THE INFLUENCE OF REMOVAL OF THE FETAL RAT BRAIN UPON INTRAUTERINE GROWTH OF THE FETUS AND THE PLACENTA AND ON GESTATION LENGTH

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Summary

In order to study the influence of the fetal rat brain on the intrauterine growth of fetus and placenta, fetal and placental weights were first determined in control rats from day 17 up to day 21 of pregnancy; fetal weight appeared to increase in nearly linear fashion between days 17 and 21. Although placental weight increased steadily up to day 21, this increase was very small during the last days of pregnancy. Subsequently a simple technique was developed to aspirate the fetal rat brain in utero.

A remarkable reduction of fetal growth was found on day 21 after fetal brain aspiration on day 18 or 19 of pregnancy. Placental weight appeared to remain static or even decreased after fetal brain aspiration on day 18 or day 19. The placental index increased after fetal brain aspiration. These effects, which appeared not to be caused by fetal blood loss, were similar to findings in pregnancies complicated by anencephalic infant.

Gestation length was not prolonged in rats treated by fetal brain aspiration, and the contraction phase started even earlier in rats who had fetal brain aspiration on day 19. Expulsion of the first fetus took place after the same gestation length in unaffected controls, sham-operated rats and rats who had fetal brain aspiration.

Fetal factors, in addition to maternal and environmental factors, seem to influence the intrauterine growth of the fetus and the placenta (for reviews see Thomson et al., 1968; Kloosterman, 1969). Observations on pregnancies complicated by anencephaly suggest that the fetal brain exerts an important influence upon the intrauterine growth of both fetus and placenta (Honnebier and Swaab, 1973). However, such studies have serious disadvantages because the "normal" intrauterine growth curves which are used for comparisons and statistical analyses are derived from unphysiological material (Kloosterman, 1970).

We describe a simple procedure for removal of the fetal rat brain in utero, and report on the effect of removal of the fetal rat brain upon the intrauterine growth of both fetus and placenta and upon gestation length.

MATERIALS AND METHODS

General procedure

Virgin female Wistar rats (T.N.O., in Zeist, Holland), weighing approximately 180 g. each
were used for this study. They were housed at 25 °C. and exposed to 12 hours of light daily (from 0700 to 1900 hours). The animals received tap water and standard rat pellets (Hope Farms) ad libitum. They were mated overnight with male rats of the same strain. The day on which spermatozoa were found in the vaginal smear was taken as day zero of pregnancy. Under our laboratory conditions delivery usually takes place late on day 21 to early on day 22 (Swaab and Veerkamp, unpublished data). The operations (carried out on day 18 or 19 of pregnancy) as well as the observations (made on day 21 of pregnancy) were performed between 0900 and 1100 hours under Hypnorm (Philips-Duphar) anaesthesia (0.05 to 0.08 ml. being given by intramuscular injection to each rat). This is a neuroleptic analgesic, containing fluanisone and the analgesic phentanylcitrate. The animals were chosen at random for the various experimental groups and were kept in individual cages after operation.

Living fetuses and their placentae were weighed with a sensitivity of 0.01 g. immediately after removal from the uterus. Fetal membranes and the umbilical cord were trimmed before the weighing. Except in experiment I (see Table II), each fetus was weighed again after emptying by suction a previously aspirated or intact cranial cavity. This was done in order to correct fetal weights for differences in cranial filling in sham-operated and brain aspirated fetuses. The weight values thus obtained are referred to as "corrected".

Normal intrauterine growth of fetus and placenta

The fetuses and placentae were weighed from day 17 to day 21 of pregnancy using five pregnant rats per pregnancy day.

Suction procedure

Fetal brains were removed by aspiration on day 18 or day 19 of pregnancy. To do this, a ventral midline incision was made in the abdomen of the pregnant rat, and both uterine horns were exposed. The brain of each fetus in the litter was removed by suction using a 1.25 × 38 mm. needle stabbed through the abplacental surface of the translucent uterine wall and the fetal membranes, and penetrating the fetal skull. Suction was achieved by a water-jet pump. The muscles and skin of the maternal abdominal wall were sutured separately. In sham operation on control animals the same procedure was used but the needle was only allowed to touch the fetal skulls. The uterine wall was not sutured. Before removing the fetuses from the uterus, the gestation sacs were examined for hydramnios.

Other operative procedures

In one group of fetuses, immediately after aspiration of the brains, the cranial cavity was filled via the same needle (measuring 1.10 × 38 mm.) with silicone rubber (Microfil, Canton Bio-Medical Products, Inc.). The brains of another group of fetuses were electrocoagulated (high frequency coagulator, ENCAR, Delft, Holland) using a 0.9 mm. copper wire that was insulated except at its sharpened tip. Sham operations were performed by merely touching the fetal skulls with the copper wire.

Gestation length

The events of parturition (Rosenblatt and Lehrman, 1963) were observed continually from 0900 hours on day 20 of pregnancy, using constant light. Animals used for this experiment were eight controls, nine rats who had fetal brain aspiration on day 18, six rats sham-operated on day 18, six rats who had fetal brain aspiration on day 19 and six rats who were sham-operated on day 19. In these animals the start of the contraction phase (Rosenblatt and Lehrman, 1963) and the moment of expulsion of the first fetus were noted.

Statistics

The differences between the various groups were tested by Student’s t test (De Jonge, 1963) and P<0.05 was considered to be statistically significant.

RESULTS

Normal intrauterine growth of fetus and placenta (Table I; Fig. 1)

All fetuses examined had survived until delivery.

A significant (p<0.001) daily increase in corrected and uncorrected fetal weight was observed between day 17 and day 21 of pregnancy.
A significant daily rise in placental weight was only seen between days 18 and 19 (p<0·001). The increases in placental weight between days 19 and 20 and days 20 and 21 were not significant (0·10<p<0·20 and 0·40<p<0·50 respectively). However, the weight increase between days 19 and 21 was significant (0·025<p<0·05). The placental index (which is placental weight divided by fetal weight) showed a daily decrease (p<0·001) from day 17 to day 21 inclusive.

Suction procedure (Table II; Figs. 1, 2 and 3)
Within one day of operation the pregnant rats were again in excellent condition. At laparotomy on day 21, the site of needle puncture in the uterine wall could not be discerned. No hydramnios was noted, only minimal amounts of amniotic fluid invariably being present on day 21. The brainless fetuses were obviously smaller than the sham operated controls, and had a cranial depression (Fig. 2). No other difference in the shape of the operated embryos could be observed macroscopically. Previous observations on brain-aspirated animals had shown that the cerebral hemispheres, hypothalamus and hypophysis cerebri always had been completely removed (Fig. 3). However, parts of the cerebellum and brain stem were sometimes present. The cranial cavity of the brain-aspirated fetuses usually contained blood-stained fluid.

The changes observed after removal of the fetal brain on day 18 or day 19 are given in Figure 1 and Table II. The decreases in fetal and placental weight and increases in placental index in the three experiments appeared to be highly significant (p<0·001), even after correction for differences in cranial filling (see Table II, corrected group). The average weights of the contents of the fetal cranial cavity in animals which were sham operated in day 18 or day 19 were 0·28 g. (SEM = 0·008) and 0·30 g. (SEM = 0·005) respectively, while it was 0·14 g. (SEM = 0·007) and 0·13 g. (SEM = 0·008) in fetuses who had brain aspiration on day 18 or day 19 respectively. The corrected body weight of brainless fetuses was 36·1 per cent less than in sham-operated fetuses. The weight increase between days 19 and 21 was 60 per cent less after brain aspiration.

After removal of the fetal brain on day 18, the placental weight on day 21 was at the same level as on day 18 (0·70<p<0·80). When the brain was removed on day 19 the placental weight had decreased by day 21 (p<0·001).

A significant increase (p<0·001) in placental index was observed after brain aspiration on days 18 or 19, even after correction for differences in cranial filling (Table II).

Other operative procedures (Table III)
The lesions caused by electrocoagulation ranged from small ones in the basal brain area to damage of the skin of the entire fetal head. However, no blood was seen in the cranial cavity or other parts of the head. The fetal and placental weights and placental indices are given in Table III. After electrocoagulation of the
brain, a highly significant (p<0.001) decrease in fetal and placental weight and an increase in placental indices were observed, even after correction for cranial filling.

In experiments in which the cranial contents were replaced by an injection of silicone rubber immediately after brain aspiration, five out of six pregnant rats died between day 19 and day 21. The results obtained in the four surviving fetuses of the only surviving mother are given in Table III, but are, of course, not capable of statistical analysis.

![Graphs showing fetal weight, placental weight, and placental index](image)

**Fig. 1**
Fetal weight, placental weight and placenta index of normal rats (●), rats who were sham operated on day 18 (■) or day 19 (▲), and rats who had a fetal brain aspiration on day 18 (□) or day 19 (△). The broken lines are drawn between the day of operation and the values observed. The graphs on the left show results based on the actual birthweight of the fetus. Those on the right (E) are based on fetal birthweight after aspiration of cranial cavity contents and are essentially similar.
**Gestation length (Fig. 4)**

The time of onset of the contraction phase in the controls was not significantly different from that of the group of animals who had a sham operation on day 18 (0·20 < p < 0·50) or day 19 (p > 0·50). No difference was found between the time of onset of the contraction phase in animals who had fetal brain aspiration or sham operation on day 18 (p > 0·50). However, the contraction phase of animals who had fetal brain aspiration on day 19 started significantly earlier (0·05 < p < 0·02) than it did in sham operated controls.

The moments of expulsion of the first fetus in controls and in sham-operated animals were similar (p > 0·50) and there was no difference in the times at which the first fetus was expelled after sham operation and after brain aspiration (0·20 < p < 0·50).

**DISCUSSION**

Although a strong positive relation seems to exist between placental and fetal weights (for reviews see Solomon and Friesen, 1968; Dawes, 1968), the growth curves of the placenta and of the fetus do not run parallel during late pregnancy (Kloosterman, 1970). In our studies, fetal weight increased progressively between days 17 and 21, showing an acceleration between days 19 and 20. The same acceleration has been reported by Csapo (1969). Although the increase was small during the last days of pregnancy, placental weight continued to increase steadily. We were thus unable to confirm other observations (for review see Kloosterman, 1970) that there was a complete arrest in placental growth during the final period of rat pregnancy. The general pattern of fetal and placental weight gain and decrease of placental index are, however, comparable to findings in late human pregnancy (Kloosterman, 1970).

The brain aspiration technique has certain advantages over the fetal decapitation technique used by Jost (1966) and by Heggestad and Wells (1965). With a litter of ten fetuses the whole procedure can be completed within 20 minutes. The technique seems less traumatic both for the uterine wall and for the fetus. Oedema or malformations of the fetus, which have been reported following decapitation (Jost, 1954), were never seen after brain aspiration and most fetuses survived for a few minutes after birth (Swaab et al., 1973). A final advantage of the study of a litter in which all fetuses are brainless is that this probably excludes the

**Table II**

<table>
<thead>
<tr>
<th>Day of pregnancy for</th>
<th>Number of living</th>
<th>Uncorrected for cranial filling</th>
<th>Corrected for cranial filling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>operation</td>
<td>observation</td>
<td>mothers</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I*</td>
<td>Sham</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Aspiration</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>II</td>
<td>Sham</td>
<td>19</td>
<td>21</td>
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<tr>
<td></td>
<td>Aspiration</td>
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<tr>
<td>III</td>
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</tr>
<tr>
<td></td>
<td>Aspiration</td>
<td>18</td>
<td>21</td>
</tr>
</tbody>
</table>

* Preliminary results of this experiment were reported in Swaab et al. (1973).
FIG. 2
Sham-operated control fetus and placenta (left) and brain-aspirated fetus and placenta (right). Both fetuses were operated upon day 19 of pregnancy. Note cranial depression in the brain-aspirated fetus.

FIG. 3
Section through the head of a sham-operated control fetus (left) and a brain-aspirated fetus (right). Observe the blood-stained fluid in the cranial cavity of the brain-aspirated fetus and the spinal cord (indicated by the arrow).
possibility of the transfer of substances from the brain or hypophysis of unoperated litter mates to the operated ones via the maternal circulation. This point is of particular importance because growth hormone might be able to cross the rat placenta (Delost, 1971; Zamenhof et al., 1966). A drawback of the suction procedure is, however, the technical problem of an operation before day 18.

During late pregnancy in the rat fetal brain appeared to have a remarkable effect upon fetal growth as judged by body weight. The reduction of the average body weight in animals that had fetal brain aspiration on day 18 or 19 was 25 or 30 per cent respectively. This was considerably more than the 5 to 15 per cent reduction reported by Jost (1966) and the 20 per cent reduction observed by Heggestad and Wells (1965). These

TABLE III
Changes in fetal weight, placental weight and placental index in sham-operated controls, after electrocoagulation of the fetal brain and after fetal brain aspiration and refilling of the cranial cavity with silicone rubber. Weights in grams. The values in brackets indicate the SEM. In the "uncorrected" group the actual weights are given, while in the "corrected" group weights were those after removing the contents of the fetal cranial cavity by suction.

<table>
<thead>
<tr>
<th>Day of pregnancy for operation</th>
<th>Number of living mothers</th>
<th>Number of living fetuses (per cent)</th>
<th>Uncorrected for cranial filling Mean weight (and SEM) fetus (g.)</th>
<th>Mean placenta weight (g.)</th>
<th>Mean placental index (and SEM)</th>
<th>Corrected for cranial filling Mean weight (and SEM) fetus (g.)</th>
<th>Mean placenta weight (g.)</th>
<th>Mean placental index (and SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>19</td>
<td>21</td>
<td>6</td>
<td>61</td>
<td>1.6</td>
<td>4.23 (0.058)</td>
<td>0.51 (0.009)</td>
<td>0.12 (0.002)</td>
</tr>
<tr>
<td>Coagulation</td>
<td>19</td>
<td>21</td>
<td>6</td>
<td>63</td>
<td>4.5</td>
<td>3.27 (0.054)</td>
<td>0.47 (0.008)</td>
<td>0.15 (0.002)</td>
</tr>
<tr>
<td>Silicone rubber replacement</td>
<td>19</td>
<td>21</td>
<td>6</td>
<td>4</td>
<td>93.6</td>
<td>3.30 (0.139)</td>
<td>0.46 (0.022)</td>
<td>0.14 (0.008)</td>
</tr>
</tbody>
</table>

(a) | (b)

FIG. 4

Time of onset of contraction phase (a), and of expulsion of the first fetus (b), in unaffected controls, animals that were sham operated, and animals who had fetal brain aspiration on day 18 (18D) or 19 (19D) of pregnancy. The vertical lines indicate the standard error of the mean.
differences may be due to the absence of oedema in our animals.

The decreased fetal and placental weights and the increased placental index observed in animals with brainless fetuses agree with available data on anencephalic infants (Honnebier and Swaab, 1973). Consequently, the associated placental weight changes are not necessarily the cause (Jones, 1955), but rather the effect of this nervous system malformation. The similarity of the normal pattern of fetal and placental growth in humans and rats, and the parallel changes observed in human pregnancy complicated by anencephaly and in rats with brain-aspirated fetuses suggest that the rat is a suitable experimental animal for further study.

Since the percentage of dead fetuses was smaller in sham-operated animals than in animals who had a fetal brain aspiration it could be that the differences in fetal and placental weight in both groups might be due to a selection of relatively light fetuses and placentae in the brain-aspirated groups caused by a higher mortality of heavy fetuses with a heavy placenta. This possibility was tested for the corrected values of experiment II and III (see Table II). Even when the heaviest fetuses and the heaviest placentae had been eliminated from consideration in the sham-operated groups so that the same percentage of fetuses was eliminated from both groups, a highly significant decrease in fetal and placental weights and increase in placental indices was observed (all values p<0.001, except for the placental changes of the day 18 group p<0.01).

In case the observed differences in fetal weight and placental index were caused by blood loss into the fetal cranial cavities and consequent circulatory changes, a number of brain aspirations was followed by silicone rubber injections but a high mortality made this an unsuitable technique. However, the few surviving fetuses showed the same low fetal and placental weight and high placental index found after brain aspiration only. Similar fetal weight and placental index changes were also found after electrocoagulation of the fetal brain. Consequently, the effects of brain aspiration could not be explained by blood loss into the fetal cranial cavity. A disadvantage of electrocoagulation (a safer procedure for the animal) is the variable size of the lesion produced and the possibility that heat may damage other important structures in the head and the neck.

Reduction of fetal body weight has also been reported after destroying the hypothalamus only (Fujita et al., 1970) but not after destroying other brain areas. Our results could be due to the absence of the hypothalamus; as the reduction in body weight can be prevented by means of growth hormone (Heggstad and Wells, 1965; for review see Delost, 1971) this reduction may be due to the lack of fetal growth hormone. The fact that growth is retarded rather than completely arrested in brain-aspirated rat fetuses might be due to the influence of maternal growth hormone (Knobil and Caton, 1953; Heggstad and Wells, 1965) and/or growth hormone-like substances of placental origin (for review see Pecile and Müller, 1966). In addition, some fetal growth occurring independently of the influence of growth hormone cannot yet be excluded.

In the present study placental weight was also apparently influenced by removal of the fetal rat brain. This finding agrees with the lower placental weight noted with anencephalic infants (Honnebier and Swaab, 1973). The mechanism by which this influence is achieved is now under investigation.

Gestation length in rats can be influenced by the time of exposure to light (Mitchell and Yochim, 1970). We therefore exposed a group of control animals to the continuous illumination which was necessary during our postoperative observations and found that their gestation length was not different to the gestation length in rats kept under our normal laboratory conditions.

The great variation in the pattern of parturition in all groups studied was remarkable. Contractions were sometimes noted for 24 hours before expulsion of the first fetus, while sometimes the first fetus appeared immediately after the first contraction. Further studies on the course of labour in fetal brain-aspirated and sham-operated rats will therefore have to include accurate methods of recording uterine activity.

The observation that gestation is not significantly prolonged in rats after brain-aspiration of the fetuses, contradicts the hypothesis (derived mainly from experimental work on sheep) that the fetal hypothalamus is of para-
mount importance in the initiation of labour (Liggins et al., 1967; Liggins, 1968 and 1969; Chard et al., 1971). Josimovich (1969 and personal communication) found that fetal adrenalectomy in rats gave varying results: after operation between days $19\frac{1}{2}$ and $19\frac{2}{3}$ of pregnancy an increasing proportion of pregnancies lasted a day beyond term, while operation between days $18\frac{1}{2}$ and $19\frac{2}{3}$ did not produce a significant increase in gestation length. Our results agree with those of Kirsch (1938), who could not demonstrate that the rat fetuses played a role in determining gestation length, and with the observations on human pregnancy with anencephalus (Honnebier and Swaab, 1973). Since hydramnios was never found in the rats with brain-aspirated fetuses, our experimental conditions presumably find their equivalent in those of our patients who had no hydramnios or excess of amniotic fluid and who were delivered of an anencephalic fetus after a labour of spontaneous onset (Honnebier and Swaab, 1973). It should be noted that the latter had a normal mean length of gestation.

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