Injection of whole posterior pituitary extracts was performed at 10 minutes intervals, and the doses administered were ranged from 1.25 to 200 mU. in 0.1 ml of vehicle.

“Tap” stimuli according to Cross (1954) was applied on the cannulated sector which was sufficiently distended to stand out from neighbouring glandular tissue.

The time of injection-ejection (latent period), and of duration was recorded. In some animals, the front and rear glands were cannulated during the course of the same experiments, in order to determine the differences in the time mentioned above.

RESULTS AND DISCUSSION

Thirty observations were made in eight lactating rabbits. Administration of “Pituitan” resulted in an ejection response after a latent period of about 10~30 sec. All responses were characterized by steep rising phase and a much slower fall as seen in Figures. 1~6 and these phenomena were easily observed by the movement of the fluid in the cannula, hence the onset of response and the duration could be measured exactly.

In order to determine whether the differences in the type of response due to the location of the cannulated that exist or not, front and rear teats were cannulated in the same animal. With doses of 25 mU. or less slight differences were observed in latent and duration period which were attributable to the teat locus, but in larger doses comparatively uniform responses occurred. (Table 1)

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<tr>
<th>Dose (mU.)</th>
<th>Time from injection to ejection (sec.)</th>
<th>Time from injection to peak of response (sec.)</th>
<th>Duration of ejection (sec.)</th>
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* Third and fifth teats on right side.

The threshold dose of “Pituitan” was about 5 mU., although in one case the response was obtained with only 1.25 mU. of “Pituitan”.

In many cases the first injection of the “Pituitan” gave a larger amplitude than subsequent equivalent doses. This observation was similar to that of Whittlestone (1954 b), but distinct explanation about the phenomenon could not be given from the present investigation.
The pauses in the flow of milk which imparted a notched appearance to the kymograph tracings occurred with the dose less than 100 mU. of “Pituitan” (Fig. 1), these notched appearances were also obtained by Cross and Harris (1953) in the doses less than 50 mU. of “Pituitrin”. It might be likely to say that these pauses were caused by the prolongation of the time required for injection as Whittlestone (1954a) suggested. The peaks of responses were obtained in 5~30 seconds after the onset of responses. The responses lasted for 35~300 seconds and any significant relationship between dose used and the time of latent or duration was not observed. Duration of response to the same dosage level was varied markedly not only from animal to animal but for each animal. Latent time, however, was comparatively uniform throughout the experiments observed. The interrelationships between these times were similar to those obtained by Cross and Harris (1953).

![Fig. 1. Kymograph records of ejection response to 50, 100, 100 mU. posterior pituitary extracts (PPE.) showing notched appearance due to the pauses in the flow of milk.](image)

![Fig. 2. Kymograph records of ejection response to repeated injection of PPE. at 10 min. intervals. Diminution of the volume of the milk ejected was postulated.](image)

The magnitude of response in kymograph seemed a little more consistant than the duration of ejection, but the data so far were not adequate for a comparison of the two as a basis for an evaluation of oxytocic activity. Repeated injection of “Pituitan” at the 10 min. intervals resulted in diminution of the volume of milk ejected which was postulated by the decrease of the magnitude in kymograph records, and occasionally the response was abolished (Fig. 2).

Figure 3 set out the response to the tap stimuli. After a latent period of about 1~2 seconds steep rise of the fluid in the cannula occurred and reached peak after 4~12 seconds. Total duration of the response was 20~40 seconds with
a few exception (110 and 60 seconds). Despite the difficulties of application of tap stimuli in constant strength, the responses were quite uniform in latency and duration in most cases, but there were also individual variations in magnitude of the responses. The records of these responses were quite alike to those obtained by the administration of 10~25 mU. “Pituitan” except for their shorter latency. When tap stimuli were applied successively at the intervals of 10 seconds, magnitude of responses decreased and the responses failed to occur finally according to fatigue of myoepithelial cells of mammary gland (Fig. 3). The short latency and the fatigue phenomenon reported here, together with the fact that these responses were not affected by adrenaline administered intravenously described below, were believed to confirm the observation described by Cross (1954, 1955,) according to which the tap stimuli directly caused the mechanical contraction of the myoepithelial cells of mammary gland and hormones of posterior lobe did not intervene.

![Figure 3](image)

Fig. 3. Kymograph records of the effect of adrenaline on the ejection response to tap stimuli and to PPE. T×6: Tap stimuli were applied 6 times successively at 10 sec. intervals.

The effect of adrenaline administration on the ejection response to whole posterior pituitary extracts and to tap stimuli was shown in Figures 4 and 5. When 5 μg adrenaline and 50 mU. of “Pituitan” administered simultaneously, the response to “Pituitan” was completely abolished 2 out of 3 cases. In the remaining, however, 10 μg adrenaline failed to suppress the response to 50 mU. “Pituitan” injected simultaneously. These responses showed small amplitudes in kymograph. When 50 mU. of “Pitaitan” was injected 130 and 140 seconds after

![Figure 4](image)

Fig. 4. 1: Simultaneous administration of 5 μg adrenaline and PPE. were followed by administration of 50 mU. PPE. (Ox), tap (T), and 50 mU. PPE. (Ox) at 60 sec. intervals. 2: Administration of 50 mU. PPE. evoked normal ejection response. Five μg adrenaline and 50 mU. PPE. were administered simultaneously 5 min. after the first injection. Fairly normal response was obtained. Fifty mU. and tap stimuli were applied alternately. Intervals between treatments were 60~90 sec.
the first administration, responses were observed with prolonged latency (49,90 sec.) and with diminution of milk flow rate. Six minutes and 20 seconds after, the response to 50 mU. "Pituitan" seemed to have equal magnitude and latency to those of the response without adrenaline priming.

After the response to tap stimuli and 50 mU. of "Pituitan" was observed, administration of 10 μg adrenaline was followed by tap stimuli and the injection of "Pituitan" at the intervals of 30~60 sec. The response to the "Pituitan" was prevented up to 4 min. after the adrenaline administration, while the response to tap applied within these period appeared normally (Figs. 5 and 6). Oxytocin provoked response barely 5~6 min. after the injection of adrenaline, but complete recovery of magnitude was not attained yet.

Numerous investigators (Peeters, Coussens and Sierens, 1949; Cross, 1953, 1954, 1955; Linzell, 1954, 1955) reported that administered adrenaline did not act directly upon the effector of the mammary gland, myoepithelial cells, but caused vasoconstriction which prevented the arrival of the effective substance to mammary gland and, in consequence, suppressed the ejection response to the whole posterior pituitary extracts. While the response to tap stimuli could not be af-

![Fig. 5. Ten μg. adrenaline administered 60 sec. before injection of 50 mU. PPE. Response to 50 mU. PPE. was obtained 250 sec. after the adrenaline administration.](image)

![Fig. 6. 1: Normal response to 50 mU. PPE. 2: T1: Normal response to 50 mU. PPE. Ten μg. adrenaline was administered (A) 30 sec. before tap stimuli (T3). Response to 50 mU. PPE. (OX1) injected 3 min. after the first adrenaline treatment was not obtained. Tap stimuli (T3) were applied 30 sec. after OX1. Response to PPE. (OX3) was obtained 330 sec. after the first injection (A).](image)
Milk-ejection responses to whole posterior pituitary extracts, to tap stimuli and the effects of adrenaline on these responses were studied in anaesthetized lactating rabbits.

Intravenous injection of the whole posterior pituitary extracts caused the ejection response after latent period of about 10–30 sec. Total duration of the response was 35–300 sec.

Latency, duration and magnitude of the responses varied markedly not only from animal to animal but for each animal.

One to 2 sec. after the application of tap stimuli, ejection responses occurred and lasted for about 30 sec. Type of these responses was quite alike to that evoked by 10–25 mU. of whole posterior pituitary extracts.

Five µg of adrenaline suppressed the ejection response to whole posterior pituitary extracts, when administered before the injection of oxytocic substances. But the responses to tap stimuli were not affected by adrenaline administration.

These observations confirmed the findings obtained by Cross (1953, 1954, 1955), and Cross and Harris (1953).

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REFERENCES