

Similarity of Congenital Malformations Produced by Hydrocortisone to those Produced by Adrenalectomy in Guinea Pigs¹

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Adrenalectomy in pregnant guinea pigs produced a syndrome of malformations which included, open eyelids, hydrocele, hematocele, abdominal eventration, umbilical hernia and scattered defects of the palate and spinal cord. (Hoar and Salem, '62).

Since the concept of congenital malformations arising from an excess or deprivation of the same compound has been well documented utilizing hypo- and hypervitaminosis A in rats (see Kalter and Warkany, '59, for a review of the subject) and since, to the best of our knowledge, such an effect has not been produced with endocrines, the question arose as to whether guinea pigs would respond to hyperadrenocorticalism in a manner similar to hypoadrenocorticalism. Accordingly, the present experiment was designed to test the effects of hydrocortisone, the principal corticoid produced by guinea pigs, on development in these animals. The results are compared with those previously obtained from our examination of the effects of hypoadrenocorticalism (adrenalectomy).

MATERIALS AND METHODS

Female guinea pigs weighing 500 gm or more were placed with males. Daily vaginal smears were obtained until sperm were found at which time the female was presumed to be mated. The day sperm were found was considered to be day zero of the gestation period. A total of 29 females received subcutaneous hydrocortisone injections (0.3 mg per 100 gm body weight twice a day) on specific days—twelve on days 10–11, nine on days 13–14 and eight on days 16–17. Twenty-six additional females received a greater amount of hydrocortisone (0.6 mg per

100 gm body weight twice a day) on days 16–17. Fourteen females served as controls. All were examined daily for vaginal openings and/or bleeding which are indicative of sterile matings, resorption of one or more of the fetuses or abortion. Embryos were collected on the thirtieth day of gestation, weighed, crown-rump length measured and examined for gross malformations. Selected malformed embryos were serially sectioned at 20 μ and stained with hematoxylin and eosin.

RESULTS

The teratogenic effects of hydrocortisone as evidenced by per cent resorptions and grossly visible abnormalities in the recovered embryos are presented in table 1. Embryos were classified as resorbed if the resorption was complete or the embryo consisted only of unidentifiable, featureless tissue. The percentage of resorptions was determined from the total number of placentas collected, while the percentages for the remaining two categories were based on actual embryos collected at the thirtieth day of gestation. Consideration of the table reveals that none of the females receiving the lower dosage of hydrocortisone (0.3 mg) aborted and their resorption rate was similar to that displayed by the controls. However, four of the females receiving the larger quantity of hydrocortisone (0.6 mg) aborted and the resorption rate (13.3%) of the remaining 22 females was almost twice that of the controls (7.1%).

The per cent of grossly visible malformations exhibited by embryos recovered

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TABLE 1
The teratogenic effect of hydrocortisone injections on guinea pig embryos as seen after 30 days of gestation

Group	Adults		Embryos			
	Treatment	Number	Abortions	Resorbed	Abnormal	Normal
Control	—	14	—	7.1% (3 of 42)	—	100% (39 of 39)
Hydrocortisone (0.3 mg per 100 gm body weight twice a day)	injected on days 10–11	12	—	5.2% (2 of 38)	40.5% (15 of 36)	59.5% (21 of 36)
	injected on days 13–14	9	—	—	50.0% (14 of 28)	50.0% (14 of 28)
	injected on days 16–17	8	—	9.0% (2 of 22)	65.0% (13 of 20)	35.0% (7 of 20)
	combined results	29	—	6.67% (4 of 60)	50.0% (42 of 84)	50.0% (42 of 84)
Hydrocortisone (0.6 mg per 100 gm body weight twice a day)	injected on days 16–17	26	4	13.3% (8 of 60)	63.4% (33 of 52)	36.6% (20 of 52)

TABLE 2
The occurrence and types of gross abnormalities seen at 30 days of gestation in guinea pig embryos following maternal adrenalectomy or hydrocortisone treatment

Group	Adults		Total % of abnormal embryos	% of the various abnormalities displayed by abnormal embryos ¹			
	Treatment	Number		Open eyelids	Subcu- taneous fluids	Ventral body wall	Other
Hydrocortisone (0.3 mg per 100 gm body weight twice a day)	injected on days 10–11	12	40.5 (15 of 36)	75.0	18.7	—	6.3
	injected on days 13–14	9	50.0 (14 of 28)	76.4	5.9	5.9	11.8
	injected on days 16–17	8	65.0 (13 of 20)	76.9	15.4	—	7.7
	Combined results	29	50.0 (42 of 84)	76.0	13.0	2.3	8.7
Hydrocortisone (0.6 mg per 100 gm body weight twice a day)	injected on days 16–17	26	63.4 (33 of 52)	—	82.3	14.7	3.0
Adrenalectomized (0.2 mg DCA daily)	18 hours post coitus	32	32.7 (16 of 49)	50	21	29	—

¹ Subcutaneous fluids = hydrocele, hematocele and edema. Ventral body wall = abnormalities ranging from abdominal eventration to umbilical hernia. Other = twisted limbs, syndactyly, spina bifida, cleft palate, myelo-meningocele and syringo-myelo-meningocele.

TABLE 3

Average weights and crown-rump lengths of embryos recovered after 30 days in utero from control and hydrocortisone-injected females

Group	Treatment	Measurements	
		C-R Length	Weights
Control	None	23.31 ± 0.3 ¹	1.89 ± 0.03 ¹
Hydrocortisone (0.3 mg per 100 gm body weight twice a day)	injected on days 10-11	24.14 ± 0.2	1.72 ± 0.03
	injected on days 13-14	23.84 ± 0.3	1.79 ± 0.06
	injected on days 16-17	23.18 ± 0.3	1.64 ± 0.04
Hydrocortisone (0.6 mg per 100 gm body weight twice a day)	injected on days 16-17	23.74 ± 0.3	1.79 ± 0.05

¹ ± Values represent the standard error of the mean.

from females receiving 0.3 mg hydrocortisone on days 10-11 was 40.5%. This percentage rose sharply to 65.0% in the embryos from animals treated on days 16-17. The increased quantity of hydrocortisone (0.6 mg) received by the second group of females on days 16-17 failed to alter these results significantly for 63.4% of the embryos recovered from these animals exhibited external abnormalities. The congenital malformations produced included open eyelid, abdominal eventration, umbilical hernia, edema, cleft palate, syringo-myelo-meningocele, syndactyly and twisted limbs. Although no attempt was made to examine all the embryos histologically the microscopic appearance of a representative few appear in figures 1-8.

The pattern of grossly visible malformations displayed by embryos in response to opposing levels of adrenal activity are presented in table 2. Data on malformations obtained by adrenalectomy in guinea pigs (Hoar and Salem, '62) have been similarly treated and included in the table. Examination of these data reveals that regardless of treatment, essentially the same pattern of malformations was displayed. Differences are present, for none of the embryos recovered from females receiving 0.6 mg hydrocortisone displayed open eyelids and adrenalectomized females exhibited a high resorption rate (48.9%) as compared with those receiving exogenous hydrocortisone (table 1).

The average weight and crown-rump length measurements of the embryos are

recorded in table 3. Embryos recovered from control and hydrocortisone-injected females had essentially the same crown-rump lengths. Although the data indicate the controls weighed more than the experimentals, statistical significance exists only between the controls and those embryos recovered from females injected on the 16-17 day with the 0.3 mg dosage of hydrocortisone ($P < 0.001$).

DISCUSSION

The evidence suggests that excess hydrocortisone does affect the development of normal offspring. There was a high percentage of abnormal young produced as well as indications that all the embryos, whether grossly abnormal or not, suffered a weight loss.

The observations are even more interesting when compared with those of other investigators. There is little doubt that cortisone injections produce malformations in mice (Baxter and Fraser, '50; and Fraser and Fainstat, '51) and rabbits (Fainstat, '54) or that hydrocortisone is equally as effective in mice (Kalter and Fraser, '52). However, neither compound appears to be teratogenic in rats (Kalter and Warkany, '59). On the other hand, the rat (Nakamura, '57) and the guinea pig (Hoar and Salem, '62) appear to be the only animals whose offspring exhibit malformations following maternal adrenalectomy. Therefore, to the best of our knowledge, guinea pigs are the only animals that exhibit congenital malforma-

tions when subjected to either a decrease in adrenocorticoids (adrenalectomy) or an excess of exogenous hydrocortisone.

This attribute of the guinea pig becomes even more puzzling when one considers the similarity in the response manifested by embryos subjected to either hypo- or hyperadrenocorticalism. This similarity existed not only in the pattern of grossly visible malformations, but also in the crown-rump lengths and weights of the recovered embryos (tables 2 and 3). A comparison of these measurements obtained from embryos recovered from adrenalectomized females (Hoar and Salem, '62) with those of embryos recovered from hydrocortisone-injected females (table 3) indicates that neither treatment affected the crown-rump length. However, the embryos from adrenalectomized females weighed significantly ($P < 0.001$) less and those from hydrocortisone-injected females marginally less than control embryos. A similar response has been reported only for the abnormal offspring of cortisone-treated mice (Kalter, '57) and cortisone-treated rabbits (Fainstat, '54) whereas the response reported here for abnormal guinea pig embryos applies to both levels of adrenal activity.

It is once more apparent that guinea pigs respond to potentially teratogenic situations involving the adrenals in a manner which is different from that of other commonly used laboratory mammals. This difference possibly is a reflection of the increased complexity of their endocrine control of pregnancy. The similarity in the teratogenic response of guinea pigs to opposite levels of adrenal activity serves not only to emphasize this difference but also the need for the development of a basic understanding of the mechanisms involved in any teratogenic response.

SUMMARY

Abnormal embryos were obtained after 30 days of gestation from guinea pigs injected subcutaneously at specific times from the tenth to seventeenth day of pregnancy with either of two dose levels of hydrocortisone.

The lower dosage (0.3 mg per 100 gm of body weight twice a day) produced no

abortions and a resorption rate of the same magnitude as the controls. Following injection on days 16 to 17, 65.0% of the embryos were abnormal whereas on days 13 and 14, 50% of the embryos and on days 10 and 11, 40.5% of the embryos were abnormal.

The higher dosage of hydrocortisone (0.6 mg per 100 gm body weight twice a day) was injected only on the sixteenth to seventeenth day and resulted in four abortions and a resorption rate (13.3%) almost twice that of the controls (7.1%). At the time of recovery, 63.4% of the embryos were abnormal. This percentage is not significantly different from that obtained with the lower dosage on days 16 and 17.

The pattern of abnormalities produced was similar in each group regardless of the timing or dosage employed and included open eyelids, edema, abdominal eventration, umbilical hernia, reduced weight, cleft palate, syringo-myelo-meningocele, syndactyly and twisted limbs.

These results are similar to those obtained previously by maternal adrenalectomy.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

All embryos were obtained after 30 days of gestation.

- 1 Coronal section of a control embryo indicating normal fusion of eyelids. $\times 12$.
- 2 Coronal section of a hydrocortisone-injected embryo indicating open eyelids. $\times 14$.
- 3 Transverse section of a control embryo; the break in the skin is an artifact due to sectioning. $\times 12$.
- 4 Transverse section of a hydrocortisone-injected embryo exhibiting subcutaneous collections of fluid. $\times 14$.

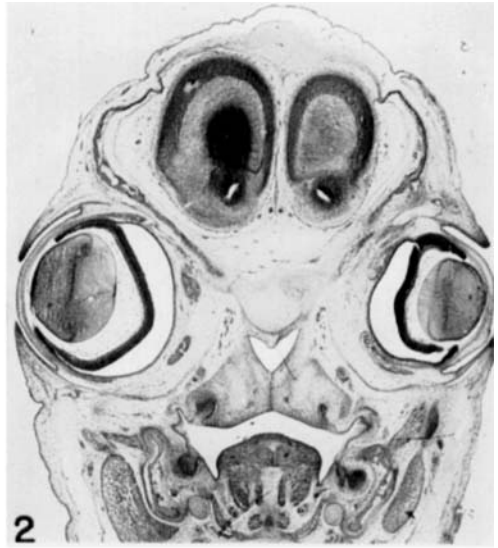
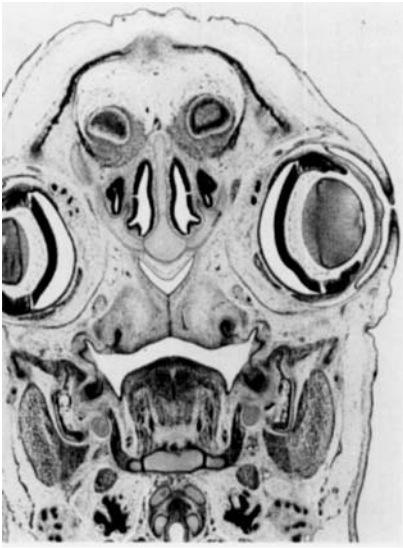


PLATE 2

EXPLANATION OF FIGURES

All embryos were recovered after 30 days of gestation.

- 5 Transverse section of a control embryo with normal umbilical cord and abdominal closure. $\times 12$.
- 6 Transverse section of a hydrocortisone-injected embryo exhibiting an umbilical hernia. $\times 14$.
- 7 Transverse section of a control with a normal abdominal closure and umbilical cord. $\times 12$.
- 8 Transverse section of a hydrocortisone-injected embryo displaying abdominal eventration. $\times 14$.

