ods, appears to be unaffected by intravenous estrogen other than by a slight increase in factor V. Following estrogen, intradermal hyaluronidase wheals persist for a longer period than noted with placebos. These changes are confined to the male. Nonspecific serum hyaluronidase inhibitor increases in both sexes following I.V. estrogen. The peak effect is noted at one to two hours, but the changes persist for approximately 8 hours, usually approaching base line levels at 24 hours. These experiments indicated that I.V. estrogens affect the ground substance. We have not attempted clinical evaluation of the use of intravenous estrogens in control of hemorrhage.

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Received January 30, 1964. P.S.E.B.M., 1964, v116.

Compensatory Hypertrophy of Right Adrenal after Left Adrenalectomy: Observations in Fetal, Newborn and Week-old Rats.* (29168)

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It has been reported that the hypophysis-adrenal axis of the rat does not respond to experimental stress during the early postnatal period(1,2) but that it does respond during the prenatal period(3). It also has been reported that volume and weight of the adrenal are reduced for a few days after birth(4,5,6) but that such reduction does not occur in a fetus which is subjected to extra time *in utero* by the experimental prolongation of pregnancy(6).

The experiments to be reported support the working hypothesis that in the rat there is a reduction in activity of the hypophysis-adrenal axis during the first 3 days after

* Aided by grant from Nat. Inst. of Child Health and Human Development.

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birth. The observations are the first of their kind in that they compare the compensatory hypertrophy of the right adrenal which occurs after left adrenalectomy in fetal rats with any which occurs after left adrenalectomy in rats of postnatal days 1 and 7.

Materials and methods. Rats of the Sprague-Dawley strain were used (Simonsen subline). Each of 4 groups of animals was composed of experimental rats (E) and of litter-mate controls (C).

Group 1 consisted of fetuses: E, left adrenalectomized *in utero*; C, unoperated. The left adrenalectomy was performed as follows. On the 20th day of pregnancy (19½ days postcoitum), a gravid female was subjected to ether anesthesia and to a midventral laparotomy. The approximate site of the left adrenal of a fetus was viewed through the translucent uterine wall and subjacent fetal membranes. At this site, the fetal skin was exposed by tiny incisions in the uterine wall and fetal membranes. The fetal skin was incised just below the lowest left rib. To keep the wound of the fetus in view, the margins of the incised fetal skin were stitched to those of the incised uterine wall by means of temporary sutures. Then the adrenal was exposed by an incision in the infracostal region. With jeweler's forceps in each hand, the adrenal was removed *en bloc*. The loss of blood was so slight that the interrupted vessels of the fetus were not ligated. The peritoneal cavity of the fetus was closed with sutures. After the skin of the fetus had been freed from the uterine wall by removal of the temporary sutures, the several remaining incisions were closed with sutures. Finally, the administration of ether was stopped and the mother returned to her cage. Two days later, the mother and the E and C fetuses were autopsied.

Each of 3 other groups (Groups 2, 3 and 4) consisted of rats from spontaneous deliveries: E, left adrenalectomized; C, sham left adrenalectomized. A young rat, still so young that it was poikilothermic, was anesthetized for the surgery by a reduction of its body temperature.

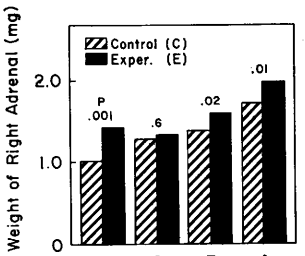
The left adrenals which were removed at surgery were cleaned of adherent fat, weighed on a Mettler balance with a sensitivity of .001 mg and then discarded. The right and left adrenals which were removed at autopsy were cleaned and individually weighed. The right glands were fixed in 10% formalin, the left discarded.

In each group, half of the E right adrenals and half of the C right adrenals were used for frozen sections which subsequently were stained with sudan Black B. The other E and C right adrenals were used for paraffin sections which were stained with hematoxylin and eosin.

With two compound microscopes equipped with a Bausch and Lomb microcomparator, the stained sections of an E adrenal were compared directly with those of the C adrenal.

Results. The data on the weights of the adrenals are shown in Fig. 1. The microscopic observations in the stained sections will be presented in the text.

In Group 1, left adrenalectomy followed by 2 days of prenatal life *in utero* caused a marked increase in weight of the right adrenal. The cortical cells and nuclei of the E right adrenals were larger than those of the C right adrenals. Sudanophilic granules (SG) in the cortices of the E right adrenals tended



Group		1	2	3	4
Period		Prenatal	— Postnatal —		
Age at Surgery (days)		20	1	1	7
Exp. Period (days)		2	3	6	3
Cases	E	15	29	15	35
	C	15	28	15	31
Wt. L. Adr., Surgery (mg)	E		1.35	1.42	1.48
Wt. L. Adr., Autopsy (mg)	C	1.07	1.34	1.48	1.96
Wt. R. Adr., Autopsy (mg)	E	1.43	1.33	1.60	1.99
	C	1.01	1.29	1.39	1.73
Hypertrophy of R. Adrenal (%)*		42	3	15	15

*Hypertrophy (%) = $\frac{\text{(Wt. R. Adr., E)} - \text{(Wt. R. Adr., C)}}{\text{(Wt. R. Adr., C)}} \times 100$

FIG. 1. Weights of right adrenals in experimental and control members of Groups 1 to 4.

to be smaller than those in the cortices of the C right adrenals. The SG in the juxtamedullary zone of the E adrenals were less distinct than those in that of the C adrenals.

In Group 2, left adrenalectomy on the first day after birth and an experimental period of 3 days did not cause any significant hypertrophy of the right adrenal nor any consistent histologic changes in the cortex.

In Group 3, left adrenalectomy on the first day after birth and an experimental period of 6 days caused a significant hypertrophy of the right adrenal. The cortical cells of the E adrenals were somewhat larger than those of the C adrenals. Both the glomerular zone and the lipid-free portion of the fasciculate zone in the E adrenals were slightly narrower than those in the C adrenals. The number and size of the SG were essentially the same in the E and C cortices.

In Group 4, left adrenalectomy on the seventh day after birth and an experimental period of 3 days produced almost the same results as those observed in Group 3. In Group 4, however, the number and size of the SG in certain E cortices were slightly reduced.

Among Groups 1 to 4, the observed ponderal and histologic changes in the E adrenals were most conspicuous in Group 1.

Discussion. The observed compensatory hypertrophy of the right adrenal during prenatal life is in keeping with the report of a response of the hypophysis-adrenal axis to the experimental stress of injected epinephrine(3). Also, it supports the earlier report that there is a reciprocal, functional relation between the hypophysis and the adrenal cortex of the fetal rat(7).

In contrast, left adrenalectomy on the first day after birth and an experimental period of 3 days did not cause any significant hypertrophy of the right adrenal, suggesting that during the first 3 days after birth the hypophysis of the rat does not respond to the experimental reduction of the amounts of corticoids. Also, it is in line with the report of negative ascorbic-acid-depletion tests during these 3 days(8).

However, left adrenalectomy on the first day after birth and an experimental period of 6 days caused a significant hypertrophy of the right adrenal. This observation, which confirms and extends an earlier report(9), would seem to mean that during the fourth to sixth days after birth the hypophysis-adrenal axis responds to the experimental reduction of the amounts of corticoids. This view is in accord with the report of positive ascorbic-acid-depletion tests from the fourth day after birth onward(8).

The hypertrophy of the fetal adrenal which follows bilateral adrenalectomy of the mother has been interpreted in terms of a decrease in the titer of circulating corticoids which, in turn, causes the fetal hypophysis to release an increased amount of ACTH(10,11). Accordingly, it would be reasonable to suppose that the cessation of supply of maternal corticoids after birth should lead to a rise in the activity of the hypophysis-adrenal axis in the newborn rat.

However, our observation of a lack of compensatory hypertrophy of the E right adrenals in Group 2 is regarded as evidence of a reduced activity of this axis during the first 3 days after birth. Also, it is known that the size of the adrenal tends to decline during the early neonatal period(4,5,6) and that this phenomenon is the reverse of that which occurs during the latter part of the prenatal period.

It has been reported that in rats during the first 3 days after birth there still exists a high concentration of corticoids in the plasma but that this concentration is less than that during the prenatal period; these observations point to the possibility that during the early neonatal period the corticoids temporarily block the hypophyseal feed-back mechanism (8). If such blockade exists and if it is augmented by corticoids from the adrenals of the mother due to the stress of parturition, the blockade should disappear as soon as the corticoids of maternal origin are used up.

Our observations support the view that in the rat some kind of blockade of the hypophyseal feed-back mechanism temporarily exists during the early neonatal period. Nevertheless, both the ponderal and the histologic observations indicate that the compensatory hypertrophy of the right adrenal during the

prenatal period is relatively greater than that during any one of the 3 postnatal periods which we studied. This difference raises the question of whether some maternal and/or placental factor(s) other than corticoids contribute(s) to the compensatory hypertrophy which occurred before birth.

Summary. In our experiments, both ponderal and histologic observations on the adrenal support the view that in the rat the hypophysis-adrenal axis begins to function before birth. After birth, there is an hiatus of 3 days during which the function is significantly reduced.

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Received January 30, 1964. P.S.E.B.M., 1964, v116.

Dietary Bile Acids and Lipid Metabolism. VI. Protective Effect of Cholic Acid in Lithocholic Acid Fed Chicks. (29169)

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Dietary lithocholic acid has been shown to depress body weight gain and increase plasma lipids and liver size of chicks(1-4). The increased liver size of lithocholic acid-fed chicks has been shown to be the result of a bile ductule proliferation(5).

The deleterious effects of lithocholic acid can be prevented, at least for a 3- or 4-week period, by simultaneously feeding cholic acid (4). Data presented here demonstrate that cholic acid protects chicks against the effects of dietary lithocholic acid for periods of up to 12 weeks.

Experimental. Male Hy-line White Leghorn chicks were fed a stock diet for 2 weeks. The chicks were then assigned to the experimental groups in such a way as to have almost identical average initial weights for all groups (128-130 g).

The chicks were reared in heated cages having raised wire floors. Food and water were supplied *ad libitum.* Body weight and food consumption were determined at weekly intervals. The basal diet employed was identical to that described previously(4). Lithocholic and cholic acids when fed were added at a level of 0.2% of the diet at the expense of glucose. Twenty-eight chicks were started in each of the 6 experimental groups as shown in Table I. Seven chicks from each group were sacrificed after having received the diets for 3, 6, 9 and 12 weeks.

At time of sacrifice, blood was obtained by cardiac puncture using a heparinized syringe. The liver was excised, blotted to remove excess blood and weighed; a slice of liver was fixed in neutral buffered formalin and the remainder was frozen and stored at —20°C for chemical analysis. Plasma and liver lipids

TABLE I. Experimental Design.

Exp group	Dietary variables
1	Control
2	.2% Lithocholic acid
3	*Idem* + .2% cholic acid
4	2% Cholesterol
5	.2% Lithocholic acid + 2% cholesterol
6	*Idem* + .2% cholic acid

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