The influence of the fetal hypothalamus and pituitary on the onset and course of parturition

D. F. SWAAB*, K. BOER* and W. J. HONNEBIER†

**Netherlands Central Institute for Brain Research, Amsterdam and †Department of Obstetrics and Gynaecology, University of Amsterdam*

Abstract The possibility that the fetal brain or pituitary either initiates parturition or influences the course of labour was studied in human and rat. The results when corticotropin or neurohypophysial hormones were injected directly into human anencephalic fetuses *in utero*, and data obtained from 147 clinical records of such fetuses, seemed to show that the fetal brain does not trigger the onset of parturition. On the other hand, the course of labour was seriously protracted in anencephalic fetuses.

Gestation length of brain-aspirated rat fetuses was not significantly longer than in sham-operated controls. However, the course of labour was protracted in the brain-aspirated fetuses. A similarly protracted expulsion patter ı was observed in Brattleboro rats homozygous for a hypothalamic form of diabetes insipidus. These dåta all pointed to the likelihood that fetal neurohypophysial hormones stimulate the course of labour. Neither oxytocin nor vasopressin could be demonstrated in the rat fetus on the last day of pregnancy, when specific immunofluorescence was used. However, a closely related compound was found that was identified as most probably being vasotocin. The hypothesis is put forward that this fetal hormone normally stimulates the course of labour.

Our research on the fetal brain and pituitary has focused on two themes. The first, which will not be elaborated on here, deals with the role of these two organs in the stimulation of intrauterine growth, a process in which α-melanotropin (α-MSH) was found to play an important part (Honnebier & Swaab 1974; Swaab *et al.* 1976). The second theme deals with the role of the fetal brain and pituitary in parturition.

The concept that the mammalian fetus might play an active role in its own delivery is not altogether new, having even been traced back 2500 years to Hippocrates. He supposed that, when the nourishment of the fetus had become inadequate, it would actively break through the membranes (cf. Kloosterman 1968). The possibility that parturition would be initiated via stimulation of the fetal pituitary has gained support on the basis of reports of prolonged preg-

nancies in humans, cattle and sheep with congenital malformations affecting the hypothalamo-hypophysial area. Experimental verification of this thesis was obtained in an elegant way for the sheep (cf. Liggins *et al.*, this volume). Our studies in humans and rats, however, failed to confirm the hypothesis that the fetal hypothalamo-hypophysial area would be the critical factor in triggering the onset of parturition in these species.

Our fellow-countryman Van Deventer (1746) was probably the first to point to the ability of the fetus to play an active role in the course of parturition. He wrote: 'a dead fetus is lacking the power to remove itself from the uterus and will thus produce a difficult course of labour'. The possibility that the fetus is actively involved in the course of labour has to our knowledge never before been tested experimentally. As will be seen, our observations in humans and rats support this hypothesis.

THE FETAL BRAIN AND PARTURITION IN THE HUMAN

Our work on the relation between fetal hypothalamo-hypophysial systems and parturition started in 1970 when Honnebier injected hormones into human anencephalic fetuses *in utero*, with the aim of terminating these pregnancies by restoring the supposed physiological defect. Endocrinologically, the anencephalic fetus is characterized by absence of the hypothalamus and by serious disturbance of the remaining pituitary functions. Since two groups of pituitary hormones were thought to be involved in the initiation of parturition, i.e. posterior lobe hormones such as oxytocin and vasopressin (cf. Chard *et al.*, this volume) and corticotropin (cf. Liggins *et al.*, this volume), both of these hormones were injected. The diagnosis of anencephaly and the purpose of our investigations were fully explained to the patients, who all gave full consent to the experiments.

In one patient, regular contractions were observed almost immediately after the second injection of oxytocin into the fetus, leading to the birth of an anencephalus 8.5 hours later (cf. Honnebier *et al.* 1974, patient 4). In contrast to this apparently prompt action of oxytocin, however, were the observations on an anencephalic fetus given five injections of posterior pituitary extract: the injections were followed by only transient uterine activity. In addition, transient fetal bradycardia (down to 40 beats/min) was recorded. Labour had to be induced by laminaria tents and by oxytocin infusion into the mother (cf. Honnebier *et al.* 1974, patient 5). Oxytocin injections into the fetus failed to induce labour in a third patient (cf. Honnebier *et al.* 1974, patient 3), even after six such injections (Fig. 1). The only effect was some weak uterine activity lasting about one hour. Various intravenous infusions of oxytocin, dose-graded

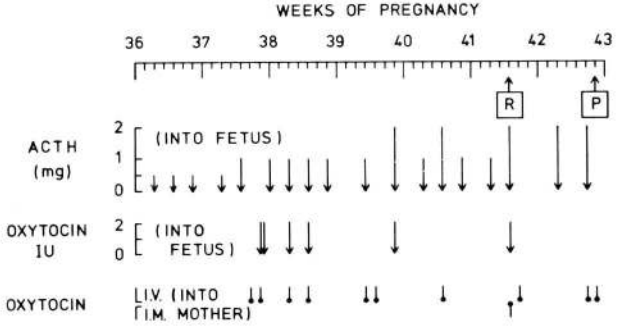

FIG. 1. Time sequence of corticotropin (ACTH) and oxytocin administration into the shoulder of a human anencephalic fetus, intravenous infusion into the mother (↓) and one series of five intramuscular injections of oxytocin into the mother (↑). ACTH = Cortrosyn-depot®, oxytocin = Piton-S® (both Organon). R = rupture of the membranes. P = parturition. For more details on this patient see Honnebier *et al.* (1974, patient 3).

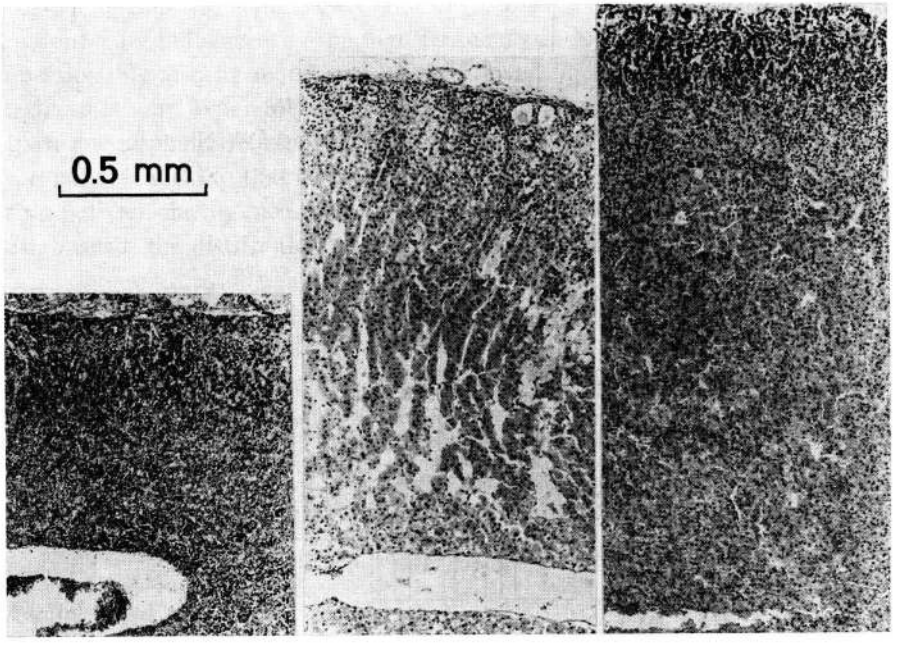

FIG. 2. Human fetal adrenals, paraffin sections of 7 μm, stained with haematoxylin & eosin. Left: small adrenal of an anencephalic fetus that was not injected with corticotropin; middle: restored adrenal of the corticotropin-injected anencephalus of Fig. 1; right: large control adrenal of a newborn with a normal nervous system. Note the central vein at the base of the figures. (Sections and photographs made by Dr A.C. Jöbsis, Dept of Pathology, University of Amsterdam, The Netherlands.)

according to the uterine reaction (cf. Theobald 1968), and one series of five intramuscular oxytocin injections, failed to induce parturition from the maternal side.

When depot corticotropin was injected into the same fetus (Fig. 1), the fetal adrenals were activated, as appeared from the rise in urinary oestrogen secretion, the relatively high concentration of dehydroepiandrosterone sulphate, the adrenal weight and the width of its fetal zone, the adrenal histology (Fig. 2)

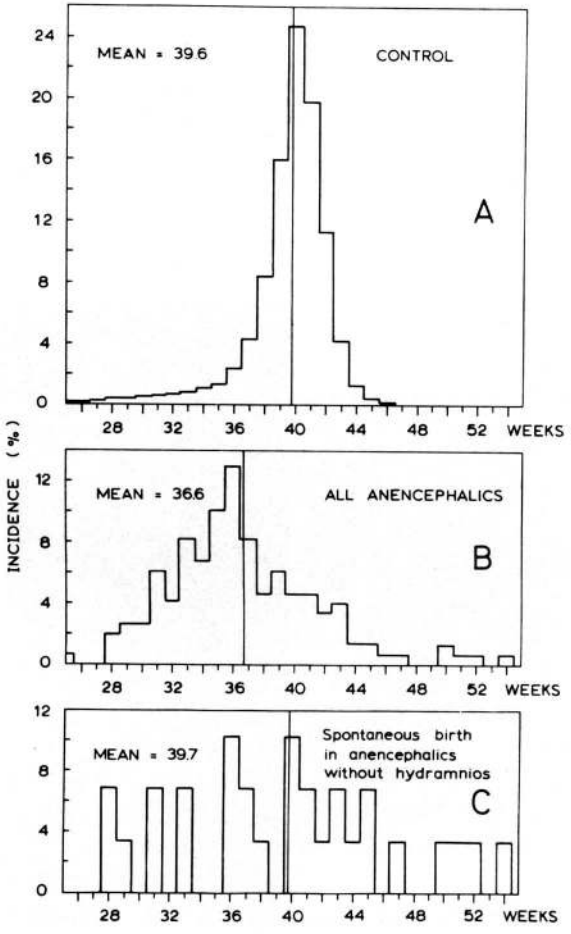

FIG. 3. Frequency distribution of gestation length: (A) for a control group of 49 996 pregnant women; (B) for mothers of all anencephalic fetuses ($n = 147$); (C) for mothers of anencephalic fetuses ($n = 29$) without hydramnios, omitting those who had stillborn fetuses with third-degree maceration, fetuses given intrauterine injections, or twins, and those in whom labour was induced. (Reproduced, with permission, from Honnebier & Swaab 1973, *J. Obstet. Gynaecol. Br. Commonw.*)

and histochemistry, and the low thymus weight (Honnebier *et al.* 1974, patient 3). Parturition did not occur before the end of the 42nd week, however, even though the membranes had ruptured nine days earlier (Fig. 1). Only after the 10th infusion of oxytocin into the mother was the fetus finally born. Another fetus injected three times with depot corticotropin was also delivered only after a maternal infusion of oxytocin (cf. Honnebier *et al.* 1974, patient 1).

Of course, such case histories never actually disprove the hypothesis that fetal posterior lobe hormones or corticotropin might normally initiate parturition. The lack of clear-cut and reproducible induction of parturition after injection of the hormone, however, brought us to reconsider the 'classical' view (e.g. Malpas 1933) of the relationship between anencephaly and prolonged gestation. We therefore decided to examine the records of 147 pregnancies with anencephaly and compare the data with a control group of about 50 000 pregnancies from the same clinic (Honnebier & Swaab 1973).

The mean gestation time in the control group was 39.6 weeks (Fig. 3*A*) but, in contradiction to our expectations, the mean gestation time in the anencephalic group was significantly shorter (36.6 weeks; Fig. 3*B*). This figure was undoubtedly influenced by the high frequency of hydramnios in anencephaly, and possibly also by maceration, fetuses given injections *in utero*, twins, and induced labour. When all such cases were ignored, the group of spontaneously born anencephalic fetuses had a mean gestation time (39.7 weeks; Fig. 3*C*) virtually identical to that of the control group. Although no apparent relationship between the absence of the fetal brain and prolonged pregnancy could be established, the distribution of the gestation lengths was certainly not similar

TABLE 1

Duration of the three stages of labour in a group of 29 anencephalic human fetuses delivered spontaneously and in which no hydramnios occurred. The 29 randomly chosen controls were matched for (*a*) period of birth [prematurity (< 38 weeks), postmaturity (> 42 weeks) or term (38–42 weeks)] and (*b*) parity (primi *vs* multi).

	Stage of labour			*Manual removal of the placenta (n)*
	I (h)	*II (min)*	*III (min)*	
Control fetuses	11.4 (1.51)	25.9 (4.08)	11.0 (1.36)	0
Anencephalic fetuses	14.3[a] (1.96)	48.1[b] (7.65)	32.5[c] (5.89)	3[d]

I, II, III: first, second and third stages of labour. [a-c] Student's *t* test: [a] $0.05 < P < 0.10$ (not significant); [b] $0.01 < P < 0.02$; [c] $P < 0.001$; [d] χ^2 test: $0.25 > P > 0.10$ (not significant).

to that of the control group. The proportion of both premature and postmature births was much larger than in the control group: 41.4% of the pregnancies were less than 38 weeks in length and 34.5% were more than 42 weeks, whereas in the control group these percentages were 12.6 and 6.2, respectively.

To see whether the fetal brain influenced the course of parturition, we compared the duration of the three stages of labour in mothers with anencephalic fetuses with that in appropriate controls (Table 1). The first stage of labour (from 4 cm up to full dilatation of the cervix) was not significantly prolonged in the anencephalic group. The second stage (expulsion), however, was nearly twice as long, and the third stage (time between birth of fetus and birth of the placenta) nearly three times as long, as in the normal population. Moreover, in the control group no manual removals of the placenta were recorded, whereas in the anencephalic group the 10% of manual removals of the placenta (because of retention, the third stage having lasted longer than 2 h) was significantly (χ^2 test: $P < 0.025$) above the mean in the university clinic (1.96% of the 8063 deliveries from 1970 up to and including 1974). Thus, observations in humans suggest that rather than the initiation it is the course of parturition that is influenced by the fetal brain.

THE FETAL BRAIN AND PARTURITION IN THE RAT

In order to see whether the findings on pregnancy length and on the course of parturition in the human anencephalic sample could be confirmed experimentally, a simple aspiration technique was developed for removing the fetal rat brain and pituitary *in utero* (Swaab & Honnebier 1973). The moment of birth of the first pup has to be considered as the time at which parturition is initiated in this kind of study, since no consistent relation could be observed between the parturient behaviour of the animal, namely the midbody peristaltic waves (Rosenblatt & Lehrman 1963), and the contractions as measured by intrauterine pressure-sensitive radio transmitters (Boer 1976). This means that changes in either the period of dilatation of the cervix or the expulsion time of the first fetus will influence the observed lengths of gestation. Pregnancy was not lengthened significantly in these brainless fetuses, however (Swaab & Honnebier 1973). On the other hand, the course of parturition was disturbed in some of the brain-aspirated animals (Swaab *et al.* 1973). Because this phenomenon agreed with the prolonged course of parturition in anencephalic human fetuses, and because our operating skill later improved, these experiments were repeated.

For this study 51 pregnant Wistar rats were allocated at random to three experimental groups. Three animals were discarded since they had litters of

fewer than seven pups (and would therefore have longer pregnancies: Boer 1976) and one animal died shortly after the brain aspiration operation. This resulted in (1) a control group ($n = 14$), (2) a sham-operated group ($n = 17$) and (3) a brain-aspirated group ($n = 16$). Brain aspiration and sham operation were performed on day 19 of pregnancy by the method of Swaab & Honnebier (1973). The animals were observed continuously from 10 a.m. on day 21. During the night (7 p.m.–7 a.m.) red light was used. All animals were in perfect condition.

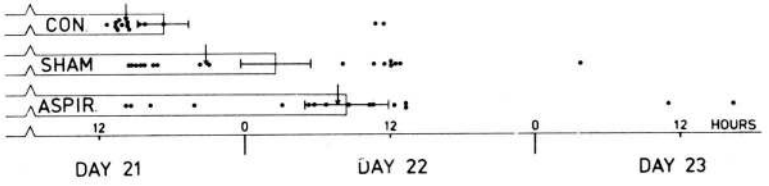

FIG. 4. Pregnancy length as determined by the moment of birth of the first pup (●) in unaffected controls (con.), sham-operated (sham) and brain-aspirated (aspir.) rats. Each point shows the length of pregnancy in one mother. The bars indicate the mean pregnancy length, the horizontal lines the standard error of the mean, and the arrows the median. According to the Mann-Whitney U-test, the pregnancy length of the control group is shorter than that of the sham-operated group and that of the brain-aspirated group ($P < 0.002$), while the difference between the sham-operated and the brain-aspirated group is not significant ($P = 0.43$).

As shown in Fig. 4, the surgery itself prolonged pregnancy. The groups with sham-operated and brain-aspirated fetuses both had longer pregnancies than did the unoperated controls. No significant difference was present between the moment of birth in the sham-operated and brain-aspirated group. However, the fact that in the latter group the median time is more than 10 h later and the mean almost 6 h later than in the sham-operated group might nevertheless point to some involvement of the fetal brain or pituitary in the initiation of parturition. This difference might, however, also be explained by other factors. Stretching of the uterus affects the induction of labour (Csapo & Wood 1969) and the decreased fetal body weight in the operated groups may therefore be of importance. In agreement with data from fetuses delivered by Caesarean section (Swaab & Honnebier 1973), the birth weight of live-born pups of the sham-operated group (5.15 g, S.E.M. $\pm$ 0.04, $n = 160$) was slightly lower (2%: Student's t test, $P < 0.01$) than that of the control group (5.27 g $\pm$ 0.03, $n = 144$), while the birth weight of the brain-aspirated fetuses (4.64 g $\pm$ 0.05, $n = 136$) was considerably less (10%) than that of the sham-operated pups ($P < 0.001$). The stillborn fetuses (brain aspiration: $n = 11$, mean weight 3.85 g, and sham operation: $n = 2$, mean weight 5.35 g) did not influence these

conclusions. The lower birth weight of brain-aspirated fetuses is probably due to there being no melanotropin in these fetuses (Honnebier & Swaab 1974; Swaab *et al.* 1976). A second factor that might lengthen pregnancy somewhat in the group with brain-aspirated fetuses is the protracted course of labour in these animals (see below).

The course of labour in the three groups was similar to that in preliminary observations. The group with brain-aspirated fetuses had a protracted course compared to the group with sham-operated fetuses, while the control group had an intermediate course (Fig. 5). This result shows again the influence of the fetal brain on the course of labour, but also indicates that the operation itself is influencing this process. The course of labour in the group with brain-aspirated fetuses is characterized by some extremely long inter-birth intervals (up to 208 min). In the group with brain-aspirated fetuses these long intervals between births were found only in animals with smaller litters (8–10 pups); the mean length of such intervals per litter was not related to litter size in either of the other groups.

From these observations on brain-aspirated rat fetuses it can be concluded, first, that the operation itself influences both length of pregnancy and course of labour. No significant prolongation of pregnancy was found, however, in mothers of brainless fetuses as compared to mothers of sham-operated pups, but the course of labour was clearly protracted. The fetal brain thus seems to be

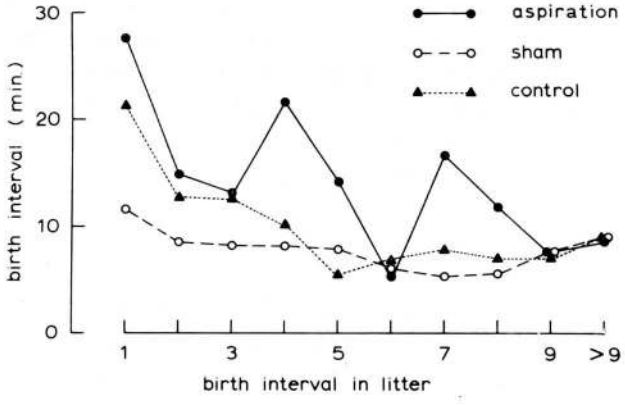

FIG. 5. Mean of each birth interval (in minutes) throughout the litter. Note the protracted course in brain-aspirated fetuses as compared to that in the sham-operated group, while the control group shows an intermediate course. The difference between the lines was tested, using Student's t test, by combining the t values of the birth intervals: the course in the brain-aspirated group was then shown to be different from that in the sham-operated group ($P < 0.01$), while the control group was not significantly different from either the sham-operated group ($0.05 < P < 0.10$) or the brain-aspirated group ($0.10 < P < 0.20$).

involved in the course of parturition rather than in its initiation, in the rat as in the human.

The data suggesting that the fetal pituitary might initiate labour (Liggins *et al.*, this volume) do not necessarily contradict the conclusion that the fetus may instead influence the course of labour. The apparently prolonged gestation of fetuses in which the hypothalamus and/or pituitary is damaged can also be explained by a greatly prolonged course of labour, with uterine contractions in such animals starting at the usual time. This possibility could be checked by measurement of uterine contractions in animals with hypothalamic or hypophysial lesions.

THE FETAL BRAIN AND THE COURSE OF LABOUR

Although a role of the fetal brain or pituitary in the onset of parturition cannot be altogether excluded by our observations in man and rat, such a role can only be of secondary importance. In both species, however, the integrity of the fetal brain appeared to be necessary for a normal course of delivery. The neurohypophysis was considered to be the probable area of importance in this respect. Extremely high concentrations of posterior lobe hormones, originating from the fetus itself, have indeed been found in human cord blood. Vasopressin concentrations were higher than oxytocin concentrations, and both hormones rose during labour (Chard 1973*a*). Anencephalic fetuses (which show a disturbed course of labour: Table 1) do not produce any posterior lobe hormones, as is clear from both the staining of neurosecretory material (e.g. Tuchmann-Duplessis & Gabe 1960; Honnebier 1974) and from hormone assays (Chard 1973*a*). Moreover, since administration of posterior lobe hormones from the fetal side induces uterine contractions (Honnebier *et al.* 1974; Nathanielsz *et al.* 1973), a role of these hormones in the course of labour seemed quite possible.

The concentrations of posterior lobe hormones in the fetal blood of the rat are not known. Although both hormones have been reported to be present in the pituitary before birth, more vasopressin would be present than oxytocin (Forsling 1973). Brain-aspirated fetuses, which of course lack these hormones, had protracted deliveries. A protracted course of labour was also observed in Brattleboro rats (Fig. 6). These animals are homozygous for a hypothalamic form of diabetes insipidus and are thus unable to synthesize vasopressin. It seemed difficult, however, to assign the fetal component for the course of labour to vasopressin alone, since this hormone could not induce any uterine contractions at term when given from the maternal side (Boer 1976). Although vasopressin is thought to potentiate the action of oxytocin on the uterus (Fuchs

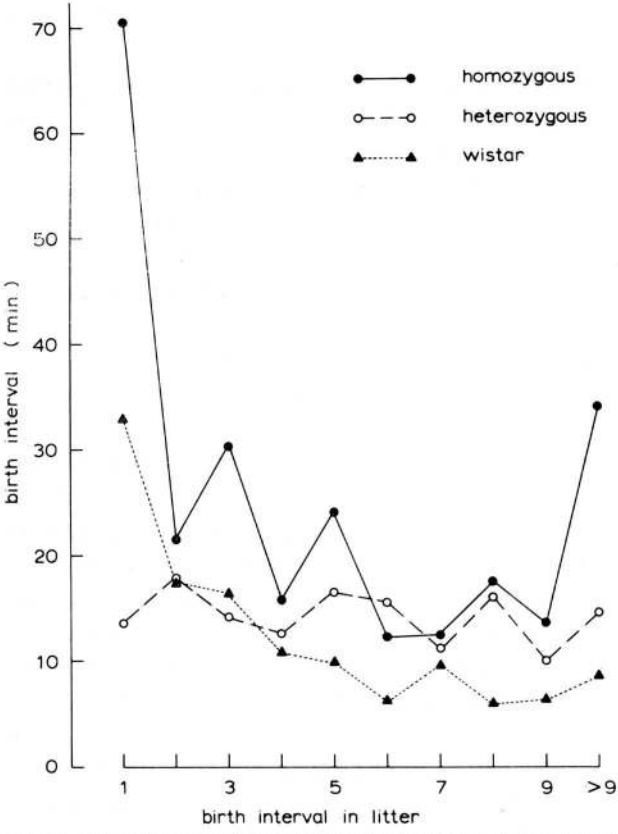

FIG. 6. Mean duration (in minutes) of each birth interval throughout the litter in Brattleboro rats either homozygous ($n = 4$) or heterozygous ($n = 6$) for diabetes insipidus, and in normal Wistar rats ($n = 8$) (for details of these groups see Boer *et al.* 1974). The difference between the lines was tested using Student's t test by combination of the t values of the birth intervals. In this way the time course in the homozygous group was shown to differ from both the heterozygous ($P < 0.01$) and the Wistar groups ($P < 0.001$). The course in heterozygous rats also differed significantly ($P < 0.01$) from that in Wistar rats. One homozygous rat failed to deliver her second pup within the following 24 h. Caesarean section at that time revealed only one more pup still living. Data from this animal were excluded from this paper.

1973), the idea about the role of fetal vasopressin or oxytocin had to be reconsidered when it appeared (see below) that neither of these hormones could be demonstrated in the fetal pituitary by immunofluorescence techniques.

IS FETAL VASOTOCIN INVOLVED IN PARTURITION?

The presence of neurohypophysial hormones in the fetal pituitary on the day of parturition was described by Forsling (1973), using radioimmunoassay. To

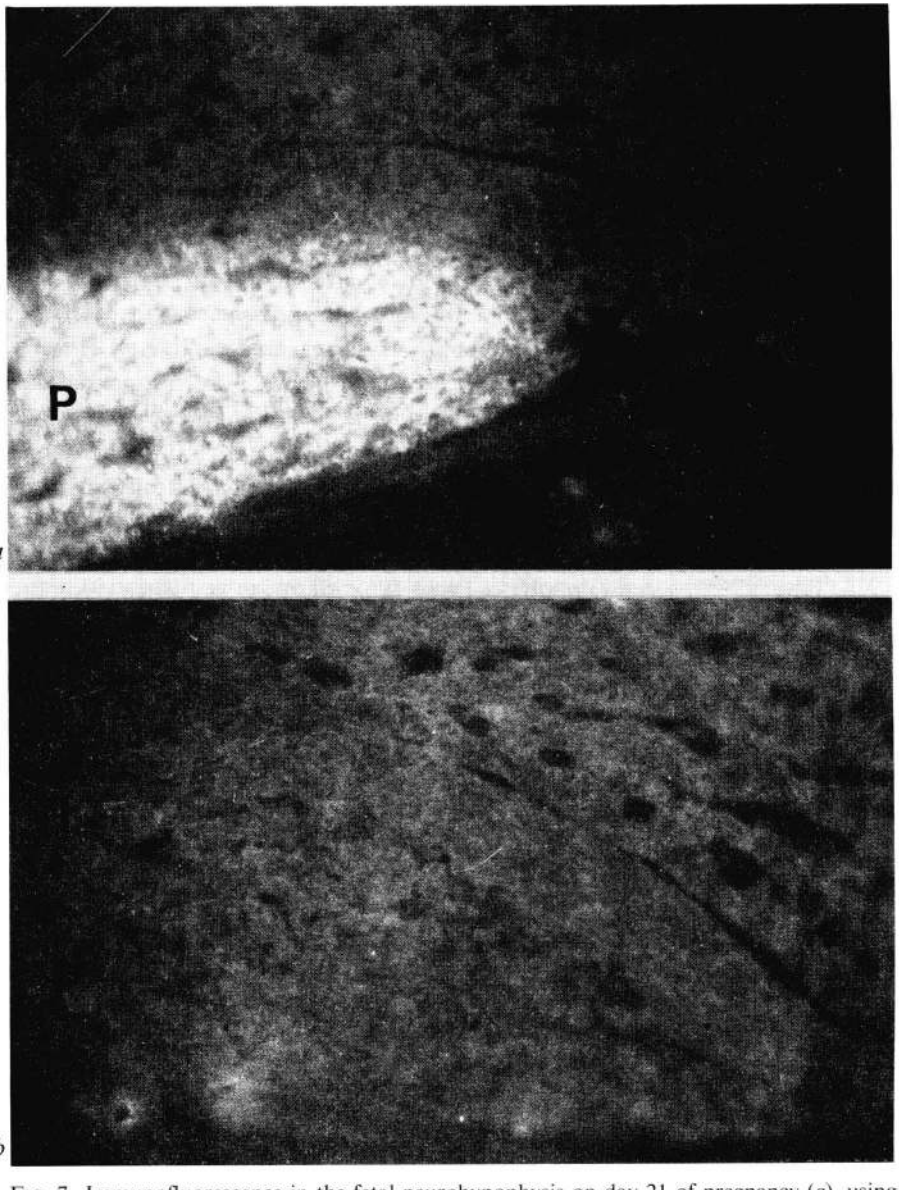

FIG. 7. Immunofluorescence in the fetal neurohypophysis on day 21 of pregnancy (*a*), using non-purified anti-vasopressin (antibody 126; Swaab & Pool 1975). This fluorescence disappeared completely after purification of anti-vasopressin on oxytocin beads (*b*). The same negative picture was observed after preincubation of anti-vasopressin with vasotocin beads or after incubation of the sections with anti-oxytocin. A = anterior lobe, I = intermediate lobe, and P = posterior lobe of the pituitary.

confirm these data we used the immunofluorescence method for localizing these hormones (for details of the technique see Swaab *et al.* 1975; Swaab & Pool 1975). Using non-purified anti-vasopressin, we saw a bright fluorescence in the fetal neurohypophysis (Fig. 7). Much to our surprise, however, no fluorescence could be found with either purified (i.e. after removal of the cross-reacting antibodies to vasopressin) or non-purified anti-oxytocin. This was an unexpected finding because, in the first place, Forsling (1973) found oxytocin in fetal pituitaries and, secondly, anti-oxytocin normally cross-reacts strongly with vasopressin (Swaab & Pool 1975). Moreover, after purification of anti-vasopressin (i.e. after removal of those antibodies that cross-react with oxytocin), the fluorescence in the fetal neurohypophysis disappeared completely (Fig. 7). We had to conclude, therefore, that not enough oxytocin or vasopressin was present in the fetal neurohypophysis near term. The compound that stained with non-purified anti-vasopressin would probably be a closely related compound. Such a compound might be arginine vasotocin. This neurohypophysial hormone of most non-mammalian vertebrates has been reported to be present also in the fetal pituitary of various mammals such as man (Skowsky & Fisher 1973; Pavel 1975), sheep (Vizsolyi & Perks 1969; Skowsky & Fisher 1973) and seal (Vizsolyi & Perks 1969). Structurally it differs by only one amino acid from both oxytocin and vasopressin, a fact which also explains why it can cross-react strongly with vasopressin in a radioimmunoassay (Chard 1973*b*). Therefore we tested the antibodies used for their cross-reactivity to synthetic vasotocin. This test was performed on vasotocin bound to agarose beads (cf. Swaab & Pool 1975), and anti-oxytocin (either purified or non-purified) indeed did not bind to vasotocin, whilst non-purified anti-vasopressin showed a strong binding that mostly disappeared after purification. The fluorescence seen in the fetal neurohypophysis with non-purified anti-vasopressin disappeared completely after removal of those antibodies that bound to vasotocin. For this removal step, non-purified anti-vasopressin was preincubated with vasotocin bound to agarose beads (cf. Swaab & Pool 1975). In addition, pituitaries of one-week-old chicks had staining capacities similar to those of rat fetal pituitaries. All of this makes it highly probable that vasotocin is present in the fetal rat neurohypophysis. In order to obtain definitive proof for this possibility, we are now preparing antibodies against arginine vasotocin, which will enable us to localize and assay this compound in fetuses and adults.

Our thesis that arginine vasotocin may well be the fetal factor that promotes the normal course of labour is supported by the demonstrated capacity of this compound to induce uterine contractions both *in vitro* (Berde & Boissonnas 1966) and *in vivo* (Fig. 8). In addition, arginine vasotocin (which is normally present in the pineal gland of adult rats) is not produced in homozygous

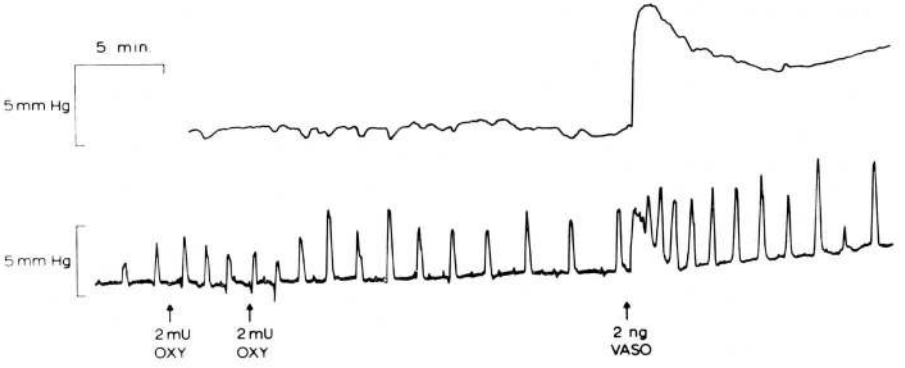

FIG. 8. Biological effect on the mammary gland (upper trace) and uterus (lower trace) of 2 ng vasotocin (VASO; Organon) as compared to the effect of 2 mU oxytocin (OXY; Syntocinon, Sandoz). Intramammary and intrauterine pressure were recorded with pressure-sensitive radio pills by the methods of Lincoln *et al.* (1974) and Boer *et al.* (1975), respectively. Note the absence of response with oxytocin and the strong response with vasotocin.

Brattleboro rats (Rosenbloom & Fisher 1975). This may explain their protracted course of labour, since in these animals we found no fluorescence with the various antibodies. The presence of still other, related, peptides in the fetal neurohypophysis of course remains possible. In general, the possibility that the fetus produces hormones that are different from those operating in the adult may have important implications for endocrinology.

ACKNOWLEDGEMENTS

We are greatly indebted to Mr B. Fisser for his valuable technical assistance and to all collaborators in the project 'Neuroendocrinology' for their help and critical remarks. The present study received financial support from the Netherlands Organization for the Advancement of Pure Research, Z.W.O., and from the Foundation, De Drie Lichten.

References

BERDE, B. & BOISSONNAS, R.A. (1966) Synthetic analogues and homologues of the posterior pituitary hormones, in *The Pituitary Gland* vol. 3 (Harris, G.W. & Donovan, B.T., eds.), pp. 624–661, Butterworths, London

BOER, K. (1976) *The Role of the Rat Hypothalamo-Neurohypophyseal System in the Onset or Maintenance of Parturition*, Med. Diss., University of Amsterdam

BOER, K., BOER, G.J. & SWAAB, D.F. (1974) Does the hypothalamo-neurohypophysial system play a role in gestation length or the course of parturition? in *Integrative Hypothalamic Activity* (Swaab, D.F. & Schadé, J.P., eds.) (*Prog. Brain Research*, vol. 41), pp. 307–319, Elsevier, Amsterdam

BOER, K., LINCOLN, D.W. & SWAAB, D.F. (1975) Effects of electrical stimulation of the neurohypophysis on labour in the rat. *J. Endocrinol. 65*, 163–176

CHARD, T. (1973*a*) The posterior pituitary and the induction of labour, in *Endocrine Factors in Labour* (Klopper, A. & Gardner, J., eds.) *(Mem. Soc. Endocrinol. 20)*, pp. 61–67, Cambridge University Press, London

CHARD, T. (1973*b*) The radioimmunoassay of oxytocin and vasopressin. *J. Endocrinol. 58*, 143–160

CHARD, T., SILMAN, R.E. & REES, L.H. (1977) The fetal hypothalamus and pituitary in the initiation of labour, in this volume, pp. 359–370

CSAPO, A.I. & WOOD, C. (1969) The endocrine control of the initiation of labour in the human, in *Recent Advances in Endocrinology* (James, V.H.T., ed.), pp. 207–234, Churchill, London

FORSLING, M.L. (1973) in *Endocrine Factors in Labour* (Klopper, A. & Gardner, J., eds.) *(Mem. Soc. Endocrinol. 20)*, p. 174 (figure), Cambridge University Press, London

FUCHS, A.-R. (1973) Parturition in rabbits and rats, in *Endocrine Factors in Labour* (Klopper, A. & Gardner, J., eds.) *(Mem. Soc. Endocrinol. 20)*, pp. 163–185, Cambridge University Press, London

HONNEBIER, W.J. (1974) *The Fetal Brain in Intrauterine Growth and Parturition, the Role of the Hypothalamo-Hypophysial Systems*, Med. Diss., University of Amsterdam

HONNEBIER, W.J. & SWAAB, D.F. (1973) The influence of anencephaly upon intrauterine growth of fetus and placenta and upon gestation length. *J. Obstet. Gynaecol. Br. Commonw. 80*, 577–588

HONNEBIER, W.J. & SWAAB, D.F. (1974) Influence of α-melanocyte-stimulating hormone (α-MSH), growth hormone (GH) and fetal brain extracts on intrauterine growth of fetus and placenta in the rat. *J. Obstet. Gynaecol. Br. Commonw. 81*, 439–447

HONNEBIER, W.J., JÖBSIS, A.C. & SWAAB, D.F. (1974) The effect of hypophysial hormones and human gonadotropin (HCG) on the anencephalic fetal adrenal cortex and on parturition in the human. *J. Obstet. Gynaecol. Br. Commonw. 81*, 423–438

KLOOSTERMAN, G.J. (1968) The obstetrician and dysmaturity, in *Aspects of Praematurity and Dysmaturity* (Jonxis, J.H.P., Visser, H.K.A. & Troelstra, J.A., eds.) *(Nutricia Symp.)*, pp. 263–280, Stenfert Kroese, Leiden

LIGGINS, G.C., FAIRCLOUGH, R.J., GRIEVES, S.A., FORSTER, C.S. & KNOX, B.S. (1977) Parturition in the sheep, in this volume, pp. 5–25

LINCOLN, D.W., BOER, K. & SWAAB, D.F. (1974) Measurement of intramammary pressure in rats by a pressure sensitive radio-pill. *Physiol. Behav. 12*, 289–292

MALPAS, P. (1933) Postmaturity and malformations of the foetus. *J. Obstet. Gynaecol. Br. Emp. 40*, 1046–1053

NATHANIELSZ, P.W., COMLINE, R.S. & SILVER, M. (1973) Uterine activity following intravenous administration of oxytocin to the foetal sheep. *Nature (Lond.) 243*, 471–472

PAVEL, S. (1975) Vasotocin biosynthesis by neurohypophysial cells from human fetuses: evidence for its ependymal origin. *Neuroendocrinology 19*, 150–159

ROSENBLATT, J.S. & LEHRMAN, D.S. (1963) Maternal behavior of the laboratory rat, in *Maternal Behavior in Mammals* (Rheingold, H.L., ed.), pp. 8–21, Wiley, New York

ROSENBLOOM, A.A. & FISHER, D.A. (1975) Radioimmunoassayable AVT and AVP in adult mammalian brain tissue: comparison of normal and Brattleboro rats. *Neuroendocrinology 17*, 354–361

SKOWSKY, R. & FISHER, D.A. (1973) Immunoreactive arginine vasopressin (AVP) and arginine vasotocin (AVT) in the fetal pituitary of man and sheep. *Clin. Res. 21*, 205

SWAAB, D.F. & HONNEBIER, W.J. (1973) The influence of removal of the fetal brain upon intrauterine growth of the fetus and the placenta and on gestation length. *J. Obstet. Gynaecol. Br. Commonw. 80*, 577–588

SWAAB, D.F. & POOL, C.W. (1975) Specificity of oxytocin and vasopressin immunofluorescence. *J. Endocrinol. 66*, 263–272

SWAAB, D.F., HONNEBIER, W.J. & BOER, K. (1973) The role of the foetal rat brain in labour. *J. Endocrinol. 57*, xxxi–xxxii

SWAAB, D.F., POOL, C.W. & NIJVELDT, F. (1975) Immunofluorescence of vasopressin and oxytocin in the rat hypothalamo-neurohypophyseal system. *J. Neural Transm. 36*, 195–215

SWAAB, D.F., VISSER, M. & TILDERS, F.J.H. (1976) Stimulation of intrauterine growth in rat by α-melanocyte-stimulating hormone. *J. Endocrinol. 70*, 445–455

THEOBALD, G.W. (1968) Oxytocin reassessed. *Obstet. Gynecol. Surv. 23*, 109–132

TUCHMANN-DUPLESSIS, H. & GABE, M. (1960) Absence de produit de neurosécrétion dans la post-hypophyse des anencéphales. *Bull. Acad. Natl. Méd. (Paris) 144*, 102–104

VAN DEVENTER, H. (1764) *Manuele Operatien, zijnde een Nieuw Ligt voor Vroedmeesters en Vroedvrouwen* [Manual operations, being a new method for man-midwives and midwives], 3rd edn., p. 223, J. en H. Verbeek, Leyden

VIZSOLYI, E. & PERKS, A.M. (1969) New neurohypophysial principle in foetal mammals. *Nature (Lond.) 223*, 1169–1171

Discussion

Chard: We have examined the same question in human cord blood at term. We used two systems of radioimmunoassay, with two antibodies to vasopressin, one of which cross-reacted with vasotocin and the other of which did not cross-react. The results were identical. One could therefore conclude that 99% of what we were measuring was arginine vasopressin. A late human fetus with vasotocin would be very interesting. One can easily picture a switch taking place.

Swaab: I would be more convinced if a specific arginine vasotocin radioimmunoassay was used. You could easily have missed considerable amounts of this hormone in umbilical cord blood, in the presence of such high concentrations of vasopressin and oxytocin as you reported.

Chard: In our experiments, the quantities would have had to be less than 1% of the amount of vasopressin there. Your results agree with the findings of Vizsolyi & Perks (1969) except that they were using some bizarre species (the seal).

Dawes: Vizsolyi & Perks (1969) first found arginine vasotocin in the sheep at about 85 days of gestation but there was less near term.

Csapo: What was the diameter of the needle you used for amniotomy, Dr Swaab? This might be critical in view of the leakage of amniotic fluid.

Swaab: The diameter we use for brain aspiration is 1.25 mm (Swaab & Honnebier 1973). The leakage of amniotic fluid is, however, not important for our results. In the first place the hole cannot be found again 24 h later. In the second place the same hole is made during the sham operation procedure, so that the same amount of amniotic fluid will leak out in the experimental and the control groups.

Nathanielsz: Dr Swaab, you used the term 'pure anencephalic' but it has

been questioned whether the pure anencephalic actually exists. I realize that we are discussing the end-point of oligohydramnios but it is relevant that if we take various endocrinological end-points there are considerable differences in the umbilical cord concentrations of somatotropin and other hormones in human anencephalics. In addition, there is a difference in their responsiveness to glucose loading and other types of intervention in the few hours they live (Grunt & Reynolds 1970). So I think it is dangerous to talk about pure anencephalics in the human clinical situation.

Swaab: By 'pure' anencephalics I meant those anencephalics that did not have hydramnios, a complication which shortens pregnancy (Honnebier & Swaab 1973). I am aware of course that every anencephalic has a different amount of hypothalamic tissue left and so differs in the degree to which function of the pituitary is retained. This is one reason why we compare all our data on humans to the rat model, where we are sure that all brain tissue and the entire pituitary are removed.

Anderson: We have a few results on cortisol concentrations in cord blood in human anencephaly (M. J. Cawson, A. B. M. Anderson & A. C. Turnbull, unpublished work) which bear out the point that there is a wide range of values. Both cortisol and cortisone were assayed by competitive protein-binding techniques after column chromatography with Sephadex LH-20. Cord cortisol concentrations ranged from 36 ng/ml, which is approaching the lower values we get at elective Caesarean section with a normal fetus, up to more than 200 ng/ml, the highest values found after normal spontaneous labour. In all these anencephalic pregnancies, labour was induced and delivery occurred vaginally. The cortisone concentrations in cord blood were extremely high, perhaps indicative of high maternal cortisol values. We find the same wide range of cortisol concentrations in the amniotic fluid with anencephaly. It seems that any concentration of cortisol is possible with this deformity. These were mixed cord blood samples but would be mainly umbilical venous blood.

Korenman: Where is the cortisone coming from?

Anderson: The cortisone in the fetus is probably a reflection of maternal cortisol which is converted to cortisone by the very active 11β-hydroxysteroid dehydrogenase (EC 1.1.1.146) in the human placenta.

Swaab: Our few measurements of corticosteroids in blood showed comparable concentrations in anencephalic umbilical cord blood and maternal blood. The corticosteroid concentration in cord blood is probably not a reliable index of fetal adrenal function but also reflects increased activity of the maternal adrenal, caused for example by vaginal delivery (Honnebier *et al.* 1974).

Csapo: The litter size is critical in the timing of the onset of labour, as we learned from studies in the rabbit (Csapo 1969), the rat (Csapo 1967; Csapo

et al. 1973*a*) and the human (Csapo & Wood 1968). To obtain a sharp Gaussian distribution curve for labour in a group of rats, we had to eliminate those with less than 6 and more than 12 fetuses (Csapo 1967; Csapo *et al.* 1973).

Swaab: We don't open them up beforehand but during the brain aspiration or sham operation we count the fetuses and discard litters which are too small, i.e. less than seven pups. In addition no litters larger than 12 were present in either the sham-operated control or the brain-aspirated group, and the mean litter sizes in the two groups did not differ significantly. So the difference in the course of labour after removal of the fetal brain is not due to differences in litter size.

Fitzpatrick: A switch such as Dr Chard mentioned must occur, because the neonatal rat has normal vasopressin production. Do you know much about the mechanism which operates the switch from arginine vasotocin to arginine vasopressin, Dr Swaab?

Swaab: From immunofluorescence microscopy it appears that at least the largest part of the fetal supraoptic and paraventricular cells contain arginine vasotocin. So it is not the glial elements that produce arginine vasotocin and the neurons that produce vasopressin or oxytocin, as is supposed by Pavel (1975), but all three hormones are synthesized in the neurons of the hypothalamo-neurohypophysial system. The observation of Skowsky & Fisher (1973) that the human fetal hypophysial content of arginine vasotocin decreases throughout gestation, while that of vasopressin increases, suggests indeed a switch of the developing neuroendocrine cell from the production of one hormone to the other. As yet, however, we have no idea exactly when or by what mechanism the neuron is switching from vasotocin to vasopressin or oxytocin production.

Fitzpatrick: It is very important because the species-specificity for vasotocin in the adult is so clear-cut. There must be something very acute and precise about this.

Korenman: This is surely the biochemical version of ontogeny recapitulating phylogeny.

Novy: Would you be willing to say that corticotropin is a sufficient tropic hormone for the fetal adrenal zone, on the basis of your findings with corticotropin injections into human anencephalic fetuses?

Swaab: We gave corticotropin depot injections directly into a human anencephalic fetus over a period of more than six weeks. This resulted in an increase in oestriol concentrations, a relatively high concentration of dehydroepiandrosterone sulphate in cord blood, and a fetal adrenal that was intermediate in size between that of normal children and that of untreated anencephalics. It is always difficult to make a firm conclusion from such a case history, but one could conclude that corticotropin is capable of stimulating fetal adrenal growth.

However, since enormous amounts of corticotropin were used and there was a relatively moderate increase in adrenal size, one or more other adrenotropic factors may be responsible for normal adrenal stimulation in the fetus. We believe that somatotropin or melanotropin might be such additional adrenotropic factors (Honnebier *et al.* 1974).

Kittinger: In chronically hypophysectomized adult rhesus monkeys we tried to regenerate the adrenal glands by daily injections of corticotropin and succeeded in doing this. We measured the cortisol concentrations from the time the injections began. It took a surprisingly long time before the adrenals became functional—a week or ten days (G.W. Kittinger, unpublished work).

Swaab: The anencephalic fetus I was referring to was treated with huge doses of depot corticotropin for more than six weeks (Honnebier *et al.* 1974).

Turnbull: It is evident that something else is required to mature the fetal adrenal in corticotropin-injected human anencephalics. In sheep, Bassett & Thorburn (1973) have shown that prolonged intravenous infusion of corticotropin into the fetus *in utero* produced a rise in plasma corticosteroids which was similar in magnitude and timing whether the fetus was intact, hypophysectomized or stalk-sectioned.

In the human we found that cortisol in amniotic fluid, which may represent fetal adrenal activity, rises over a six-week period from about 34 weeks onwards. Six weeks might be the preparation time that is required. We just do not know.

Anderson: Prostaglandin E was reported as being absent from a sample of amniotic fluid taken during advanced labour in a patient with an anencephalic fetus (Keirse & Turnbull 1973) and before labour the concentrations of PGF appear to be much lower in amniotic fluid with anencephaly than where the fetus is normal (M.D. Mitchell, unpublished work). These findings raise the possibility that an intact fetal hypothalamo–pituitary–adrenal axis may be necessary for the synthesis and/or release of prostaglandins in human pregnancy.

Dawes: The question was raised earlier of whether, under extreme circumstances, sufficient prostaglandin was released for it to act not only as a local but also as a general hormone. Is there the possibility of positive feedback?

Swaab: The only information I know of on the influence of prostaglandins on the pituitary concerns their effect on the adult hypothalamo–neurohypophysial system (Singh & Sebuwufu 1973; Vilhardt & Hedquist 1970). I am not aware of any data on the effect of prostaglandins on the fetal pituitary.

Turnbull: Have increased concentrations of PGE been measured in the circulation of the human fetus?

Challis: I don't think they have. In patients in active labour the cord plasma concentrations of PGF increase to above the concentrations in patients at elective section.

Chard: With pharmacological infusion of prostaglandin, for example, there is release of oxytocin, but the relevance of that to physiology is uncertain.

Kittinger: Dr Anderson, have you looked at the histology of the adrenals in anencephalics?

Anderson: Not in any detail.

Kittinger: The cortisol : cortisone ratio in anencephalics suggests to me that the cortisone is of maternal origin. The placenta is extremely active in the conversion of cortisol to cortisone, whereas the liver of the fetus has some activity but nowhere near as much as the placenta. So apparently this steroid has been processed by the placenta.

Anderson: We have some unpublished evidence that the anencephalic placenta has an active 11β-hydroxysteroid dehydrogenase, at least *in vitro*, and so would be able to convert maternal cortisol to cortisone, just as in normal placental tissue. I agree that the cortisone in anencephalic cord plasma may well be largely of maternal origin.

Thorburn: Cortisol concentrations in cord blood in cases with congenital adrenal aplasia have been reported (Pakravan *et al.* 1974) and were found to be quite significant. Were they in the normal range?

Challis: They were the sort of concentrations that one should get from the kinetics data on how much maternal cortisol is transferred across the placenta unchanged.

Nathanielsz: Dr Anderson, you mentioned the 11β-hydroxysteroid dehydrogenase in the anencephalic placenta. Have any other aspects of biochemical function of the anencephalic placenta been studied? Are there any marked changes in ultrastructure?

Anderson: Not that I know of. We only looked for some of the steroid-metabolizing enzymes and in that respect the anencephalic placenta appears to possess *in vitro* the same enzymes as a normal placenta.

Swaab: The wet weight of anencephalic placentas is less than in normal fetuses of similar ages (Honnebier & Swaab 1973). The same decrease in placental weight was found in rat fetuses which were made anencephalic (Swaab & Honnebier 1973). Placental weight was increased by injecting rat somatotropin or α-melanotropin into these brain-aspirated fetuses (Honnebier & Swaab 1974).

Dawes: This raises the general question of whether the development of the placenta is also under the control of the fetal pituitary.

Novy: In experimental anencephaly of rhesus monkey fetuses, the raw weights of the placenta and membranes do not differ from those of normal controls. There is frequently a lot of oedema in the membranes and in the cord itself. If one removes the membranes and the cord and weighs only the placental

tissue, it is possible to demonstrate that placentas are significantly smaller in anencephalic fetuses.

Swaab: The placentas I referred to were measured without membranes or umbilical cord, both in humans and in rats.

Kittinger: Some of our data suggest that steroidogenesis in the experimental anencephalic placenta is affected. The concentration of oestradiol in the plasma of the mothers, which is presumably of placental origin, is decreased, as is progesterone production in the *in vitro* work I reported earlier (this volume, pp. 235–249).

Heap: Have you any information about enzymic activities of the corpora lutea towards the end of pregnancy in your experimental animals with decapitated fetuses? We discussed earlier (see Heap *et al.*, this volume, p. 134) the induction of the 20α-hydroxysteroid dehydrogenase (EC 1.1.1.149) in the corpus luteum of the rat by fetoplacental removal.

Swaab: We did not look at that.

Turnbull: In anencephalic pregnancies without hydramnios we found that many were prolonged, but not all (Anderson *et al.* 1969). We only had eight cases in 10 years. When we looked at the histology, those delivered at term had small adrenal glands, weighing about 2 g instead of the normal 8–12 g. Those delivered at six weeks past term had adrenals weighing 0.2 g with virtually no fetal zone present, whereas the fetuses delivered at term had at least some fetal zone. There were also differences in things like the weight of the thymus, the gland being small (7–11 g) in anencephalics delivered at term but huge (up to 33 g) where the pregnancy was prolonged, implying that the prolongation of pregnancy was associated with much less fetal pituitary–adrenal activity than at term. In your human anencephalics, Dr Swaab, was there evidence of a gradient between those delivered before term and those delivered after term?

Swaab: We have no such evidence. We collected the adrenals but we saw no consistent trend.

Gennser: Would you say that the difference between the adrenals was a cause or an effect of the prolongation of pregnancy?

Turnbull: It is very difficult to know. In prolonged human pregnancy there are suggestions of less cortisol in amniotic fluid, lower adrenal weights, and so on. But I would not know whether that means the fetal adrenal was taking longer to reach the norm in prolonged pregnancy, or whether, having reached the norm, it then regressed. My hunch would be that it was developing more slowly.

Swaab: Jost *et al.* (1970) did not find any change in adrenal weights of anencephalics during the course of gestation. In addition, in our group of anencephalics with no hydramnios, all fetal adrenals were hypoplastic, and no

relationship between the size of the adrenal cortex and gestation length could be demonstrated (Honnebier & Swaab 1973).

Turnbull: I have unpublished evidence that past term there are quite significant reductions in fetal adrenal weight. I would have thought it was not established that there was no change in weight.

Korenman: I assume that those fetuses were damaged in some way?

Turnbull: A lot of attention has to be given to the circumstances in which they are collected. The series I am quoting were collected by my wife about 1952 (E.P.N.Turnbull, unpublished work). They were from babies who died suddenly in labour, which used to occur more often than it does now.

Novy: Laverty *et al.* (1973) reported 11 cases of congenital adrenal hypoplasia. Delivery of the fetus was either at term or premature, but severe pre-eclampsia was present in 9 of the 11 mothers.

Chez: Do you have more information about the subcategory of anencephaly with hydramnios, Dr Swaab? What was the mean delivery time and the range for that time in this group? It would be interesting to have an explanation for the aetiology of hydramnios, and for why the presence of hydramnios does not result in premature labour. The physical act of swallowing seems incomplete as an explanation and I wonder if an endocrinopathy is involved.

Swaab: The mean gestation length for the human anencephalic group with slight hydramnios was 34.9 weeks and that with severe hydramnios was 36.6 weeks, while that of anencephalics without hydramnios was 39.7 weeks, which was similar to that of the control group (39.6 weeks). We omitted the groups with hydramnios because we thought then that the Liggins' hypothesis on the important role of the fetus in the onset of labour, as was shown in sheep, would also hold true for human pregnancy, and we were afraid that prolonged pregnancy in anencephalics would be obscured by the cases with hydramnios. But the mean gestation length appeared not to be prolonged in anencephaly, even after omission of the hydramnios groups. What was different from the control group, however, was the large percentage of anencephalics born premature and postmature. So the exact timing of the initiation of labour appeared to be lost in anencephalics (Honnebier & Swaab 1973).

References

ANDERSON, A.B.M., LAURENCE, K.M. & TURNBULL, A.C. (1969) The relationship in anencephaly between the size of the adrenal cortex and the length of gestation. *J. Obstet. Gynaecol. Br. Commonw. 76*, 196–199

BASSETT, J.M. & THORBURN, G.D. (1973) Circulating levels of progesterone and corticosteroids in the pregnant ewe and its fetus, in *The Endocrinology of Pregnancy and Parturition* (Pierrepoint, C.G., ed.), pp. 126–140, Alpha Omega Alpha Publishing, Cardiff

CSAPO, A.I. (1967) The 'ovariectomy syndrome' and the initiation of labor, in *International Symposium on Prenatal Life: Biological and Clinical Perspectives*, Thomas, Detroit

CSAPO, A.I. (1969) The four direct regulatory factors of myometrial function, in *Progesterone: its regulatory effect on the myometrium (Ciba Found. Study Group No. 34)*, pp. 13–55, Churchill, London

CSAPO, A.I. & WOOD, C. (1968) The endocrine control of the initiation of labor in the human, in *Recent Advances in Endocrinology* (James, V.H.T., ed.), pp. 207–239, Churchill, London

CSAPO, A.I., CSAPO, E.F., FAY, E., HENZL, M.R. & SALAU, G. (1973) The delay of spontaneous labor by naproxen in the rat model. *Prostaglandins 3*, 827–837

GRUNT, J.A. & REYNOLDS, D.W. (1970) Insulin, blood sugar and growth hormone levels in an anencephalic infant before and after intravenous administration of glucose. *Pediatrics 76*, 112–116

HONNEBIER, W.J. & SWAAB, D.F. (1973) The influence of anencephaly upon intrauterine growth of fetus and placenta and upon gestation length. *J. Obstet. Gynaecol. Br. Commonw. 80*, 577–588

HONNEBIER, W.J. & SWAAB, D.F. (1974) Influence of α-melanocyte-stimulating hormone (α-MSH), growth hormone (GH) and fetal brain extracts on intrauterine growth of fetus and placenta in the rat. *J. Obstet. Gynaecol. Br. Commonw. 81*, 439–447

HONNEBIER, W.J., JÖBSIS, A.C. & SWAAB, D.F. (1974) The effect of hypophysial hormones and human chorionic gonadotrophin (HCG) on the anencephalic fetal adrenal cortex and on parturition in the human. *J. Obstet. Gynaecol. Br. Commonw. 81*, 423–438

JOST, A., DUPOUY, J.P. & GELOSO-MEYER, A. (1970) Hypothalamo-hypophyseal relationships in the fetus, in *The Hypothalamus* (Martini, L., Motta, M. & Fraschini, F., eds.), pp. 605–615, Academic Press, New York

KEIRSE, M.J.N.C. & TURNBULL, A.C. (1973) E prostaglandins in amniotic fluid during late pregnancy and labour. *J. Obstet. Gynaecol. Br. Commonw. 80*, 970–973

LAVERTY, C.R.A., FORTUNE, D.W. & BEISCHER, N.A. (1973) Congenital idiopathic adrenal hypoplasia. *Obstet. Gynecol. 41*, 655–664

PAKRAVAN, P., KENNY, F.M., DEPP, R. & ALLEN, A.C. (1974) Familial congenital absence of adrenal glands: evaluation of glucocorticoid, mineralocorticoid and estrogen metabolism in the perinatal period. *J. Pediatr. 84*, 74–78

PAVEL, S. (1975) Vasotocin biosynthesis by neurohypophysial cells from human fetuses. Evidence for its ependymal origin. *Neuroendocrinology 19*, 150–159

SINGH, U. & SEBUWUFU, P.H.S. (1973) Prostaglandins and neurosecretion: a histochemical study. *E. Afr. Med. J. 50*, 199–206

SKOWSKY, W.R. & FISHER, D.A. (1973) Intermittent idiopathic, inappropriate vasopressin secretion in a child. *J. Pediatr. 83*, 62–68

SWAAB, D.F. & HONNEBIER, W.J. (1973) The influence of removal of the fetal rat brain upon intrauterine growth of the fetus and the placenta and on gestation length. *J. Obstet. Gynaecol. Br. Commonw. 80*, 589–597

VILHARDT, H. & HEDQVIST, P. (1970) A possible role of prostaglandin E_2 in the regulation of vasopressin secretion in rats. *Life Sci. 9*, 825–830

VIZSOLYI, E. & PERKS, A.M. (1969) New neurohypophysial principle in fetal mammals. *Nature (Lond.) 223*, 1169–1171