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STUDIES ON THYROID GLANDS, ADRENAL GLANDS, AND REPRODUCTIVE SYSTEMS OF ACEPHALIC HAMSTER FETUSES[1, 2]

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Extensive literature has accumulated dealing with acrania in the human fetus. The underlying causes for this anomaly have been discussed by Warren (1951). There have been reports on the effects of fetal hypophysectomy in a number of mammalian species since methods have been developed to produce experimental acrania. Reviews of fetal endocrinology have been given by Moore (1950) and Jost (1953). The purpose of the present study is to report the effects of decapitation and the resulting hypophysectomy on the thyroid gland, the adrenal gland, and the reproductive system of the fetal hamster.

Method

A total of 141 fetuses from 26 female hamsters were decapitated at an average age of 12 days, 15 hours, the earliest age at which the position of the fetus could be readily observed through the uterine wall. Fetal age was calculated from the time of observed copulation. Usually five fetuses of a litter were decapitated, one fetus removed from the uterus intact and preserved for future study, and any additional fetuses were allowed to continue development. Of the 141 fetuses decapitated, 22 were recovered alive at an average of 51.4 hours following the operation (table 1).

Pregnant hamsters were anæsthetized with nembutal, and the horn of the uterus withdrawn through an incision in the body wall. Using a no. 7 straight sewing needle and white cotton thread, no. 50, a triangular suture was made through the muscle of the uterus on the antimesometrial side over the head of the fetus. The needle was loosed from the thread so that the two free ends lay together at the apex of the triangular suture, and one thread was looped through the other. A small incision was made through the uterus in the center of the triangle through which the head of the fetus usually protruded. When the head was completely outside, the loose ends of the thread were grasped with forceps and drawn tight, severing the head in the neck region, and at the same time closing the incision in the uterine wall. The horn of the uterus was replaced and the incision in the body wall of the animal closed.

Fetuses were fixed in Bouin's solution, sectioned serially at 15 microns, and stained in hematoxylin and eosin. Measurements were taken from the cross sections using an ocular micrometer, and size and volume of organs were calculated from these measurements.

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TABLE 1.—SEX DISTRIBUTION, LENGTH OF CONTROL AND ACEPHALIC FETUSES, AND TIME OF ACEPHALIC DEVELOPMENT

Fetal Age		Controls			Acephalics				Time without heads (hours)
Days	Hours	M	Length (mm) crown-rump	F	Length (mm) crown-rump	M	Length (mm) neck-rump	F	Length (mm) neck-rump	
11	7	1	8.5	..	....	..	....	..	....	..
11	15	1	9.0	..	....	..	....	..	....	..
13	1	1	13.0	..	....	..	....	..	....	..
13	13	1	12.0	..	....	..	....	..	....	..
13	17	..	....	1	16.0	2	14.0*	..	....	48
15	1	..	....	1	20.0	1	14.0	1	13.0	48
15	2	..	....	..	....	1	12.0	..	....	49
15	8	1	18.0	..	....	1	15.0	..	....	48
15	12	..	....	..	....	3	15.5*	1	15.5	51
15	13	..	....	..	....	3	15.0*	1	15.0	42
15	14	1	19.0	..	....	1	14.3	2	14.3*	44
15	15	2	18.2*	2	18.2*	2	15.0*	2	15.0*	75
15	16	..	....	2	17.5*	..	....	1	15.0	50

* Average length.

RESULTS

Control and acephalic fetuses were the same size, excluding the head of controls, and showed an increase in length of approximately 80% between the 12th and 16th day (figs. 4, 5).

Thyroid Gland.—Of the 22 acephalic fetuses recovered alive, studies on thyroid glands of 17 are considered here. In five cases the gland had apparently been removed during the decapitation process.

The thyroid of 12-day-old control fetuses was composed of irregular masses of closely grouped cells with an occasional follicle already formed, and with some cells in a circular pattern as though forming follicles. Areas between the masses of cells were filled with connective tissue and red blood cells. The glands of older control fetuses showed considerable differentiation and many follicles, but without colloid (figs. 6, 7). Follicles were round or oval in form and showed little variation in size, and many interfollicular cells were present.

In acephalic fetuses some follicles were formed, but these were rela-

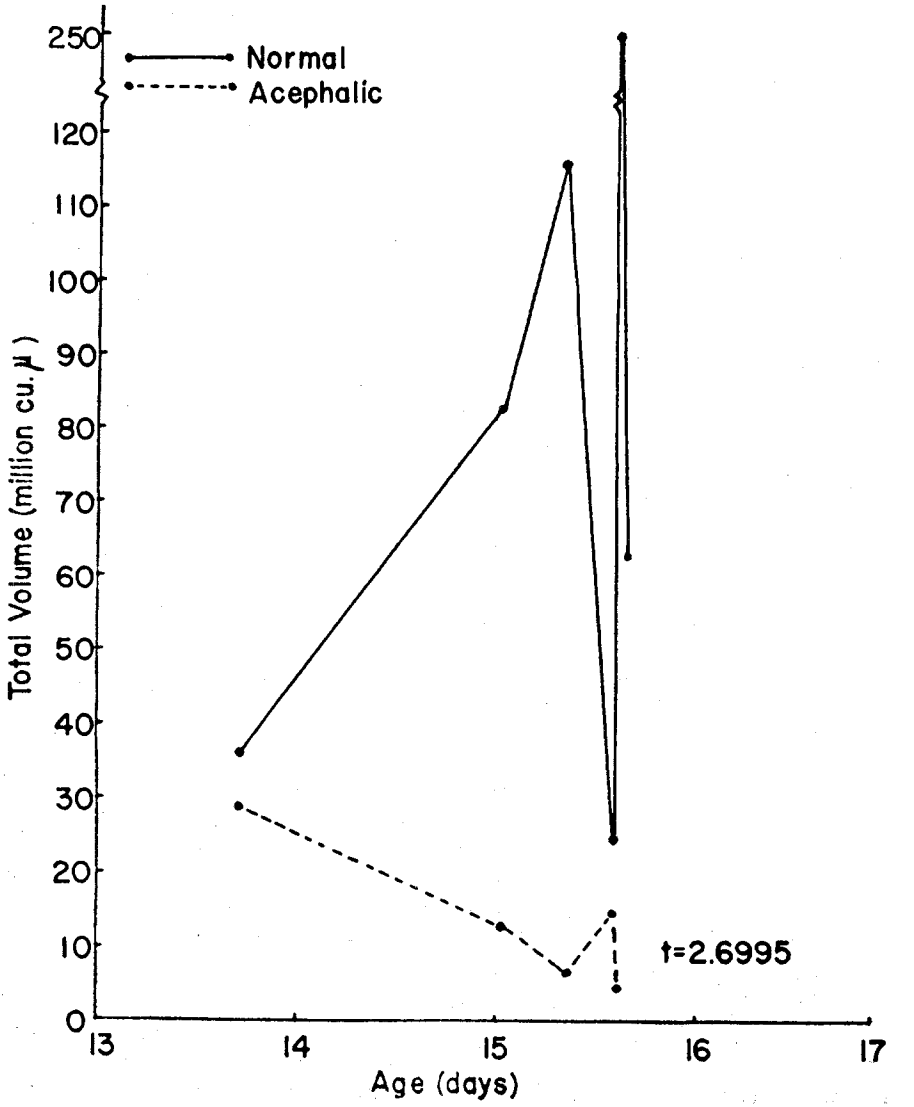

FIG. 1.—Volumes of thyroid glands of control and acephalic fetuses between ages of 13 and 16 days.

tively large, and few interfollicular cells were present. The follicles contained no colloid (figs. 8, 9). The thyroid glands of these fetuses were much smaller than glands of controls of the same age. Glands of controls showed an average increase in length of 79.3% compared to glands of younger controls, whereas in acephalic fetuses there was only a 27.6% increase. The volume of the thyroid of the oldest controls

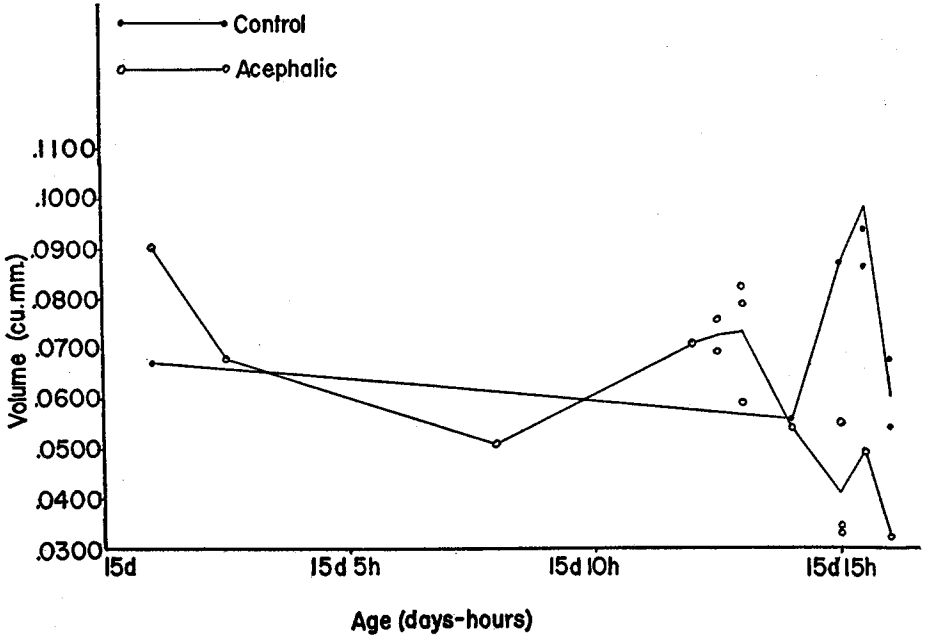

FIG. 2.—Total of average volumes of right and left adrenal glands of control and acephalic fetuses between ages of 15 days, and 15 days, 16 hours.

was 287% greater than that of acephalics (fig. 1). The smaller volume of the thyroid of headless fetuses seemed to be due to a smaller number of follicles and interfollicular cells.

Adrenal Gland.—At 10 days the medullary and cortical components of the adrenal gland could not be readily identified, but by 11 days, 7 hours the cortex had formed from coelomic mesothelium, and primitive medullary cells could be seen between the sympathetic ganglia and the masses of cells which form the cortex of the gland (fig. 10). The primitive medullary cells were arranged in rows adjacent to the cortical cells and at some points were between the cortical cells.

By 13 days, 17 hours the fetal cortex had increased in thickness and its cells could be readily distinguished from the cells of the medulla. The capsule of the gland, made up of flattened cells, was distinct.

In controls the cortex of the gland increased in thickness up to 15 days, 15 hours, but in the oldest controls observed, at 15 days, 16 hours, the width of the cortex appeared to decrease. In acephalic fetuses a more marked decrease in thickness of the cortex occurred at 15 days, 15 hours. In older fetuses of both groups small groups of cells with darkly-stained nuclei were dispersed throughout the central portion of the gland and on the ventro-medial

surface between the cortex and the capsule. Zonation of the cortex was not apparent (figs. 2, 11, 12, 13).

The development of the adrenals of acephalic and control fetuses appeared to be similar, but when total volumes of glands of 8 controls and 15 acephalic animals, between 15 days and 15 days, 16 hours old, were determined and treated statistically there was a significant difference in volumes of glands of the two groups (fig. 2). The average volumes of right and left adrenals together showed a statistically significant difference between control and acephalic fetuses (Level of Significance, P_t was 0.03). When left adrenals of the two groups were compared as to volume, a P_t value of 0.001 was found, while a comparison of right adrenals gave a P_t value of 0.2. Thus, the average volumes of left adrenals were significantly different, while no significant difference was found for the right glands. No significant difference was found when the volumes for right and left gland of controls were compared, nor was there a difference between right and left glands of acephalic fetuses. Average volumes of glands for control and acephalic fetuses are given in table 2.

Reproductive system.—Sex distribution for the control group was 9 males and 6 females, as compared to 14 males and 8 females in the acephalic group. The gonads of the fetuses did not vary appreciably in size or degree of development, but measurements of primary germ cells and interstitial cells of male fetuses indicated that these cells in acephalic fetuses were smaller in size and fewer in number than those of controls.

Wolffian ducts were present at 11 days, but lumina of the ducts were not continuous, and junction with the urogenital sinus was made as a solid cord of cells. Lumina of ducts were continuous with the urogenital sinus at 15 days, 8 hours in control males. In female fetuses of the control group the Wolffian ducts appeared as solid cords of cells adjacent to the urogenital sinus. Rem-

TABLE 2.—VOLUMES OF ADRENAL GLANDS OF CONTROL AND ACEPHALIC FETUSES BETWEEN AGES OF 15 DAYS AND 15 DAYS, 16 HOURS

Number of fetuses	Average volumes of right adrenals (cu. mm.)	Average volumes of left adrenals (cu. mm.)	Total of average volumes of both adrenals (cu. mm.)
Controls..... 8	.0363	.0415	.0778
Acephalics. 15	.0302	.0298	.0600
Control Males..... 5	.0339	.0413	.0752
Acephalic Males.....12	.0302	.0311	.0613
Control Females..... 3	.0406	.0420	.0826
Acephalic Females..... 3	.0304	.0247	.0551

nants of the Wolffian ducts remained in this area as late as 15 days, 15 hours, but in no case were the ducts continuous with the urogenital sinus.

In acephalic male fetuses the Wolffian ducts were well developed by 13 days, 17 hours. In all acephalic males the Wolffian ducts were shorter and narrower than those of control males of comparable ages, and showed discontinuous lumina (fig. 3). At 15 days, 1 hour, the Wolffian ducts were connected to the urogenital sinus by continuous lumina, but this condition did not persist in older fetuses of this group.

In females of the acephalic group the Wolffian duct retrogressed in a manner similar to that in control females.

In control males the Mullerian ducts were present as solid cords of cells near the anterior portion of the Wolffian ducts at 11 days, 7 hours. By 11 days, 15 hours the Mullerian ducts had increased in length with lumina throughout, and by 13 days they were well developed and longer than the Wolffian ducts. By 13 days, 13 hours the Mullerian ducts had started to retrogress, but were present in all older fetuses in the region of the urogenital sinus.

In acephalic males the Mullerian ducts were well developed at 13 days, 17 hours, but appeared to be quite small by 15 days. Remnants of the Mullerian ducts were present in all males up to 15 days, 15 hours.

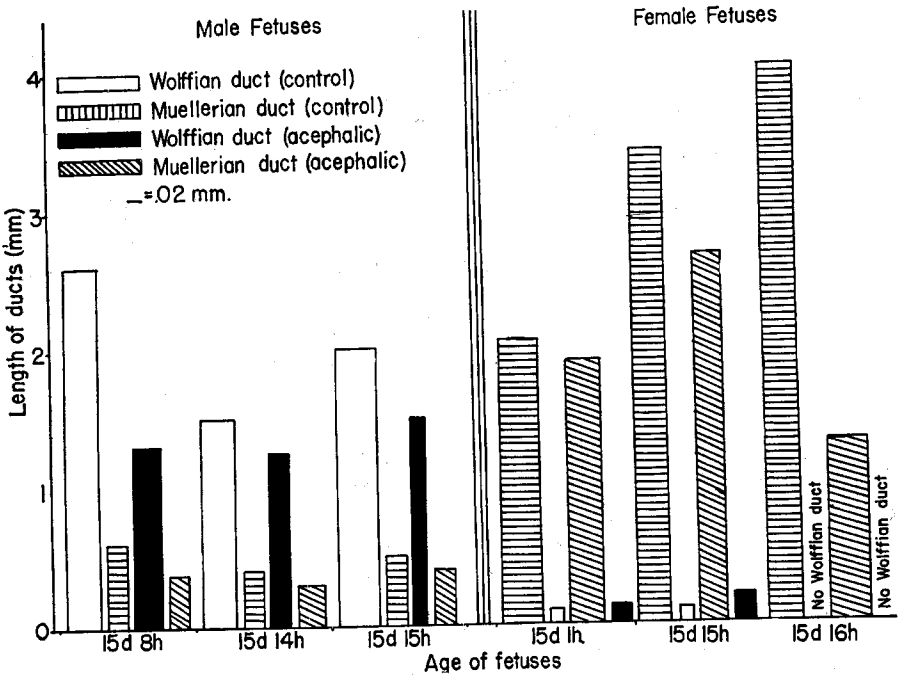

FIG. 3.—Lengths and widths of reproductive ducts of male and female fetuses of control and acephalic groups between 15 days and 15 days, 16 hours.

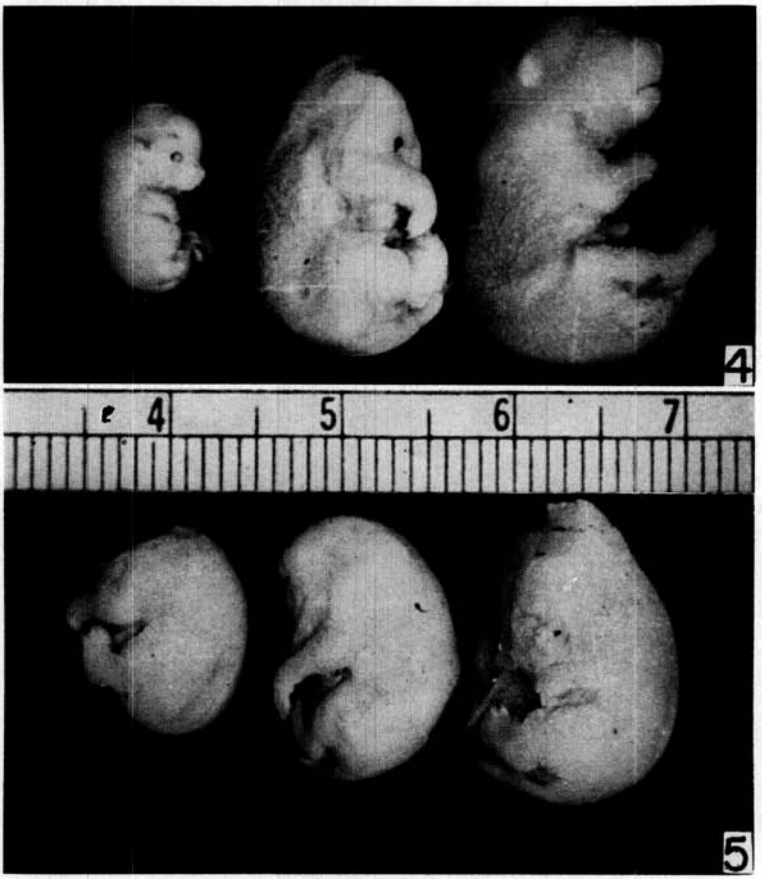

FIG. 4.—*Left*, control fetus, age 12 days, 16 hours; *center*, acephalic fetus, age 15 days, 13 hours; *right*, control fetus, age 15 days, 15 hours. FIG. 5.—Littermate acephalic fetuses, age 15 days, 13 hours. Thyroid gland is missing in the fetus at left.

In control females the Mullerian ducts were present at 13 days, 17 hours, and were fused at their posterior ends as solid cords. By 15 days, 16 hours the posterior ends of the ducts had formed the utero-vaginal canal.

The Mullerian ducts in acephalic females never attained the length or degree of development that they did in control females. Even at age 15 days, 16 hours the ducts were much shorter than in control females and the utero-vaginal canal had not formed.

Accessory reproductive structures.—Seminal vesicles were present in males 15 days, 8 hours old as outpocketings from the Wolffian ducts. Ortiz (1945) found that they first appear at 14½ days. However, in controls, these structures showed no marked increase in length until 15 days, 15 hours. In

acephalic males these structures were present at 15 days, 1 hour and showed varying conditions of development up to 15 days, 15 hours.

During the 15th day, in control males, the prostate, bulbourethral, preputial, and coagulating glands and the ejaculatory ducts appeared. Although there was considerable variation in development of these accessory structures in acephalic males, they were markedly smaller than in control males.

The only accessory reproductive structures observed in females were the bulbourethral and preputial glands. They appeared to follow the normal pattern of retrogression.

Discussion

Techniques for the removal of the fetal hypophysis by decapitation have been reported for the rat by Wells(1950a), and Domm and Leroy(1951), and for the rabbit by Jost(1947). The technique described above for decapitating hamster fetuses was used by Foote and Foote (1949) for a preliminary report on effects of decapitation on the thyroid of the hamster fetus. Raynaud and Frilley (1947) used X-rays to "hypophysectomize" mouse fetuses.

In this study the average period of time the hamster fetuses developed in an acephalic condition was 51.4 hours or 13.5% of the total gestation period. The shortest development period was 42 hours and the longest was 75 hours.

From histological observations on the thyroid gland it appears that by the 12th day a few follicles have formed, but no pronounced follicular development occurs until the 15th day. Since the thyroids of acephalic fetuses show fewer and larger follicles and few interfollicular cells, it would seem that any influence of the hypophysis would be on the production of interfollicular cells, and only indirectly on the development of follicles.

Raynaud and Frilley (1948) and Raynaud (1950) found the thyroid small and with little colloid near time of birth. Jost, Morel, and Marois (1949, 1952) injected I^{131} into decapitated rabbit fetuses, and found little difference in thyroid activity of decapitated fetuses and controls on day 22, but at day 28 there was a marked decrease in thyroid activity. Jost (1953) found there was a delay in the appearance of colloid in the thyroids of decapitated rabbit fetuses.

Atrophy of the adrenal cortex in "hypophysectomized" mice has been reported by Raynaud (1943), and Raynaud and Frilley (1950), in decapitated rats by Wells (1947, 1948), Jost (1951a), Domm and Leroy (1951), and Kitchell and Wells (1952), and in decapitated rabbits by Jost (1948). Raynaud (1950) found that the adrenals of mice are supressed by "hypophysectomy" only during the last day of prenatal life. In the present study there is an indication of a significant difference in the size and volume of adrenal glands of control and acephalic fetuses, shown particularly in the left adrenals and in considering the total volume of both adrenals. While there is some evidence for a relationship between the hypophysis and the adrenal cortex in the fetal hamster, one cannot discount the possibility that other fac-

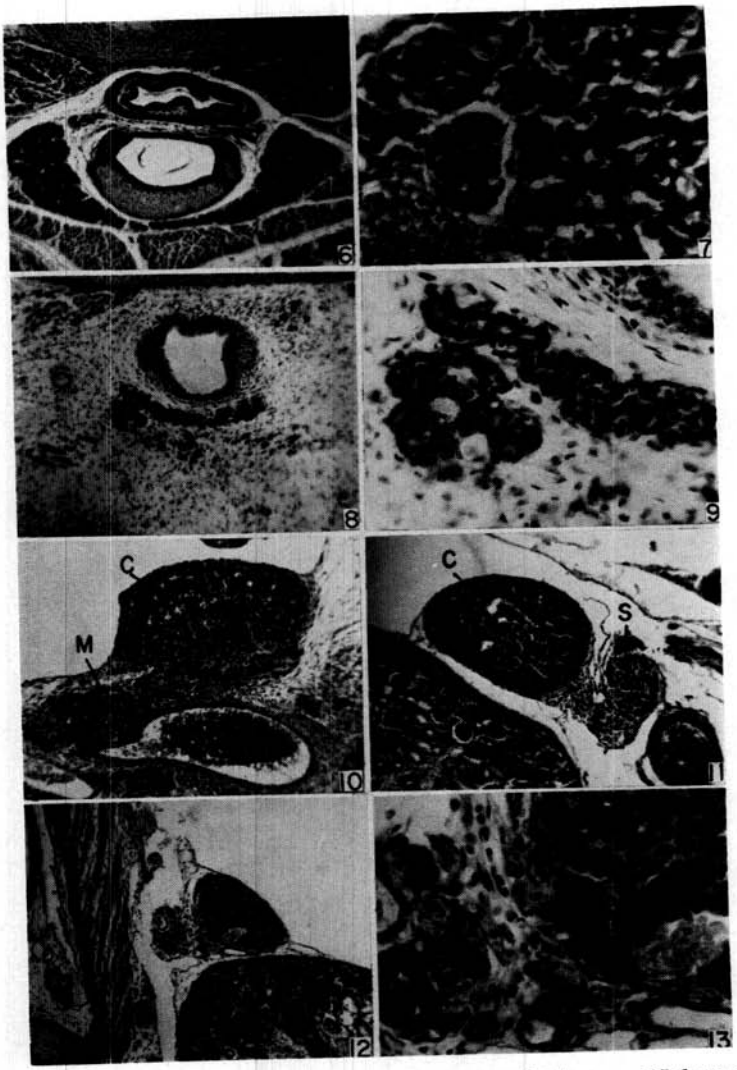

FIG. 6.—Cross section through neck region of control fetus, age 15 days, 16 hours, showing thyroid gland, esophagus, and trachea. X 33. FIG. 7.— Cross section of thyroid gland shown in fig. 6. X 170. FIG. 8.—Cross section through neck region of acephalic fetus, age 15 days, 8 hours, showing thyroid gland and trachea. X 42. FIG. 9.—Cross section through thyroid gland shown in fig. 8. X 170. FIG. 10.—Cross section through region of left adrenal gland of control fetus, age 11 days, 7 hours, showing cortical cells (C), medullary cells (M), and dorsal aorta. X 50. FIG. 11.—Cross section through region of left adrenal gland of control fetus, age 15 days, 8 hours, showing cortical (C) and medullary components, sympathetic ganglion (S), dorsal aorta, and kidney. X 50. FIG. 12.—Cross section through region of left adrenal gland of acephalic fetus, age 15 days, 2 hours, showing cortical and medullary components of adrenal gland, and kidney. X 50. FIG. 13.—Cross section through median portion of adrenal gland shown in fig. 12. X 200.

tors affect adrenal size, particularly the severity of the operation.

Gonads of acephalic male fetuses showed variations in number and size of interstitial and germ cells, and the accessory reproductive structures showed certain deviations from the normal development pattern. Similar effects have been reported by others. Raynaud (1950) and Raynaud and Frilley (1947) reported a reduced number of gonocytes in gonads of "hypophysectomized" mice. Wells (1947, 1950b) found a reduction in size and number of testicular interstitial cells in decapitated rat fetuses. Jost and Colonge (1947) and Jost (1953) found that some accessory organs were reduced in fetuses killed three days after decapitation, but this difference was not apparent after longer periods of development.

Although differences in size and structure of thyroids, adrenals, and reproductive systems of acephalic hamsters indicate some relationship with the hypophysis, the drastic operation of decapitation results in high mortality of fetuses and could account for some variations from normal in fetuses which survive the operation. In the present investigation control fetuses were littermates of decapitated animals and were allowed to complete development intact. No fetuses which had been submitted to surgery, other than acephalic animals, were studied. Wells (1950a) has discussed surgical methods and survival in fetal rats, and has reviewed the work in this field.

Summary

1. A method for decapitating 12-day old hamster fetuses is described.

2. The thyroid glands of acephalic fetuses were smaller than those of controls and showed little increase in volume between the 12th and 16th day of gestation. Although follicles were formed no colloid was observed in glands of control or acephalic fetuses.

3. The cortex of adrenal glands of control and acephalic fetuses decreased in thickness during the last part of the 15th day, but the total volume of adrenals of acephalics was less than the volume of glands of controls.

4. The Wolffian ducts of acephalic males and the Mullerian ducts of acephalic females were shorter and smaller in diameter than these ducts of controls. The accessory structures in acephalic fetuses showed greater variation than did those of controls.

5. Results indicate that the fetal hypophysis may have some controlling influence on development of thyroid glands, adrenal glands, and reproductive systems of the hamster, but consideration must be given to the possibility of variation due to the drastic surgery of decapitation.

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