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Histochemical Studies of Effects of Cortisone on Fetal and Newborn Rats.*† (21935)

LEONARD ROSS‡ AND E. D. GOLDSMITH.

From the Department of Anatomy, Medical College and School of Dentistry, University of Alabama, Birmingham, and the Department of Histology, College of Dentistry, and Graduate School of Arts and Science, New York University, New York City.

There is a growing body of experimental evidence demonstrating the effect of cortisone on enzyme systems(1-3). These investigations, for the most part biochemical in nature, have been carried out on a variety of cellular enzymes(4-6). Much emphasis has been placed on the effect of cortisone on alkaline phosphatase. A number of workers(7), utilizing histochemical methods, have observed an increase in the alkaline phosphatase content of the brush border of the kidney tubule after injection with cortisone. Moog(8-10), obtained a precocious increase of intestinal phosphatase upon injection of fetal or newborn mice with cortisone. She interprets these results as indicating that cortisone influences the basic metabolic processes which are responsible for phosphatase synthesis.

Ribonucleic acid (RNA) has also received much attention as a substance involved in cortisone action. Decreases in hepatic RNA after cortisone injection have been observed by several investigators(11), although there is some conflict on this point(12,13). One group(7) noted an increase in renal tubule RNA after cortisone injection.

In view of the widespread interest in the mode of action of cortisone, it was decided to extend and correlate the above-cited findings. Because of the importance of the functional relationships existing between alkaline phosphatase, RNA and polysaccharide-protein complexes, histochemical methods were employed to follow the changes induced by cortisone on these materials.

Materials and Methods. Two series of animals were employed. The first series consisted of 24 female Wistar rats. Each female was placed with a male and daily vaginal smears were made until fertilization occurred (as evidenced by sperm in the smear). Beginning with the seventh day of pregnancy, the mothers were divided into 3 equal groups. Group I animals received 75 mg of cortisone per kilo of body weight daily throughout pregnancy; Group II animals received 100 mg and Group III served as controls. The cortisone was divided into 2 daily doses, given 8 hours apart.

Each of the above groups was subdivided into 2 sub-groups of equal size. In one Caesarian sections were performed on the fourteenth day and in the other on the sixteenth day of gestation. The uterine horns were exposed under moderate nembutal anesthesia, a tally of the number of fetuses present was taken and one-half of the number was removed. The incisions were then closed and

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cortisone treatment was continued. A second Caesarian section was performed 4 days after the first, in each case yielding 18 and 20 day fetuses respectively. All fetuses were weighed on a torsion balance and fixed *in toto* at 4°C for 24 hours in Bouin's, Carnoy's or 95% ethyl alcohol. A total of 201 fetuses was so obtained.

In the second series of experiments, a total of 110 post-partum rats was used. Litters were kept with the mother and beginning with 24 hours after the hour of birth, the litter mates were divided into 2 groups. The first group received a daily injection of 0.1 mg of cortisone while the second group served as vehicle-injected controls. Injections continued until 24 hours before autopsy. At least 2 complete litters were autopsied on each of the following days of age: 3, 5, 7, 9, 11, 13, and 15. Liver, intestine and kidney were fixed as in the first series.

In addition to a routine hematoxylin and eosin stain, the following histochemical methods were employed. Alkaline phosphatase was demonstrated by the Ca-Co method of Gomori(14). Both DNA and RNA were visualized by methyl green-pyronin Y used according to the methods of Taft(15) and Kurnick(16). To substantiate the specificity of the stain, alternate sections were digested with a solution of ribonuclease (Worthington) in distilled water. Mucopolysaccharides were identified by the periodic acid-Schiff method of McManus(17). Glycogen was identified by malt diastase digestion.

Results. As was to be expected, at all ages the cortisone-injected animals showed a significant inhibition of weight gain as compared to the controls. This was evidenced by a marked smallness in size of the fetal rats and by a general debilitated appearance in the post-partum rats. There was no difference in response between the fetal rats whose mothers received the 75 mg dosage and those of mothers receiving the 100 mg dosage.

Fetal Rats. In the fetal rats, beginning with the 16th day of intra-uterine life, the hepatic parenchyma of the cortisone injected animals showed increases in glycogen deposition and decreases in RNA content when compared with controls of the same age. This has been a constant finding in all previous studies in this area(11-13). No differences became observable in the intestinal epithelium and renal tubule epithelium of the hormone-treated animals until the 16th day of intra-uterine life. At this age, there is the beginning of a response to hormone treatment which becomes accentuated at 18 days of age. By the 18th day, there is a higher RNA content in the supra-nuclear regions of the intestinal epithelium in the hormone-treated fetuses than in the controls of the same age. This increase of RNA is even more pronounced at 20 days of age, where the controls show only a moderate RNA content (Fig. 1a) while the experimentals demonstrate quite marked accumulations of RNA (Fig. 1b).

Similarly, in the brush border of the renal tubules there is an increased deposition of RNA in the cortisone-treated animals. This increase begins at 16 days of age and is quite pronounced at 18 days; the controls showing only a moderate RNA brush border content (Fig. 2a) while the treated animals demonstrate heavy deposits in the equivalent loci (Fig. 2b). This difference is no longer evident at 20 days of age.

As far as PAS-positive materials are concerned, the only significant differences are found in the renal tubule epithelium of the 18-day-old fetuses. In this site, there is evidence of increased glycogen deposition in the treated fetus (Fig. 3b) as compared with the control (Fig. 3a).

Along with these increases in RNA, concomitant increases in alkaline phosphatase were evident. Signs of increased alkaline phosphatase activity of the striated border could be seen in the 18-day-old treated fetuses. This heightened enzyme activity in the cortisone injected animal was more outstanding at 20 days of age. In the control animal of this age, the intestinal striated border shows only a slight alkaline phosphatase activity (Fig. 4a). The hormone treated animal, on the other hand, exhibits a marked activity which extends into the supra-nuclear cytoplasm (Fig. 4b). Both of these slides were incubated in the substrate for 10 minutes.

In the renal tubule epithelium, the cortisone treated animals exhibit an increased alkaline

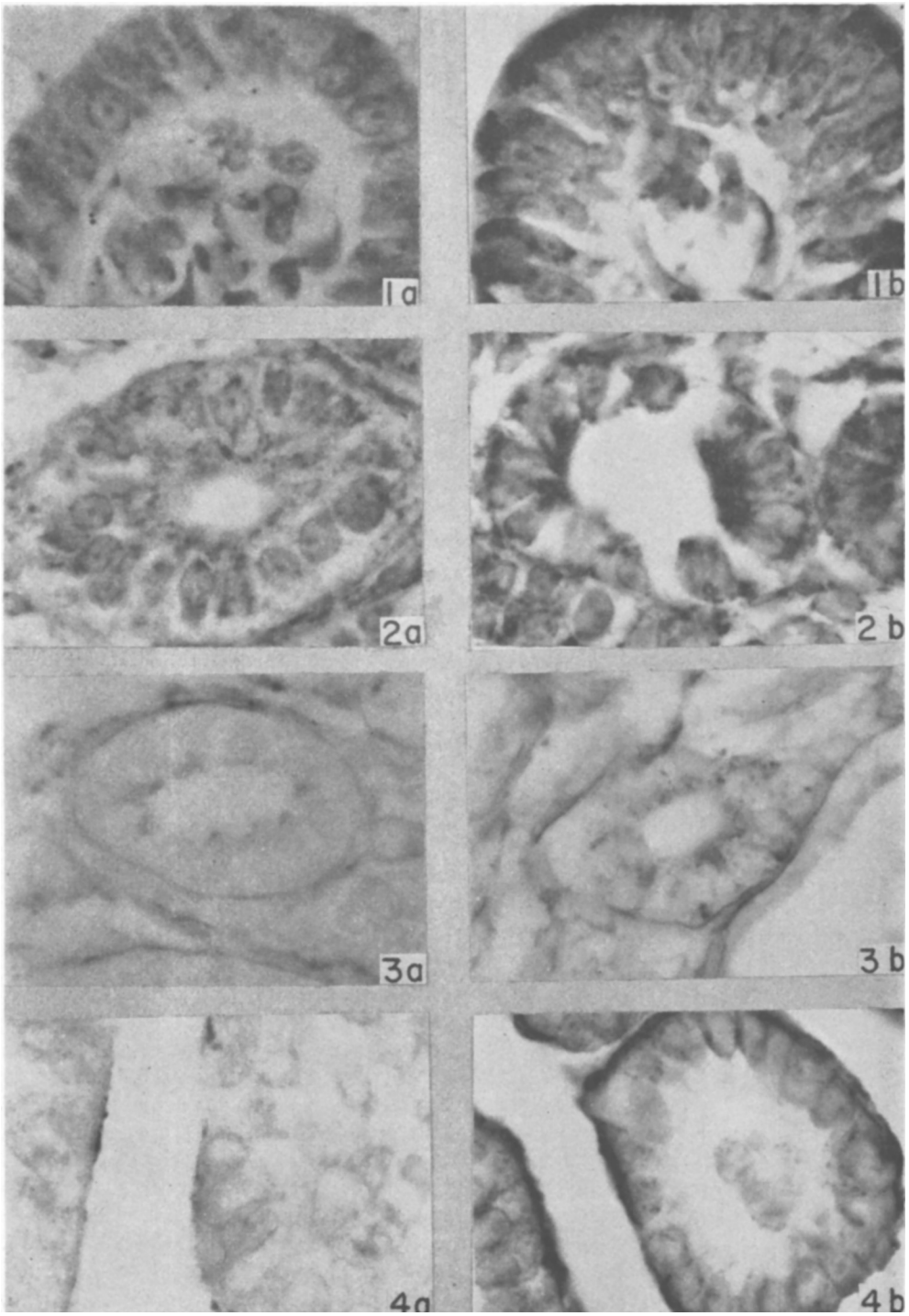

FIG. 1. Intestinal epithelium, 20-day-old fetal rat. Methyl green-pyronin. a, control; b, experimental. Note marked deposition of supra-nuclear RNA in treated fetus. ×600.

FIG. 2. Renal tubule, 18-day-old fetal rat. Methyl green-pyronin. a, control; b, experimental. Note increase in brush border RNA in b. ×600.

FIG. 3. Renal tubule, 18-day-old fetal rat. PAS. a, control; b, experimental. Note deposition of glycogen in and adjacent to brush border in b. ×600.

FIG. 4. Intestinal epithelium, 20-day-old fetal rat. Alkaline phosphatase, ten min. incubation time. a, control; b, experimental. Note in treated fetus (b) greater alkaline phosphatase activity as compared to control (a). ×600.

phosphatase content beginning with 16 days of age. This difference is even more exaggerated at 18 days. At this time, the controls show very little brush border alkaline phosphatase activity (Fig. 5a). The treated animals, on the other hand, demonstrate a very marked enzyme activity in the same site (Fig. 5b).

Post-partum Rats. In the post-partum rats, the cortisone injected animals at all ages exhibited a marked increase in hepatic glycogen deposition and a decrease of RNA content. As far as RNA is concerned, while the controls showed heavy deposits of RNA, usually in the form of rodlets or spheroids (Fig. 6a), the experimental animals demonstrated only scattered remnants of this material (Fig. 6b).

As far as the intestine and kidney are concerned, no effects of cortisone treatment were observed until the 9th day of age. At this time, significant increases in alkaline phosphatase appeared in the treated animals. This became more marked in the 1-day-olds (the oldest age group that survived the cortisone treatment). There were no such corresponding increases in RNA and PAS-positive materials. The intestinal epithelium of the 11-day-old control animals showed a moderate striated border alkaline phosphatase activity (Fig. 7a). Treated animals of the same age, on the other hand, show a more marked activity in the same site (Fig. 7b). The renal tubule brush border activity is similarly enhanced, the controls showing only moderate alkaline phosphatase deposition (Fig. 8a), while the experimental animals demonstrate a strong activity (Fig. 8b).

Discussion. Essentially, the results herein presented are in accord with the findings of Karnofsky and his group(18) on the injection of cortisone into the chick embryo and with Moog's(6-8) experiments on the influence of cortisone on the fetal and post-partum mouse.

In our work, we have observed that there were no histochemically demonstrable effects of exogenous cortisone administration in the fetus until the 16th day of intra-uterine life. Karnofsky and his group noted that the chick embryo did not show a significant response to cortisone administration until after the 10th day of development. They maintained that the cortisone, in its observable effects, influences the utilization of a new substrate which is provided for cell growth as a result of the shift in embryonic metabolism occurring at this stage. The same line of reasoning might be applied to the present work on the rat, wherein a sensitive indicator of cortisone action, the liver, showed no response until the 16th day of fetal life.

Our observations have indicated that the RNA, alkaline phosphatase and glycogen of the renal tubule brush border are increased in the treated fetus as compared with the controls beginning with the 16th day and that this difference is no longer in evidence by the 20th day. The striated border of the intestinal epithelium of the treated animals did not show any increases in alkaline phosphatase and RNA until the 18th day. This increased response, however, was obtained through the 20th day. In both the renal tubule and intestinal epithelium the cortisone served to bring about a precocious acquisition of RNA.

In the post-partum rats a similar picture was observed. A response of the intestinal and renal tubule epithelium to cortisone could not be demonstrated in rats younger than 9 days of age. As in the fetuses, the response was exhibited as a precocious increase in the amounts of alkaline phosphatase and RNA. Moog has suggested that the intestinal epithelium passes through a transitory state of sensitivity to cortisone as a stage in its normal developmental sequence. The addition of excess exogenous cortisone merely hastens the basic metabolic response processes involved in the functional differentiation of both the intestinal and renal tubule epithelium.

Thus, there would appear to be 2 stages of sensitivity to cortisone in the rat. The first

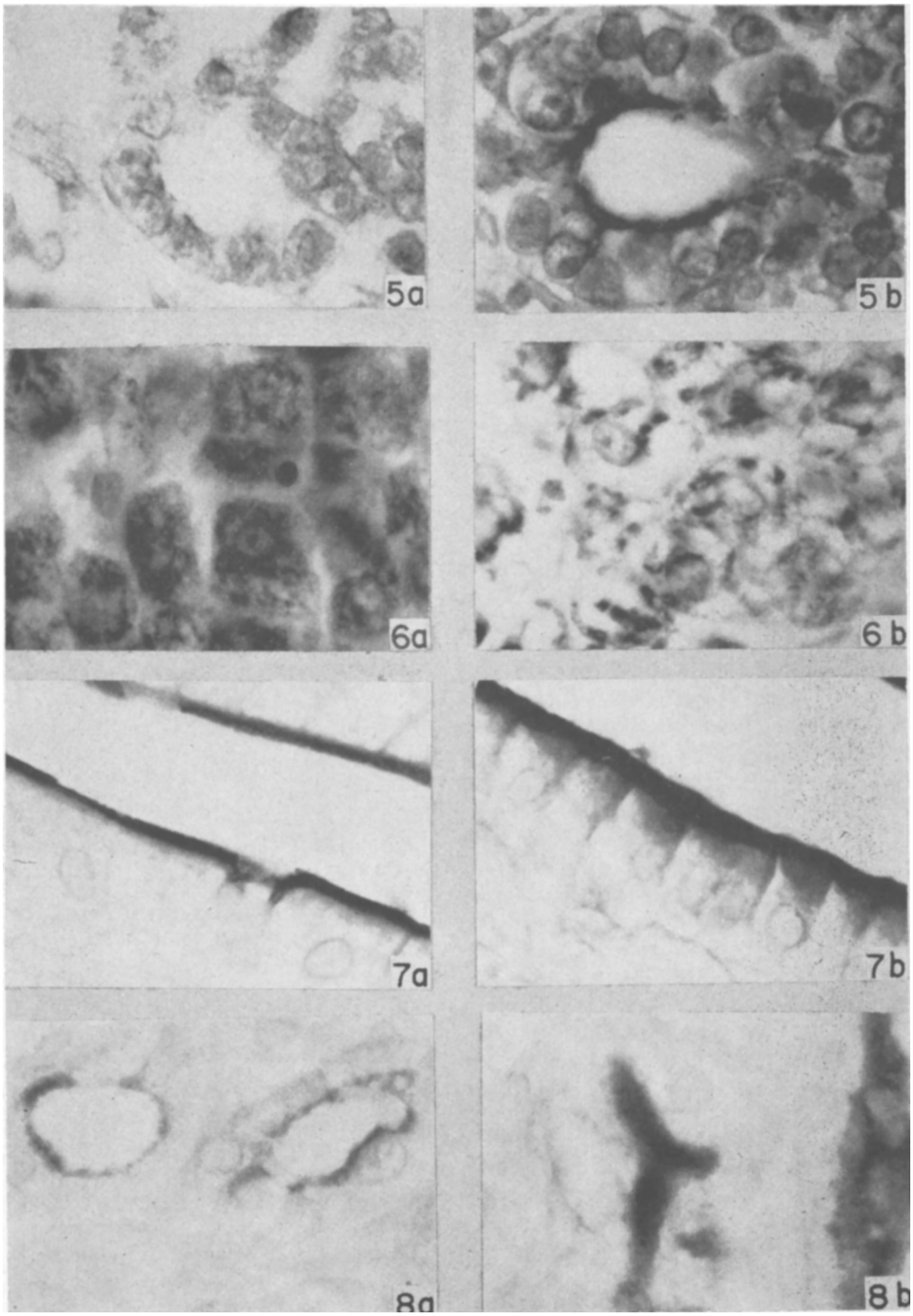

FIG. 5. Renal tubule, 18-day-old fetal rat. Alkaline phosphatase, 10 min. incubation time. a, control; b, experimental. Note very strong enzyme reaction in treated fetus (b) and contrast this with control (a). ×600.

FIG. 6. Liver, 11-day-old post-partum rat. Methyl green-pyronin. a, control; b, experimental. Marked depletion of RNA is very prominent in b. ×600.

FIG. 7. Intestinal epithelium, 11-day-old post-partum rat. Alkaline phosphatase. Ten min. incubation time. a, control; b, experimental. Treated rat (b) shows a strong enzyme reaction evident even in supra-nuclear cytoplasm. ×600.

FIG. 8. Renal tubule, 11-day-old post-partum rat. Alkaline phosphatase, 10 min. incubation time. a, control; b, experimental. Note greater brush border phosphatase activity in b. ×600.

appears in the embryo at 16 days of fetal life for the kidney tubule and at 18 days for the intestinal epithelium. The former is lost by the 18th day, whereas, the latter is still evident at 20 days. In the post-partum rat, the stage of sensitivity first appears at 9 days of age for both kidney and intestinal epithelia.

Baker and Bridgman(19), who studied the gastric and intestinal mucosa in adrenalectomized adult rats, noted a decrease in cytoplasmic RNA and striated border alkaline phosphatase activity. Following the injection of cortisone into these animals the RNA was reconstituted but the amount of alkaline phosphatase present was not affected. These findings lend further support to our observations as well as those of Moog(9) that after a certain stage of development cortisone is apparently no longer able to influence the formation of alkaline phosphatase in the intact animal.

It should, however, be emphasized that had Baker and Bridgman used whole adrenocortical extract, they might have altered the alkaline phosphatase activity in the adrenalectomized animal.

Summary. A histochemical study of the effect of cortisone on the liver, intestine and kidney of fetal and post-partum rats was made. There were no histochemically observable effects of cortisone on the fetus until the 16th day of age. Beginning with this day it was found that cortisone caused an increase in glycogen deposition and a decrease in RNA content of the hepatic parenchyma. In the post-partum rats these liver effects were noted at all ages. The intestinal striated border demonstrated a precocious acquisition of alkaline phosphatase and RNA as compared to controls of the same age. In the renal tubules these effects were manifest in the 16- and 18-day-old fetuses, while the intestinal effects were obtained in the 18- and 20-day-old fetuses. Similar precocious increases in renal and intestinal alkaline phosphatase and RNA were noted in the cortisone-treated newborns beginning with the ninth day of age.

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