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THE MILK EJECTION REFLEX IN THE PIG

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SUMMARY

1. The milk ejection reflex in response to suckling was studied in conscious sows by continuous recording of intramammary pressure, radioimmunoassay of plasma concentrations of neurohypophysial hormones, and observation of the behaviour of the sows and piglets.

2. A regular pattern of nursing, suckling and milk ejection was observed. The mean duration of the suckling period was 6·3 min. Over 144 suckling periods, 113 milk ejections were recorded. Each milk ejection was characterized by a sudden rise in intramammary pressure reaching 20–49 mmHg, and lasting 8–41 sec. Milk ejections occurred only once per suckling period, at a mean interval of 44·3 min.

3. Each milk ejection occurred with a mean latency of 2·4 min from the onset of a period of initial massage of the udders by the piglets, and was coincident with a period of quiet suckling when the piglets were consuming milk. The onset of nursing was signalled by the sows grunting in a rhythmic manner. In most cases, the frequency of grunts, at first low, increased suddenly 23 sec before milk ejection.

4. During eighteen suckling periods leading to milk ejection, neurohypophysial hormone assays performed on serial blood samples showed an increase in plasma concentration of oxytocin up to 30 sec before milk ejection. The concentration of lysine–vasopressin did not rise above basal levels.

5. In 21·4% of the suckling periods, no rise in intramammary pressure was observed. In these 'incomplete sucklings', the sow usually failed to grunt rapidly, and the piglets obtained no milk. For three of these periods, hormone assay showed no increase in oxytocin or vasopressin concentrations in blood.

6. Oxytocin given intravenously produced variations in intramammary pressure which depended on the dose and the rate of injection. Rapid injections of 25–50 m-u. oxytocin, caused milk ejections similar to those induced by suckling. When oxytocin was administered at different rates, the faster the injection, the shorter the latency and the higher the amplitude of the response. Plasma concentrations of oxytocin after injection of 25 m-u. were similar to those observed during reflex milk ejection.

7. Trains of electrical pulses were applied to the posterior pituitary of four anaesthetized sows. At frequencies of stimulation above 10 Hz, a rise in intramammary

The experiments were carried out at the Instutit für Tierzucht und Tierverhalten, Mariensee.

pressure and an increase in plasma oxytocin and vasopressin concentrations were observed. At frequencies of stimulation of 30–50 Hz, the response of the mammary gland and the time course of the variations in oxytocin plasma concentrations were similar to those observed during natural reflex milk ejection.

8. It is concluded that reflex milk ejections during suckling in the pig are caused by the intermittent and spurt-like release of about 25 m-u. oxytocin, without concomitant vasopressin release. It is postulated that the release of oxytocin is probably precipitated by a brief and massive activation of oxytocin-secreting neurones in the hypothalamus. Central mechanisms controlling the intermittent release of oxytocin are discussed.

INTRODUCTION

The milk ejection reflex in response to suckling has been studied and reviewed extensively (Bisset, 1974; Cross & Wakerley, 1977; Poulain & Wakerley, 1982). Studies performed in several species, in particular the rat, have permitted the essential sequence of events characterizing the reflex to be established: upon stimulation of the mammary gland, oxytocin-secreting neurones in the hypothalamus show by an intermittent and burst-like electrical activation, which in turn causes the release of oxytocin; a rise in the intramammary pressure and ejection of milk then follow. The general applicability of this model can be questioned in view of the wide variety of species differences. For example, in some species, such as the rat, the young suckle for most of the day (Lincoln, Hill & Wakerley, 1973); in others, such as the rabbit, they remain detached for most of the day, and there may be only one milk ejection a day (Cross & Harris, 1952; Zarrow, Denenberg & Anderson, 1965). In some animals, single offspring are nursed; in others, whole litters, and in marsupials, young of different ages can be nursed simultaneously. In addition, the mammary glands themselves differ between species, depending on whether or not cisternae exist to store milk. It might be expected therefore that all these species differences are reflected in the physiological organization of the milk ejection reflex.

The pig is one species in which the female possesses numerous mammary glands without cisternae, and gives birth to a large litter. A number of aspects of the milk ejection reflex in the pig have been described, such as the behaviour of the mother and her piglets (Gill & Thomson, 1956; Fraser, 1973, 1975*a*, *b*; Whittemore & Fraser, 1974; Whatson & Bertram, 1980), the effect of oxytocin or vasopressin on the mammary gland (Whittlestone, 1952, 1953, 1954*a–c*) or the plasma concentration of oxytocin during suckling (Folley & Knaggs, 1966; Forsling, Taverne, Parvizi, Elsaesser, Smidt & Ellendorff (1979). The present study was designed to give a more comprehensive picture of the milk ejection reflex in the sow. To this end, a technique of continuous recording of the intramammary pressure in the non-anaesthetized animal was devized, so that naturally occurring reflex milk ejections could be detected and related to the release of posterior pituitary hormones and to the behaviour of the sow and piglets. In addition, to understand further the mechanisms underlying reflex milk ejection, the effects of I.V. injections of oxytocin and of electrical stimulation of the posterior pituitary were investigated. Some aspects of

these experiments have been reported in short communications (Bruhn, Ellendorff, Forsling & Poulain, 1981; Ellendorff, Forsling & Poulain, 1981; Poulain, Rodriguez & Ellendorff, 1981).

METHODS

Fifteen lactating German Landrace sows, 150–200 kg body weight, were used during their first lactation, 1–4 weeks after parturition. They were kept under a 12 hr light/12 hr dark schedule, in air-conditioned quarters at an ambient temperature of 21 °±2 °C and a relative humidity of 50–70 %. They received a standard pig chow and water *ad libitum* and were caged in standard commercial farrowing crates on straw. Up to eight sows with or without litters (adjusted to 7 to eight piglets) were kept simultaneously in the experimental unit, and no special precautions were taken against noise. However, each animal was accustomed to the presence of the experimenter and to experimental handling. Within 48 hr of parturition, a silastic catheter used for blood sampling (dead space: 1·5–2 ml.) was implanted under anaesthesia into the external jugular vein without interruption of the blood flow (Ellendorff, Parvizi, Elsaesser & Smidt, 1977). In some cases, a double catheter comprising two catheters mounted side by side was implanted, so that one cannula, used for injection, was placed 10–15 cm further down from the cannula used for blood sampling.

Intramammary pressure recordings

A polyvinyl catheter (Porter PP 50) was introduced the evening before the experiment into one of the two ducts of a nipple, usually of one of the first three anterior teats. The catheterization was performed under brief (5–10 min) anaesthesia (sodium methohexital, Brevimytal, Eli Lilly). The technique involved no dissection. After cleaning the nipple with 70 % ethanol, a sterile catheter was inserted for about 3 cm through the opening of the duct. It was then fixed to the nipple with adhesive tape, and further secured on the skin surface as far as the dorsal mid line and held in place by a belt made of elastic bandage to protect it from the piglets, In some cases, the two ducts of a teat (each one draining a separate mammary gland), were catheterized. In other cases, one duct of an anterior teat and one of a posterior teat were catheterized. As soon as the sow had recovered from anaesthesia, the piglets were returned to their mother.

On the following morning (8–9 a.m.) the catheters were connected to a pressure transducer (Statham P-23) located outside the farrowing crate, and a continuous recording of the intramammary pressure was obtained on a polygraph (Beckman R411). After reference calibration with a mercury manometer, single I.V. injections of oxytocin (10–50) m-u. dissolved in 1 ml. isotonic saline) were given and rapidly flushed into the circulation with 3 ml. of saline, in order to test the sensitivity of the mammary gland. Subsequent I.V. injections of oxytocin were systematically performed during the recording session and at the end of the experiment. At the end of the day, the catheter was removed from the mammary gland, and the animal was givn an I.V. injection of antibiotics (Reverin, Hoechst, 110 mg) to prevent the risk of infection resulting from the cannulation.

Behavioural observations

During each experiment, the behaviour of the sow and her piglets was observed and carefully noted, either directly from the experimental room, or by video recording. In addition, a microphone was installed in front of the farrowing crate to record the sow's vocalization on a cassette recorder. Recording was limited to the nursing periods, and the vocalization was analysed in terms of the number of grunts per unit of time.

Blood collection

Blood sampling in the conscious animal was performed via an 80 cm Silastic catheter connected to the jugular catheter. In order to detect possible transient release of hormone ten to fifteen successive blood samples of 8–10 ml. each were collected, the syringes being exchanged at 15 sec intervals. The samples were collected on ice, immediately centrifuged (2000 rev/min at 4 °C, 20 min), and the plasma stored at −20 °C for subsequent hormone analysis.

Intravenous injections of oxytocin

The response of the mammary gland to I.V. injections of various doses of oxytocin was investigated in the conscious animal and also more systematically under anaesthesia to ensure steady conditions of intramammary pressure recording. Anaesthesia was induced with an initial dose of 5 ml. methomidate HCl (Hypnodil, Janssen, Düsseldorf), and maintained for no more than 3 hr by further 2 ml. injections whenever required.

Electrical stimulation of the posterior pituitary

The four sows of this study were initially deeply anaesthetized with azaperone methomidate HCl (Stresnil and Hypnodil, Janssen, Düsseldorf), then provided with a tracheal catheter which was connected to a closed anaesthesia system (Ellendorff *et al.* 1977). The animal was ventilated with a mixture of nitrous oxyde and oxygen in a ratio of 4:1, at a rate that allowed spontaneous respiration to be maintained. Anaesthesia was further supported with Hypnodil injected at a rate of 2 ml. every 5–10 min. Under additional local anaesthesia, a large craniotomy was performed, and the posterior pituitary exposed by partial aspiration of frontal and medial brain structures up to the mesencephalon so that the animal could continue to breathe spontaneously. An electrode holder was then fixed to the occipital bone with screws and dental cement, and a bipolar concentric stimulation electrode (o.d., 0·2 mm; tip separation, 0·5 mm; Rhodes Medical Instruments SNEX-100) was lowered into the posterior pituitary under visual control. Electrical stimulation delivered from a Grass S88 stimulator and two isolation units (Grass PSIU 6), consisted of biphasic pulses (width, 1 msec; intensity, 1 mA) applied in trains of various frequencies and durations. In these experiments, intramammary pressure was recorded continuously, and for some electrical stimulation periods blood samples were collected in a similar manner to that described for natural reflex milk ejections. These samples served for the analysis of plasma concentrations of oxytocin and vasopressin.

Hormone analysis

Oxytocin analysis was carried out as described previously for the pig (Forsling *et al.* 1979), based on the method of Chard & Forsling (1976). The minimum concentration detectable was dependent on the exact volume extracted but was approximately 0·25 μu./ml. plasma. The recovery for oxytocin was $69 \pm 6{\cdot}5\%$ (S.E. of mean). The interassay variation was 14·9% and the intrassay variation was 9·9%. Data were corrected for recovery.

Lysine–vasopressin was determined (as described by Forsling, Aziz, Miller, Davies & Donovan, 1980) by radioimmunoassay using plasma extracted with Fuller's earth (Skowsky, Rosenbloom & Fisher, 1974). Synthetic lysine–vasopressin (Ferring, Malmö) was used as standard and for preparation of labelled peptide by the lactoperoxidase method.

RESULTS

(1) *The milk-ejection reflex in response to suckling in the non-anaesthetized sow*

The milk-ejection reflex during suckling was studied in twelve sows during seventeen experimental sessions lasting 6–12 hr. As described by other authors (Gill & Thomson, 1956; Fraser, 1973, 1975*a*, *b*, 1977; Whatson & Bertram, 1980), we observed a complex behavioural interaction between the sow and piglet. For the sake of clarity, we shall first describe the most common pattern of behaviour of the mother and her young, then relate this pattern to the changes in intramammary pressure and to variations in plasma concentration of neurohypophysial hormones that occurred at that time. In a later section, we shall consider a number of departures from the usual pattern.

(*a*) *Nursing and suckling behaviour.* In nine animals, during twelve experimental sessions, we observed a stereotyped pattern during most of the nursing periods, which recurred at fairly regular intervals and culminated in milk let-down. Nursing was at

times initiated by the mother calling her litter, in other instances by the piglets which signalled their wish to suckle by squealing in the vicinity of the mother's head or by stimulating the teats. At this point the sow took up a position lying on her side, which could be different from one nursing period to another, and presented her teats to the piglets. Eventually the whole litter vigorously butted the mammary glands, with or without attaching themselves to the nipples, often jostling for position and squealing. During this period the sow's grunts became rhythmic. The frequency of grunts was

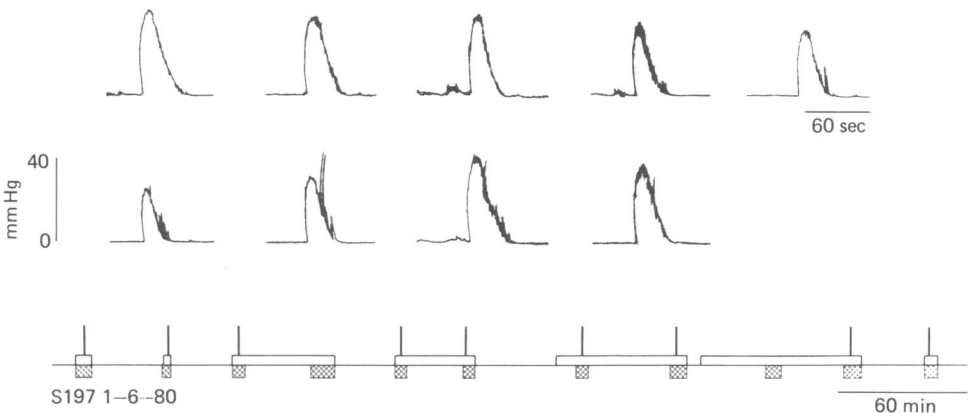

Fig. 1. Variation of the intramammary pressure during nine successive milk ejections. The record below the pressure recordings indicates the relation between milk ejections and the behaviour of the mother and the piglets. Each milk ejection is signalled by a vertical bar. The open horizontal block indicates periods when the mother was lying on her side. The hatched blocks indicate periods when the piglets were suckling. Note that two suckling periods failed to trigger milk ejection ('incomplete suckling').

at first rather low, then in most cases became quite rapid. When the vocalizations were sufficiently loud to be recorded and analysed, the maximum rate of grunting was found to be $9{\cdot}3 \pm 0{\cdot}3$ grunts/5 sec ($n = 70$), while the rate during the slow grunting period reached $3{\cdot}8 \pm 0{\cdot}2$ ($n = 5$); $P < 0{\cdot}05$, Student's t test). While rapid grunting was still in progress the whole litter became suddenly quiet, each piglet sucking a nipple. This period, which lasted 7–38 sec, was followed by another phase of active stimulation with butting and nosing at the udders. This latter period lasted from less than one minute to several minutes, until the piglets detached themselves from the mother, fell asleep or engaged in other activities, or until the mother put an end to nursing by getting up or turning to another position.

(*b*) *Intramammary pressure recordings.* 113 milk ejections were recorded in the nine animals (mean: $9{\cdot}4 \pm 0{\cdot}7$ S.E. of mean per session). Each milk ejection was characterized by a sudden and sharp increase in intramammary pressure, rapidly reaching 20–40 mmHg, then gradually returned to base line levels (Figs. 1, 2 and 4). The exact duration of the milk ejection was not always easy to determine because of numerous variations. Where it could be evaluated, the duration from the onset of the rise to the return to base line levels was 21 ± 1 sec (mean $\pm$ S.E. of mean range

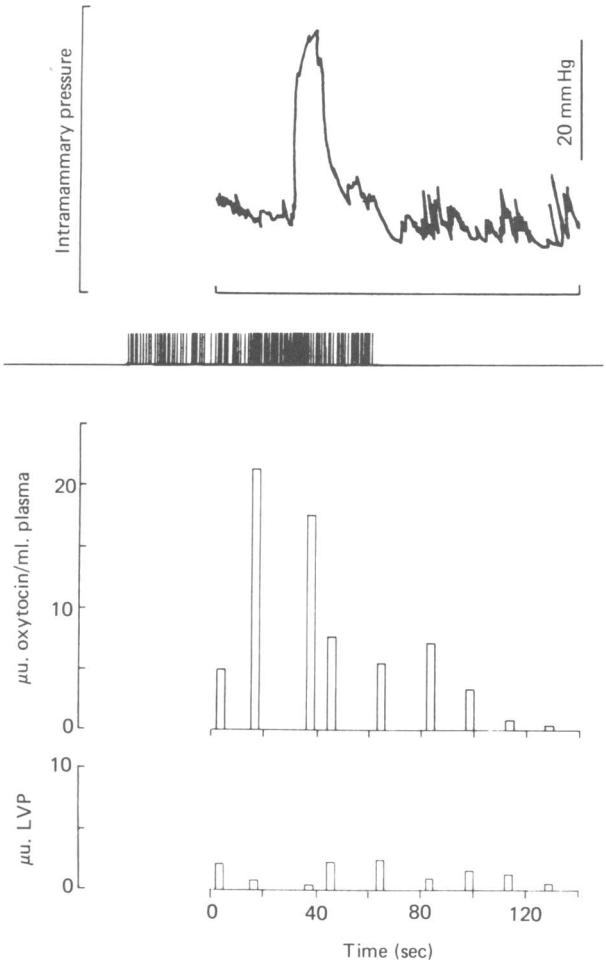

Fig. 2. Relation of milk ejection to grunting and neurohypophysial hormone release. Top: variation of the intramammary pressure during milk ejection. Below: maternal vocalization: each vertical bar represents a grunt. Note the increasing frequency of grunts before milk ejection. Bottom: plasma concentration of oxytocin and lysine–vasopressin (*LVP*). Note the rise in oxytocin concentration before milk ejection, while vasopressin concentration remained unchanged.

8–41 sec, $n = 98$). In each of the animals, the changes in intramammary pressure were highly reproducible from one milk ejection to another (Fig. 1).

Taking the time of onset of the rise in intramammary pressure as a reference point, the behavioural events during nursing and suckling also showed a high degree of reproducibility. It was only when most of the litter simultaneously stimulated the udders that a milk ejection occurred. The initial phase of suckling, consisting of butting the udders, lasted $2{\cdot}4 \pm 0{\cdot}1$ min (mean $\pm$ S.E. of mean range: 1–6 min,

$n = 113$). Fast grunting (Figs. 2 and 4) preceded milk ejection by $23 \pm 0{\cdot}8$ sec ($n = 57$). In all the milk ejections observed, the period of quiet suckling, with the young consuming milk, was always synchronous with the rise in intramammary pressure. The later period of active stimulation of the udders was contemporaneous with the return to base-line levels of the intramammary pressure. The whole sequence of the suckling behaviour leading to milk ejection lasted $6{\cdot}3 \pm 0{\cdot}3$ min (mean $\pm$ S.E. of mean range: 2–16 min, ($n = 113$) and recurred at a mean intervals of $44{\cdot}3 \pm 1{\cdot}4$ min (range 21–92 min, $n = 101$), calculated from milk ejection to milk ejection. We never observed a spontaneous milk ejection in the absence of suckling (Fig. 1).

(*c*) *Oxytocin and lysine–vasopressin levels in relation to milk ejection.* The plasma concentrations of neurohypophysial hormones were determined for eighteen suckling periods in five animals. Sequential blood sampling, comprising ten to fifteen

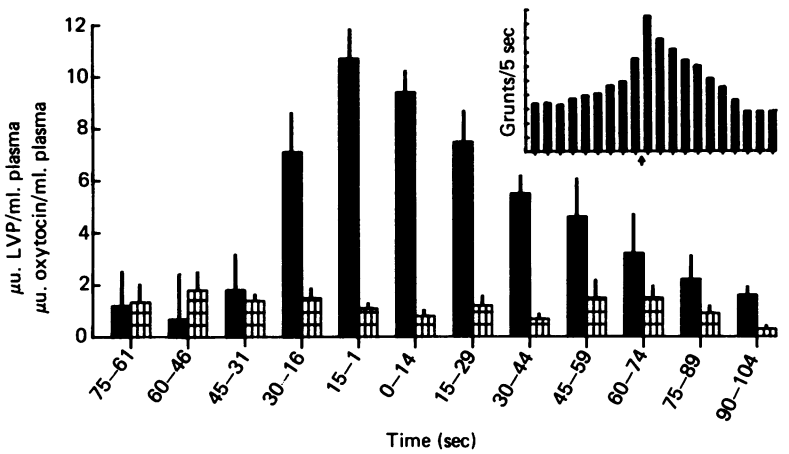

Fig. 3. Plasma oxytocin and lysine–vasopressin (LVP) (mean $\pm$ S.E. of mean) concentrations from 75 sec before to 104 sec after the onset of rise in intramammary pressure (time 0). Full bars represent oxytocin, hatched bars vasopressin. The grunting pattern is indicated in the insert, taken over 5 sec intervals; arrow corresponds to time zero in major histogram.

successive samples taken over a 15 sec period each, was started during the period of slow grunting when the young were suckling, and was continued at least until the intramammary pressure returned to base-line level (Figs. 2 and 4). As shown in Fig. 3, plasma concentrations of oxytocin increased and reached a peak before milk ejection, then gradually declined down to base-line values, taking much longer than the intramammary pressure itself took to return to base-line. For each suckling period, the hormone concentration in plasma rose from a low value (range: $< 0{\cdot}2$–$6{\cdot}0$ μu./ml.) to a single peak (range: $3{\cdot}9$–$21{\cdot}2$ μu./ml.) detected in samples taken 30–16 sec before the rise in intramammary pressure ($n = 8$), or 15–1 sec before ($n = 5$) or at the time of milk ejection ($n = 5$). In contrast to oxytocin, lysine–vasopressin levels showed no significant change during any part of suckling or milk ejection (Figs. 2 and 3).

(*d*) *Other patterns of milk ejection.* As mentioned earlier, the sow and her piglets showed a highly repeatable pattern of nursing, suckling and milk ejection, that could clearly be distinguished from other forms of behaviour. In some cases, however, the piglets were particularly restless, and made frequent attempts to suckle. These attempts differed from the actual periods of suckling in that the piglets did not synchronize their efforts and the mother did not grunt. In these instances we never observed a milk ejection.

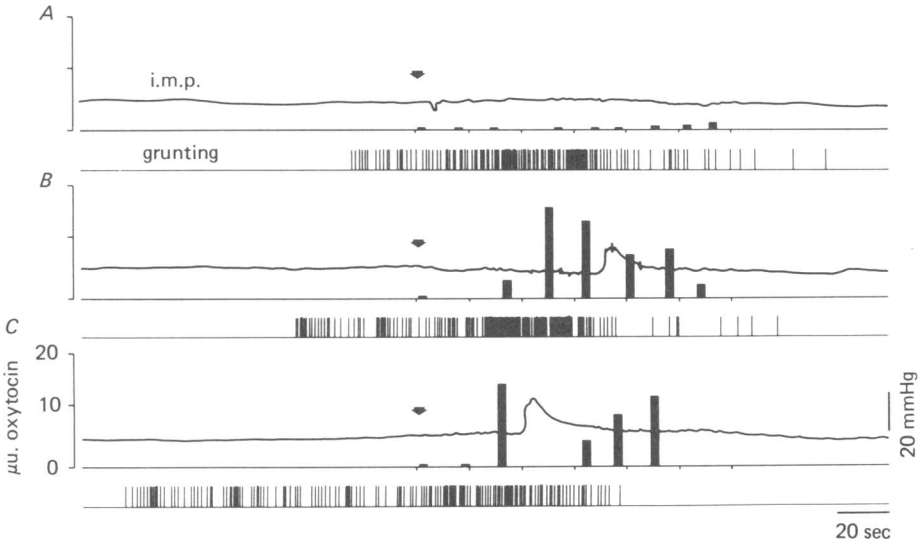

Fig. 4. Relation of milk ejection to grunting and plasma oxytocin concentration during three periods of suckling in the same sow. The intramammary pressure (i.m.p.) is plotted together with the concentration of oxytocin in the blood (vertical blocks) and the grunts, signalled by vertical bars. The arrows indicate the beginning of blood sampling. *A*, example of an 'incomplete suckling' during which no milk ejection occurred. Although the mother nursed and grunted in a normal way, there was no increase either in intramammary pressure, or in plasma concentration of oxytocin. *B*, *C*, two successful sucklings which led to milk ejection, signalled by a rise in intramammary pressure. Oxytocin levels increased before milk ejection. In *B*, there was a clear acceleration in grunting, which was not apparent in *C*.

One of the most notable deviations from the usual pattern was 'incomplete suckling', which has been defined as apparetly normal suckling which does not lead to milk ejection (Barber, Braude & Mitchell, 1955; Whittemore & Fraser, 1974). Identification of 'incomplete suckling' has so far been based on behavioural observations or by attempts to milk the udder manually. In our experiments, intramammary pressure recordings permitted us to identify unambiguously a number of 'incomplete sucklings' (Figs. 1 and 4). In the nine sows which showed a fairly regular pattern of milk ejection, no rise in intramammary pressure was observed in thirty-one (21·5 %) out of 144 recordings performed when all behavioural components of nursing and suckling were present. During these periods, slow grunting was

present. The rapid grunting period was usually, but not always absent (Fig. 4*A*). Oxytocin values were determined during three of these periods and never exceeded the base-line levels found in the periods between milk ejections (Fig. 4). Plasma vasopressin concentrations remained at base-line levels ($< 1{\cdot}4$ μu./ml.). 'Incomplete suckling' seemed to occur especially when the piglets attempted to suckle too early after a milk ejection. In sixteen cases, when an 'incomplete suckling' was preceded by two successful sucklings, the interval between the two successful sucklings was $40{\cdot}6 \pm 3{\cdot}1$ min, while the latency from the last milk ejection to the incomplete suckling was $23{\cdot}0 \pm 2{\cdot}2$ min (mean $\pm$ S.E. of mean, $n = 16$, $P < 0{\cdot}001$, Student's paired t test).

A high proportion of 'incomplete suckling' was particularly evident when the sow seemed to be disturbed. This occurred in five experiments involving four animals, one of which had shown a regular pattern of nursing in another session. For example, in two cases, the piglets were removed for 2 hr before the recording session in the hope of facilitating suckling by a hungry litter. The mothers became restless and after the piglets were returned, despite numerous suckling attempts, no milk ejection occurred until very late in the evening. In another case, the sow had its mammary gland catheterized without anaesthesia, which can be done without restraint while the animal is lying quietly. However, the sow became disturbed as the catheter was fixed with the elastic bandage aroung the body. Seven suckling attempts followed, occurring with a mean interval of 61 min. Injections of oxytocin given I.V. between the second and third incomplete suckling showed that the mammary gland was capable of responding and that the pressure recording system was fully functional. In these five experiments only ten reflex milk ejections occurred, that were comparable to those observed under usual conditions.

(2) *Effect of I.V. injections of neurohypophysial hormones*

In non-anaesthetized sows that had been observed during suckling, exogenous oxytocin was given systematically by rapid injection through the jugular catheter in order to evaluate the amount of oxytocin released during reflex milk ejections. The minimum dose to which the mammary gland reacted was 10 m-u., but in most animals 25 m-u. was necessary to obtain a change in intramammary pressure similar to that observed at natural milk ejection (Fig. 5).

In seven animals, a more systematic study was performed after anaesthesia to avoid recording artifacts resulting from the movements of the sow and to allow comparison between successive responses. As shown in Fig. 5, the latency, the shape, the amplitude and the duration of the response depended both on the amount and on the rate of oxytocin administration. When injected rapidly, in less than 2 sec, the amplitude of the rise in intramammary pressure depended on the dose of hormone. As in the non-anaesthetized animal, the threshold dose was 10–25 m-u. No reaction was observed when 5 m-u. were injected. With 10 m-u., four animals responded (fourteen out of sixteen injections) in a manner similar to that observed during reflex milk ejections. One animal gave a small response, the two others did not react. With 25 m-u. all animals but one responded by a rise in pressure of 30–40 mmHg. With very high doses of oxytocin (> 1 u.), multiple rises in intramammary pressure were observed. The latency of the response also depended on the dose injected. At doses of 10–50 m-u., the latency was $15{\cdot}5 \pm 1{\cdot}0$ sec ($n = 26$), which was significantly higher

than the latency of 8·9 ± 1·7 sec($n = 23$) for doses > 100 m-u. ($P < 0·001$, Mann–Whitney U-test). The latency also depended on the position of the mammary gland. In three animals, one front and one rear teat, separated by about 80 cm, were cannulated. For the rostral gland, the latency of the milk ejection was significantly shorter than for the caudal gland (12·4 ± 0·4 *versus* 16·2 ± 0·8, $n = 17$, $P < 0·001$, Student's paired t test). However, when the two galactophorous ducts of one nipple, which drain two different mammary glands, were cannulated, the intramammary

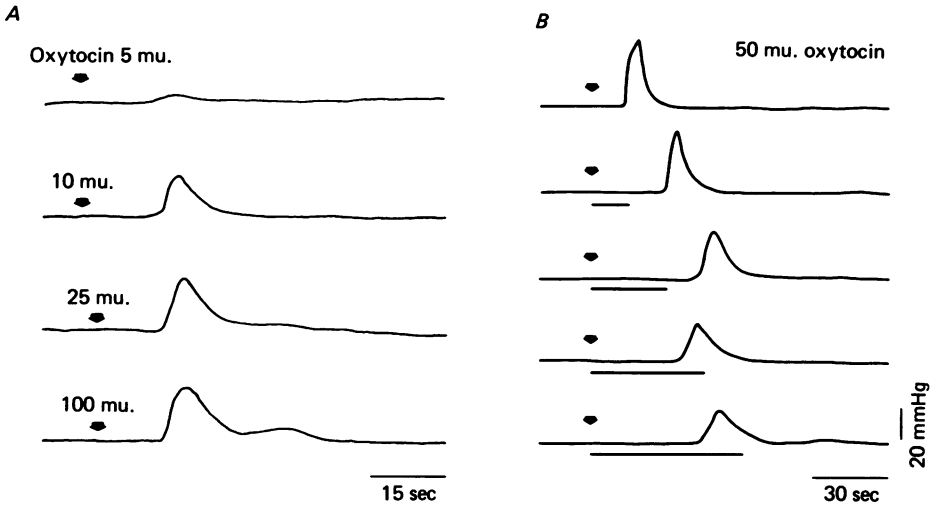

Fig. 5. Effect of I.V. injections of exogenous oxytocin on the intramammary pressure of an anaesthetized sow. *A*, note the increasing amplitude of the response with increasing doses of hormone. *B*, the same amount of hormone was injected at different rates. The horizontal line indicates the duration of injection. Note the similarity in the steepness of the rising slope between rapid injection (top) and natural milk ejection (Figs. 1 and 2).

pressure rose simultaneously in the two glands, both after oxytocin injection and during natural milk ejection.

When the same dose of oxytocin was injected at different rates, the greater the time for injection, the longer the latency of the increase in intramammary pressure, the slower its rise, and the lower its amplitude and duration (Fig. 5).

The level of plasma oxytocin was also assayed after injection of a dose of 25 m-u. of exogenous oxytocin. A maximal value of hormone (23 μu./ml.) was reached within 10–15 sec after the injection. Return to base-line concentration, (< 2 μu./ml.) was noted by 4 min 25 sec. The response of the mammary gland to the injection was similar to that observed during a reflex milk ejection. Lysine–vasopressin, injected in a dose of 25 m-u. caused no change of the intramammary pressure at any time. However, the plasma concentration of vasopressin rose from a basal level of < 1·4 μu. to 3·3 μu./ml. within 10–15 sec following injection, then to a second peak (5·1 μu./ml.) at 2 min 10 sec. Return to base-line levels had taken place within 2 min 55 sec.

(3) *Effect of electrical stimulation of the posterior pituitary*

Electrical stimulation of the posterior pituitary, performed under anaesthesia, produced changes in intramammary pressure in four animals. In two of the sows, the effect of varying the stimulus frequency while applying the same number of stimuli was investigated in detail.

As seen in Figs. 6 and 7 the threshold frequency required to evoke a mammary response was 10 Hz. The greatest increase in intramammary pressure was obtained

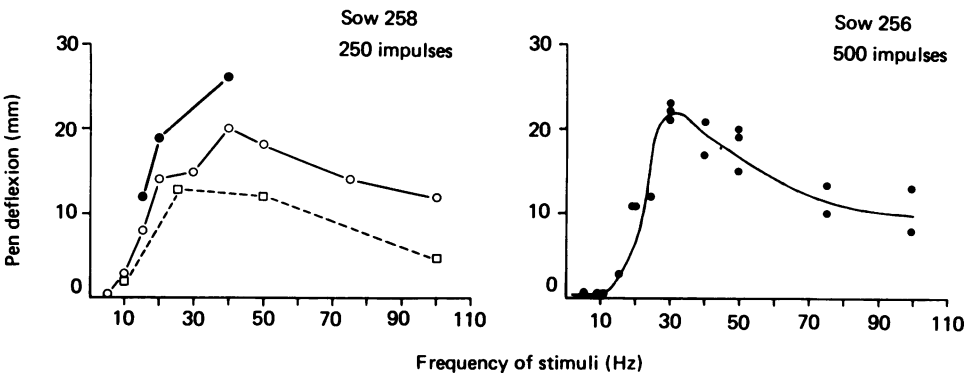

Fig. 6. Effect of electrical stimulation of the neurohypophysis on intramammary pressure. In anaesthetized sows, a constant number of electrical pulses was applied in trains of different frequencies, while monitoring the intramammary pressure on a polygraph recorder. For each train of stimuli, the maximal pen deflexion was measured as an index of the mammary gland response. Left, the three series of stimulation correspond to three different positions of the electrode into the posterior pituitary. Right: the position of the stimulating electrodes was always the same; each point corresponds to one train of stimulation.

with 30–50 Hz, while at frequencies of 75 or 100 Hz, the responses were relatively smaller. For frequencies between 25 and 50 Hz, the response of the mammary gland was very similar in shape, amplitude and duration to that observed during suckling-induced milk ejections. The latencies of the responses depended also on the frequency of stimulation, so that the responses with the highest amplitude had the shortest latency. Thus, for the range of frequencies 25–50 Hz, the latencies of the response were $28{\cdot}8 \pm 0{\cdot}6$ sec, $n = 22$, while for frequencies below or above this range the mean latency was $33{\cdot}1 \pm 1{\cdot}3$ sec, $n = 18$ ($P < 0{\cdot}005$, Student's t test). After prolonged periods of electrical stimulation, the intramammary pressure showed repeated increases reminiscent of the pattern obtained with large doses of oxytocin.

Both oxytocin and vasopressin were discharged into the circulation after electrical stimulation (Figs. 7 and 8). In ten cases, where electrical stimulation induced a mammary gland response, sequential blood sampling at 15 sec intervals showed that plasma oxytocin started to increase in some cases within the first 15 sec after the start of electrical stimulation and reached a peak between 15–45 sec. Usually, the maximal values of oxytocin were obtained with the range of frequencies that gave maximal

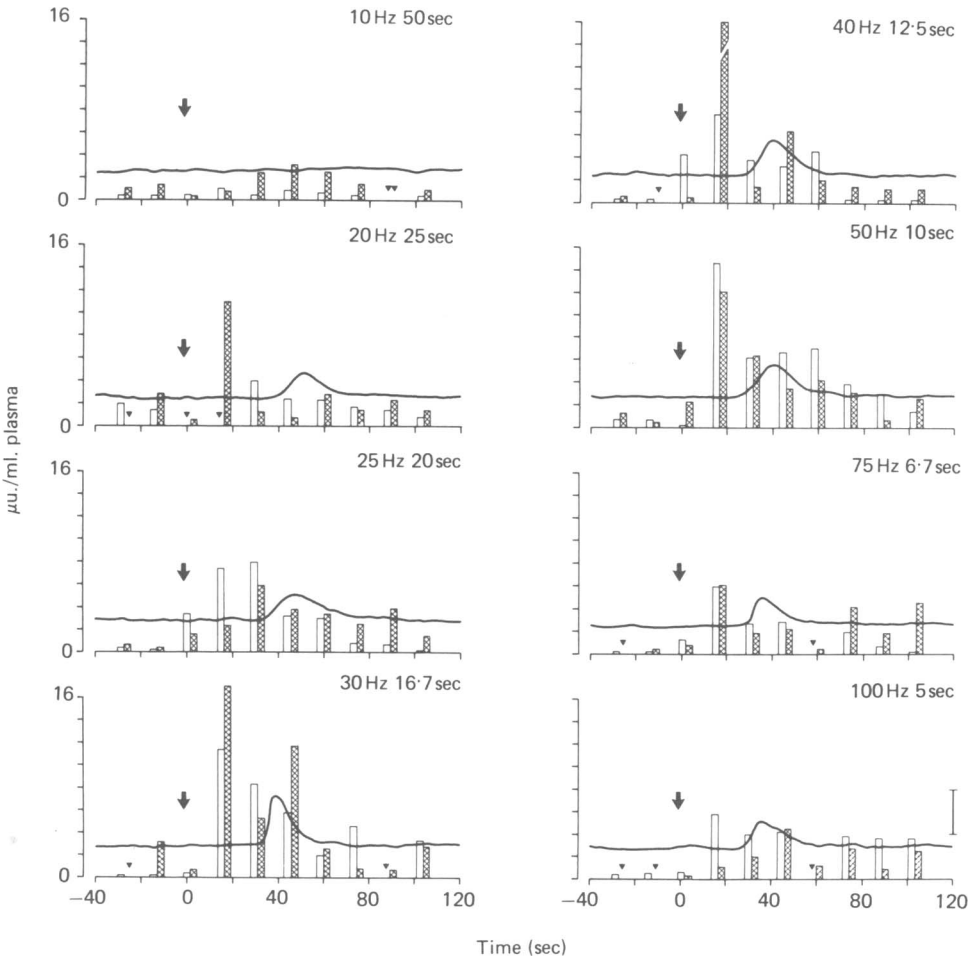

Fig. 7. Effect of electrical stimulation of the neurohypophysis on plasma concentrations of oxytocin and vasopressin and on intramammary pressure. The data were obtained from sow 256 of Fig. 6. The vertical arrows indicate the beginning of the train of stimulation. Blood samples were collected every 15 sec, and both oxytocin (open bars) and vasopressin (hatched bars) were assayed in the same samples. Black triangles indicate samples for which hormone assay was not possible. Note that the increase in plasma concentration of both hormones preceded the response of the intramammary gland (continuous lines; vertical calibration bar = 20 mmHg).

intramammary pressure. Statistically, plasma oxytocin was significantly higher in the samples taken 0–29 or 15–44 sec after the onset of stimulation as compared with pre-stimulation levels ($P < 0{\cdot}001$, Mann–Whitney U test, Fig. 8). Plasma vasopressin concentrations changed in parallel with those of oxytocin, although there was no increase in the first post-stimulus sample. In comparison to the pre-stimulatory levels, vasopressin increased significantly in the samples taken between 0 and 29 sec ($P < 0{\cdot}01$) or between 15 and 44 sec ($P < 0{\cdot}001$, Mann–Whitney U test) (Fig. 8). In

one trail where electrical stimulation was below threshold for evoking a mammary gland response, concentrations of the neurohypophysial hormones did not increase above basal levels (Fig. 7).

DISCUSSION

Intramammary pressure changes in relation to endocrine and behavioural events during suckling

Our experiments demonstrate that long-term intramammary pressure recordings can be performed in the non-anaesthetized sow without difficulty. The animals showed no sign of being disturbed by cannulation of one or more glands, and as the

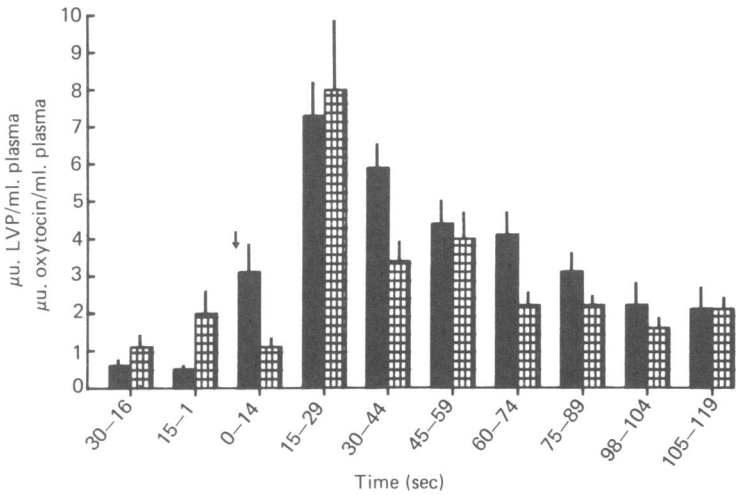

Fig. 8. Cumulative response of plasma oxytocin (filled columns) and lysine–vasopressin (hatched columns) mean ± before and after the onset (arrow) of electrial stimulation above threshold, i.e. when the intramammary pressure increased.

technique involved no dissection the experiments could be repeated in the same animals at an interval of a few days. Continuous recording of the intramammary pressure thus allowed detection of each reflex milk ejection which could be unambiguously distinguished from artifacts due to the movements of the piglets or of the mother (Fig. 1 and 2).

The technique also permitted a degree of analysis of the events leading to milk ejection in the unanaesthetized pig that had heretofore not been possible. Briefly, each milk ejection was observed only after a period of suckling that was initiated either by the mother or by the piglets. As seen by other workers (Gill & Thomson, 1956; Folley & Knaggs, 1966; Fraser, 1977; Whatson & Bertram, 1980), we could broadly distinguish several phases in the suckling behaviour of the piglet: first, the whole litter started jostling for the teats and nosing vigorously at the udders; then a phase of quiet suckling followed, which gave place to final phase of rapid suckling and nosing at the udders, at the end of which the piglets usually went away from their mother.

Our records showed unequivocally that the beginning of the phase of quiet suckling was coincident with the onset of the rise in intramammary pressure. Milk ejection appears therefore to follow the phase of initial massage of the udders, as suggested by some authors (Gill & Thomson, 1956; Folley & Knaggs, 1966) and not as behavioural observations indicated, the phase of quiet suckling (Whittemore & Fraser, 1974). The brief period of quiet suckling, lasted 7–38 sec (Folley & Knaggs, 1966, 13–58 sec; Whittemore & Fraser, 1974; 4–45 sec) and corresponded to the duration of the alterations in the intramammary pressure (range 8–41 sec). Quiet suckling therefore, takes place when the piglets are consuming milk. This may explain why this phase is so well synchronized in the whole litter, a phenomenon that puzzled Whittemore & Fraser (1974). A similarly synchronized behaviour in a litter can be observed during suckling in the rat, at the time of the stretch reaction of the pups, which occurs when the rise in intramammary pressure is maximal (Lincoln *et al.* 1973). In the sow, the initial phase of nursing was usually characterized by a period of slow grunting, followed by a phase of fast grunting. The duration of these two phases, as well as the frequency of grunts, were similar in our experiments to those previously reported (Whittemore & Fraser, 1974). However, we found that fast grunting started about 23 sec before the rise in intramammary pressure, compared with the estimate of Fraser (1977) of 25–35 sec. However, Fraser considered the end of the quiet suckling period as the beginning of milk ejection. The whole sequence of events during suckling recurred at fairly regular intervals of 44·3 min, similar to those previously reported (53·2 min; Fraser, 1977).

Blood sampling for hormone determination during a number of milk ejections was facilitated by the ability to relate the events of nursing to the rise in the intramammary pressure. Once the period of slow grunting was established, serial samples were collected until the milk ejection and/or the suckling period were finished. A rise in plasma oxytocin has been reported (Folley & Knaggs, 1966), but its precise relation to milk ejection, detected by visual observation only, could not be clearly established. In the present study, hormone assays showed that rise in intramammary pressure was preceded by some 30 sec by the appearance of large amounts of oxytocin in the jugular vein. In contrast, no vasopressin increase was observed at milk ejection (Figs. 2 and 3). Thus suckling in the pig induces no vasopressin release, as was reported in the rabbit and the rat (Cross, 1955; Bisset, Clark & Halder, 1970; Wakerley, Dyball & Lincoln, 1973), whereas electrical stimulation of the neurohypophysis causes the release of both neurohypophysial hormones.

The pulsatile nature of oxytocin release during suckling

Oxytocin release during milk ejection appears pulsatile in the pig. First, the variations in intramammary pressure observed during the reflex milk ejections could also be produced either in response to I.V. injection of oxytocin or in response to a brief train of electrical stimuli applied to the neurohypophysis. Protracted I.V. injections of oxytocin, or prolonged periods of electrical stimulation of the neurohypophysis, produced repeated increases in the intramammary pressure, not seen in the course of the normal reflex. Furthermore, during reflex milk ejection, we observed a single peak of oxytocin release before milk ejection. In a few cases of 'incomplete suckling', when there was no rise in intramammary pressure there was no increase

in plasma oxytocin. The shape, duration and amplitude of the variations of the intramammary pressure recorded in response to suckling, to I.V. injections of oxytocin or to electrical stimulation, are remarkably similar to those recorded in the rat (Wakerley & Lincoln, 1973). The necessity of oxytocin release for milk let-down in the two species may be due to the fact that, in both cases, the mammary gland has no cisternae for milk storage, in contrast to other species such as the cow or the goat, where milk can be obtained by the young in part by passive removal (for review see Bisset, 1974).

It is difficult to estimate from the data obtained the amount of hormone discharged from the neurohypophysis, but comparisons of the peak concentrations and the rate of decrease of the hormone between natural milk ejections and milk ejections due to I.V. injection of oxytocin, suggest that it is in the range 10–25 m-u. oxytocin.

The fact that the increase in oxytocin in blood outlasted the response of the mammary gland by several minutes is compatible with a spurt-like release of the hormone, since the same phenomenon was observed after a rapid injection of oxytocin. It is possible that once the hormone is diluted in the whole plasma volume the concentration is too small to stimulate the gland further. It is also possible that the procedures for hormone assay detect inactivated oxytocin, whereas the mammary gland would react only to free/intact oxytocin (Fabian, Forsling, Jones & Lee, 1969).

Stimulatory and inhibitory mechanisms of the milk ejection reflex

As shown in other species (Cross & Harris, 1952; Wakerley & Lincoln, 1973), electrical stimulation of the pig neurohypophysis caused oxytocin release and a subsequent milk ejection. Although the frequency capable of increasing plasma concentration was as low as 10 Hz, the most effective range was 30–50 Hz. This provides additional evidence for facilitation of hormone release within a limited range of high frequency of stimulation (Wakerley & Lincoln, 1973; Mikiten & Douglas, 1965; Ishida, 1970; Dreifuss, Kalnins, Kelly & Ruf, 1971; Nordmann & Dreifuss, 1972). The relative attenuation of the responses at very high frequencies has also been observed *in vivo* or *in vitro* and has been attributed to a failure of small fibres to conduct action potentials at a high rate (Douglas & Ritchie, 1962).

Since the optimal parameters of electrical stimulation correspond to those found in the rat, the final mechanisms leading to the release of oxytocin in response to suckling in the pig could consist of a sudden activation of the oxytocin-secreting neurones as described for the lactating rat (Wakerley & Lincoln, 1973; Lincoln & Wakerley, 1974). In this species, each reflex milk ejection is caused by a high-frequency discharge of action potentials occurring synchronously in the whole population of oxytocin-secreting neurones of the paraventricular and supraoptic nuclei of the hypothalamus. This discharge consists of a brief burst of potentials, lasting 2–4 sec and reaching a peak rate of 60–80 spikes/sec.

The afferent mechanisms responsible for the activation of oxytocin neurones appear to be complex. Suckling seems essential and a number of maternal factors also appear to be necessary. Of the maternal factors characteristic of successful suckling, much attention has been given to grunting and in particular to the change from a slow to a fast rhythm at the time of milk ejection (Whittemore & Fraser, 1974). Fast grunting however appears to occur after oxytocin release, for the latency from the onset of

fast grunting to the rise in intramammary pressure was shorter in our experiments than the latency from electrical stimulation to the rise in intramammary pressure. This may explain why removing the piglets did not prevent milk ejection once the fast grunting phase had started (Fraser, 1977). The mechanisms responsible for the acceleration in grunting are unknown. They may be common with those allowing the activation of oxytocin neurones. Alternatively, the massive electrical discharge of oxytocin neurones could facilitate grunting, since oxytocinergic fibres have been described in the dorsal and dorsomedial vagal nucleus (Swanson & Sawchenko, 1980; A. Weindl, personal communication), which *inter alia* innervates the larynx. It must be noted, however, that in a few cases of incomplete suckling, rapid grunting took place, with no milk ejection (Fig. 4).

Reflex milk ejection appears to occur only if the sow is in a state of relaxation. When the sows were disturbed they often refused to nurse, and even if they did there were many failures to eject milk. In the rat, reflex milk ejections occur when the animal is in a state of somnolence, characterized electroencephalographically by a rhythm of slow-wave sleep and it has been suggested that sleep was necessary for the expression of the reflex (Voloschin & Tramezzani, 1979; Lincoln, Hentzen, Hin, van der Schoot, Clarke & Summerlee, 1980). This is not the case in the pig, as during the period from the onset of nursing to the milk ejection the electroencephalographic rhythm is always that of wakefulness (Poulain *et al.* 1981).

Provided that a normal pattern of nursing and suckling occurs, the time course of events suggest that there is, first, a latent period from the onset of stimulation to the onset of neuronal activation, and secondly, a refractory period due to central inhibitory factors. It is noteworthy that there is a delay of 1–6 min from the onset of suckling to milk ejection, a period longer than that for hormone released to act on the mammary gland, which is estimated to be 30 sec from our electrical stimulation experiments. In the lactating rat, a delay of several minutes is also observed from the onset of suckling to the first milk ejection. During this time and in the intervals between successive milk ejections although the young rats continue the suckle, the electrical activity of oxytocin neurones is similar to that observed in the absence of suckling. It is thus likely that in the pig as well as in the rat, a mechanism interrupts the afferent impulses from the stimulated mammary gland before they reach the hypothalamic nuclei. The sudden activation of oxytocin cells would take place only after summation of afferent impulses and opening of a gate (Lincoln *et al.* 1973).

The intermittent nature of milk ejection reflex in the pig is in part due to behavioural factors since the piglets do not suckle all the time. However, there does seem to exist a refractory period after each milk ejection which lasts much longer than the actual period of suckling. As we and others (Fraser, 1975*b*; Whatson & Bertram, 1980) have observed, 'incomplete suckling' in apparently unstressed sows occurs frequently when the young try to suckle earlier than usual, even though nursing, including grunting, is apparently normal. That several milk ejections are not observed during one suckling period cannot be attributed to tachyphylaxis, since I.V. injections of oxytocin given 1 min after a natural milk ejection caused a response. It is also unlikely that prolonged inhibition of oxytocin release occurs at the level of the neurohypophysis. Brief electrical stimulation could repeatedly release oxytocin at intervals of 4–8 min, and prolonged stimulation caused prolonged mammary gland

responses. The inhibitory mechanism therefore seems to be located centrally within the afferent arc of the milk ejection reflex, as was initially proposed for the rabbit by Cross (1955).

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