SOME EFFECTS OF EXOGENOUS HYDROCORTISONE ON PREGNANCY IN THE RAT

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EIGHT FIGURES

During the past two decades a number of agents and dietary regimens have been reported to be teratogenic when administered to pregnant mammals. The diversity of the teratogenic agents and the similarity of the congenital defects induced have led to considerable speculation regarding the mechanisms involved. Since most procedures subjected the maternal organism to a certain degree of stress, it was suggested by Seyle ('50, '51) and Fraser et al. ('51a, '54) that the products of hyperactive maternal adrenal cortices might be the primary causal factor in many of the forms of induced congenital anomalies. The reports of Fraser and associates ('51a, '51b, '53a, '53b, '54), and Kalter and Fraser ('52, '53) on the production of cleft palate in the offspring of mice treated with cortisone, hydrocortisone or adrenocorticotropin have provided considerable support for this general concept. In addition, Fainstat ('54) recently reported the appearance of cleft palate in the offspring of rabbits treated with cortisone.

In view of the important implications in the reports of Fraser and his associates the following investigation was undertaken in order to ascertain whether the normal course of pregnancy in the rat could also be altered by the administration of an exogenous adrenal steroid.

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METHODS AND MATERIALS

Normal female rats (Long-Evans strain) were bred with stock males and placed on this laboratory's diet I[2] on the first day of gestation (i.e. day of finding sperm). All females were weighed regularly and vaginal smears were examined daily for the presence of erythrocytes, the placental sign.

The steroid employed was hydrocortisone [3] which was diluted with physiological saline so that 1 ml of the solution contained 10 mg of steroid. This solution was employed in all experimental groups except B10 (see table 1); in this group the material was used in an undiluted form. Control solution consisted of 0.9% benzyl alcohol in physiological saline. In dosages of 10 mg or more, the hydrocortisone was administered in multiple subcutaneous locations in such a manner that not more than 5 mg was administered in any one site.

One hundred female rats were divided into three control groups and 16 experimental groups based upon the treatment each received (see table 1). Since most teratogenic agents have been reported to be effective in the rat at some interval of time during midpregnancy, a large proportion of the animals used in these experiments were subjected to various dosages of hydrocortisone during the second week of gestation.

Experimental and control animals treated during the first week of pregnancy had one uterine horn removed on the 11th day of gestation. This procedure made it possible to observe any interference with implantation that may have occurred as a result of treatment during the first week of pregnancy.

The pregnant rats were sacrificed on the 21st day of gestation, approximately 36 hours prior to normal parturition. The maternal abdominal wall was incised, fetuses removed by

[2] Ground whole wheat, 67.5%; casein, technical, 15.0%; whole milk powder, 10.0%; hydrogenated vegetable oil, 5.25%; calcium carbonate, 1.5% and NaCl iodized, 0.75%.

[3] Merck's Hydrocortone (17-hydroxycorticosterone-21 acetate) in a microcrystalline form (25 mg/ml) suspended in a vehicle of distilled water containing the following: 0.9% benzyl alcohol, 0.9% sodium chloride, 0.4% polyoxyethylene sorbitan monleate, and 0.5% carboxymethyl cellulose.

TABLE 1

Group designations, dosages and timing employed in experimental and control rats

ANIMAL GROUP	NUMBER OF ANIMALS	SUBCUTANEOUS DOSAGE/DAY
Females treated during the first week of pregnancy (day 1–7)		
SERIES A		
Control	5	1 ml control solution
A10	5	10 mg [1]
A15	5	15 mg
A20	5	20 mg
Females treated during the second week of pregnancy (day 7–14)		
SERIES B		
Control	5	1 ml control solution
B0.6	6	0.6 mg
B1.25	9	1.25 mg
B2.5	9	2.5 mg
B5	5	5.0 mg
B7.5	5	7.5 mg
B10 [2]	5	10.0 mg
B15	5	15.0 mg
B20	5	20.0 mg
F10ip [3]	6	10.0 mg
Females treated during the third week of pregnancy (day 14–21)		
SERIES C		
Control	5	1 ml control solution
C10	5	10 mg
C15	5	15 mg
C20	5	20 mg

[1] Milligrams hydrocortisone.

[2] Females in this group received undiluted hydrocortisone.

[3] Females in this group received intraperitoneal injections.

uterine section, and the number of implantation and resorption sites recorded. Offspring from each litter were weighed collectively; exceptionally large or small fetuses were weighed individually. Examination of fetal oral cavity, brain, eyes, heart, great vessels, and other viscera was accomplished by dissection and "macroscopic sections" (Wilson, '54a).

Although the primary concern of this investigation was to ascertain the teratogenic effects of hydrocortisone administered to pregnant rats in the fashion described above, the following important correlative observations were made on mother, placenta and fetus.

All maternal viscera was examined at autopsy and organs appearing in any way abnormal were removed and preserved for microscopic studies. Dissection of the injection sites in animals receiving 10 mg of hydrocortisone, or more, revealed white subcutaneous areas believed to contain a residue of the administered steriod. In order to ascertain the relative effects of the presumed residual steroid on maternal adrenal cortices at the time of autopsy, the maternal adrenals from females receiving more than 10 mg of steroid/day were removed, weighed and subjected to histological examination.

Placentas were removed by manual manipulation and divested of the extraembryonic membranes and umbilical cord. These organs were immediately weighed (individually) to the nearest milligram on a torsion scale and cross sections of the placentas from the area of the central veins were examined histologically.

The left adrenal glands were removed from six fetuses (3 males and 3 females) in each litter of the control groups and all experimental groups except B1.25, B2.5 and B7.5 (see table 1). Because of their small size it was necessary to weigh six glands together and calculate the average weight of a single gland. The microscopic structure and relative cortical widths were determined in histological sections of these glands.

In order to determine the relative significance of the observations on organ and body weights, significance of differ-

ences of mean values was tested by Fisher's method ('38) for the distribution of "t" values. Values of $p < .02$ were considered as of probable significance, $p < .01$ as highly significant and $p > .02$ were considered not significant.

The classification of the experimental animals which was presented in table 1, will be used to facilitate the description of results observed in the above described experiment. Observations and measurements of the effects of hydrocortisone administered to the pregnant rat were made in three locations, and will be presented in the following order; the mother, the placenta and the fetus.

RESULTS

Maternal reactions to experimental treatment

Body weight changes. Mean net maternal body weight gains or losses (gross body weight minus the weight of the conceptuses) in all experimental groups were significantly lower than the weights observed in the control groups (table 2). Comparison of the mean net weight of groups receiving more than 5 mg of steriod/day, however, revealed no significant differences which could be related to the dosages of hydrocortisone employed.

The rate of body weight loss during treatment, as determined by the slope of plotted curves (fig. 1) demonstrated only slight variation in all groups of Series A or C. This rate appeared to be the same for all groups in Series B which received 5 mg, or more, of hydrocortisone each day, when administered subcutaneously in a diluted form. Undiluted hydrocortisone administered in a subcutaneous site (Group B10) produced a less severe effect on the final mean net body weight and on the rate of body weight loss during treatment. An increase in the number of subcutaneous injection sites did not appear to increase or retard the effect of the steroid on maternal body weight.

All animals receiving 10 mg, or more, of hydrocortisone each day revealed at autopsy a white subcutaneous mass at the

injection sites which was believed to contain a residue of the steroid. A failure of the rate of gross weight change to return to control levels during the last week of pregnancy was exhibited by animals of Series A and B which received 5 mg/day, or more, of the steroid (fig. 1). On the other hand, the average rate of weight change in animals which received intraperitoneal injections, returned to the control rate after the period of treatment. At autopsy a steroid residue was not found in

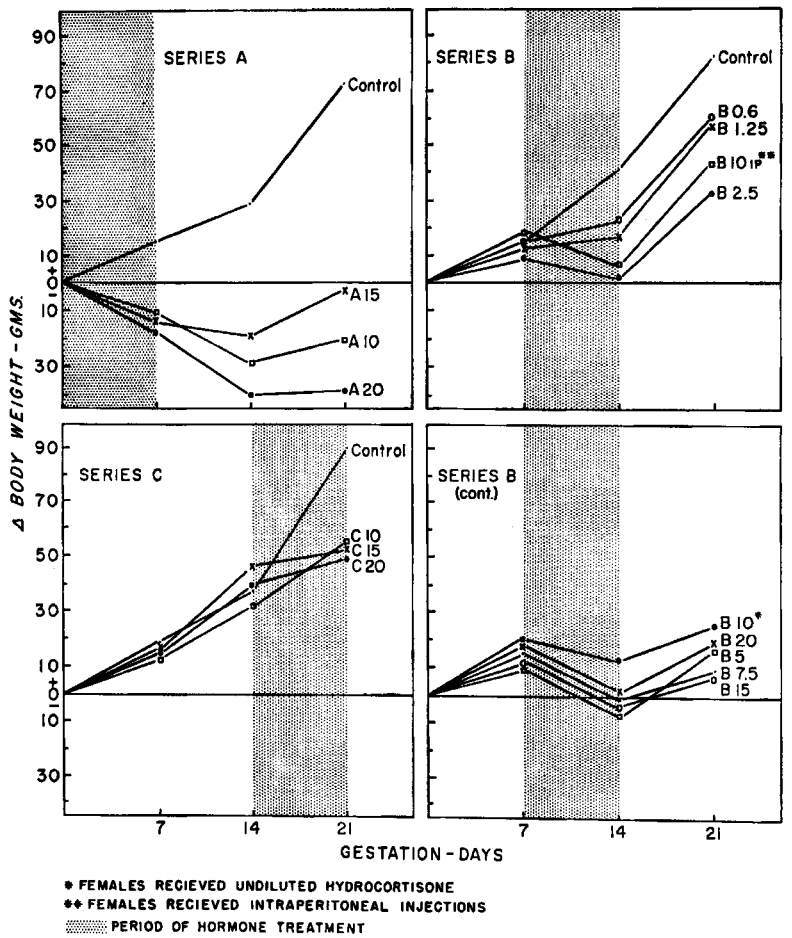

Fig. 1 Average maternal gross body weight changes during gestation.

the peritoneal cavity of these animals. It appears that the continued decrease in net maternal weight and a failure to return to the control rate of gross weight increase in the animals of Series A and B which received subcutaneous dosages of more than 5 mg/day, indicated that an active steroid

TABLE 2

Some effects of exogenous hydrocortisone and control solutions on pregnant rats

EXPERIMENTAL GROUP	RATS BRED	AV. NET WT. CHANGE DURING GESTATION	AV. ADRENAL WT.	VAGINAL EBC	RESORPTION OF IMPLANTATION
	no.	*gm*	*mg*	*day*	*%*
Females treated during the first week of pregnancy (day 1–7) SERIES A					
Control	5	42.6	49		3
A10	5	—24.5			42
A15	5	—19.1	25		36
A20	5	—54.4	25		40
Females treated during the second week of pregnancy (day 7–14) SERIES B					
Control	5	50.0	49	14.5	12
B0.6	6	35.3		13.3	31
B1.25	9	24.7		13.8	16
B2.5	9	8.7		13.0	23
B5	5	—12.7		13.0	27
B7.5	5	—21.7		13.6	26
B10[1]	5	5.2		13.4	34
B15	5	—17.5	22	13.5	20
B20	5	—10.2	26	13.8	4
B10ip[2]	6	25.2	38	12.5	58
Females treated during the third week of pregnancy (day 14–21) SERIES C					
Control	5	56.0	50	14.2	5
C10	5	17.1		14.2	0
C15	5	13.5	30	14.4	13
C20	5	15.6	28	14.8	7

[1] Females received undiluted hydrocortisone.

[2] Females received intraperitoneal injections.

was still being released from the injection sites for the duration of pregnancy, even though treatment had been stopped.

Maternal death and general reactions to treatment. The ventral midline adbominal incision made to remove the uterine horn in animals of Series A healed very poorly in all experimental females. Pyemia at suture sites was common and complete closure of the wound had not occurred by time of autopsy. Control animals, on the other hand, demonstrated no infections and wounds were well healed within four to five days.

Two animals of Series A died before time of autopsy, one from Group A15 (18th day of gestation) and one from Group A20 (17th day of gestation). Prior to death these animals appeared lethargic and lost 66 grams and 74 grams of body weight respectively. Another animal in Group A20 lost 84 grams but survived until autopsy at which time all implantation sites were resorbing.

At autopsy, each of these animals revealed a number of white nodules which appeared grossly to be on the surface of the heart, lungs and kidneys. Histological examination of the lesions revealed necrotic foci extending deeply into the stroma of the organs replacing normal tissues. Similar lesions have been reported elsewhere (Antopol, '51) as a result of cortisone treatment: a detailed account of their morphology will not be presented here. These lesions were never observed in the fetuses.

Maternal adrenal glands. The adrenal glands of all females from experimental groups receiving subcutaneous dosages of 15 to 20 mg/day, and all control adrenals were weighed at autopsy. Regardless of the period of gestation during which the hormone was administered, the treated rats had significantly smaller adrenals than did the control females (table 2). The mean adrenal weights for animals of Series A and B did not vary significantly from the mean weights observed in Series C. Animals of Group B10ip, injected intraperitoneally during the second week of pregnancy, had maternal adrenal

weights averaging 38 mg (26–50), a value not significantly different than the control mean adrenal weight (i.e., $p > 0.02$).

Microscopic studies revealed that the morphology of the adrenal cortex from females of Group B10ip was similar to the controls. All other experimental adrenals examined revealed a reduction in cortical width when sections from approximately the same position were compared with the controls. There was also a reduction of cytoplasm in the cells of the zona reticularis and zona fasiculata with a resultant apparent increase in the number of nuclei observed in any given field. It was concluded that the subcutaneous residues, observed at the injection sites at autopsy, were releasing an active steroid as evidenced by the reduction in adrenal weights and the observed cortical involution.

Reproductive performance. The reproductive performance of animals used in the above experiments was determined by an arbitrary measure (R/Ix100) based on the total number of resorption sites (R) in relation to the total number of implantation sites (I) for each group of animals, (table 2). The percentage of resorptions in all animals administered hydrocortisone subcutaneously was 39% for Series A, 19% for Series B and 7% for Series C. Increased dosages did not significantly alter the percentage of resorptions.

Early administration of hydrocortisone to pregnant rats (day 1–7) did not adversely affect implantation. The average number of implants counted in both uterine horns on the 11th day of gestation varied by no more than 0.7 sites for any one experimental group as compared with the controls. Since in Series A 39% of the litters were resorbed by day 21, the causal factor for resorption must make its appearance at a later time.

Placental reactions to experimental treatment

Placental weight. All experimental groups receiving 5 mg/day, or more, of hydrocortisone subcutaneously had significantly smaller placentas than did the controls (fig. 2). In

Series B mean placental weights decreased with increases in steroid dosage, although no two successive groups exhibited a significant difference in weight. The mean weight of the placentas from Group B10ip was significantly heavier than the control placental weight, possibly a result of the reduced litter size. Series A demonstrated an increase in mean placental weight with an increase in dosage and this too appears to be the result of factors other than the hormonal tratment.

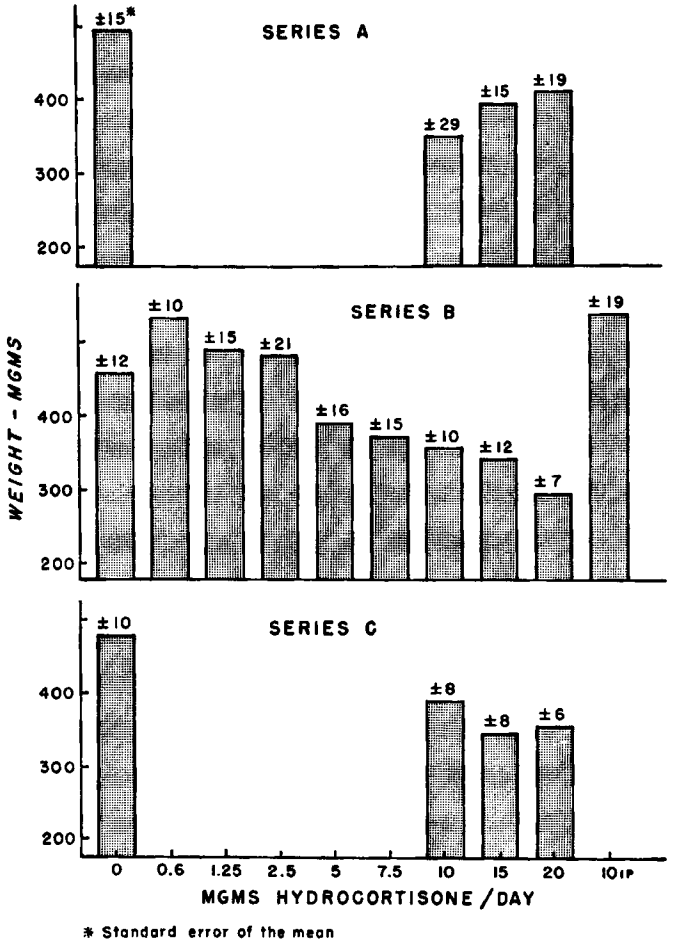

Fig. 2 Average placental weights.

Placental structure. Histological examination of the placentas from treated animals revealed a general reduction in size of all components as compared with the controls (fig. 6 cf 5). The morphologic characteristics of the spongiotrophoblast appeared most altered, when compared with the controls, in those groups receiving the highest dosage of the steroid. Cells in this area observed in the placentas from Group B20 and to a lesser extent in other groups receiving 15–20 mg of steroid each day, exhibited a reduction in cytoplasm, hyperchromic nuclei, and in some instances, pyknosis, (fig. 7 cf 8).

Fetal reactions to experimental treatment

Fetal Weight. Average fetal weights were significantly below control values in litters taken from females treated with 10 mg or more of hydrocortisone each day during the first or second week of pregnancy (fig. 3). The litters of animals

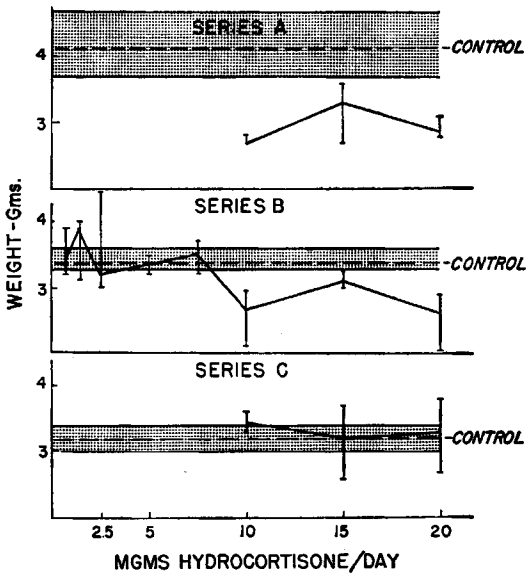

Fig. 3 Average fetal weight/litter.

Note: (1) Stippled area represents range of control fetal weights; (2) Vertical bars represent range of experimental fetal weights.

which received intraperitoneal injections of 10 mg/day of steroid had litters that averaged 4.1 gm per fetus (3.8–4.8), a weight significantly greater than the control group. The steroid appeared to have little effect on fetal weight when administered during the third week of gestation, the period of most active growth. Average fetal weights in these groups were equal to or larger than the control group. A regular correlation between placental and fetal weight could not be demonstrated, although an inverse relationship between litter size and fetal and placental weight frequently appeared (i.e., smaller litters result in heavier placentas and fetuses).

Malformations. Two of the 563 fetuses exhibited gross malformations and 12 others were smaller (< 2.0 gm) than could be anticipated in normal litter variation. Two of the stunted fetuses were found in Series A (Group A15), 7 in Series B (Groups B7.5, B10, B15, B20), and one in Series C (Group C20). In addition, two dead and stunted fetuses were found in litters from Groups B1.25 and B20.

The two grossly malformed fetuses both occurred in litters from Series B (Groups B7.5 and B10). These offspring exhibited defects in the caudal portions of their bodies. The malformed fetus from Group B7.5 exhibited the following external defects; micromelia and retarded digital development in hindlimbs, shortening of the lumbo-sacral region of body, absence of skin surrounding the umbilicus, umbilical hernia, patent vaginal orifice, very small urogenital tubercle and mild bilateral hydronephrosis and hydroureters. The fetus from Group B10 exhibited absence of tail and an imperforate anus.

Studies of cleared fetuses from experimental Group B10ip revealed defects of the thoracic vertebral bodies in 6 of the 21 offspring. The defective offspring possessed anomalous 10th or 8th thoracic vertebral bodies and the two or three vertebral bodies caudal to this defect also demonstrated deviation from the normal pattern. In all instances the malformations consisted of dumbell shaped or elongated ossification centers.

Studies of offspring from other experimental groups and control offspring did not reveal similar findings.

Viability. It was repeatedly observed that initial respiratory activity failed to appear when fetuses were removed from the uteri of experimental females. This occurred with greater frequency at the higher dosages (> 7.5 mg/day). All control

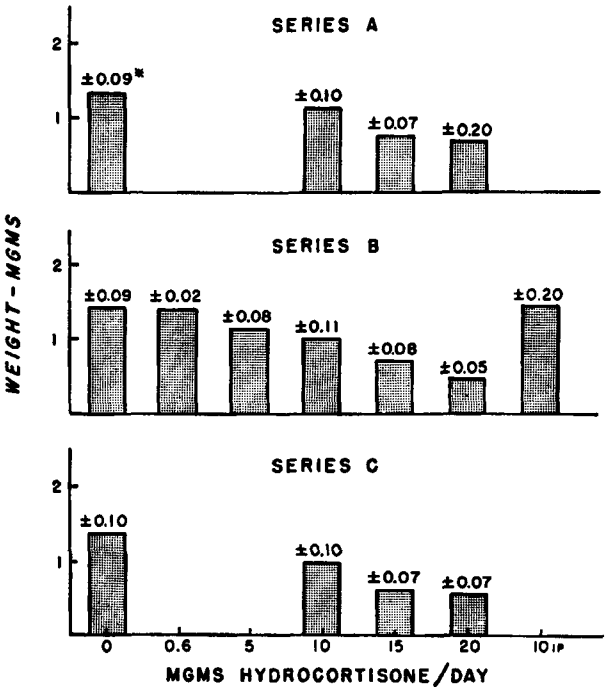

Fig. 4 Average fetal left adrenal weights.

fetuses, on the other hand, demonstrated active respiratory movements shortly after they had been freed of their investing membranes. The duration of response to applied external stimuli was noticeably shorter in offspring from the experimental series.

Fetal adrenals. Average fetal left adrenal weights showed a marked change, in an inverse ratio to the dosage of hormone employed (fig. 4). A reduction in weight accompanied each

increase in dosage in all experimental groups except B10ip. The fetal adrenals from this group exhibited a mean adrenal weight of 1.46 mg (1.25–1.80), which was essentially the same as the mean adrenal weight for the controls. The mean adrenal weight of all fetuses taken from mothers which received 15 or 20 mg of hydrocortisone/day, was significantly lower than the controls. A non-significant difference ($p. < 0.05$) was displayed by the adrenals of fetuses from Groups A10, B5, B10, and C10.

Fetal adrenal morphology. Studies were made of hemotoxylin and eosin stained preparations of fetal adrenals from each experimental group. Only the fetal glands from experimental groups which received 15 or 20 mg of steroid/day, during the second or third week of pregnancy, demonstrated a striking difference when compared with the controls. The major alterations observed consisted of a reduction of cytoplasm in the cortical cells of the inner zones and hence, a reduction of cortical width. The most severe alterations in morphology were found in the fetal adrenals from Groups B20 and C20.

DISCUSSION

A complete correlation of the many facets of the experiments presented above will not be attempted here. Instead, the effects of hydrocortisone on the conceptus and pregnant rat will be correlated with other reports on experimental mammalian teratology.

Although only two malformed fetuses were found in the present experiments, the most severe effects on the developing offspring were in those animals treated during the second week of pregnancy. This observation lends support to the many reports on the susceptibility of the rat embryo to a variety of agents administered during this phase of pregnancy (see reviews by Fraser and Fainstat, '51a, Warkany, '53, and Nelson, '54).

The variation of response to teratogenic agents in fetuses or embryos from a single litter is not peculiar to the experi-

ments reported here. Fraser and associates ('53b), Cohlan ('53), Nelson ('54) and Wilson ('55), to mention a few, have witnessed the production of both malformed or resorbed and normal offspring in a single litter of a treated rat. Explanations of this phenomenon in the past have been hypothetical for the most part and the experiments presented here in no way resolve this problem.

The severe effects on the pregnant rat which were produced by exogenous hydrocortisone in the above described experiments induced few morphologically evident alterations in the developing embryo and fetus. This observation corroborates Wilson's ('56b) suggestion that the effectiveness of a teratogenic agent cannot be determined by the severity of its activity in the mother, rather, certain key metabolic processes or metabolites appear to be involved. Results presented here indicate that the maternal metabolic patterns altered by exogenous hydrocortisone do not necessarily influence development in the embryonic and fetal rat.

Further investigation is necessary, however, before hydrocortisone can be totally dismissed as a teratogenic agent in the rat. The increased percentage of resorptions observed in animals treated during the first or second week of pregnancy may be the result of fetal malformations of such severity that death ensues. Such was the case in Waddington's and Carter's ('53) observations on the effects of trypan blue administered to pregnant mice. Although the effects of hydrocortisone on placental weight and morphology indicate that this organ could be the causal factor in hydrocortisone induced resorptions, an examination of the early development of the offspring of treated rats is obligatory before any conclusion can be reached.

The adverse effects of hydrocortisone on fetal viability must also be considered. Although these animals are not morphologically malformed, it is quite possible that they are physiologically abnormal and, consequently, in a functional sense, anomalies. The particular manner in which hydrocortisone reduces fetal viability in the rat was not determined, although

it was observed frequently that a failure to initiate respiration resulted in death after the offspring were divested of their embryonic membranes. Offspring were not allowed to survive, however, and hence the ultimate test of viability was not employed. The fetuses from treated mothers also exhibited a less vigorous response to external stimuli, possibly the result of an anesthetic effect which has been ascribed to large dosages of adrenal steroids (Selye, '42). This phenomenon could also be associated with the damage to neuroblasts observed by Hicks ('54) in offspring of cortisone treated rats.

Browne ('52) suggested that the teratogenic effects of cortisone in mice, as observed by Fraser and associates ('53), might be attributed to a suppression of the secretions of fetal adrenal cortex by the exogenous cortisone administered to the mother. Morphological evidence of fetal adrenocortical suppression has been presented here and in the investigations of Davis and Plotz ('54). Since a large number of morphologically normal fetuses with reduced adrenal weights and cortical size were observed in both investigations, it would appear that, in the rat, Browne's hypothesis is not valid. Indeed, Tobin ('39) has demonstrated that adrenalectomy of the rat fetus 5–6 days prior to parturition did not alter fetal growth or survival in utero.

The route of adrenal steroid administration considerably alters the effect of hydrocortisone and cortisone in treated rats and mice. The transitory slight maternal body weight loss in mice treated with intramuscular injections of cortisone (Fraser, '54) is similar to the reduced effect on body weight loss in pregnant rats treated with intraperitoneal injections of hydrocortisone (reported here) and in non-pregnant rats treated with intraperitoneal injections of cortisone (Greenspan, et al., '53). This suggests that the maternal metabolic disturbance induced by exogenous adrenal steroids administered by intramuscular or intraperitoneal injections differs from that observed in animals treated with subcutaneous injections. The latter more closely reproduces the effects

resulting from continuous subcutaneous injections of adrenocorticotropin (Ingle, '51) and hence simulates more accurately the effects of a hyperactive maternal adrenal cortex. Greenspan and associates ('53) have suggested that intraperitoneal injections produce a high but transitory level of circulating steroid and that a sustained level is necessary to induce the metabolic alterations which result in weight loss and adrenal and thymic involution. Jost ('56) has recently reported that the administration of 1–3 mg of cortisone directly into the fetal abdominal cavity before the 16th day of gestation produced cleft palates. It appears, therefore, that a high level of circulating steroid is necessary to induce alterations in embryonic development and that such levels were not attained in the above described experiments. The results presented here clearly indicate, however, that alterations in maternal metabolism which resulted from the administration of exogenous hydrocortisone to the pregnant rat were not teratogenic. The pregnant rats described above exhibited physical symptoms which have been attributed to hyperadrenalcorticism and it is doubtful that more serious alterations in maternal metabolism could be attained short of death.

SUMMARY

Hydrocortisone was administered to pregnant rats for seven days during the first, second or third week of pregnancy. Dosages employed ranged from 0.6 to 20 mg/day. The effects of this treatment were studied in the mothers, the placentas and the fetuses.

It was observed that exogenous hydrocortisone when administered to pregnant rats will alter the outcome of pregnancy, especially if the steroid is administered during the first or second week of gestation. This steroid appeared to be an impotent teratogenic agent in the rat, but on the other hand, it was observed to seriously alter maternal metabolism, increase the number of resorptions, reduce placental size, reduce fetal size, and exert a detrimental effect on fetal viability.

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PLATE 1

EXPLANATION OF FIGURES

5 A section of control placenta cut in the vicinity of the central vein. The dark staining spongiotrophoblast is located on the left and the lighter staining labyrinth on the right. Periodic acid-Schiff's reagent and hematoxylin, 8 ×.

6 A section of placenta from a rat treated with 20 mg of hydrocortisone/day during the second week of pregnancy (Group B20). Location of section and orientation of photograph same as that of the controls in fig. 5. Note general reduction in size as compared with the control and relatively greater decrease in amount of spongiotrophoblast. Periodic acid-Schiff's reagent and hematoxylin, 8 ×.

7 A higher magnification (340 ×) of the spongiotrophoblast in the same section of control placenta demonstrated in fig. 5. A small portion of the labyrinth can be observed at the top of the photograph. Note the large vesicular nuclei and abundance of cytoplasm in the spongiotrophoblast cells. Basophilia of the cytoplasm in these cells provides an even grainy appearance.

8 A higher magnification (340 ×) of the spongiotrophoblast from the same section of experimental placenta demonstration in fig. 6. The borders of the spongiotrophoblast are indicated by the labyrinth at the top of the photograph and the giant cell at the bottom. Note the small hyperchromic nuclei, the reductions in cytoplasm, the presence of perinuclear vacuoles and the loss of a uniform basophilia observed in the control.


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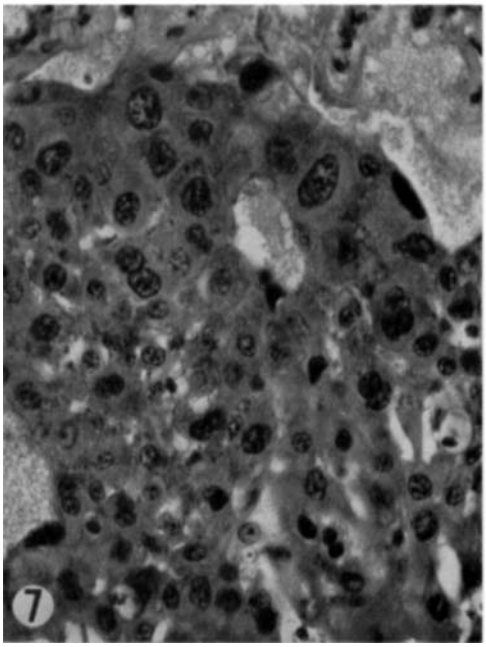
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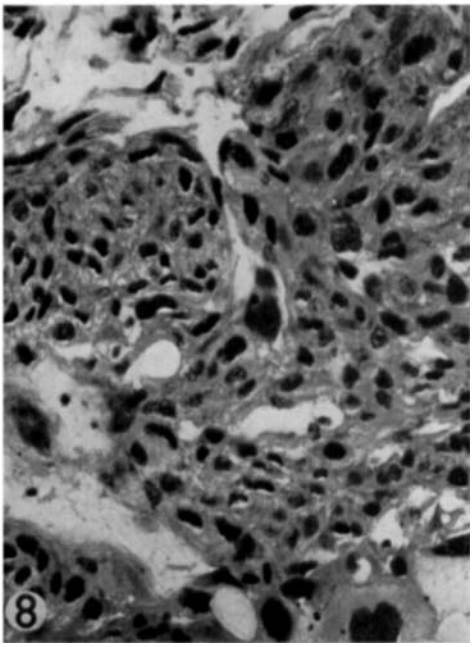
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