HYPOPHYSIS-ADRENAL SYSTEM IN THE FETAL RAT

EFFECTS OF HYDROCORTISONE, CORTISONE, DCA, ADRENALECTOMY AND MATERNAL HYPOPHYSECTOMY UPON THE HYPOPHYSIS [1,2,3]

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FIVE FIGURES

INTRODUCTION

The existence of a functional reciprocal relation between the fetal hypophysis and adrenal cortex has been suggested (Kitchell and Wells, '52a, '52b). In the rat fetal hypophyseoprivia retards the growth of the adrenal cortex, an effect which is prevented by injected corticotrophin (Wells, '47, '48, '49). Unilateral adrenalectomy of the fetus induces a compensatory hypertrophy of the remaining adrenal (Kitchell, '50), as does unilateral destruction of the adrenal by cautery (Tobin, '39). This hypertrophy can be prevented by implanted cortisone (Kitchell and Wells, '52a).

The appearance of chromophilic granules in the hypophysis before birth could be regarded as morphological evidence of functional activity of the fetal hypophysis. In the hypophysis

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of the newborn rat, the chromophiles are few, however, and they exhibit only a weak staining reaction (Siperstein, Nichols, Griesbach and Chaikoff, '54). This may be correlated with the observation of lack of response to injections of epinephrine; in rats younger than 8 days this does not reduce the ascorbic acid content of the adrenal (Jailer, '50). This was thought to be due to a failure of the hypophysis to secrete sufficient corticotrophin since the adrenals are capable of response to injections of corticotrophin.

The present work was designed to expand the study of the functioning of the hypophysis-adrenal system before birth by examining the fetal hypophysis. Some of these observations have been reported in abstract (Coetzee, '56). An earlier account was presented in a dissertation (Coetzee, '55).

MATERIALS AND METHODS

A total of 201 pregnant rats of the Sprague-Dawley strain was used; the pregnancies were dated from the observed mating. One hundred fifty-three experimental fetuses (E) and 122 control fetuses (C) were collected for study (groups A to I, table 1). Thirteen of the pregnant rats were normal females which yielded control fetuses from day 14 to day 22 (near term). The litter was usually the experimental unit in that the experimental and control fetuses were litter-mates of the same sex and of approximately the same body weight. Group I was exceptional since the controls were obtained from normal mothers.

The length of treatment varied from group to group, depending on the type of treatment (2 to 8 days, table 1), but was as nearly uniform as possible within any particular group. Five kinds of treatment were administered: subcutaneous injection of an aqueous suspension of cortisone (groups C and D) or of a solution of desoxycorticosterone acetate (DCA) in sesame oil (group F); subcutaneous implantation of pellets of hydrocortisone, cortisone or DCA (groups A, B, B′, C, E and E′); sham implantation (groups

Volumetric data

FETUSES			TREATMENT			BODY WEIGHT (gm)	HYPOPHYSIS			
Group	Number [1]	Sex	Kind	Dose (mg) [2]	Duration (hrs)		Volume ($mm^3 \times 10^2$)	$\left(\frac{\text{Volume } (mm^3 \times 10^2)}{\text{Body wt. (gm)}}\right)$ Ratio	E/C index [3]	P_t [4]
A	7E	M	Hydrocortisone (implanted)	4 (1.5)	69	3.01	12.67	4.23	109	.57
	7C	M				5.08	20.23	4.01		
B	8E	M	Hydrocortisone (implanted)	1 (0.4)	69	3.31	13.69	4.16	92	.055
	8C	M				4.91	22.41	4.56		
B′	4E	F	Hydrocortisone (implanted)	1 (0.4)	69	3.06	12.80	4.18	89	.04
	4C	F				4.46	20.91	4.69		
C	10E	M	Cortisone (implanted)	4 (1.7)	70	4.18	18.93	4.54	100	.50
	10C	M				4.99	22.68	4.56		
D	6E	M	Cortisone (injected)	1	52	3.32	20.34	6.17	118	.04
	6C	M				3.86	20.22	5.28		
E	3E	M	DCA (implanted)	4 (2.4)	69	4.18	20.38	4.96	132	.32
	3C	M				5.18	20.30	3.93		
E′	7E	F	DCA (implanted)	4 (3.0)	69	4.51	20.57	4.57	105	.31
	7C	F				4.83	21.17	4.37		
F	8E	M	DCA (injected)	1	51	3.64	20.64	5.78	109	.055
	8C	M				3.97	20.89	5.28		
G	7E	M	Sham implantation		69	4.78	20.81	4.38	107	.57
	7C	M				4.85	20.96	4.40		
G′	3E	F	Sham implantation		69	4.51	22.72	5.15	107	.35
	3C	F				4.78	22.70	4.77		
H	3E	2M 1F	Fetal adrenalectomy		51	4.37	25.30	5.78	125	.048 [5]
	3C	2M 1F				4.75	22.13	4.64		
I	6E	M	Maternal hypophysectomy		195	3.98	21.06	5.37	123	.007
	6C [6]	M				5.29	23.12	4.38		

[1] E, experimental; C, control.

[2] Numbers in parenthesis indicate weight of desiccated pellets removed at autopsy.

[3] E/C index = E/C ratio × 100.

[4] "$P_t = 0.01 - 0.05$" suggested that difference was significant. P_t less than 0.01, highly significant.

[5] Male and female fetuses used in t-test, see "observations."

[6] Controls from unoperated mothers.

G and G′); fetal bilateral adrenalectomy (group H); and maternal hypophysectomy (group I).

The subcutaneous injections were initially performed on fetuses which had been "extrauterinized" (Wells, '50), but all subsequent injections were performed *in utero* (groups D and F). In the latter procedure, the uterus of the anesthetized pregnant rat (ether) was exposed by incising the ventral abdominal wall at the linea alba. One uterine horn was partially exteriorized, and the second fetus from the cervix was selected. The hypodermic needle was introduced successively through the uterine wall, fetal membranes and fetal skin. Fluid in the amount of 0.05 cm^3 was injected. In the opposite horn, the second fetus from the cervix was similarly treated. The surgical procedure was terminated by suturing the maternal abdominal wall. A series of 4 injections, at intervals of 12 hours, was given. No injection was made on the last day of pregnancy, day 22.

The implantation of pellets of hormone was performed *in utero* (Yakaitis and Wells, '56). The pellets were prepared from crystals, desiccated for 48 hours and weighed. A 4 mg dose, or a 1 mg dose (in either case from 1 to 12 pellets), was used (groups A, B, B′, C, E and E′).

Sham implantation was used as a "control experiment." This procedure was identical with that of actual implantation except that no pellets of hormone were introduced (groups G and G′).

Bilateral adrenalectomy of the fetus was performed in group H (Kitchell and Wells, '52b). In a group not presented in table 1, the adrenalectomized fetuses were subjected to implantation of pellets of hydrocortisone, cortisone, or DCA. Just prior to suturing the skin incision, the pellets were subcutaneously implanted. None of these fetuses survived long enough for special consideration.

In group I, the mothers were hypophysectomized by a modification of the parapharyngeal approach of Thompson ('32). The modification was two fold: a tracheal cannula was em-

ployed and a binocular dissecting microscope was used to facilitate the exposure and removal of the hypophysis.

All experiments were terminated 21 days and 15 hours after the observed mating (day 22). The mother was killed by decapitation, and the experimental and control fetuses were removed by Caesarean section. Within the litter, the control fetus was usually taken from the same uterine horn as the experimental fetus, but the immediate neighbor of the treated fetus was not selected. The removed fetuses were individually weighed and then killed by cutting the heart. The fetal calvarium and the brain were removed, and the hyophysis exposed. The hypophysis and the sphenoid bone were removed in a block. Those blocks with hypophyses which were to be used for the volumetric analysis were placed in Bouin's solution, 5 days in this solution leading to decalcification of the bone. Several hypophyses from fetuses given each type of treatment were reserved for special cytological study, and were fixed in Zenker's, Regaud's, or Maximow's modification of Helly's solution.

During the autopsy the implanted pellets of hormone were removed, wherever possible, and placed in a desiccator, and after 48 hours were weighed. In the hypophysectomized mothers, the region of the hypophysis was removed and then fixed in Bouin's solution for 15 days (for decalcification). In the adrenalectomized fetuses, the regions adjacent to the two kidneys were removed and placed in Bouin's solution (10 days).

The tissues thus obtained were prepared for microscopical study. The hypophyses fixed in Bouin's solution were sectioned at 6 μ and mounted in perfect series. The other fetal hypophyses were sectioned in series at 4 μ. The blocks of tissue from the hypophysectomized mothers were sectioned at 20 μ The blocks of tissue adjacent to the kidneys of adrenalectomized fetuses were sectioned at 10 μ. All sections were stained with hematoxylin and eosin, except the 4 μ sections of hypophyses, which were stained with basic fuchsin and aniline blue (Fain and Wolfe, '44).

The volume of the anterior lobe of the hypophysis was determined by the paper-weight method (Hammar, '14; Boyden, '40). Every 5th section was projected with an Edinger projector and drawn.

The stained 6 μ and 4 μ sections of the hypophysis were examined microscopically with the aid of a microcomparator. This instrument permitted the simultaneous viewing of parts of two sections, experimental and control. Only transverse sections through the center of the hypophysis and those adjacent to the center were used.

In measuring the greatest diameter of epithelial cells of the anterior hypophysis (table 3), the 6 μ sections were chosen. Every 5th section was marked for use, if needed. Each cell to be measured showed a sharply-defined cell membrane; it had been sectioned through what was deemed the equator of the nucleus. Using an ocular micrometer, a 7.5x ocular and a 95x objective, 20 cells per hypophysis were measured.

OBSERVATIONS

Control fetus of 13 days and 12 hours (day 14). One fetus of this age was studied to determine the approximate stage of development of the hypophysis at the time of performing the maternal hypophysectomy. The whole fetus, weighing less than 1 gm, was fixed in Bouin's solution and serially sectioned at 10 μ. The development of the hypophysis was equivalent to that of a human embryo of Horizon XVII (Streeter, '48). The infundibulum, posterior lobe and Rathke's pouch were present. The posterior lobe was a rounded, knob-like structure "suspended from" the infundibulum. The anterior lobe was a part of Rathke's pouch, an ellipsoidal structure in which the longitudinal axis and the cavity were placed transversely. The pouch still retained its connections with the stomodeum. In the anterior lobe, the epithelial cells did not yet show a cord-like arrangement, as the blood vessels had not penetrated this lobe. The epithelial cells of the anterior lobe were tightly packed. They were of uniform size, and their cytoplasm was scant. Numerous mitoses were observed.

Controls from 18 days and 12 hours to 19 days and 13 hours of age (days 19 and 20). The hypophyses of 8 fetuses illustrated the stages of development at times when the remaining experiments were begun (table 2; duration of treatment from 51 to 70 hours, table 1). In glands of the 19th day, the capsule was a thin layer of fibroblasts and loose connective tissue. The anterior lobe showed some small sinusoids and many epithelial cells arranged in irregular cords and acini, these acini being most prominent at the periphery of the lobe. Most epithelial cells were tightly packed, and had a scant amount of cytoplasm. Their nuclei were usually small and round, but were sometimes large and oval. A few large epithelial cells bordered the sinusoids. Numerous mitoses were noted.

TABLE 2

Volumetric data on control hypophyses

FETUSES	AUTOPSY	SEX	BODY WEIGHT	VOLUME ($mm^3 \times 10^3$)	Vol. ($mm^3 \times 10^3$) / Body wt. (gm)
	da: hr		*gm*		
3	18: 12	—	1.34	13.05	9.69
2	18: 20	M	1.38	9.32	6.77
3	19: 13	M	2.17	12.99	5.95
3	20: 14	M	3.01	16.23	5.41
4	21: 15	M	5.60	25.07	4.48

In glands of the 20th day, the increase in the size and number of the sinusoids was a striking feature of the anterior lobe. These sinusoids were wider and longer than before, and anastomosed with each other. In consequence, the epithelial cells showed a definite cord-like arrangement throughout the lobe. These cells were larger and had more cytoplasm than those of the 19th day.

Controls of 21 days and 15 hours (day 22) and an overview of all controls. From 90 control hypophyses of the 22nd day, the anterior lobe of a typical gland may be described. Two major trends in development were a marked increase in vascularity and an increase in the number of large epithelial cells which were located along the sinusoids (fig. 2). In such

cells, the nuclear chromatin was denser than in younger glands. In the 4 μ sections of hypophyses stained by the Fain and Wolfe method ('44), only one or two chromophilic cells per section were faintly visible. No cytoplasmic granules were seen, but the cytoplasm appeared to have a more pinkish hue, or a more bluish hue, than that of the other cells.

When all controls from day 18 to day 22 were considered together, the "hypophysis volume/body weight" ratio steadily decreased as the determined age of the fetus increased (table 2). During this period the volume of the anterior hypophysis had doubled, whereas the body weight had increased 4 times. It was difficult to account for the discrepancy between the first two volumes in table 2, 13.05 and 9.32, respectively. One possible explanation is that the actual age of fetuses could not be determined from the available material, as this would depend upon the times when the mated females had ovulated and when fertilization had occurred; it is known that in the rat ovulation occurs 7-11 hours after the beginning of "sexual receptivity" (Blandau and Soderwall, '41). The largest increase in the volume of the hypophysis appeared to occur during the last 25 hours of the considered fetal period (from 16.23 to 25.07, 54%).

The scattergraph of volumetric data on 90 control hypophyses of the 22nd day (fig. 1) shows that the spread within one litter of 13 fetuses was less than that in the 77 control fetuses from different litters. In the latter fetuses, all those whose body weight was in the range of 3.0 to 4.5 gm, with only two exceptions, were litter-mates of treated fetuses which had received 4 subcutaneous injections; the mothers had been anesthetized and operated upon 4 times; this was in contrast to those controls from litters in which the experimental fetuses were given a single treatment. There appeared to be an overall direct relation between the body weight and the volume of the hypophysis. Consequently the plan of using control fetuses and experimental fetuses from the same litter and of

selecting a control fetus in which the body weight was approximately equal to that of the treated litter-mate, was probably a good one.

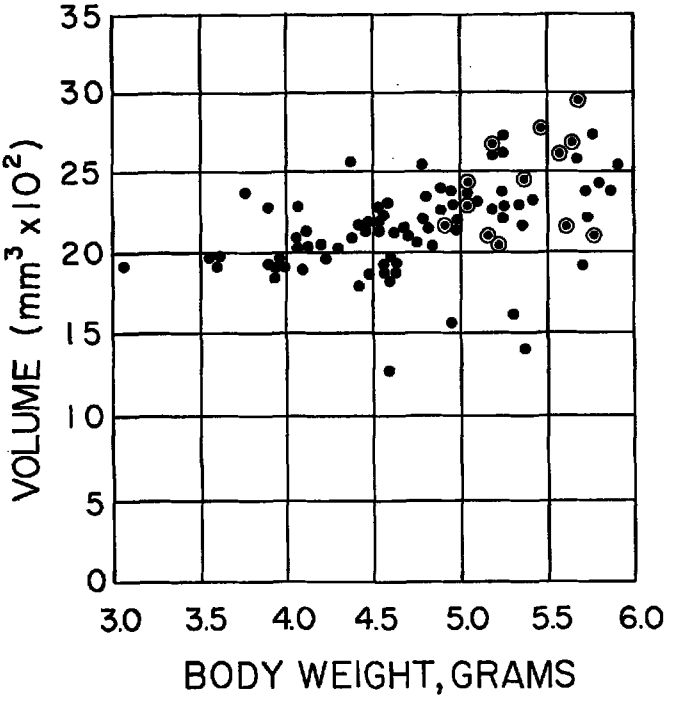

Fig. 1 Scattergraph of the volumetric data on the anterior hypophysis in 90 control fetuses which were killed at the age of 21 days and 15 hours (day 22). Each volume is plotted against the body weight of the fetus. The circled dots indicate 13 fetuses of one litter.

Histologically and volumetrically, the anterior hypophyses of males were similar to those of females. In the 13 fetuses from one litter the "hypophysis volume/body weight" ratio for 6 males was 4.6 and that for 7 females was 4.4. Nevertheless, male and female fetuses were not considered together within any particular group, except in group H in which there were only three experimental fetuses, two males and one female. In making a statistical study of the volumetric data,

the fetuses of group H were considered together, regardless of sex.[4]

Change in weight of implanted pellets of hormone. The weight of the pellets removed at autopsy was always less than that of the same pellet(s) at the time of implantation (table 1). This suggests that there was some absorption of the hormone. The average weight after recovery was less than one-half the average original weight for all implantation experiments except those where DCA was implanted (groups E and E'). This suggests that DCA was less rapidly absorbed than either hydrocortisone or cortisone, in agreement with the observation of Kallman and Gordon ('54) that DCA, when implanted, is absorbed at a slower rate than cortisone. However, in the present work, the variation in the loss of weight of pellets in the members of any particular group was so great that no significant conclusion could be drawn as to the actual quantity of hormone absorbed. In addition the pellets often fragmented upon implantation, making an accurate recovery impossible.

Effects of 4 mg implanted hydrocortisone (group A). The absolute volume of the experimental anterior hypophysis (E) was considerably smaller than that of the corresponding litter-mate control hypophysis (C) in 6 of the 7 pairs of fetuses. The average value of E was approximately equal to that of 19-day controls (tables 1, 2). The average body weight of the E fetuses was larger (40%) than that of 19-day controls, but considerably smaller than that of litter-mate controls of the 22nd day. This suggests that both the growth of the hypophysis and that of the fetus were retarded.

However, when the "hypophysis volume/body weight" ratios were considered, the differences were less apparent. The E value was less than the C in only 4 of the 7 pairs of

[4] As to the extent of the "experimental error" in determining the volume of glands by the paper-weight method, duplicate determinations on the right adrenal of each of 4 fetuses were made; the difference between the original data and the duplicate set of data was about 1% (Kitchell and Wells, '52b).

fetuses. The E/C indexes were 85, 85, 93, 98, 123, 127 and 153.[5] The data were not statistically significant (P_t of 0.57).

Despite the lack of statistical significance of the volumetric data, histological examination revealed consistent differences between the E and C anterior hypophyses. In E the cord-like arrangement of the epithelial cells was almost indistinguishable, as the sinusoids were not evident except under high magnification (fig. 3). These sinusoids were identified by the presence of their lining endothelial cells since very few blood cells were noted. (It so happens that the illustrated control

TABLE 3

Diameter of epithelial cells of the hypophysis

FETUSES		TREATMENT	LARGEST DIAMETER OF CELLS
Members of group	Number [1]		
			μ
A	2E	4 mg hydrocortisone	8.0
	3C		9.4
B	4E	1 mg hydrocortisone	7.9
	3C		9.4
H	3E	Fetal adrenalectomy	9.2
	3C		8.7

[1] E, experimental; C, control.

tissue, figure 2, shows no blood cells.) The epithelial cells were tightly packed, and this, in addition to the lack of visible blood cells, gave the E hypophysis a very compact appearance. The cytoplasm of the epithelial cells was scanty. In most cases some of the nuclei seemed to lack a definite chromatin pattern except near the nuclear membrane. The large cells in E were mainly identified by their nuclei, the cytoplasm being less abundant than that in C. Very few mistoses were noted. The measured largest diameter of epithelial cells was smaller in E than in C (table 3).

These observations, namely histological and volumetric, seem to indicate that the treatment had retarded the growth

[5] E/C index = E/C ratio × 100.

of the hypophysis. In addition the growth of the fetus appears to have been retarded. These differences seem to be due to the hydrocortisone, since similar changes were not observed in fetuses subjected to a sham implantation ("control experiment," groups G and G′, table 1).

Effects of 1 mg implanted hydrocortisone (groups B and B′). In all 12 pairs of fetuses comprising groups B and B′, the volume of the E hypophysis was less than that of the C (table 1). The "hypophysis volume/body weight" ratio was less in E than in C in all cases except one. The E/C indexes were 74, 81, 91, 94, 95, 96, 96 and 108 for the males and 84, 85, 90 and 97 for the females. The data for the males were just beyond the limit of statistical significance (P_t of 0.055), whereas those for the females were statistically significant (P_t of 0.04). The body weight of the E fetus was considerably less than that of the C (as was also noted in group A).

Microscopical examination showed consistent differences between the E and C anterior hypophyses (the E hypophyses were similar to those of group A). The arrangement of the epithelial cells in irregularly anastomosing cords was not apparent in E, due in part to the reduced size and number of the sinusoids. The latter were observed only under such high magnification that the lining endothelial cells could be identified. The epithelial cells were tightly packed, and the cytoplasm was scanty and poorly differentiated. The nuclei were of varying shapes, mostly round, small and shrunken. The chromatin was located around the edges, giving the nuclei a hollow appearance. An occasional mistosis was observed. The measured diameter of epithelial cells was smaller in E than in C (table 3).

It was concluded that the 1 mg dose of hydrocortisone is as effective as the 4 mg dose in retarding the growth of the hypophysis and the growth of the fetus as well. The retardation of the growth of the hypophysis was more pronounced than that of the fetus.

Effects of implanted cortisone (group C). The volume of the E hypophysis was less than that of the C in all 10 pairs of fe-

tuses comprising this group. The "hypophysis volume/body weight" ratios showed no consistent differences between E and C; the value of E was less than that of C in only one-half of the cases. The E/C indexes ranged from 78 to 114, and the average was 100. The data were not significant (P_t of 0.50).

Histologically, however, some consistent differences were noted between the E and C hypophyses. In E the epithelial cells were more closely packed, and the sinusoids were evident, although the sinusoids were not as large or as numerous in E as in C. The cytoplasm was less abundant in E than in C, particularly in the large cells situated along the sinusoids. The nuclei of E were similar to those of C. Fewer mitoses were noted in E than in C.

Despite the lack of statistical significance of the volumetric data, the cytological observations suggest that the implanted cortisone did exert some effect in retarding the development of the hypophysis. This effect was not as clearly defined as in the fetuses given hydrocortisone.

Effects of injected cortisone (group D). The absolute volume of the E hypophysis was approximately equal to that of the C in all pairs of fetuses studied. The "hypophysis volume/body weight" ratio for the E fetus was larger than that of the C in all cases. The E/C indexes were 101, 110, 112, 115, 123, and 147. The data were statistically significant (P_t of 0.04).

One might erroneously conclude from the data that the cortisone injections had stimulated the growth of the hypophysis. However, it seems that the body weight of the fetus was more affected than the hypophysis. The body weight of the E fetus was less than that of C, which in turn was less than that of other controls from unoperated mothers. This reflected the fact that these treated fetuses were subjected to 4 subcutaneous injections, so that the mother had been operated upon 4 times. Although this may have been a factor contributing to the small body weight of both the E and C fetuses, it would seem that the action of the cortisone had reduced the body weight of the E fetuses.

The cytological observations supported this view. The E hypophysis was similar to the C in all respects. The cellular cords of epithelium and the sinusoids were equally well differentiated in the E and C hypophyses.

The lack of controls receiving 4 injections of distilled water was balanced to a certain degree by the treatment of sham-implantation (compare groups G and G'). However, this sham-implantation was a single treatment.

The present work is not adequate to determine why the injected cortisone failed to influence the morphology of the hypophysis. Three possible factors could be considered. First, the dose of 1 mg may have been too small for effectiveness during the experimental period of 52 hours; a dose of 4 mg over a period of 69 hours was only effective to a slight degree. Secondly, the cortisone preparation was a suspension, and it is possible that some of the particles were too large to pass through the needle. Thirdly, the injections were performed *in utero* in 4 of the 6 cases, and it is possible that some of the hormone preparation escaped from the puncture wound upon withdrawal of the needle. The other two experimental fetuses of this group were extrauterinized, and the puncture hole was sealed with celloidin to prevent any loss of fluid. In each case the volume of the E hypophysis was smaller than that of the C. The E/C indexes for these two pairs of fetuses were smaller than for those of the other 4 pairs.

Effects of DCA (groups E, E' and F). The volumetric data on the three groups did not indicate any consistent differences which could be attributed to the DCA. In the "hypophysis volume/body weight" ratios, any differences between the E and C data were probably attributable to differences in the body weight. The cytological examination supported this view since the E glands were similar in all respects to the C glands. It was not possible to determine whether the reduced body weight in the E fetuses was attributable to the treatment as a whole or to a specific action of the DCA.

Effects of bilateral adrenalectomy (group H). Due to the severity of the treatment, only three living experimental fe-

tuses were obtained, two males and one female. The completeness of the adrenalectomy was verified by a microscopic examination of the serial sections of the areas adjacent to the kidneys. The operation had been bilaterally complete in the two males. In the female fetus approximately 5–10% of the right adrenal had not been removed. This was considered too small an amount to be endocrinologically significant.

The absolute volume of the E hypophysis was larger than that of the C in two of the three pairs of fetuses. In the third pair, males, the E and C values were approximately equal. Regarding the "hypophysis volume/body weight" ratios, the E value was larger than the C value in all three pairs. The E/C indexes were 118, 118 and 140. The data were statistically significant (P_t of 0.048).[6]

Histological observations revealed that in cellular arrangement and in sinusoidal pattern the three E hypophyses were well developed (figs. 4 and 5). There appeared to be a preponderance of large epithelial cells with an abundant cytoplasm. Measurements showed that the E cells were somewhat larger than the C cells (table 3). In one case the E hypophysis seemed to bulge the hypophyseal capsule outwards.

These volumetric and histologic observations are subject to the limitation of the small size of group H. Nevertheless, they suggest that the absence of the fetal adrenals stimulated the growth of the fetal hypophysis.[7]

Effects of maternal hypophysectomy (group I). The experimental fetuses were selected from hypophysectomized mothers in which the completeness of hypophysectomy had been verified by a microscopic examination of the sections of

[6] Certain liberties were taken with the "t-test" since males and females were considered together; it had been found, however, that the hypophyses of male controls were similar to those of female controls (see OBSERVATIONS, controls of the 22nd day).

[7] In 11 litters not presented in table 1 (22 adrenalectomized fetuses), two fetuses per litter were adrenalectomized as usual and then one of these fetuses was given subcutaneously a 1 mg pellet of hydrocortisone or of cortisone. These experiments were designed to determine if the implanted hormone would prevent any changes in the hypophysis produced by the adrenalectomy. None of the 22 fetuses survived.

the hypophyseal region. The controls were from normal mothers, and were taken at 21 days and 15 hours after the mating.

The "hypophysis volume/body weight" ratio of the E fetus was larger than that of the C in 5 of the 6 pairs of fetuses studied. The E/C indexes were 91, 115, 115, 130, 143 and 146. The data were statistically significant.

Cytological examination did not reveal any consistent differences between the E and C anterior hypophyses. Mitoses of epithelial cells were noted in both E and C hypophyses.

Even though the volumetric data suggested that maternal hypophysectomy had produced a slight stimulatory effect upon the developing hypophysis (average E/C index of 123, table 1), the cytological observations did not support this view. It is possible, however, that a stimulating effect might have occurred and that this effect was too small to be detected cytologically. In any event the E hypophyses were from fetuses in which the body weight was relatively small.

DISCUSSION

The foregoing observations are regarded as two new lines of evidence for a functional reciprocal relation between the fetal hypophysis and the adrenal cortex. The first is that implanted hydrocortisone retards the growth of the anterior hypophysis. The manifestations of this retardation are: the volume of the anterior lobe is reduced; the cords of epithelial cells are poorly defined; the cytoplasm of the epithelial cells is scanty; some of the nuclei are distorted; mitoses are few; the largest diameter of the epithelial cells is reduced; the sinusoids are small and scarce. Our view that this retarded growth was associated with a decreased corticotrophic activity of the anterior hypophysis is supported by the simultaneous observation (in a companion study) that the growth of the adrenals in these same hydrocortisone-treated fetuses was likewise retarded (Yakaitis and Wells, '56). This effect upon the adrenal is similar to that in the fetal rat following hypophyseoprivia (Kitchell and Wells, '52b).

The second line of evidence is that in the fetus bilateral adrenalectomy seems to stimulate the growth of the anterior hypophysis. The treatment increases: the volume of the anterior lobe, the number of large epithelial cells, the diameter of these cells and the amount of cytoplasm in the cells. Our view is that the adrenalectomy decreased the concentration of an adrenal cortical hormone in the fetal blood and that this deficiency stimulated the fetal hypophysis to produce an extra quantity of corticotrophin. The attempt to prevent such effects of adrenalectomy by implanting hydrocortisone, or cortisone, was unsuccessful since none of these adrenalectomized fetuses was alive at outopsy.

Our several observations are in harmony with those of certain other workers: (1) with the compensatory hypertrophy of the remaining adrenal in unilaterally adrenalectomized fetuses (Kitchell, '50),[8] (2) with the prevention of this hypertrophy by implanted cortisone (Kitchell and Wells, '52a), (3) with the atrophic changes in the adrenal cortex following fetal hypophyseoprivia (Wells, '47, '57; Jost, '48, '57; Rayaud and Frilley, '50; Domm and Leroy, '51; Cohen, '55), (4) with the prevention of these changes by injected ACTH (Wells, '48, '49; Kitchell and Wells, '52b) and (5) with the atrophic changes in the adrenals of monkey fetuses from mothers which were subjected to injections of ACTH during the pregnancy (Schmidt and Hoffman, '54).

In these fetal monkeys studied by Schmidt and Hoffman, the hypophysis was usually unaltered in size and in histological appearance; one hypophysis did show a decrease in the number of basophilic cells of the beta type. It is likely that the injected ACTH caused the secretion of an excess of maternal cortical hormones, that these hormones entered the fetal circulation and that they brought about a reduced adreno-

[8] A similar hypertrophy was observed in fetal rats in which one of the adrenals had been destroyed by cautery, but this might have been in part a result of hemorrhage since an attempt to destroy both adrenals had been made (Tobin, '39); from a study of the hypophyses of these fetuses, Tobin reported a degranulation of the basophils, a decrease in the number of acidophils and a corresponding increase in the number of chromophobes.

corticotrophic output of the fetal hypophysis. In any case, this interpretation would account for the atrophic changes in the fetal adrenals.

In our work, the effects of cortisone upon the anterior hypophysis were less pronounced than those of hydrocortisone. In studies of rats after birth, it has been reported that cortisone and hydrocortisone are about equal in effecting an inhibition of the corticotrophic function of the hypophysis (Sayers and Sayers, '48). There is, however, a lack of agreement as to the type of effect of cortisone upon the morphology of the hypophysis. Certain workers report varying changes in the basophils and in the size of the hypohysis (Laqueur, '50; Winter, Silber and Stoerk, '50; Tuchmann-Duplessis, '51; Golden and Bondy, '52; Kallman and Gordon, '54), while other workers report none—and this in spite of the deleterious effect upon the adrenals (Halmi and Barker, '52).

Hydrocortisone (and to a lesser extent cortisone) retarded the growth of the anterior hypophysis much more than it retarded the growth of the fetus (cf. Coetzee, Yakaitis and Wells, '57). It is known that in mammals large doses of these hormones may interfere with organic metabolism (Ingle, '50). Leroy and Domm ('51) did not note any effect of injected cortisone upon the body weight of fetuses.

Our histological preparations failed to show in the anterior hypophysis any well-defined chromophilic cells (cf. Siperstein, Nichols, Griesbach and Chaikoff, '54).[9] On transplantation of the fetal hypophysis into the anterior chamber of the eye of an hypophysectomized male, however, the grafted hypophysis has the capacity of corticotrophic function (Goldberg, Knobil and Greep, '55).

The present observations were made in spite of any possible influence of maternal hormones and of placental hormones. It has been postulated: (1) that in the rat given massive

[9] Basophils and acidophils were reported in human embryos (Romeis, '40), in fetal pigs (Rumph and Smith, '26; Nelson, '33; Aron, '29) and in fetal guinea pigs (Kirkman, '37); using rabbit fetuses and a histochemical method for staining glycoproteins in basophils of the adult hypophysis (periodic acid-Schiff method), a positive reaction was reported in the fetal hypophysis (Jost and Danysz, '52).

amounts of ACTH some of this ACTH can cross the placental barrier (Jones, Lloyd and Wyatt, '53), and (2) that in the hypophysectomized pregnant monkey a placental corticotrophin may be the factor which prevents the maternal adrenals from undergoing as much retrogression as that occurring in the hypophysectomized non-pregnant female (Smith, '55).[10]

Our work suggests that the hypophysectomy of the mother did not markedly affect the growth of the fetal hypohysis. Although the "hypophysis volume/body weight" ratios in the experimental fetuses were increased, the experimental and control hypophyses did not show any consistent histologic differences. Maternal hypophysectomy did not modify the growth of the fetal adrenals (Yakaitis and Wells, '56; Knobil and Briggs, '55; Greep, '57).

The importance of our observations is not minimized by the fact that there is a lack of agreement with respect to the functioning of the hypophysis-adrenal system in young rats, in young mice and in premature human infants. In the published records, the physiologic tests involved such "stressful" stimuli as laparotomy and injected epinephrine; they involved such responses as a depletion of the ascorbic acid in the adrenals, a drop in the number of circulating eosinophilic leucocytes and an increase in the output of urinary glucocorticoid. Certain tests were positive (Rinfret and Hane, '55a, '55b; Thompson and Blount, '54; Venning, Randall and Gyorgy, '49), and other tests were negative (Jailer, '50; Jailer, Wong and Engle, '51).

The DCA treatment failed to exert any detectable effect upon the anterior lobe of the hypophysis. This obseravtion is in accord with the view that the hypophysis does not influence the secretion of DCA and with the published observations of other workers that in mammals the size of the hypophysis

[10] Certain hormones may be extracted from placental tissue—a hormone with adrenal cortical activity (Johnson and Haines, '52), cortisone and hydrocortisone (De Courcy, Gray and Lunnon, '52) and a hormone with corticotrophic activity (Jailer and Knowlton, '50; Opsahl and Long, '51; Assali and Hamermesz, '54); these facts alone do not necessarily show that such hormones influence the physiology of the fetus.

and the cellular appearance of it remain unchanged after DCA treatment (Carnes, Ragan, Ferrebee and O'Neill, '41; Kallman and Gordon, '54.[11]

SUMMARY AND CONCLUSIONS

Implanted hydrocortisone retards the growth of the anterior lobe of the hypophysis. The characteristics of this retardation are: a reduced volume of the lobe, poorly-defined cords of epithelial cells, a reduced diameter of these cells, a scant amount of cytoplasm in the cells, a distortion of some of the nuclei, a scarcity of mitoses and a reduced size and number of sinusoids.

Implanted cortisone produces the same type of histological changes, but the magnitude of the changes is less than that caused by hydrocortisone.

Bilateral adrenalectomy of the fetus seems to induce an enlargement of the anterior lobe of the fetal hypophysis and an enhanced development of the hypophyseal cells and sinusoids.

Maternal hypophysectomy may have a slight stimulatory effect upon the developing anterior lobe, especially when judged by the volumetric data alone, but these data are inadequate to solve this problem.

Desoxycorticosterone acetate (DCA) does not exert any effect upon the anterior lobe.

These observations constitute new evidence for the existence of a functional reciprocal relation between the fetal hypophysis and the fetal adrenal cortex in the rat.

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[11] The functioning of the zona glomerulosa in the adrenals during postnatal life is a related problem in which the particulars, in our opinion, are not completely understood (see Yakaitis and Wells, '56).

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PLATE 1

EXPLANATION OF FIGURES

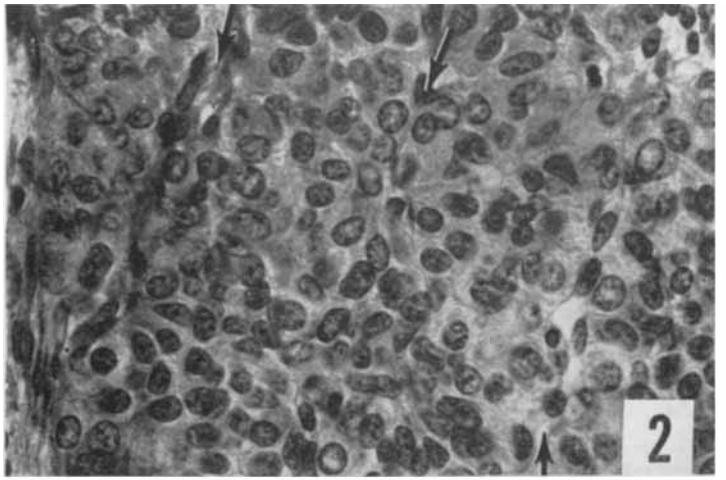
Photographs 2–5 show transverse sections of the anterior hypophyses from 4 fetal rats which were killed at the age of 21 days and 15 hours. The photographs are so oriented that the capsular region of each anterior lobe is at the observer's left. The arrows indicate sinusoids which in turn mark out the cords of epithelial cells—cords which are most easily seen in figures 2 and 4. The hypophyses had been fixed in Bouin's solution, sectioned at 6 μ and stained with hematoxylin and eosin. $\times$ 550.

2 Anterior hypophysis of a female control (fetus 1834f).

3 Hypophysis of an experimental male fetus which had been subjected to 4 mg hydrocortisone (fetus 1654e, group A).

4 Hypophysis of an experimental male fetus which had been subjected to bilateral adrenalectomy (fetus 1847e, group H).

5 Hypophysis of a control male from the same litter (fetus 1847c, group H).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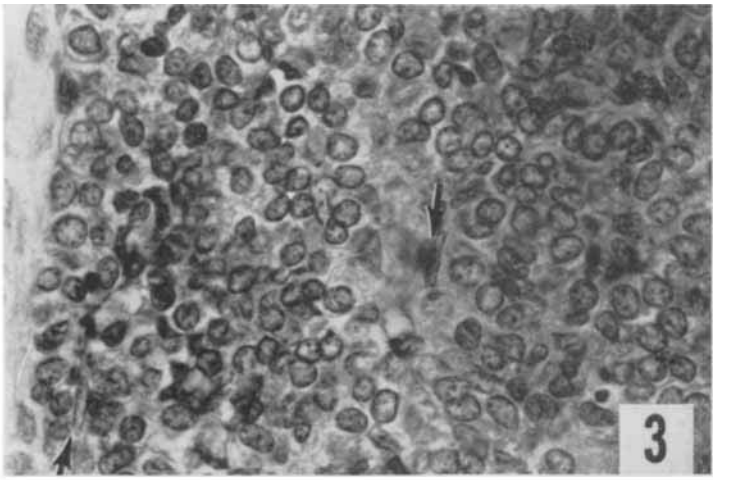
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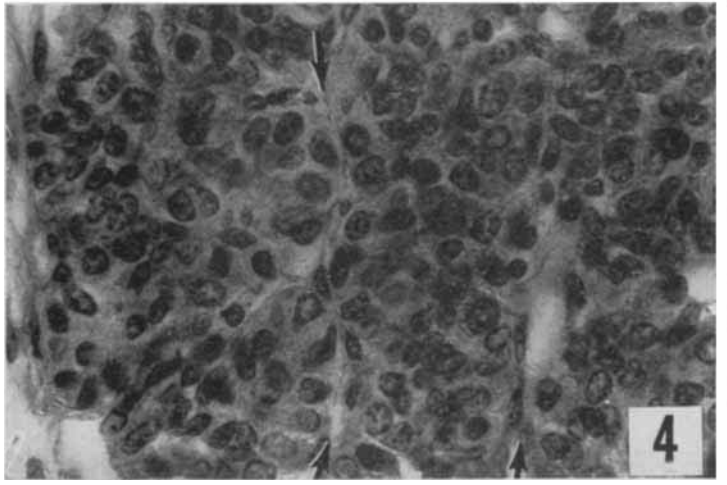
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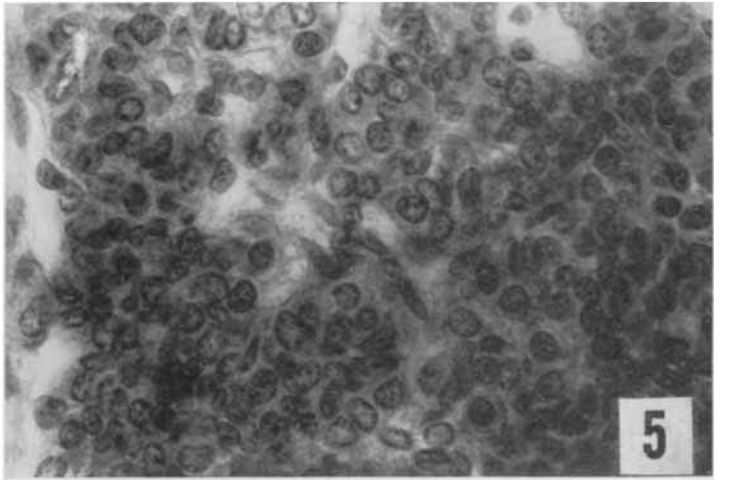
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