Hormonal Aspects of Glycogen Accumulation in Fetal and Neonatal Rat Liver[1] (34917)

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(Introduced by C. A. Schneyer)

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There are numerous reports showing that a newborn rat does not mobilize ACTH upon the administration of various stressful stimuli (1–9). In this young animal, however, the adrenal is responsive to exogenous ACTH. Moreover, the pituitary glands of such animals contain physiologically active ACTH, which can be secreted under certain circumstances (10, 11). It has been argued, therefore, that in this species the hypothalamic regulatory mechanisms are not completely functional at birth (12). According to another view (3) this unreactivity might be due in fact not to hypofunction but to hyperfunction of the axis. This hyperfunction could be the result in part of the stress of parturition or of the sudden onset of new conditions of extrauterine life.

In this early period of rat life even the peripheral tissues do not exhibit normal reactivity toward the administration of some hormones. Thus, for example, newborn rat shows an extreme resistance to the action of cortical steroids (13). Furthermore, a powerful lymphocytolytic hormone—cortisol only slightly reduces the weight of the newborn rat lympatic organs (N. Avdalovic, unpublished). It appears, therefore, that besides the studies of the rat pituitary–adrenal responsiveness in the early postnatal period, a careful investigation of the peripheral tissue reactivity is indicated.

The present experiments were designed to determine whether or not fasting newborn rat could accululate glycogen in the liver following the administration of cortisol. The first series of experiments deals with the measurements of fetal liver glycogen under normal as well as under changed hormonal and nutritional conditions. From the present work evidence is presented that fetal and newborn rat are fully capable of synthesizing glycogen from dietary sources. Also, results obtained show that cortisol-treated newborn rats do not accumulate glycogen in the liver during fasting.

Materials and Methods. Random bred albino rats of Wistar origin were used throughout the experiments. The time of conception was determined by the following procedure: at 6 p.m. five females, 2–3 months old, were put in the cage together with one male rat, a proved breeder. On the following morning at 7 a.m. all the female rats were examined. A copious finding of live spermatozoa in the vaginal smear was accepted as a positive sign of conception. The day of positive vaginal findings were considered as the first day of pregnancy. On the day of delivery half of the litter was used as a control group and the other half for experimental. Only 6 babies were left with their mothers in order to maintain a constancy of litter size during the experiment. Adrenalectomy was performed under light ether anesthesia using a dorsal approach (14). Liver glycogen was determined according to the method of Seifter *et al.* (15). Cortisol was administered to newborn rats 3 times a day. (Cortisol was supplied from Prolek-Beograd as a microcrystallinic suspension.)

Results. Glycogen concentration in fetal and neonatal rat livers. Early in gestation the glycogen concentration tends to be so low that it could not be measured by the method used. The fetal animal starts to accumulate large amounts of glycogen in the latter part of gestation (Fig. 1). During the last 2 days of intrauterine life liver glycogen is approx-

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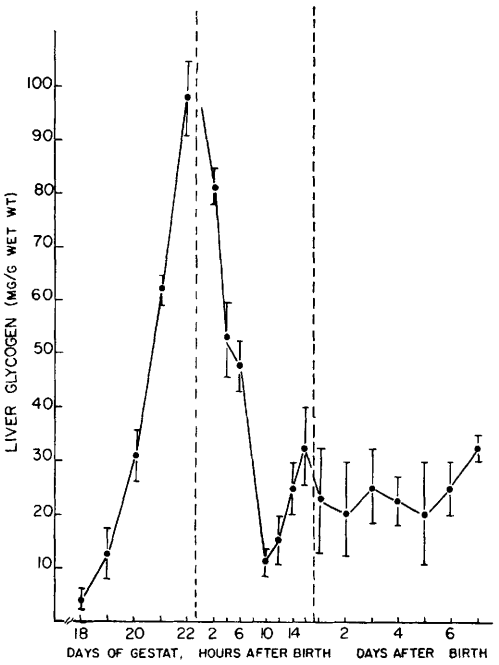

FIG. 1. Glycogen accumulation in the fetal and neonatal livers: Each point represents the mean ± standard error from 15 to 20 individual determinations.

imately 10–12% of the wet weight of the organ. Values of this magnitude are rarely seen in normally fed adult rats. The liver glycogen concentration falls very rapidly after birth in spite of the fact that all newborn rats had, without exception, a full stomach of milk (Fig. 1). It remains low for 1 or 2 weeks and then rises gradually toward the adult levels. Regularly fed pregnant mothers have a liver glycogen level that is about 3–4% of liver weight (Fig. 1).

Adrenalectomy of pregnant mothers and its effect on the fetal liver glycogen content. Adrenalectomy was performed at various stages of pregnancy. The results of the experiment on mothers adrenalectomized at 13th day of gestation are presented in Table I. It is evident that the adrenal gland of the mother contributes to some extent to the ultimate level of fetal liver glycogen. However, the rapid accumulation at the end of pregnancy is still preserved in spite of the adrenalectomy.

The effect of fasting and feeding during gestation on the fetal liver glycogen. At various stages of pregnancy, rats were fasted for 48 hr. The control group was in the same stage of gestation but animals had free access to food. Both groups of rats were killed at the same time and the fetal livers were compared for glycogen content. It is obvious from data in Table II that fasting of pregnant animals produced a significant decrease in fetal liver glycogen content, although at the end of pregnancy fetal glycogen was still above or at least within the range of liver glycogen in normally fed adult rats. The phenomenon of abrupt rise is still preserved and the only difference exists in the ultimate degree of glycogen accumulation.

TABLE II. Liver Glycogen in Fetuses from Fed and Fasted Pregnant Rats.

Day of gestation	Liver glycogen (mg/g of tissue wet wt)[a] Fed mothers[b]	Fasted mothers[c]	p
18	4.10 ± 0.63 (8)[d]	0.14 ± 0.04 (13)	<0.001
20	30.20 ± 4.88 (7)	9.12 ± 1.38 (9)	<0.001
21	65.29 ± 2.13 (20)	37.33 ± 3.02 (14)	<0.001

[a] Values are means ± SE.
[b] Pregnant rats fed *ad libitum*.
[c] Pregnant rats fasted for 48 hr.
[d] Number of animals is shown in parentheses.

In view of the rapid and free exchange of

TABLE I. Liver Glycogen Levels in Fetuses from Intact Pregnant Rats and Those Adrenalectomized at Day 13 of Gestation.

Group	No. of animals	Day of gestation	Liver glycogen (mg/g of tissue wet wt)[a]	p
Adrenalectomized pregnant rats	21	22	61.50 ± 2.31	
Intact pregnant rats	10	22	96.39 ± 4.03	<0.01

[a] Values are means ± SE. All rats were killed at day 22 of pregnancy.

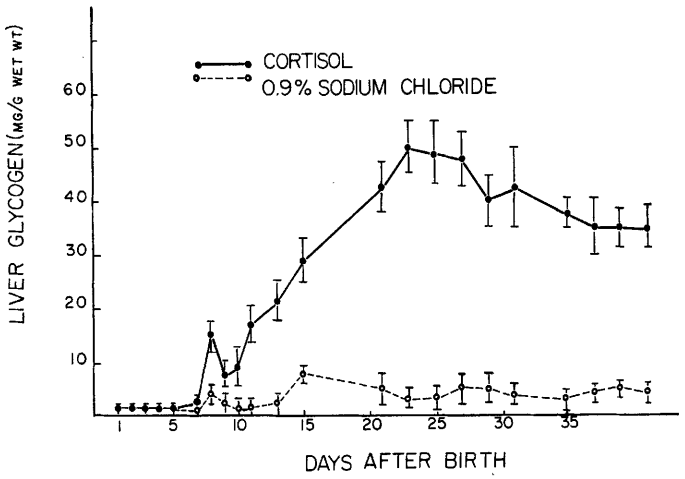

FIG. 2. Response of the rat to cortisol administration (3× daily, 1 mg/100 g of body wt during 48 hr): At varying intervals young rats belonging to the same litter were divided into two groups. Both groups were fasted for 48 hr but only one received cortisol. Each point represents the mean from 20 to 30 individual livers.

glucose between fetus and mother (16), this result points to an extraordinary autonomy of the fetal liver. This autonomy is clearly manifested in the fact that the fetal rat succeeds in maintaining a very high liver glycogen concentration under the conditions when, concomitantly, there is not detectable glycogen in the liver of the mother.

Effect of cortisol administration on the liver glycogen content during the early neonatal period. In order to determine whether peripheral tissues could be activated by the exogenous administration of corticosteroids during early postnatal life, the following experiments were performed: young rats belonging to the same litter were divided into two groups at varying times after birth. Both groups of rats were fasted for 48 hr but only one group received 6 injections of cortisol. The single dose was 1 mg/100 g of body weight. This dose proved to be effective in producing a substantial accumulation of glycogens in fasting adult rats.

In young animals (Fig. 2), fasting provoked a significant depletion of the liver glycogen stores. Administration of cortisol in the first week of extrauterine life, however, was without effect as far as glycogen accumulation was concerned. Liver glycogen rose very quickly in fetuses which, upon fasting, were returned to their mothers. In agreement with data from the literature (17), this experiment shows that during the first week of neonatal life young animals are capable of synthesizing and concentrating glycogen in the liver from dietary sources. Meanwhile, the capability to form glycogen upon cortisol administration becomes fully expressed around days 14 to 21 after birth. This period of time (days 21–22) corresponds to normal time of weaning.

Discussion. The present data are in agreement with reports in the literature (16, 17), and show that fetal rat is fully capable of synthesizing liver glycogen during the last 5 days of intrauterine life. The degree of accumulation of fetal liver glycogen depends on many factors such as the nutritional and hormonal status of the mother (18). In the present work, it was found that in the fasting neonatal rat accumulation of liver glycogen does not occur upon administration of cortisol. The following explanations are suggested: (a) Cortisol metabolism: A young rat may be capable of metabolizing cortisol to a greater extent than an adult rat. However, since the turnover of cortisol in newborn babies is two to four times slower than in adults (19), this is not the likely explanation. (b) Impaired gluconeogenesis in newborn rats: The unresponsiveness noted can not be attributed to low activity of the gluconeogenic pathway. Since in fact, an increased capacity for glucogenesis compared with the adult

has been reported in the neonatal animal (20, 21). Furthermore, the principal gluconeogenic enzymes are present in the neonatal liver in concentrations higher than in the adult rat (22–24). The high activity of glucose-6-phosphatase in this early period of rat life is of importance as a possible explanation of present results. Thus, it is possible that the peripheral tissues of neonatal rat respond to cortisol administration in the same manner as adult tissues do, with the only difference being a greater conversion into free glucose than into the glycogen. Consequently, in spite of active gluconeogenesis there is no detectable accumulation of glycogen in the fasting, cortisol-treated newborn rats. (c) Defective reactivity of peripheral tissues toward cortisol: It might appear that the finding of cortisol unresponsiveness in early neonatal life is simply incompatible with the fact that these animals have actually an increased capacity for gluconeogenesis. However, the capacity for gluconeogenesis may not necessarily show a direct correlation to the ability of cortisol in mobilizing the endogenous substrates for gluconeogenesis. Thus, while extrahepatic tissues are still unreactive, exogeneously supplied substrate could promote the gluconeogenesis. Since milk contains a rather high concentration of necessary precursors, this above seems as a very likely explanation. Milk is the only source of the food in the first 2–3 weeks of life in rats and, hence, these animals do not receive the same supply of carbohydrates as they used to get *in utero*. The neonatal rat must be, therefore, in a state of intensive synthesis of glucose from noncarbohydrates precursors like amino acids and glycerol. In this period, exogenously administered corticosteroids might not be able to mobilize enough substrates for the otherwise hyperactive gluconeogenic pathway.

Summary. The results are presented showing the changes in the fetal liver glycogen level during gestation of the rat. The effect of the nutritional and hormonal status of the pregnant mothers on the fetal liver glycogen has been examined. It was found that both of these factors influence the degree of glycogen accumulation in the fetal liver but do not change the pattern itself. Also, it was found that the fasting newborn rat, treated with cortisol, does not accumulate glycogen in the liver at any faster rate than the control animals.

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1. Jailer, J. W., Endocrinology **46**, 420 (1950).
2. Eskine, I. A., Ann. Endocrinol. **18**, 343 (1957).
3. Milkovic, K., and Milkovic, S., Ann. Endocrinol. **37**, 301 (1959).
4. Levine, S., Alpert, M., and Lewis, G. W., J. Comp. Physiol. Psychol. **51**, 774 (1958).
5. Irwin, E., Buchanan, A. R., Longwell, B. B., Holtkamp, D. E., and Hill, R. M., Endocrinology **46**, 526 (1950).
6. Gregoire, A., C. R. Acad. Sci. **244**, 2420 (1957).
7. Bacq, Z. M., and Fisher, P., Rev. Belg. Pathol. **25**, 384 (1956).
8. Grosjean, O., and Lemaire, R., C. R. Soc. Biol. **256**, 962 (1962).
9. Schapiro, S., Geller, E., and Eiduson, S., Proc. Soc. Exp. Biol. Med. **109**, 935 (1962).
10. Harris, G. W., "Neural Control of the Pituitary Gland." Arnold, London (1955).
11. Rinfert, A. P., and Hane, S., Endocrinology **56**, 341 (1955).
12. Glydon, R. S. J., J. Anat. **91**, 237 (1957).
13. Field, E. J., J. Anat. **91**, 278 (1957).
14. Farris, E. J., and Griffith, J. Q., "The Rat in Laboratory Investigation." Lippincott, Philadelphia (1942).
15. Seifter, S., Dayton, S., Novic, B., and Muntwyler, E., Arch. Biochem. **25**, 191 (1950).
16. Dawes, G. S., and Shelley, H. J., *in* "Carbohydrate Metabolism and Its Disorders" (F. Dickens, P. J. Randle, and W. J. Whelan, eds.), Vol. 2, p. 133. Academic Press, London/New York (1968).
17. Walker, D. G., *in* "Carbohydrate Metabolism and Its Disorders" (F. Dickens, P. J. Randle, and W. J. Whelan, eds.), Vol. 1, p. 465. Academic Press, London/New York (1968).
18. Jost, A., Harvey Lect. **55**, 201 (1961).
19. Migeon, C. J., J. Pediat. **55**, 280 (1959).
20. Ballard, F. J., and Oliver, I. T., Biochim. Biophys. Acta **71**, 578 (1963).
21. Yeung, D., and Oliver, I. T., Biochem. J. **103**, 744 (1967).
22. Ballard, F. J., and Hanson, R. W., Biochem. J. **104**, 866 (1967).
23. Yeung, D., and Oliver, I. T., Biochem. J. **105**, 1229 (1967).
24. Vernon, R. G., and Walker, D. G., Biochem. J. **106**, 321 (1968).

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