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Some Effects of Cortisone on the Mother and on the Fetus When Administered During Pregnancy in the Albino Rat

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SOME EFFECTS OF CORTISONE ON THE MOTHER AND ON THE FETUS WHEN ADMINISTERED DURING PREGNANCY IN THE ALBINO RAT

by

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APPROVAL SHEET

The thesis submitted by Robert J. Novak has been read and approved by three members of the faculty of the Graduate School.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fullfillment of the requirements for the Degree of Master of Science.

May 31, 1956
Date

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LIFE

Robert John Novak was born in Oak Park, Illinois, October 16, 1930.

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He began his graduate studies in the Department of Anatomy, Stritch School of Medicine, Loyola University, in September 1952, and in September 1954 he entered the Stritch School of Medicine, Loyola University.

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INTRODUCTION

Despite the current wide use of Cortisone both clinically and experimentally, little is known concerning its effect on mother and fetus when administered during pregnancy. The extensive use of Cortisone in the field of medicine, and the range of the observed effects has lead to an immense amount of investigative work involving many of the commonly used experimental animals. The complexity of the adrenocortical steroids both structually and functionally, their mode of action and their range of effects serve to make the problem particularly diversified. In view of the wide interest in and its ready availability and the importance of gaining more information concerning its behavior in pregnancy, we decided to study the effects of Cortisone on mother and fetus when administered prior to and during various stages of pregnancy as well as on the young when adminstered in the neonatal period.

Cortisone is a crystalline steroid compound, originally fractionated from extracts of the adrenal cortex. It is now prepared synthetically. It is supposedly similar to one of the hormones produced by the adrenal cortex. There is evidence to indicate that some adrenocortical compounds have androgenic properties, since hirsutism and other masculinizing effects have been observed following prolonged injections.

If Cortisone has androgenic properties, then there is the possibility that sufficiently high doses may affect the testes as well as other sex characters. What then would be the affect on such structures of fetuses in utero where Cortisone is administered to the mother? There is the possibility that Cortisone does not pass the placental barrier or if it does that the amount that gets across is too small to cause noticeable or significant changes in the fetus. There is also the possibility that Cortisone is metabolized by the mother and that the metabolite may not pass the placental barrier or that the metabolite has been modified to the extent that it is no longer effective.

REVIEW OF THE LITERATURE

Cortisone is one of a group of crystalline steroid compounds isolated from extracts of the adrenal cortex by Dr. E. C. Kendall and his coworkers at the Mayo Foundation. This substance, originally called compound E, was described by Mason, Myers, and Kendall in 1936. It was also described by Wintersteiner and Pfiffner as their compound F. (1936) and independently by Reichstein as substance Fa. Cortisone was first used on patients by Dr. Phillip S. Hench and his associates at the Mayo Clinic in 1948.

Cortisone is a steroid hormone thought to be like substances produced by the cells of the zona fasiculata of the adrenal cortex. Being a steroid-sterid it is a phenanthrene type derivitive or more correctly it has a cyclopentaneperhydrophenanthrene structure. Chemically is is shown as 17-hydroxy 11-dehydro corticosterone.

Although there is no positive experimental evidence to show that

the adrenocorticotropic hormone (ACTH) from the anterior pituitary (beta cells) increases the liberation of a Cortisone-like substance from the adrenal cortex, it appears from the experiments of Ingle and Kendall (1937) that a high level of certin in the body fluids suppresses the secretion of ACTH. This is evidenced by the cortical atrophy which occurs when large doses of Cortisone are administered to albino rats.

An analysis of the chemical structure of Cortisone indicates the great similarity between its chemical structure and the chemical structure of some of the sex hormones. Some of the latter have been isolated from steroid compounds and through the use of bio-assay it has been found that some of them are produced by the adrenal cortex. Because of the similarity in chemical structure which exists between Cortisone and the sex hormones it is not surprising that some of the effects of adrenal hyperplasia, neoplasia, and atrophy, (Addisons Disease, Cushings Syndrome, etc.), are revealed in the secondary sexual characteristics of the individual.

The studies of Wells and Kendall (1940) showed that compound E exerts a profound inhibitory effect on the growth and development of young rats. In studying the relationships of Cortisone when administered in pregnancy, Courrier and Delonge (1951) found that the administration of Cortisone to pregnant rabbits in high dosages (25 mg.) causes resorption, abortion and death of fetuses. DeCosta and Abelman (1952) observed that Cortisone interferes with pregnancy in the rabbit by causing fetal degeneration, fetal abortion and neo-natal deaths. They also reported that Cortisone does not interfere with the normal mating urge of the mature female rabbit and

that it decreases fertility especially when administered prior to mating.

Damm and Leroy (1951) also observed that high dosages of Cortisone injected in pregnant rats had a noticeable effect on fetuses, causing increased mortality and loss of weight, whereas lower dosages had little or no effect. All injections were begun on or after the 10th day of pregnancy. Leroy and Damm (1951) also showed that a direct subcutaneous injection of 500 gamma of Cortisone into the fetus between the 11th and 16th days of gestation killed all the fetuses, whereas following injections made between the 16th and 19th days, fetuses usually survived and were normal in size. This experiment was unique in that injections were made directly into the fetus through the semi-transparent uterine wall.

Jones, Lloyd and Whatt (1952) provided significant information by showing that administration of Cortisone in pregnant rats produced a rise in fetal adrenal cholesterol. It is felt by most workers today that the cholesterol nucleus is the precursor of the adrenocortical compounds. These workers concluded that Cortisone, or a metabolite of Cortisone freely entered the fetal circulation in rats when injections were made during pregnancy. Davis and Plotz (1954) using the albino rat showed that Cortisone administration in pregnancy caused a high mortality rate among the newborn and a decrease in the fetal adrenal weight. They found that some control animals gained more than 50% in body weight during gestation, whereas administration of 3.0 mg. of Cortisone produced a striking inhibitory effect on the normal weight increase of pregnant rats. They observed no effect on weight gain when only 1.0 mg. of Cortisone was administered daily.

Robson and Sharaf (1951) utilizing 5 mice, 11 or 12 days pregnant, injected 2.0 mg. of Cortisone per day, in 2 doses, for periods up to 5 days. In four of these resorption of all fetuses occurred.

The lack of agreement on the effects of Cortisone in pregnancy reported in previous experiments indicated a need for further information on this subject and prompted the present investigation.

MATERIALS AND METHODS

Albino rats of the Sprague-Dawley strain were selected as the experimental animal for this investigation. An inbred colony was started in April 1953 and the colony was enlarged by appropriate matings to approximately 350 rats from which almost any age animal was available for experimentation. Females were mated with specifically chosen males of good sturdy stock. All animals were given water and "Fox-chow" pellets "ad-libitum".

The Cortisone employed in these experiments was Cortone Acetate generously supplied to Doctor L. V. Domm through the courtesy of Merck and Company, Inc. All injections were made subcutaneously in the inguinal region utilizing a finely graduated tuberculin syringe and a number 25 needle.

Because of the possible androgenic properties of some of the adrenocortical compounds, the fetal testis was chosen as one of the organs likely to show modifications if Cortisone or some metabolite of Cortisone passed the placental barrier. The testes of Cortisone-injected newborn were also studied microscopically in order to determine if there was any similarity in structure between these testes and those of young born of

mothers having received injections of Cortisone during the gestation period.

The testes were removed under a binocular dissecting microscope with the aid of two dissecting needles and a fine eye forceps. Care was taken to leave a part of the epididymis and ductus deferens attached to the testes so as not to injure them in manipulations with the forceps, which because of their very small size was otherwise difficult to avoid. The testes were fixed in either Bouin's or a 10% calcium-formalin solution. They were dehydrated in a graded series of alcohols and imbedded in paraffin. Sections were cut at 7, 8, and 10 microns and subsequently stained with hematoxylin and eosin.

The results of administering Cortisone in vaious dosages and for varying periods before mating as well as during various stages of pregnancy were observed. Weights were taken on some of the animals to check possible weight changes during pregnancy. Some observations were also made on neonatal young injected with Cortisone for varying periods of time and the weights recorded. The young were carefully examined at birth for anomalies and some of them sacrificed for histological study. Those remaining were allowed to mature in order to observe possible modifications in growth and survival rate.

Before being placed in fixative the calvarii of fetuses and newborn were incised and the anterior abdominal walls opened form the xiphoid process to the pubic symphysis by means of a number 11 Bard Parker scalpel blade. The fixative employed was either Bouin's or a 10% calcium-formalin fluid. A total of 112 pregnant rats were employed in these experiments and

687 fetuses and newborn were recovered and utilized in our study.

EXPERIMENTAL RESULTS

1. Mating Response

In a series of 17 mature female rats, Cortisone (2.5, 3.5, 5.0 mg.) was administered daily for varying periods (21 to 58 days) prior to and during mating. (Table I) Observations were made on mating response and copulation. No diminution in the mating response of female animals receiving Cortisone prior to, and during mating was observed. These animals appeared to react toward the male and to receive the male in a manner similar to the females of the control group.

These studies would seem to indicate that the prolonged administration of Cortisone has no effect on the mating response of the mature female rat. The sexual behavior and receptivity of treated females appeared to be essentially normal.

2. Fertility

We were also interested in the effect of Cortisone on fertility. A study was made in a series of 11 mature female animals in which Cortisone (3.5, and 5.0 mg. dosages) had been administered prior to copulation and for periods of from 22 to 58 days. Copulations ensued but pregnancy did not follow. Vaginal smears were not made but vaginal plugs were noted in 4 of the 11 animals (Table II). Various methods were employed to promote pregnancy. This included changing males and in putting as many as four

TABLE I

SHOWING THE EFFECTS OF PROLONGED CORTISONE ADMINISTRATION ON THE MATING RESPONSE OF THE FEMALE RAT

	Rat No.	Mg. inj. per day	No. of injs.	Total Amt. Inj. (mg.)	Day of Expt. Male Introd.	Total Days Cohabitation Without Preg.
1.	R6	2.50	21	52.5	11	10
2.	R9	2.50	27	67.5	5	22
3.	R11Y11	2.50	31	77.5	7	24
4.	R18	2.50	50	125.0	9	41
5.	R18Y25	3.50	21	73.5	1	20
6.	R6	3.50	36	126.0	1	35
7.	R10	3.50	58	203.0	7	51
8.	R18Y20	3.50	46	161.0	1	45
9.	R20Y11	3.50	36	126.0	5	31
10.	R20Y12	3.50	36	126.0	5	31
11.	R20Y13	3.50	36	126.0	5	31
12.	R20Y14	3.50	36	126.0	5	31
13.	R20Y15	3.50	36	126.0	5	31
14.	R20Y16	3.50	36	126.0	5	31
15.	R18	5.00	22	110.0	5	17
16	R1	5.00	27	135.0	15	12
17.	R12Y22	5.00	27	135.0	4	24

TABLE II

SHOWING THE EFFECTS OF PROLONGED DAILY ADMINISTRATION OF CORTISONE ON FERTILITY

	Rat No.	Mg. Inj. per day	No. of Injs.	Total amt. inj. (mg.)	Day male introd.	Days foll. last inj. o deliv'd	No. of young born Alive	Dead
1.	R10	3.5	58	203.0	7	0	0	0
2.	R18Y23	3.5	46	161.0	1	44	11	0
3.	R20Y11	3.5	36	126.0	5	37	6	0
4.	R20Y12	3.5	36	126.0	5	42	7	0
5.	R20Y13	3.5	36	126.0	5	41	9	3
6.	R20Y14	3.5	36	126.0	5	38	8	0
7.	R20Y15	3.5	36	126.0	5	35	2	5
8.	R20Y16	3.5	36	126.0	5	42	11	0
9.	R18Y21	5.0	22	110.0	9	38	5	0
10.	R1	5.0	27	135.0	15	31	7	1
11.	R12Y22	5.0	27	135.0	3	35	12	1

In each of these rats copulation was observed during period Cortisone was administered, but pregnancys did not ensue until after the injections were discontinued. Note the time lapse following termination of injections when young were delivered.

males with a single female. It is interesting to note however, that all animals with but one exception became pregnant within 10-25 days after cessation of injections, and delivered normally.

3. Observations on Weight

A decrease in the normal weight gain was observed in our rats following the administration of Cortisone during pregnancy. Daily weights were kept on pregnant animals receiving varying dosages of Cortisone (2.5, 3.5, and 5.0 mg.) and a striking decrease in weight gain was observed. (Figures 1, 2, 3). Our observations show that injections of Cortisone not only caused a definite decrease in weight gain during pregnancy but also a noticeable fluctuation in weight. The lower weight gain of treated rats is apparently not caused by a decrease in the size of litters, nor a decrease in the weight of the average fetus, but by a loss in body fluids of the mother through an as yet unknown mechanism.

4. Mortality Rates In Utero

Another finding of interest was the effect of Cortisone administration on the mortality rate of fetuses of the pregnant rat. Davis and Plotz (1954) working with the albino rat showed that the administration of Cortisone in pregnant rats caused a high mortality rate among newborn. They administered 3.0 mg. of Cortisone for 16 to 19 days and observed a mortality rate of 47.70%.

We were interested in determining whether mortality rates vary with different dosages, hence we injected 2.5, 3.5, 5.0 mg. of Cortisone

for varying periods during the gestation period. The data (Table III) indicate that Cortisone increased the mortality rate of fetuses born of Cortsone treated mothers. Although some inconsistency occurs, our data indicate that the mortality rate is in general proportional to the period of administration. This is particularly evident in those animals which received Cortisone for a period of from 14 to 21 days. This evidence naturally does not take into consideration any fetuses which may have died in utero.

5. Gross Anomalies

All newborn rats were carefully examined for signs of anomalies. No evidence of any gross anomalies was observed in any of the young. Fraser (1952) working with special strains of mice found that treatment with 1.25 to 2.50 mg. of Cortisone begun on the 10th or 11th day of gestation caused cleft palate in 79% of the offspring. The etiology of this anomaly is unknown. Fainstat (1954) administered 25.00 and 30.00 mg. of Cortisone daily for 4 days to pregnant rabbits. He observed median post-alveolar cleft palates in 17 of 35 embryos whose mothers had received Cortisone. In 36 control embryos no indications of this condition were observed.

6. Birth-Weights

Some of the young were weighed at birth and no difference was noted in the birth weight of experimentals when compared with the birth weight of the controls. Davis and Plotz (1954) found that 3.0 mg. of Cortisone injected daily for 21 days in pregnant rats did not affect the average body weight of young at birth. Domm and Leroy (1951) in similar experiments found but a

TABLE III

SHOWING MORTALITY RATES OF YOUNG AT BIRTH IN A SERIES OF CORTISONE TREATED AND NORMAL RATS

	Mg. inj. per day	No. of litters	Total No. born	Avg. per litter	% born alive	% born dead
Last seven days	2.50	9	82	9.11	91.5	8.5
	3.50	0	0	0.00	00.0	0.0
	5.00	1	11	11.00	91.0	9.0
Last fourteen days	2.50	2	21	10.50	42.8	57.1
	3.50	7	75	10.43	79.4	20.0
	5.00	3	29	9.66	37.9	62.0
Over fourteen days to whole gestational period (21 days)	2.50	1	7	7.00	100.0	00.0
	3.50	4	38	9.50	55.2	44.7
	5.00	4	33	8.25	42.4	57.5
Controls	0.00	24	229	9.54	95.0	5.00

The majority of these were observed and recorded at birth. In a number of cases mortality was not recorded until some time had elapsed but in no case was this period more than 14 hours.

slight decrease in the weight of fetuses but this was only in cases where very high dosages had been administered. They utilized dosages of 6.25 mg. and 5.0 mg.. In another experiment they administered dosages of 3.00 mg. and observed no decrease in the weight of fetuses. Our observations are therefore in agreement with those of Davis and Plotz (1954) and Domm and Leroy (1951) in that the growth retarding effect of Cortisone observed in newborn rats is not found in the fetuses of Cortisone treated rats.

7. Newborn Survival Rate

Comparisons were also made on the survival rate of newborn rats, from mothers which had received different dosages of Cortisone for varying periods of the gestation period. The newborn were kept under observation up to 100 days after parturition. The findings indicate that practically all animals not sacrificed following parturition grew to maturity. (Table IV). In a series of 137 experimental animals a survival rate of 94.30% was observed. The survival rate of the controls was 93.10%. It is apparent from these results that the young of Cortisone treated mothers showed no decrease in survival rate in our experiments.

8. Growth Pattern

In the animals observed up to 100 days of age there was no perceptible modification of the growth pattern. The experimental animals appeared no different from animals in the control groups. They showed no noticeable precocity in body habitus nor any observable retardation in body growth.

TABLE IV

SHOWING RELATIVE SURVIVAL RATES OF YOUNG BORN OF CORTISONE TREATED AND NORMAL RATS

	Rat No.	Mg. inj. per day	No. of inje.	Total amt. inj. (mg.)	No. born	No. killed*	No. surv. 100 days
Experimentals							
1.	R3	2.50	6	15.0	12	0	12
2.	R12	2.50	7	17.5	14	0	11
3.	R6	2.50	21	50.5	7	4	3
4.	R11Y12	2.50	6	15.0	10	6	4
5.	R9	2.50	27	67.5	8	6	1
6.	R11Y11	2.50	31	77.5	12	5	6
7.	R18Y21	3.50	10	35.0	10	6	4
8.	R18T22	3.50	14	45.5	11	5	6
9.	R18Y24	3.50	16	56.0	10	6	4
10.	R18Y25	3.50	21	73.5	8	4	4
11.	R11	3.50	13	45.5	8	4	4
12.	R12	3.50	10	45.0	10	6	4
13.	R3	3.50	12	42.0	12	0	12
14.	R2Y12	5.00	4	20.0	11	4	7
15.	R13	5.00	9	45.0	10	8	2
16.	R3Y11	5.00	10	50.0	12	3	9
17.	R18Y24	10.00	4	40.0	12	0	12
18.	R18Y22	10.00	4	40.0	12	0	12
19.	R9	10.00	2	20.0	15	0	14
Controls							
1.	R12Y22				10	4	6
2.	R2Y11				9	3	6
3.	R9				15	4	11
4.	R11Y21				8	5	3
5.	R20				11	4	7
6.	R12Y23				6	3	2
7.	R3Y11-12				7	1	4
8.	R11Y22				9	0	9
9.	R3				11	1	9
10.	R12Y35				12	1	11
11.	R3Y11-12				8	0	8
12.	R3Y23				7	0	5

* Column shows number in each litter killed at birth for histological study

9. Effect on the Testes of the Fetus

As stated previously, because of the androgenic-like properties of certain adrenocortical compounds, the testes were chosen as one of the organs most likely to show modifications if Cortisone or a metabolite of Cortisone passed the placental barrier. Masculinizing effects following prolonged administration of Cortisone have been noted in the clinical literature. Leroy (1952) observed that the administration of Cortisone caused an increase in the weight of the testes of rats and hamsters, and he noted that this effect was not modified by adrenalectomy or hypophysectomy. We were interested in determining if there were any histological modifications in the testes of fetuses whose mothers had been treated with Cortisone. In figures 4, 5, 6 are shown some of the modifications that have occurred. In the young of mothers receiving 3.5 mg. of Cortisone for 12 consecutive days some type of cellular degeneration has occurred in the testis. The tubules appeared to be absent in the central part of the testis of such individuals while the connective tissue stroma and loose cells remained. The peripheral tubules maintained their normal structure and showed no evidence of degeneration. This medial degenerative or necrotic area did not show evidence of leucocytic infiltration nor any giant cells. The supporting connective tissues appeared the same as those in the control group. There is no evidence of hyperchromatism of any of the cells. The testes of fetuses whose mothers received 5.0 mg. of Cortisone for the last 18 days of gestation showed a much wider area of degeneration. The tubules appeared to be widely separated and degeneration extended more peripherally with large areas lacking con-

voluted tubules. Whether any effect on spermatogenesis or a modification in the Sertoli or Leydig cells occurred was not determined. From the above it would seem that some type of cellular modification has occurred in some of the testes of fetuses of Cortisone treated mothers having received injections for most of the gestation period. On the other hand however, there is the possibility that this so-called area of degeneration may be the mediastinum of the testis, and that the areas that are devoid of seminiferous tubules represent the stroma of the mediastinum coursing longitudinally through the testis. Further experiments will be undertaken to clarify this point.

10. Effects of Cortisone on the Newborn Rat

In this experiment 14 litters consisting of 139 newborn rats were used. Individual litters were divided so that some of the young received Cortisone, and the rest served as controls. No distinction was made between males and females. Experimentals and controls were weighed en mass at each injection and averaged. The experimentals in five litters received 250 gamma of Cortisone daily while in 9 other litters 500 gamma was injected. The duration of injections was 6 to 14 days. In 12 of the above 14 litters mortality rates of experimental animals ran as high as 77.70%. (Table V). At the end of experiments it was observed that control rats outweighed the surviving experimental rats by 2 to 3 times. (Table VI). The most striking effect however was the inhibition of growth in newborn rats receiving Cortisone. The control littermates were 2 to 3 times larger than the exper-

imentals.

The condition of the experimentals became progressively more precarious during the course of the experiments. Diarrhea and clonic seizures were observed. The fur of the experimental rats appeared ruffled and their general appearence was that of malaise and debilitation. In some cases the experiment was concluded ahead of schedule because it was felt that experimental animals would not survive.

We were able to confirm the observations of Domm and Leroy (1955) on the precocious eruption of incisors. These authors fould that newborn rats treated with Cortisone showed a precocious eruption of the incisors. They maintain that this is due to a decreased development of the bones of the face and the tissues of the gingiva rather than to the direct action of Cortisone on the dental papillae or the organic constituents of the teeth.

We also observed in the course of our experiments that the eyelids of experimental rats opened before those of controls.

In comparing the testes of control rats and those receiving 250 and 500 gamma of Cortisone respectively the testes of the latter appeared to be slightly smaller (Figures 7, 8, 9).

DISCUSSION

Whether Cortisone, or some metabolite of Cortisone passed the placental barrier to bring about the observed testicular modifications, the increased mortality rate, and the other effects observed in our experiments remains to be determined. The effects observed in the fetuses of Cortisone

TABLE V

SHOWING MORTALITY RATE IN CORTISONE TREATED AND NORMAL YOUNG RATS

Experimentals

Series No.	Amt. inj. (gamma)	Days of expt.	Total amt. inj. (mg.)	Initial no. of animals	Terminal no. of animals	Mortality rate %
Experimentals						
1.	250	10	2.50	5	2	60.0
2.	250	13	3.25	4	2	50.0
3.	250	8	2.00	5	4	20.0
4.	250	10	2.50	5	2	60.0
5.	250	6	1.50	5	4	20.0
6.	500	6	3.00	8	8	00.0
7.	500	8	4.00	8	8	00.0
8.	500	8	4.00	6	2	66.6
9.	500	8	2.00	7	2	71.4
10.	500	11	2.50	9	2	77.7
11.	500	12	2.50	7	6	14.2
12.	500	9	2.00	5	2	60.0
13.	500	14	3.00	8	3	62.5
14.	500	7	2.00	8	2	75.0
Controls						
1.		10		3	2	33.3
2.		13		3	3	00.0
3.		8		3	3	00.0
4.		10		3	3	00.0
5.		6		4	4	00.0
6.		6		5	5	00.0
7.		8		4	4	00.0
8.		8		3	3	00.0
9.		8		3	3	00.0
10.		11		4	4	00.0
11.		12		3	3	00.0
12.		9		2	2	00.0
13.		14		3	3	00.0
14.		7		4	4	00.0

TABLE VI

SHOWING WEIGHT DIFFERENCES IN CORTISONE TREATED AND NORMAL YOUNG RATS

Series no.	Amt. inj. (gamma)	No. of inj.	Total amt. inj. (mg.)	Days of expt.	Average initial weight expts'	Average initial weight controls	Average terminal weight expts'	Average terminal weight controls
1.	250	10	2.50	10	5.82	5.30	8.40	29.00
2.	250	13	3.25	13	8.05	7.60	9.80	28.47
3.	250	8	2.00	8	6.56	7.47	7.05	20.13
4.	250	10	2.50	10	5.80	5.67	8.40	15.00
5.	250	6	1.50	6	6.44	6.40	7.13	12.00
6.	500	6	3.00	6	6.50	6.50	7.19	11.64
7.	500	8	4.00	8	9.50	9.56	11.00	20.00
8.	500	8	4.00	8	6.54	6.13	8.00	18.20
9.	500	4*	2.00	8	7.69	7.80	6.25	10.00
10.	500	5*	2.50	11	6.09	6.38	7.90	24.00
11.	500	5*	2.50	12	7.09	7.27	10.00	24.77
12.	500	4*	2.00	9	8.60	8.90	11.80	21.70
13.	500	6*	3.00	14	7.80	8.33	14.33	32.00
14.	500	4*	2.00	7	6.98	7.30	5.60	15.70

* All injections except these were made daily

In each experiment controls and treated were littermates of animals

The weight above is represented in grams

treated pregnant rats would appear to support the assumption that Cortisone did cross the placental barrier. Other workers have also obtained substantial evidence in favor of this assumption. Davis and Plotz (1954) showed that the adrenals of fetuses in which the mother had been treated with 3.0 mg. of Cortisone acetate daily showed a decrease in the width of the adrenal cortex and a decrease in fetal adrenal weight. Additional support is obtained from the findings of Jones, Lloyd and Whatt (1953) who showed that Cortisone administration in pregnant rats caused an increase of 70% in fetal adrenal cholesterol. In explaining their results these investigators maintain that Cortisone or some metabolite of Cortisone freely passed the placental barrier causing suppression of the pituitary-adrenal axis and thereby causing the observed rise in the fetal adrenal cholesterol. Schmidt and Hoffman (1954) working with pregnant monkeys administered ACTH and found that the maternal adrenals responded typically by hypertrophy of the fascicular zone and a loss of their cytoplasmic vacuolation. Fetal adrenals on the contrary were small with seemingly enlarged glomerular zones and atrophic fasciculer zones, changes similar to those produced in adult adrenals by Cortisone administration. This they believed showed that an excessive amount of cortical steroids from the enlarged maternal adrenals passed into the fetal circulation and inhibited the activity of the fetal adrenals.

On the other hand, it is believed at the present time that Cortisone may be freely administered in humans during pregnancy without deleterious effects. Katzenstein and Morris (1953) maintain that, despite the contradictory experimental evidence from animals, their few clinical obser-

vations suggest that Cortisone therapy during pregnancy does not result in any damage to the infant. Abelman and DeCosta (1952) also state that in their opinion Cortisone may be administered freely during pregnancy in the human. In other words, an increased mortality and other modifications were found in the rat and other experimental animals while in the human, according to these investigators, no noticeable effects were noted in the fetus when mothers were treated during pregnancy. If the above observations are correct what then is the basis for the differences in response between the human and lower mammals ? The following is suggested as a possible explanation for the differences observed.

It is known that the human has a placenta (Haemochorialis) in which the chorionic villi are free and float in cavernous spaces and are bathed by the maternal blood. Mossman (1937) working with various rodents observed that the rats' placenta appeared to be hemochorial, probably becoming hemoendothelial.If this istrue then the placenta of the rat is the closest approach to an actual intermingling of fetal and maternal blood. The fetal capillaries are separated from maternal trophoblast channels in the labyrinth by an endothelial layer. Hence possibly Cortisone, or one of its metabolites may pass more readily from mother to fetus in the rat thus accounting for the modifications and effects observed.

On the other hand, however, Wislocki, Deane, and Dempsey (1945) observed in their work on the rat that a basement membrane existed around each of the fetal capillaries, and this would indicate that the rats' placenta is not hemoendothelial in the strict sense of the term. Bridgman (1948)

substantiates these findings by observing that the cytoplasm of the labyrinthine trophoblast persists until term and does not break down in sections leading to a hemoendothelial type of placenta. Recently, however, Wislocki and Dempsey (1955) utilizing the electron microscope studied the placentae of rats. They observed that the placenta of the rat throughout gestation possesses a placental barrier which is composed of " (1) Two or three thin, overlapping sheets of cytoplasm of individual trophoblastic cells, (2) a basement membrane supporting the trophoblast and (3) the wall of a fetal capillary consisting of a basement membrane bearing a lining of endothelial cells. Thus the placental barrier is not hemoendothelial but hemochorial in character, and the trophoblast is also cellular instead of syncytial in structure." This would seem to indicate then that the human and rat would have the same type of placenta (hemochorial) and passage of substances across these placental barriers might be essentially similar.

Flexner and Gelhorn (1942) working with different compounds have shown that the passage and rate of transfer of substances from the blood of the mother to that of the fetus increases as the layers to be passed decreased. Even if the trophoblast layer persists in its entirety, the placental barrier in the rat is one of the thinnest encountered. Thus it would appear possible that substances could pass its placenta more readily than the human placenta. On the other hand the overlapping cellular character of the trophoblast of the rat placenta as shown by the electron microscope studies may have far different transfer properites than the single layer syncytium of the human placenta. But does Cortisone follow this pattern?

Its chemical structure is infinitely different than any of the compounds heretofore used in transference studies. Besides, the substances used in placental transference studies diffused across a gradient which Cortisone could probably not do. Work with radio-active Cortisone should provide interesting results here.

It is important to note that the amount of Cortisone administered in pregnant animals is much higher on a body weight basis than the equivalent amounts administered to pregnant women. (Human 50-150 mg. per day). Abelman and DeCosta (1952) gave daily dosages of 15.0 mg. of Cortisone to rabbits weighing from 3.5 to 5.0 kilograms. On the basis of weight the equivalent dosage in a pregnant woman (60 kilo) would be in the range of 180 to 250 mg. per day. Courrier administered 25 mg. daily in rabbits which on a weight basis would be 300 mg. daily, for a 60 kilogram woman.

In our work on the rat we injected a maximum of 5.0 mg. daily which on a weight basis would be the equivalent of 1500 mg. of Cortisone daily for a 60 kilogram woman. There exists the possibility therefore that the dosage used in our experiments was beyond the therapuetic level, and that some of the results obtained may be more pathological than physiological. If this is true it would appear tehn that high dosages of Cortisone in the rat act as a lethal substance causing the high mortality rate of fetuses. The positive site of this lethal action has not been identified. It is possible that the metabolic degradation products of Cortisone may cause adverse effects on the sensitive fetal tissues. There is also the possibility that Cortisone acts on the placenta itself, or on the placental-fetal relationship.

Great weight loss and inability to gain much weight, plus extreme debilitation results in newborn when large amounts of Cortisone are administered directly. This is not seen in the fetuses whose mothers had received Cortisone. This would lead one to postulate that Cortisone in its original form does not pass the placental barrier, but rather a metabolite of Cortisone. If this is true then the metabolite is capable of causing fetal adrenal suppression and fetal mortality as well as other effects, but it is not capable of causing weight loss and fetal debilitation. However, whether Cortisone, or its metabolites act directly on the fetus to cause death, as in the case where Domm and Leroy (1951) injected fetuses directly in utero or whether it causes changes in the placental-fetal relationship remains to be determined

The decrease in weight gain observed in our pregnant rats gave evidence of the occurrence of some type of Cortisone effect on electrolyte balance within the tissues of the body. Apparently Cortisone causes sodium excretion which in turn would remove water from the body thus causing a decrease in weight. The manifestations of this electrolyte imbalance could disturb metabolic pathways.

Several theories have been advanced to account for the loss of weight observed in Cortisone treated animals. Gaunt, and Eversole (1949) maintain that Cortisone suppresses posterior pituitary activity thus preventing the action of ADH on sodium and water retention, thereby causing weight loss due to sodium and water diuresis.

Bland (1952) explains weight loss following Cortisone administration by assuming that Cortisone inhibits the pituitary release of ACTH and consequently the production of adrenocortical compounds is suppressed. This would mean that desoxycorticosterone, which acts somewhat as an antodiuretic substance, could no longer help control sodium and water preservation in the kidney tubules and therefore an excess renal loss of sodium and water would occur causing the observed decrease in weight. This theory seems to be invalidated by other workers who find that ACTH has no control over the production of desoxycorticosterone by the zona glomerulosa of the rat adrenal gland but rather that the amount of sodium and water present in the body (autonomously) helps control desoxycorticosterone production and thereby helps control sodium and water retention and excretion.

It is also the contention of some workers that the loss of weight due to Cortisone administration is due to body water being removed from the mesenchymal-derived tissues, resulting in an increase in sodium and water diuresis. The observations made on the cause of weight loss and gain are quite conflicting. The opinions on etiology by various authors on this subject are diverse. At this time no conclusive evidence can be presented to substantiate any one claim.

The relationship between Cortisone and the testes presents an interesting problem. It is true that a direct relationship exists between the testes and the adrenal cortex. Removal of testes in a newborn rat or mouse results in persistence of the X-zone of the adrenal cortex. The administration of androgen causes disappearance of the X-zone. Hyperplastic, or

neoplastic growths of the adrenal cortex are associated with a masculinization in both males and females. This Adrenogenital Syndrome may be reversed by extirpating the adrenal cortex which produces the excessive amounts of hormone. Another proof of the existing relationship of adrenal gland and testis is found in the physical findings associated with Cushing's Syndrome.

Yet experimental evidence is far more contradictory concerning androgenicity and estrogenicity of adrenocortical compounds. Talalay, Dobson, Ebersole, and Huggins (1952) in their work on the albino rat found that " Cortisone acetate is neither androgenic, nor is it an antagonist to testosterone propionate with respect to the prostate epithelium of the dog. We were able to confirm earlier observations that Cortisone acetate is without effect on the weight of the uterus of the castrate immature female rat. When given in conjunction with diethystilbestrol, Cortisone acetate partially suppresses the stimulating effect of this estrogen on the uterus in what appears to be a competitive manner."

Yet in our work on the rat we observed the appearance of some type of testicular degeneration in fetuses whose mothers had received large amounts of Cortisone. The etiology of this degeneration is rather obscure. Moore (1953) in his work on rats observed that the administration of Cortisone failed to demonstrate an effect " on the accessory reproductive glands of castrated or intact males or females, of either young or older ages. Gonadal changes in relative weight or histology were indefinite in the male, but in the female the ovaries were constantly heavier in the treated animals."

He concluded that " Cortisone Acetate in doses up to 5.0 mg. per day does not exhibit an androgenic, estrogenic or gonadotropic effect in young or adult rats."

We know that in adrenalectomy sexual functions are impaired and that degeneration of the testis occurs.. A possible theory then to account for testicular modifications is as follows: Cortisone inhibits the production of ACTH by the pituitary. Because of this diminution of ACTH the fetal adrenal atrophies and the production of adrenocortical substances is diminished thereby causing degeneration of testis. Another possibility is that Cortisone inhibits the beta cells of the pituitary which are responsible for the production of gonadotropic hormones. The diminution of gonadotropic hormones results in testicular degeneration.

In our experiments on the administration of Cortisone in newborn rats we were unable to find the type of testicular modification observed in the fetuses of treated mothers. It is possible therefore that the substance which caused the fetal testicular modifications is a metabolite of Cortisone produced by the mother, since Cortisone apparently did not cause the same effects in treated newborn as were observed in the fetuses of treated mothers.

SUMMARY

1. In 17 mature female rats administration of Cortisone, from 21 to 58 days had no effect on the mating response.
2. Our evidence indicates that prolonged administration of Cortisone caused a decrease in the fertility of mature female rats.
3. A lower gain in weight was observed in all pregnant rats treated daily

with 2.5, 3.5, and 5.0 mg. of Cortisone

4. Our results indicate that Cortisone increased the number of stillborn in Cortisone treated mothers and that the rate increased with prolonged Cortisone administration.

5. The young of Cortisone treated mothers showed no visible anomalies.

6. The decrease in weight observed in Cortisone treated young rats was not observed in the newborn of Cortisone treated mothers.

7. A normal postnatal survival rate was observed in young rats whose mothers had received daily injections of Cortisone during pregnancy.

8. The young of Cortisone treated mothers showed a normal postnatal growth pattern.

9. The testes of newborn from pregnant rats having received dosages of Cortisone beyond 3.5 mg. for periods of 12 or more days were modified. There appeared to be fewer seminiferous tubules in the medial portion of such testes.

10. In comparing the testes of newborn rats receiving 250 and 500 gamma of Cortisone with controls the former appeared to be slightly smaller.

11. Cortisone administration in newborn rats caused an increase in mortality.

12. A reduced growth rate and weight gain were observed in newborn rats receiving Cortisone. Diarrhea and clonic seizures were observed. The fur was ruffled and the general appearance was one of debilitation.

13. It is concluded that Cortisone, or a metabolite of Cortisone crossed the placental barrier causing the effects observed in the young of treated pregnant rats.

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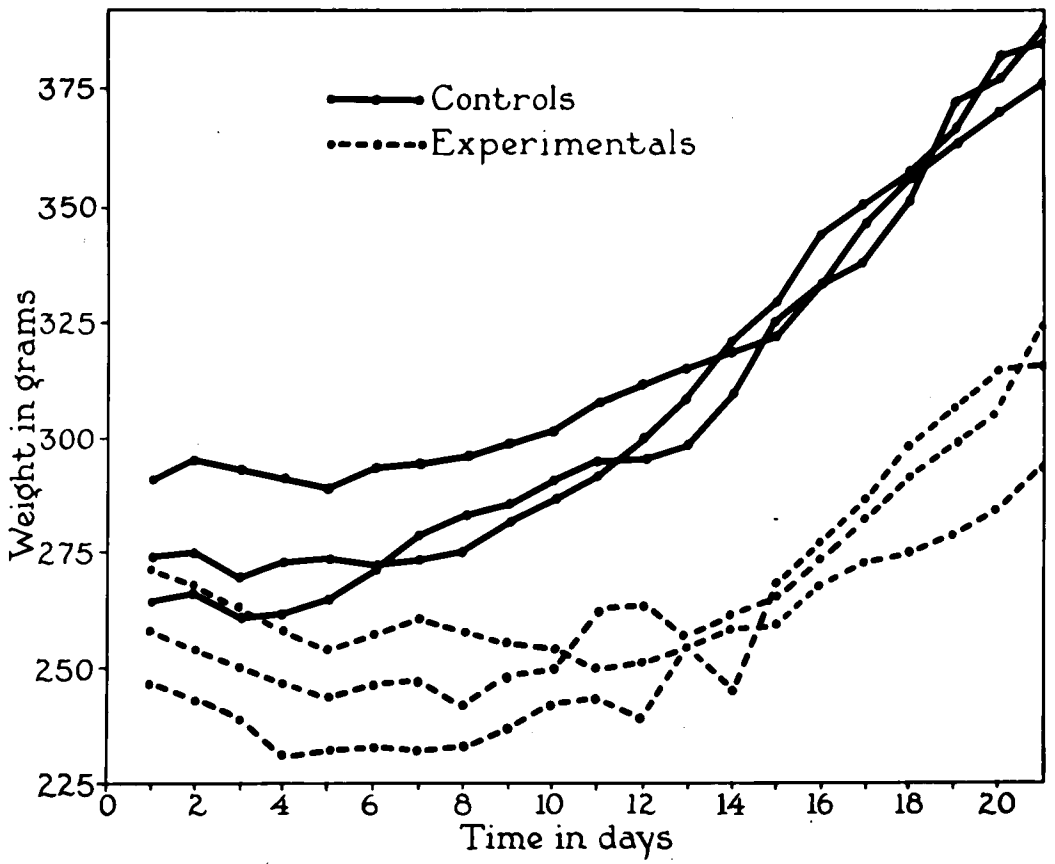
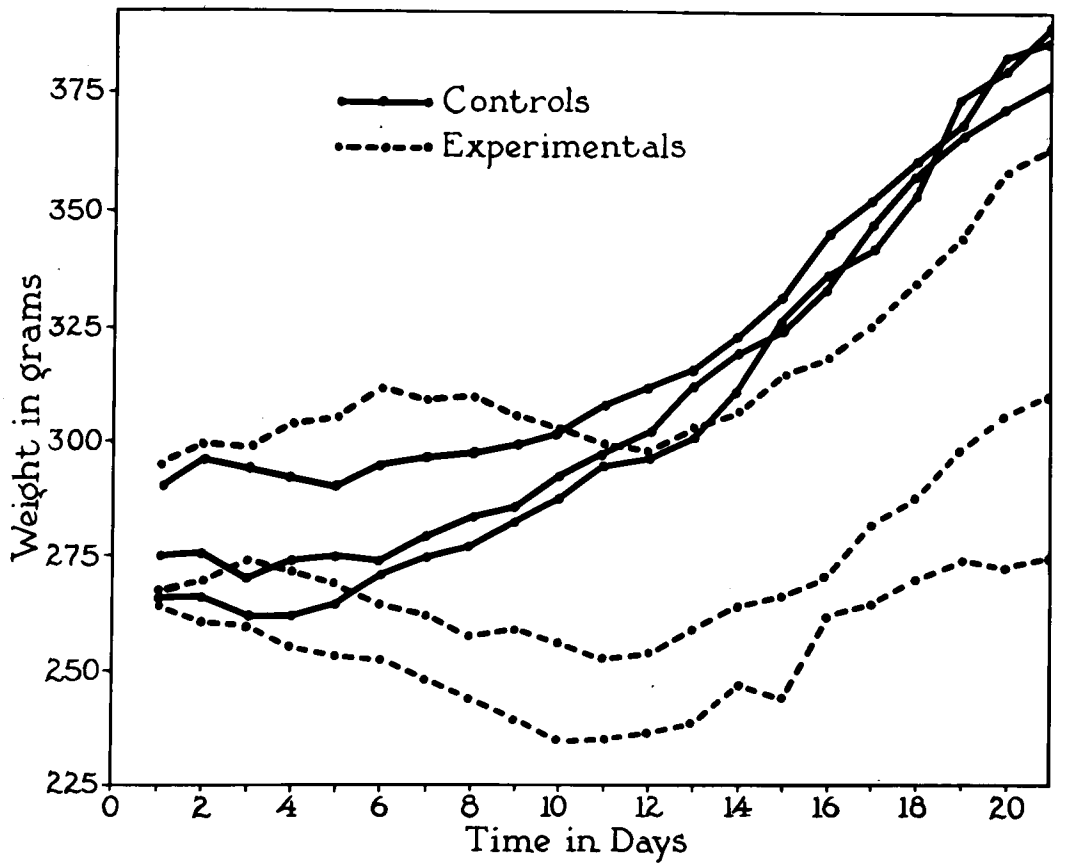


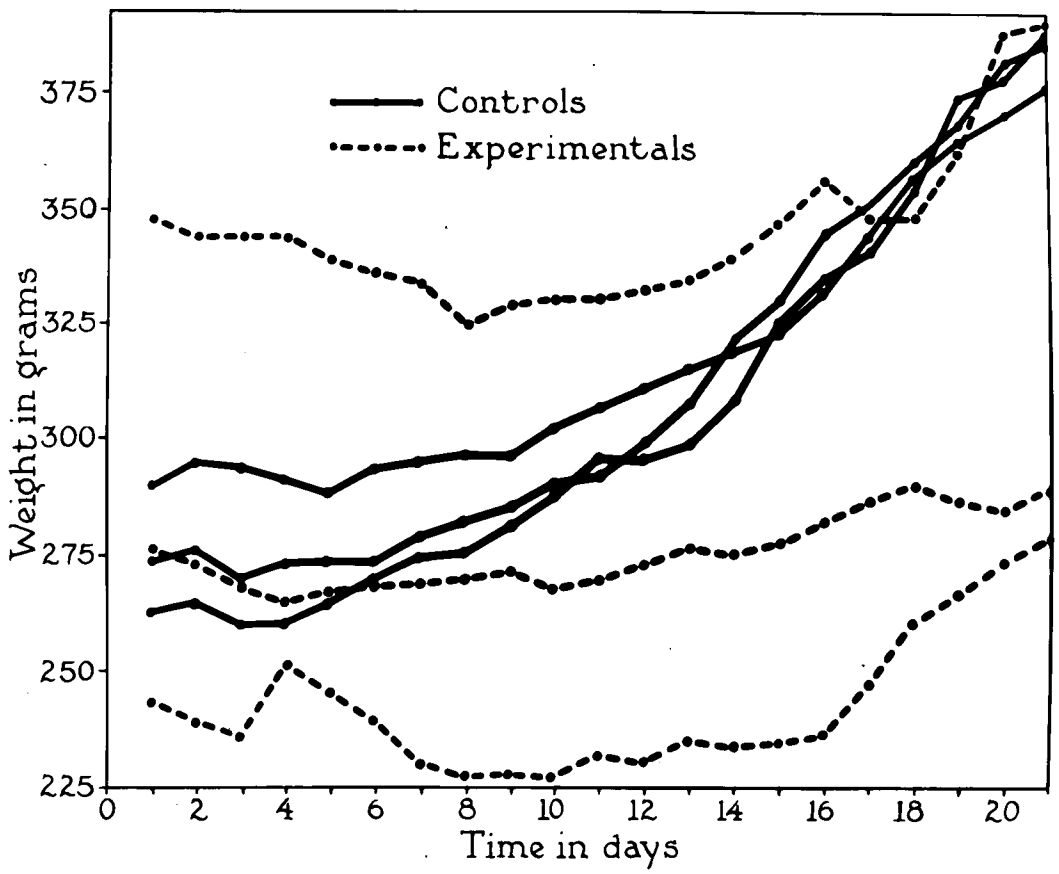
Graph showing decrease in weight gain in pregnant rats that had received daily injections of 2.5 mg. of Cortisone during gestation.

Figure 1



Graph showing decrease in weight gain in pregnant rats that had received daily injections of 2.5 mg. of Cortisone during gestation.

Figure 2



Graph showing decrease in weight gain in pregnant rats that had received daily injections of 5.0 mg. of Cortisone during gestation.

Figure 3

PLATE I

Figure. 4. Testis from newborn whose mother had received injections of 3.5 mg. of Cortisone from the 10th day to the 21st day of gestation. Newborn was killed at birth. Notice the capsule, seminiferous tubules, and the lack of tubules in the center of the testis. Testis was fixed in Bouins fluid. Stained with hematoxylin and eosin. Magnified. 85X.

Figure 5. Testis from newborn whose mother had received injections of 3.0 mg. of Cortisone from the 4th to the 21st day of gestation. Newborn was killed the day of birth. Notice that the medial portion of the testis shows an increased lack of seminiferous tubules. Testis was fixed in Bouin's fluid. Stained with hematoxylin and eosin. Magnified 85X.

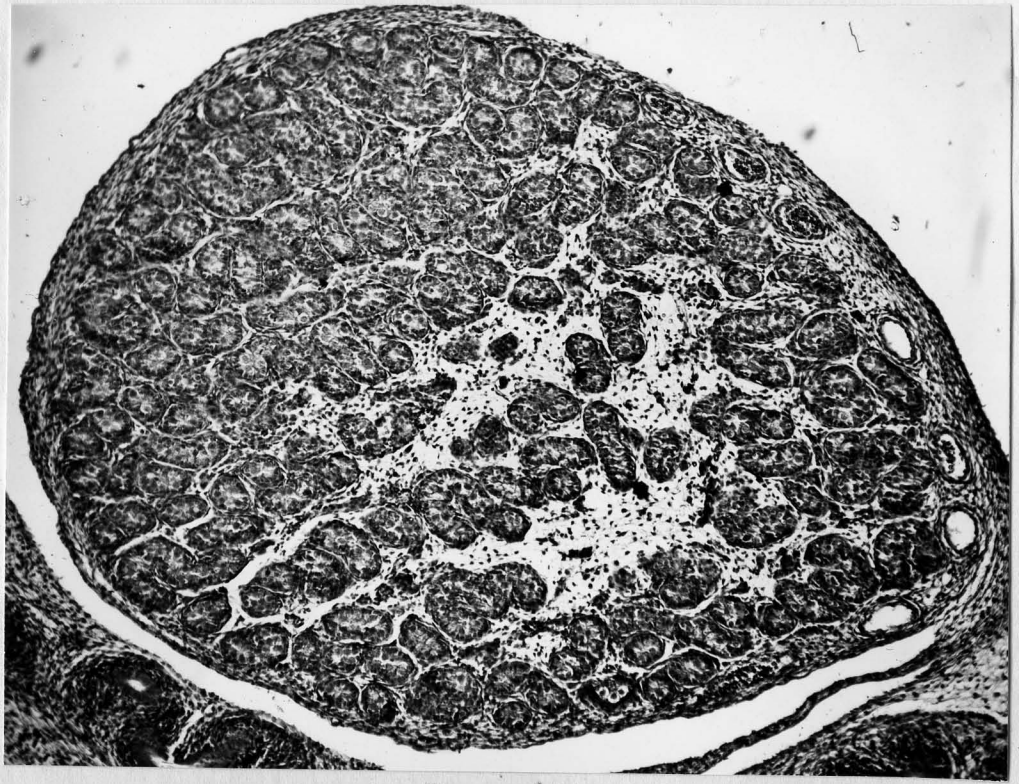


Figure 4

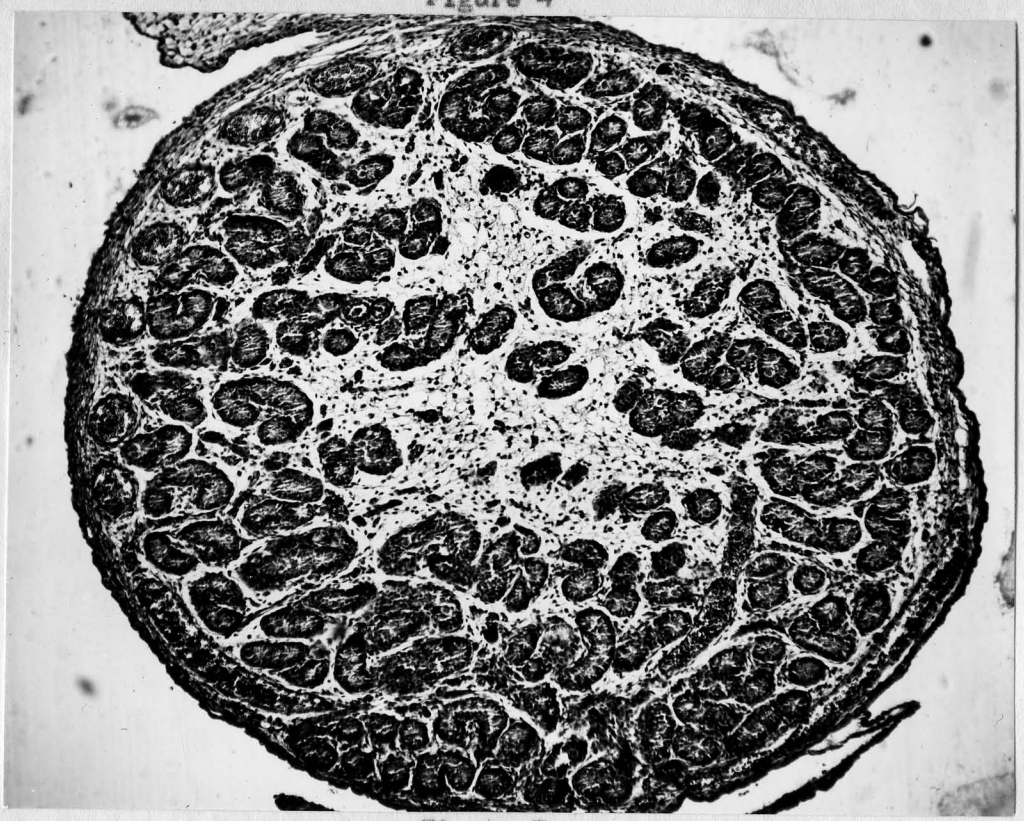


Figure 5

PLATE II

Figure 6. Newborn control testis. Newborn killed on day of birth. Notice the capsule, and seminiferous tubules. Testis was fixed in Bouin's fluid. Stained with hematoxylin and eosin. Magnified 85X.

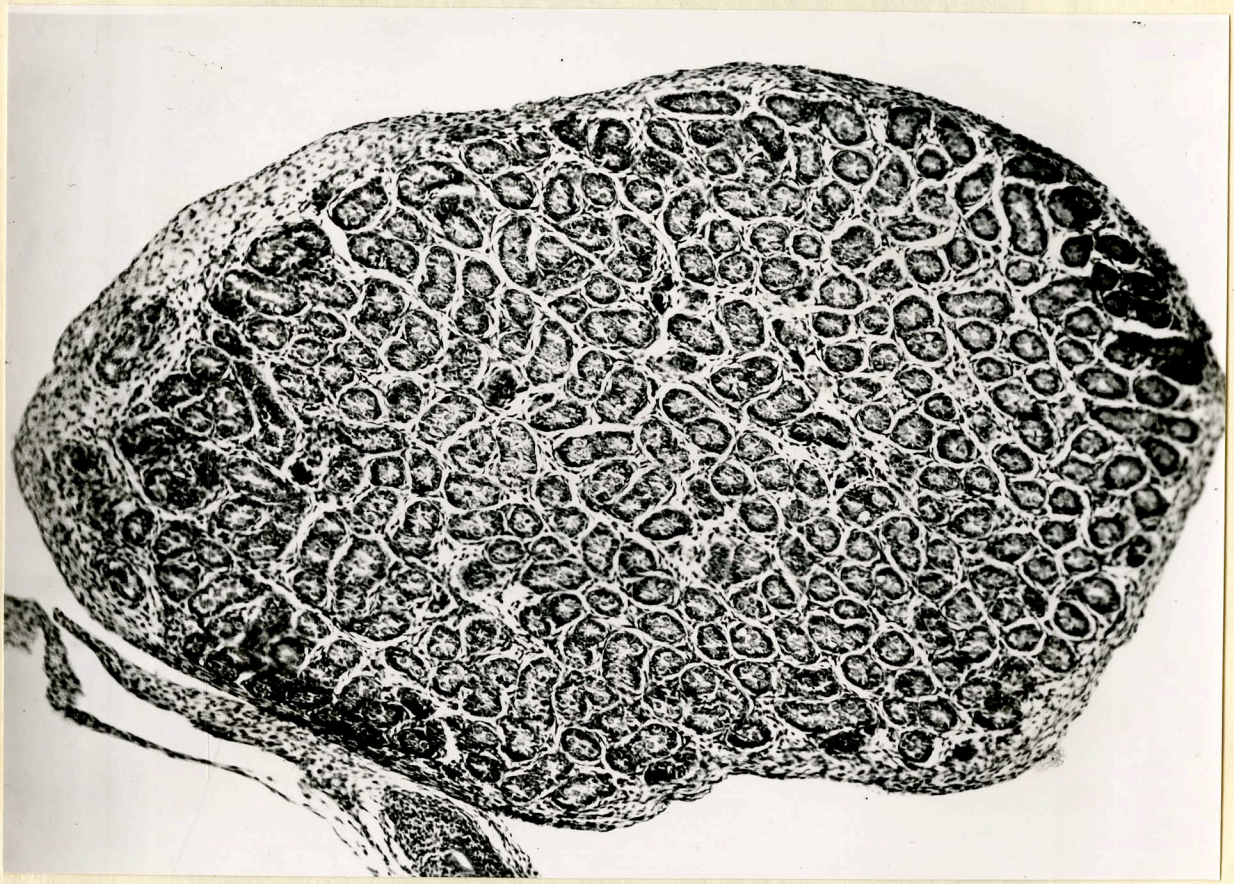


Figure 6

PLATE III

Figure 7. Testis from newborn rat that received injections of 250 gamma of Cortisone daily for 10 days beginning on the 2nd day after birth. Killed age 12 days. Notice the capsule and seminiferous tubules. Testis was fixed in Bouin's fluid. Stained with hematoxylin and eosin. Magnified 85X.

Figure 8. Testis from newborn rat that received injections of 500 gamma of Cortisone daily for 6 days beginning on the 3rd day after birth. Killed age 9 days. Notice the capsule and seminiferous tubules. Testis was fixed in Bouin's fluid. Stained with hematoxylin and eosin. Magnified 85X.

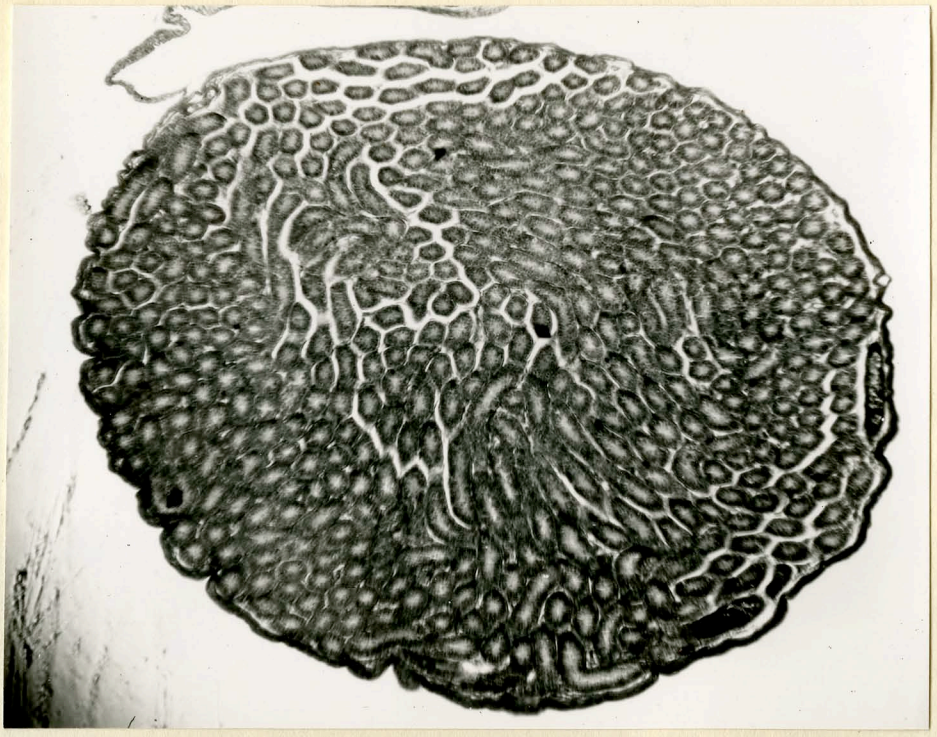


Figure 7

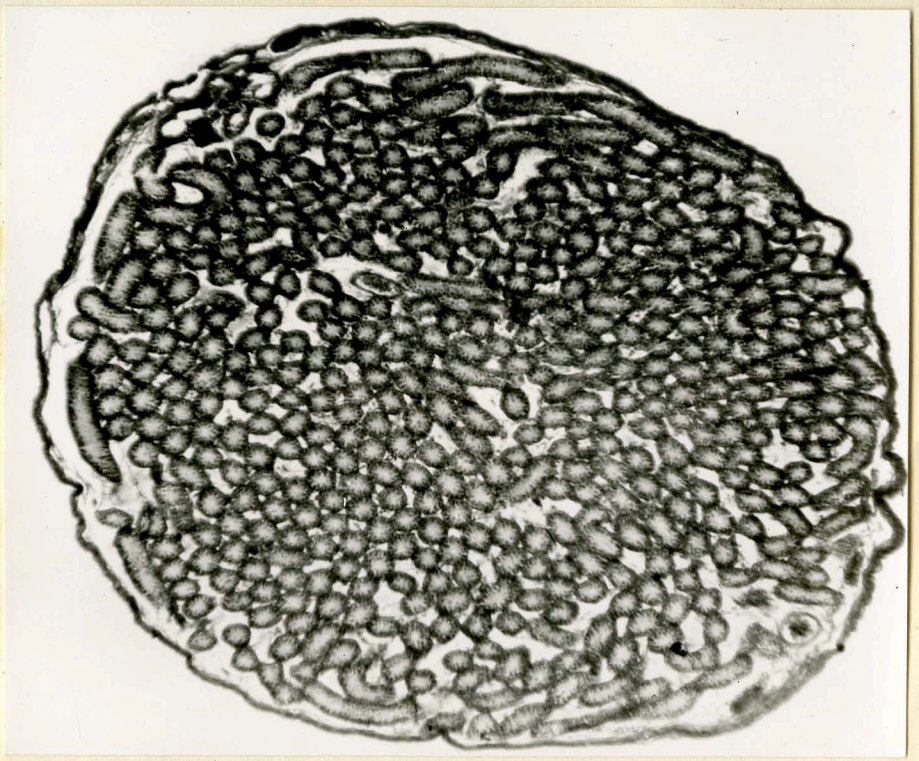


Figure 8

PLATE IV

Figure 9. Testis from newborn control sacrificed when 12 days old. Notice the capsule and seminiferous tubules. Testis was fixed in Bouin's fluid. Stained with hematoxylin and eosin. Magnified 85X.

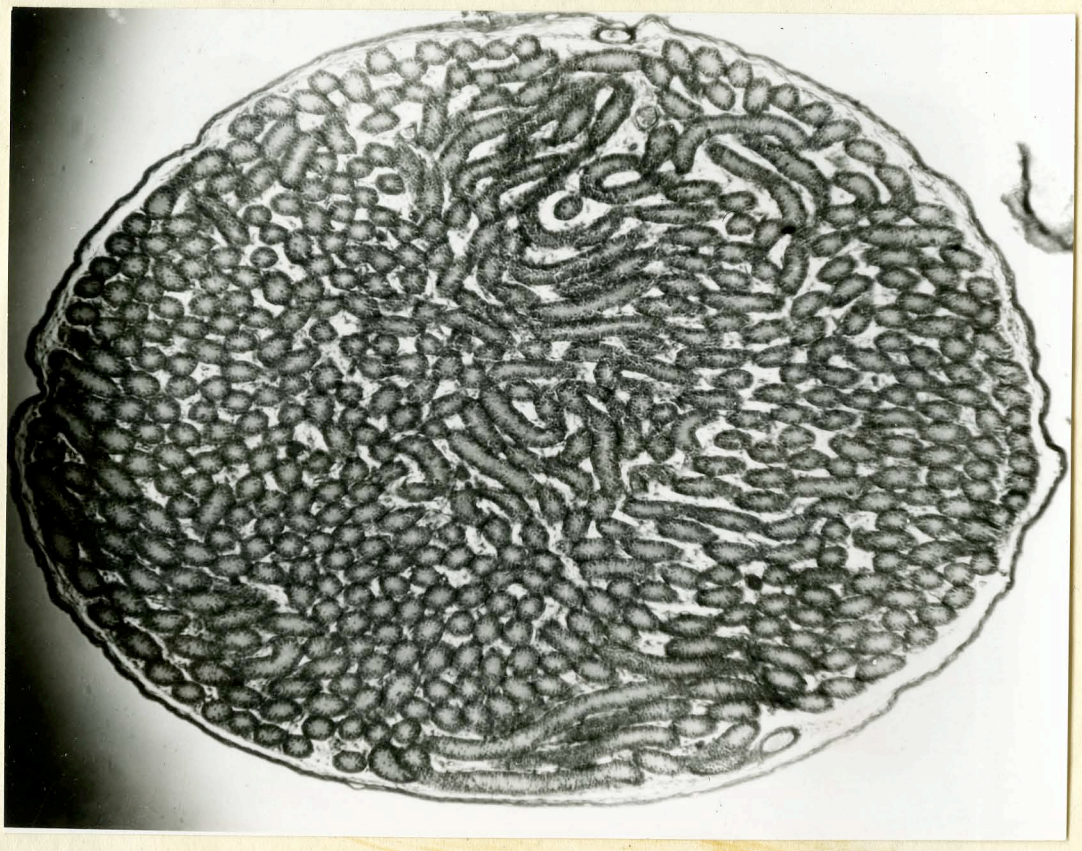


Figure 9