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Enhancement of Prenatal Development in the Rat by Operative Restriction of Litter Size

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Abstract. The number of fetuses in the rat has been operatively restricted between 50 and 90% of normal by destruction of the other implantation sites by cauterization on the 8th day of pregnancy. The surviving offspring had significant increases in placental weights, body weights, cerebral weights, cerebral protein and cerebral DNA (cell number). Placental weights followed the extent of restriction.

Key Words
Rat
Prenatal litter restriction
Placenta
Cerebral increases
DNA
Protein

Introduction

In the previous paper [1] we have demonstrated that when mature rats were subjected to unilateral ligation of one uterine horn in order to increase nutrient supply to the other horn, they gave birth to half the normal number of offspring which were heavier at birth and had higher cerebral weight, protein and DNA (cell number) than the controls. A subsequent study [2] in the rabbit revealed that litter restriction to one results in even more pronounced increases in the above parameters, as well as in placental weight.

In the present work, we have used a new technique of litter restriction in the rat and have demonstrated that the increase in placental weights essentially follows the extent of restriction.

Materials and Methods

Albino rats used in the present experiment and their maintenance were similar to those in the previous work [1].

The operative restriction of litter size was performed as follows. Animals were anesthesized with an inhalent (methoxyflurane), and an abdominal midline incision of approximately

2 cm was made. Both uterine horns were gently lifted out avoiding unnecessary trauma as well as unnecessary handling of the actual implantation sites. During the entire procedure the horns were kept moist with saline. This surgical procedure was done on the 8th day of pregnancy and the number of implantation sites and their location was noted. A Bantam Bovie (Cincinnati, Ohio) electrosurgical unit with a needle electrode was used to destroy the implantations. It was found that inserting the needle electrode through the uterine walls directly into the implant and cauterization for approximately 5 sec was most effective. Since the surgical unit was used on the coagulating position, a minimum of bleeding is encountered. The uterine horns were then returned into the abdominal cavity and muscle and skin tissue were closed with single stitch.

The offspring were delivered by cesarean section approximately 6 h before term in order to recover the placentas. The offspring were weighed and killed by decapitation. The cerebral hemispheres, without cerebellum and olfactory lobes [3], were immediately removed and weighed. They were then frozen and subsequently used for the following colorimetric determinations: DNA, by a modification of the diphenylamine method [4–6]; and protein, by a modification of the Folin method [7]. From the individual DNA values, the number of total cerebral cells could be calculated by dividing by a (constant) DNA content per rat cell (6×10^{-6} μg) [4]. Such calculation is based on the assumption that the cells in the cerebral hemispheres of the newborn rat are essentially diploid.

Results und Discussions

The effect on operative restriction of the litter size on newborn parameters are represented in table I. It can be seen that this restriction resulted in highly significant mean increases in body and placental weight, as well as cerebral weight, DNA and protein. (Compare also [2].) It is of interest that the highest increases (up to 62%) were for placental weight and they followed the degree of litter restriction (first column). It has been suggested that prenatal brain development is correlated with placental weight [8] and therefore presumably with the flow of nutrients to the fetus.

As shown in the previous paper [1], in the rat the neonatal parameters normally do not depend on the size of the litter. On the other hand, in the case of operative litter restriction all neonatal parameters did increase (table I). It thus appears that the pregnant female had a potential for supporting the growth of a larger number of fetuses than actually produced, and this potential was not subject to regulatory decrease after surgery: hence each surviving fetus might have received more nutrients and oxygen, or might have had a more efficient removal of waste materials. This might have been facilitated by the concomitant increases in placental weights.

The effects of the amount of ovarian and adrenal tissue present per fetus during litter restriction will be the subject of a subsequent publication [9].

Table I. Effect of operative restriction of the number in the litter on parameters of the newborn

Litter restricted to	Number of rats		Offspring weights, g ± standard deviation			Offspring cerebral content ± standard deviation	
	mothers	total born	body	placenta	cerebrum	DNA, μg	protein mg
Control no restriction	9	73	5.8 ± 0.4	0.536 ± 0.066	0.156 ± 0.013	593 ± 36	8.23 ± 0.86
10%, fraction remaining 11		11	6.7 ± 0.6 (+16%)[1]	0.865 ± 0.124 (+62%)	0.194 ± 0.026 (+25%)	642 ± 28 (+8%)	9.81 ± 1.49 (+19%)
16%	4	8	6.8 ± 1.0 (+17%)	0.783 ± 0.100 (+46%)	0.195 ± 0.036 (+25%)	636 ± 33 (+7%)	9.91 ± 1.85 (+20%)
23%	2	6	6.8 ± 0.3 (+17%)	0.643 ± 0.160 (+20%)	0.914 ± 0.022 (+25%)	642 ± 34 (+8%)	10.19 ± 1.49 (+24%)
42%	2	10	6.6 ± 0.7 (+14%)	0.620 ± 0.137 (+16%)	0.205 ± 0.012 (+32%)	630 ± 28 (+6%)	11.03 ± 1.24 (+34%)
48%	2	12	6.3 ± 0.5 (+9%)	0.595 ± 0.092 (+11%)	0.175 ± 0.009 (+13%)	612 ± 33 (+3%)	8.74 ± 0.64 (+6%)
Mean restriction 20%			6.6 ± 0.8 (+14%)	0.666 ± 0.178 (+24%)	0.191 ± 0.029 (+23%)	629 ± 39 (+6%)	9.86 ± 1.82 (+20%)
p			< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[1] Increase over control.

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References

1 VAN MARTHENS, E. and ZAMENHOF, S.: Deoxyribonucleic acid of neonatal rat cerebrum increased by operative restriction of litter size. Exp. Neurol. *23:* 214 (1969).
2 VAN MARTHENS, E.; GRAUEL, L., and ZAMENHOF, S.: Enhancement of prenatal development by operative restriction of litter size in the rabbit. Life Sci. *11 part I:* 1031 (1972).
3 ZAMENHOF, S.; VAN MARTHENS, E., and MARGOLIS, F. L.: DNA (cell number) and protein in neonatal brain. Alteration by maternal dietary protein restriction. Science *160:* 322 (1968).
4 ZAMENHOF, S.; BURSZTYN, H.; RICH, K., and ZAMENHOF, P. J.: The determination of deoxyribonucleic acid and of cell number in brain. J. Neurochem. *11:* 505 (1964).
5 MARGOLIS, F. L.: DNA and DNA-polymerase in chicken brain regions during ontogeny. J. Neurochem. *16:* 447 (1969).
6 ZAMENHOF, S.; GRAUEL, L.; VAN MARTHENS, E., and STILLINGER, R. A.: Quantitative determination of DNA in preserved brains and brain sections. J. Neurochem. *19:* 61 (1972).
7 LOWRY, O. H.; ROSEBROUGH, N. J.; FARR, A. L., and RANDALL, R. J.: Protein measurement with the Folin phenol reagent. J. biol. Chem. *193:* 265 (1951).
8 ZAMENHOF, S.; GRAUEL, L., and VAN MARTHENS, E.: Study of possible correlations between prenatal brain development and placental weight. Biol. Neonate *18:* 140 (1971).
9 VAN MARTHENS, E. and GRAUEL, L.: Ovarian and adrenal effects on fetal and placental development during litter restriction. Nutr. Metabol. (submitted for publication).

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