Does the Hypothalamo–Neurohypophyseal System play a role in Gestation Length or the Course of Parturition?

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INTRODUCTION

During parturition the rat hypothalamo–neurohypophyseal system (HNS) shows signs of activation at the level of the supraoptic (SON) and paraventricular nuclei (PVN) (Swaab and Jongkind, 1970) and at the level of the neurohypophysis (Stutinsky, 1957, for reference see Sloper, 1966; Boer et al., 1973). Moreover, parturition is accompanied by a release of oxytocin and vasopressin (for reviews see Heller and Ginsburg, 1966, and Swaab, 1972). Whether this activation of the HNS causes the initiation of parturition, or is itself caused by the process of parturition is still a matter of dispute.

Parturition can be induced by oxytocin infusion (e.g. Fuchs and Proklete, 1970; Fuchs and Saito, 1971) in rat, or by electrical stimulation of the infundibulum and median eminence (Cross, 1958, Lincoln, 1971) in the rabbit. Such observations, however, do not give much information about the normal activities of the HNS in parturition. In addition, neither mechanical (Gale and McCann, 1961; c.f. Fitzpatrick, 1966) nor immunological elimination of HNS function (Kumaresan et al., 1971) gave conclusive evidence that the HNS initiates parturition. Assays suggest that the endogenous blood levels of oxytocin are very low in human during spontaneous labor (Chard et al., 1970; Boyd and Chard, 1973).

In order to test the hypothesis that the HNS is involved in the initiation or further course of parturition, delivery and its distribution during 24-h periods has been observed in rats after various treatments. The HNS was activated by water deprivation, while its inhibition was induced by alcohol administration (see below). Parturition was also studied in the Brattleboro strain of Long–Evans rats, since these animals display a hereditary hypothalamic diabetes insipidus involving an absence as well as an activation of certain HNS functions. The influence of two anesthetics on parturition was also examined. In the first place a review will be presented of the current state of knowledge concerning each of the conditions used in the present investigation.

HNS and alcohol

Alcohol induces a diuresis (Van Dyke and Ames, 1951; Fig. 2) which is supposed to

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be caused by inhibition of the HNS (Millet et al., 1968; Cobo and Quintero, 1969; Fuchs and Wagner, 1963a, b). On the other hand, a low blood level of alcohol seems to have a stimulatory effect on oxytocin release (Cobo and Quintero, 1969). In support of the action of alcohol on the HNS, changes were found in nuclear size of the neurons of the SON and PVN and in the neurohypophyseal content of neurosecretory material (NSM) (Hirvonen et al., 1966). The parameters used in that study, however, do not allow the conclusion that alcohol inhibits hormone synthesis in the SON or PVN (e.g. Swaab, 1970). Preliminary data obtained in our department (Van Leeuwen, unpublished) failed to show any inhibition of neurosecretory activity, as measured by the TPP-ase distribution (Swaab and Jongkind, 1970), following various schedules of alcohol administration.

Effects of ethanol on HNS activity were generally found at blood concentrations which approach those found in alcoholic coma (Cobo and Quintero, 1969). Ethanol might therefore not cause a specific inhibition of the HNS (Cobo and Quintero, 1969), as has often been supposed (Millet et al., 1968), but will probably affect other central and peripheral neural systems as well (Eidelberg and Wooley, 1970).

Effects of ethanol upon parturition have been reported for rabbit and human. In rabbit it was possible to postpone delivery for about 30 h, while uterine sensitivity for oxytocin was unimpaired (Fuchs, 1966a). In human ethanol was effective in treatment of premature parturition, possibly owing to its inhibitory effect on uterine activity during labor (Zlatnik and Fuchs, 1972; Bieniarz et al., 1971; Luukkanen et al., 1967). No information is available for the rat in this respect. The dose administered in the present study by means of a stomach tube is reported to cause complete inhibition of milk ejection in rats when given intraperitoneally (Fuchs, 1969), although this is in dispute again (Lincoln, 1973).

**HNS and water deprivation**

Water deprivation is a strong stimulus for the activation of the SON, PVN and the neurohypophysis, as has been shown by morphological and enzymatic studies (e.g. Ortmann, 1951; Zambrano and De Robertis, 1966; Jongkind, 1969; Boudier et al., 1970; Swaab, 1970; Boer et al., 1973).

In the rat, water deprivation induces an increased protein synthesizing capacity in the perikarya of the SON (Norström, 1971) and also an increased transport of NSM along the stalk to the infundibular process (Norström and Sjöstrand, 1972). Neurohypophyseal content of vasopressin and oxytocin decreases during water deprivation (Jones and Pickering, 1969) while the plasma level of vasopressin (Little and Radford, 1964), as well as urinary excretion of vasopressin (Noble and Taylor, 1953) increase. On the basis of these results it would appear that the serum oxytocin level will also be elevated during water deprivation, but so far no direct data are available concerning blood and urine levels of oxytocin after such a treatment.

Jones and Pickering (1969) reported a 30% lower level of both nonapeptides in the neural lobe within 24 h after the start of water deprivation, while after 5 days the content was almost depleted. Moreover, Miller and Moses (1971) observed that, on
the very first day of water deprivation, urinary vasopressin excretion in the rat reaches already its highest level, and remains so throughout at least a 4-day period. Therefore water deprivation periods of 1, 2 and 4 days were used in the present study.

**HNS and Brattleboro rats**

Diabetes insipidus (DI) in the Brattleboro rat is not due to absence of the HNS. Both the magnocellular nuclei and the neurohypophysis are morphologically intact (Sokol and Valtin, 1965; Scott, 1968; Kalimo and Rinne, 1972). Morphological and enzymatic studies showed in fact a highly activated HNS, just like that in normal rats deprived of water (Sokol and Valtin, 1965, 1967; Scott, 1968; Kalimo and Rinne, 1972; Swaab et al., 1973). Together with a lower neurohypophyseal oxytocin content (Valtin et al., 1965) and a higher neurophysin serum level (Cheng et al., 1972) this suggests an increased synthesis and release of HNS products. Although this has not been measured directly, the serum level of oxytocin in the Brattleboro strain has been suggested to be elevated as compared to that in normal Long–Evans rats (Valtin et al., 1965; Cheng et al., 1972). With respect to the characteristics mentioned above, Brattleboro rats heterozygous for DI are intermediate between normal Long–Evans or Wistar rats and homozygous DI animals (Sokol and Valtin, 1965; Cheng et al., 1972; Swaab et al., 1973). The electrical activity of the SON, however, appears to be the same for both heterozygous and homozygous Brattleboro rats (Dyball, 1973).

In one of the early papers on the Brattleboro rats by Valtin and Schroeder (1964), reproductive abnormalities were mentioned for these animals. Although a lessened reproductive capability is well-documented for 5 generations of Brattleboro’s by Saul et al. (1968), no explanation for this is known at present.

**MATERIALS AND METHODS**

For the present study virgin female rats weighing approximately 200 g were used: Wistar rats and Brattleboro rats, homo- and heterozygous for diabetes insipidus (DI), all obtained from T.N.O. (Zeist). The Brattleboro’s were all of the same age (ca. 3 months). The animals were kept in individual cages at 25 °C and exposed to 12 h light daily (from 7 a.m. to 7 p.m.). They received tap water and standard chow (Hope Farms) *ad libitum*. Pregnant animals were obtained by mating females overnight: Wistar, homo- and heterozygous Brattleboro females with corresponding males. The day on which spermatozoa were observed in the morning vaginal smears was called day zero of pregnancy. After pregnancy had been confirmed by palpation, the Wistar rats were divided at random into the experimental groups.

Twenty-four hour observation of parturition was started at the latest by 9 a.m. on day 21. Red light was used during the dark period (c.f. König and Martin, 1968). For each rat the exact time of each delivery of a pup was noted, the pups were removed and weighed. Observations ended with the last delivery.

The first series of experiments included 4 groups of 8 rats each: one control group

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(C1), two water-deprived groups (W4D and W2D, that did not receive water respectively from midday of day 17 or day 19 until the last pup was born), and one group that received ethanol (10 ml 10% v/v in tap water) every 12 h by a stomach tube as long as no pup was delivered, starting at 9 a.m. on day 21 (ALC 12/9). Beside these Wistar groups, the first series included the two Brattleboro groups: 6 rats homozygous for DI (HOM DI) and 7 rats heterozygous for DI (HET DI). Whether they were homo- or heterozygous for DI was determined prior to pregnancy by their daily water intake (Swaab et al., 1973).

The second series of experiments, performed approximately one month later, included 4 groups of Wistar rats: a control group of 8 rats (C2), a water-deprived group of 7 rats (from midday of day 20 until the last pup was born; W1D), and 2 groups of 7 rats that received ethanol (10 ml 10% v/v in tap water) every 6 (ALC 6/3) or 12 h (ALC 12/3) for as long as no pup was born. Ethanol was administered by means of a stomach tube, beginning at 3 a.m. of day 21.

In addition to these experiments, two groups (each consisting of 5 female Wistar rats) received, at 9 a.m. on day 21 of pregnancy, a single s.c. injection of either urethane (UR) (1.2 g/kg) or sodium pentobarbital (PENT) (0.12 g/kg). Another group of 6 Wistar rats served as control (C3). Here the observations were made at 2-h intervals. Urine output per hour was measured, using metabolism cages (Jongkind, 1964), in 5 non-pregnant female Wistar rats which received ethanol (10 ml 10% v/v in tap water) twice, by means of a stomach tube, at an interval of 6 h. Whenever the amount of urine was adequate, the osmolality of the urinary sample was determined by means of freeze-point determination (Knauer Type M).

Statistical differences between the various observations were tested by the Student-t-test (De Jonge, 1963). A level of $P < 0.05$ was considered to be statistically significant.

RESULTS

**Time distribution (Fig. 1)**

The distribution of the onset of parturition in time is given for the two control groups (C1) and (C2). Thirteen of the 16 animals started delivery between 0.50 and 6.30 p.m. on day 21 (Fig. 1).

![Fig. 1. Distribution of the onset of parturition in the Wistar controls (C1 and C2) over the 24 h of a day.](image-url)
Alcohol (Figs. 2, 3 and Table 1)

After administration of 10 ml 10% ethanol by stomach tube, an increased diuresis starts during the second hour and declines within the following 2 h. The urine osmolality gave a reciprocal picture (Fig. 2). Repeating the ingestion of alcohol 6 h after the first one, the rats reached a level of anesthesia in which they did not react to painful stimuli such as foot-pinching.

Only in the alcohol group ALC 12/3 a significant increase in gestation length was observed. This change was also significant as compared to animals from the ALC 6/3

![Graph showing urine production and osmolality](image)

Fig. 2. Urine production and osmolality of Wistar females after ethanol ingestions (ALC: 10 ml 10% in tap water). The first volume was given at 09.00 a.m. Vertical lines indicate ± S.E.M. (5 animals). The number of the animals that produced enough urine per hour for osmolality measurement is given between brackets.

### TABLE 1

<table>
<thead>
<tr>
<th>Code*</th>
<th>Pup weight (g)**</th>
<th>Change***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>5.12 ± 0.05 (89)</td>
<td>n.s.</td>
</tr>
<tr>
<td>ALC 12/9</td>
<td>5.17 ± 0.06 (74)</td>
<td>n.s.</td>
</tr>
<tr>
<td>C2</td>
<td>5.15 ± 0.04 (84)</td>
<td>n.s.</td>
</tr>
<tr>
<td>ALC 12/3</td>
<td>5.16 ± 0.05 (57)</td>
<td>n.s.</td>
</tr>
<tr>
<td>ALC 6/3</td>
<td>4.80 ± 0.07 (33)</td>
<td>− 7%</td>
</tr>
<tr>
<td>Water deprivation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>5.15 ± 0.04 (84)</td>
<td>n.s.</td>
</tr>
<tr>
<td>W1D</td>
<td>5.02 ± 0.05 (62)</td>
<td>n.s.</td>
</tr>
<tr>
<td>CI</td>
<td>5.12 ± 0.05 (89)</td>
<td>− 8%</td>
</tr>
<tr>
<td>W2D</td>
<td>4.73 ± 0.05 (74)</td>
<td>− 38%</td>
</tr>
<tr>
<td>W4D</td>
<td>3.16 ± 0.05 (81)</td>
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</tbody>
</table>

* For code see legend Fig. 3.
** Expressed in grams ± S.E.M. (number of pups).
*** Only mentioned if statistically significantly different at the $P < 0.05$ level as compared to their appropriate control (C). n.s. = not significantly different.

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Fig. 3. Gestation length, duration of parturition and interval between births for Wistar rats on different schedules of alcohol treatment and different periods of water deprivation. Alcohol was given (10 ml 10% v/v by stomach tube) every 12 h starting at day 21, 3 a.m. (ALC 12/3) and 9 a.m. (ALC 12/9) or every 6 h starting at day 21, 3 a.m. (ALC 6/3). Water deprivation periods respectively 1 (W1D), 2 (W2D) and 4 days (W4D) before midday of day 21 of pregnancy. Controls in the two series of experiments are C1 and C2. Horizontal lines indicate ± S.E.M. Numbers between brackets are the numbers of females and number of intervals respectively.

* Difference statistically significant at the $P < 0.05$ level as compared to the controls of the same series.

group (0.01 < $P < 0.02$). Three animals of the ALC 6/3 group delivered before the third alcohol ingestion. Of the other 4 animals in this group which were unconscious, 2 died after 4, and the others after 9 alcohol administrations. Immediate post-mortem examination of the animals showed that all the pups were still alive. In order to calculate gestation length in these cases the time at which the mother died was regarded as the end of gestation. Combining the values for gestation length of all 7 females of the ALC 6/3 group, a mean gestation length of 530.1 h (SEM 5.2) could be calculated, which is not significantly different (0.10 < $P < 0.20$) from the control value of 522.0 h (SEM 2.1).

By administration of alcohol every 6 h (ALC 6/3) the duration of parturition and the birth interval was increased. In the group ALC 12/3, on the other hand, only the birth interval was increased.

Water deprivation (Fig. 3 and Table I)

One day of water deprivation (W1D) prior to the mean delivery time of the control animals had no effect upon any of the parameters tested. Two and 4 days of water
deprivation (W2D and W4D) postponed delivery by 12.5 and 11 h respectively. No differences in duration of parturition and birth intervals were seen between the water-deprived and the control groups, except for a shorter duration of parturition in the W2D group. A gradual decrease in pup weight was observed between 1 and 4 days of water deprivation, up to 38% in the W4D group.

*Brattleboro rats (Fig. 4 and Table II)*

No difference in gestation length could be observed between heterozygous and homozygous Brattleboro rats. The same holds true for duration of parturition, possibly caused by the great variance in the HOM DI group. The mean birth interval for HOM DI was increased by about 70% as compared to HET DI.

One homozygous DI female which started parturition failed to deliver a second pup within the following 24 h. Cesarian section at that time revealed only one more still living pup (weight 7.38 g). This animal was excluded from the calculations for mean

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Fig. 4. Gestation length, duration of parturition and interval between births for Wistar (C1) and heterozygous (HET DI) and homozygous (HOM DI) Brattleboro rats. Horizontal lines indicate ± S.E.M. Numbers between brackets are respectively the numbers of females and the intervals between births. * Difference statistically significant at the $P < 0.05$ level as compared to HET DI.

**TABLE II**

MEAN PUP BIRTH WEIGHT AND LITTER SIZE FOR WISTAR (C1) AND FOR HETEROZYGOUS (HET DI) AND HOMOZYGOUS (HOM DI) BRATTLEBORO RATS

<table>
<thead>
<tr>
<th></th>
<th>Pup weight</th>
<th>Litter size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>grams ± S.E.M. (number of pups)</td>
<td>± S.E.M. (number of females)</td>
</tr>
<tr>
<td>Wistar (C1)</td>
<td>5.12 ± 0.05 (89)</td>
<td>11.1 ± 0.5 (8)</td>
</tr>
<tr>
<td>HET DI</td>
<td>5.75 ± 0.04 (64)</td>
<td>9.1 ± 1.1 (7)</td>
</tr>
<tr>
<td>HOM DI</td>
<td>5.89 ± 0.07 (45)</td>
<td>7.7 ± 1.7 (6)</td>
</tr>
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</table>

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duration of parturition and mean birth interval as given in Fig. 3 and Table II. Including these 24 h in the values for HOM DI, the mean duration of parturition was calculated at 6.53 h (SEM 3.59), and the birth interval at 296 min (SEM 29.4). Using these values too, the duration of parturition did not differ significantly from the HET DI level (0.2 < P < 0.3, instead of 0.1 < P < 0.2), while the significance of the birth interval increased (P < 0.001, instead of 0.01 < P < 0.02).

No difference in pup weight was found between the two groups. Litter size was significantly lower for homozygous as compared to heterozygous Brattleboro rats.

Anesthesia (Table III)

No influence upon gestation length was caused by pentobarbital; urethane in contrast postponed delivery for about 18 h.

<table>
<thead>
<tr>
<th></th>
<th>Gestation length (expressed in 2 h intervals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3</td>
<td>261.2 ± 1.5 (6)*</td>
</tr>
<tr>
<td>PENT</td>
<td>261.0 ± 1.3 (5)</td>
</tr>
<tr>
<td>UR</td>
<td>270.2 ± 0.9 (5)**,§</td>
</tr>
</tbody>
</table>

* Mean ± S.E.M. (number of animals).
** Including two animals which had not delivered on day 22 at 6 p.m.
§ P < 0.001.

DISCUSSION

The concept that the HNS, and in particular oxytocin, is involved in the onset or course of parturition is based upon indirect evidence (Fuchs, 1966a; Fitzpatrick, 1969; Fuchs and Poblete, 1970; Burton and Forsling, 1971; Chard et al., 1971; Fuchs and Saito, 1971; Lincoln, 1971). Until now, even the measurement of oxytocin levels in the blood during parturition (McNeilly et al., 1972; Burton et al., 1972) failed to give a decisive answer in this respect, due to the low levels (Chard et al., 1971) and the intermittent spurt-like release (Gibbens et al., 1972) of oxytocin. The determination of the start of the contraction phase by visual observation, as described by Rosenblatt and Lehrman (1963), appeared to be too inaccurate to rely upon (Fuchs, 1966b). In the present study the onset of the first delivery was therefore chosen for the onset of parturition.

The distribution per 24 h of the parturition in the two combined control groups
(C1 and C2) is closely related to the daily fluctuation of the neurohypophyseal oxytocin content, found in the male Wistar rat by König and Martin (1968). Most parturitions occurred at the time of day when these authors had measured a low oxytocin content. Although the relationship is very indirect, this agrees with the possibility of a supposed triggering role of oxytocin with respect to parturition. In contradiction to the expectation, 2 and 4 days of water deprivation did not shorten gestation length, but caused a postponement of the first delivery. Since, however, fetal weight influences the length of gestation (Csapo, 1969), one might be inclined to ascribe this increase in gestation length directly to the decreased fetal weights. This cannot be the only explanation, however, since the mean birth weight of the pups in the 4 day water-deprived group was 33% lower than that in the 2 day water-deprived group, whereas their mean gestation lengths were not different at all.

Alcohol too caused increased gestation length in some of the schedules used. In spite of a different method used for administration of ethanol and the other species in this study, the results obtained in the present study confirm those of Fuchs (1966a) in the rabbit. Under alcoholic conditions too, however, lower fetal weight might play some role, since the birth weights of pups in the group receiving alcohol every 6 h (ALC 6/3) were lower than normal. This effect of alcohol agrees with the growth-inhibiting action of alcohol in the post-natal period (Ratcliffe, 1972). The group receiving alcohol every 12 h from 3 a.m. on day 21 on (ALC 12/3) had pups of normal weight, but the gestation length was prolonged. This also points to a relatively lower intra-uterine growth rate during the alcohol period as compared to normal.

Although ethanol is claimed to specifically inhibit the HNS (Millet et al., 1968), it definitely has a severe anesthetic effect. Each successive alcohol ingestion further increased the anesthetic level, which resulted in complete unresponsiveness to painful stimuli such as foot-pinchings, and finally in the death of 4 out of 7 rats from the group that received alcohol every 6 h. Therefore it was investigated whether anesthesia by pentobarbital (Nembutal®) or urethane was effective in postponing delivery. It appeared that only urethane gave a similar postponement as occurred in the ALC 6/3 group. The ineffectiveness of pentobarbital might be ascribed to the shorter anesthesia as compared to urethane (10 and 27 h respectively if one considers as the end of anesthesia the time that the animals were able to move after handling). Preliminary results obtained from 4 rats revealed that injection of the same amount of pentobarbital, now given at midday of day 21, gave prolonged gestation for about 21 h.

These data suggest that the anesthetic effect of alcohol is an important factor in the prolongation of gestation in this experiment. This is also supported by the fact that the diuresis after ingestion of ethanol (10 ml 10% in tap water) is only increased for 2 h and the urine is hypotonic only for 1 h. This means that if release of hormones from the neural lobe is necessary for the onset of parturition, its possible inhibition by alcohol is too short to cause a postponement. In the Brattleboro rats no difference was found in gestation length between the rats homo- or heterozygous for DI. There was, however, a striking difference between them and normal Wistars (P < 0.01). In order to know whether this difference has any significance, this study needs to be repeated using Brattleboro rats, showing no hereditary DI.

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An acceleration of the course of parturition was caused only by water deprivation lasting 2 days, 1 and 4 days water deprivation failing to produce any effect. This difference does not seem to be important, however, since it is ruled out when the litter size is included by calculating the mean interval between births. No influence of water deprivation upon the interval between births was found in any of the groups.

In some of the groups ethanol prolonged the duration of parturition, which was even more obvious when the interval between births was calculated. This is in agreement with the idea that oxytocin is needed for the normal course of delivery (Kumaresan et al., 1971). On the other hand, the mothers were rather dull during parturition, which means that some influence of anesthesia cannot be ruled out.

The Brattleboro groups, whether homo- or heterozygous for DI, had the same duration of parturition. The mean interval between births, however, is longer in the homozygous than in the heterozygous group. This difference is accompanied by a decreased litter size in the homozygous group, but no relationship seems to exist between litter size and duration of parturition in this group of animals (unpublished data). The smaller litter size for the homozygous rats confirms the findings of Saul et al. (1968). The longer interval between births might be explained by a hypertonia of the uterus, caused by a high level of oxytocin (Poseiro and Noriega Guerra, 1961), which would be expected in the Brattleboro rat (Valtin et al., 1965).

Recapitulating, we must conclude that the results of our study are more in conflict with, than in support of the hypothesis that posterior lobe hormones are important for the onset or course of parturition in rats.

**SUMMARY**

In the present study the hypothesis was tested that the rat hypothalamo–neurohypophyseal system (HNS) is involved in the initiation or further course of parturition. For this purpose the influence upon length of gestation and duration of parturition following water deprivation, alcohol administration, Nembutal and urethane anesthesia was investigated. All these treatments are presumed to influence HNS activity. For the same reason, gestation length and duration of parturition were studied in rats of the “Brattleboro-strain”, both homo- and heterozygous for hypothalamic familiar diabetes insipidus.

None of these treatments resulted in a shortening of gestation length. Alcohol administration in one of the schedules used as well as both anesthetics caused a postponement of delivery. Gestation length in the Brattleboro strain was about one day longer than in the Wistar rats used in the rest of the study. No difference was observed, however, between Brattleboro’s which were homo- or heterozygous for diabetes insipidus.

Duration of parturition, when corrected for litter size, was not shortened by any of the experimental situations. The duration was longer in 2 out of the 3 alcohol groups, however. The homozygous Brattleboro’s were found to have a longer duration of parturition than the heterozygous ones.
These results are more in conflict with than in favor of the concept that the posterior lobe of the pituitary is important for the onset of parturition in rats.

REFERENCES


BURTON, A. M. AND FORSLING, M. L. (1971) Hormone content of the neurohypophysis in foetal, new-born and adult guinea-pigs. J. Physiol. (Lond.), 221, 6–7P.


DYBALL, R. E. J. (1973) Single unit activity in the supraoptic nucleus of Brattleboro rats. J. Physiol. (Lond.), 231, 39–40P.


DISCUSSION

FORBES: The observations on parturition during anesthesia have confused me. You have not mentioned the possibility of an influence of the fetuses being under anesthesia either by one of the anesthetics or by alcohol. Under anesthesia the fetuses are possibly not able to respond in a normal way, that will presumably initiate parturition in the first place.

G. BOER: As Dr. Swaab has demonstrated (this volume), we do not think any more that the rat fetuses have a role in the initiation of parturition. Moreover, in the animals which died after several alcohol injections, immediate autopsy revealed that the pups were alive and in good condition.

LINCOLN: In our colony of Brattleboro rats in Bristol we have also noticed a lot of difficulties in the course of parturition of the homozygous Brattleboro rats. Have you already tried whether vasopressin treatment will restore the normal pattern of parturition?

G. BOER: No, that is what we intend to do, but it is a lot of work because the 24 h of observation are a powerful stress for the observer.

K. BOER: We want to wait for the 'normal' Brattleboro, that means, Long–Evans rats having no diabetes insipidus, since these are in our opinion the only good control animals.