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be accepted as fulfilling this part of the requirements for the degree of DOCTOR OF PHILOSOPHY

Approved by:

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THE EFFECT OF CHRONIC FORMALDEHYDE IRRITATION ON PREGNANT ALBINO RATS

A dissertation submitted to the
Graduate School of Arts and Sciences
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by

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TABLE OF CONTENTS

INTRODUCTION

The effect of stressor agents on the female reproductive system has been investigated by Selye ('39, '47, '50a, '51). He has found that ovarian atrophy and an interruption of normal vaginal cyclic changes are the most obvious effects in the nonpregnant female rat. If, however, the stress is prolonged until a sufficient adaptation has been established, the ovary will return to normal and vaginal cycles will be resumed. It was found that during the period of ovarian atrophy the ovary would respond to extraneous gonadotropins, and the vagina would respond to injected estrogens. These results indicated a decreased secretion of gonadotropins by the anterior pituitary. In contrast to this apparent decrease, an increased production of ACTH was noted as indicated by the hypertrophy of the adrenal cortex. The above response was explained on the basis of a "shift in the anterior pituitary hormone production"; i. e., in response to stress, the pituitary of an animal produced more ACTH, essential for existence during stress, at the expense of a normal elaboration of other more expendible hormones.

The above observations were concerned with the reactions of nonpregnant animals. The question then arises: "what is the reaction of the pregnant animal under condi-

tions of stress?" This question becomes especially significant when one observes that recently several investigators have considered stresses and strains of diverse kinds imposed upon pregnant women as principal factors in the precipitation of toxemias of pregnancy, and have classified these toxemias as diseases of adaptation (Parviainen '50; Garrett '50a, '50b and Selye '50a, '51).

Selye ('46, '47, '51) made observations on the effects of stressors upon pregnant rats, but his results were recorded in a generalized form only. He found that there was a tendency for abortion and resorption to occur, and attributed these reactions to the "shift in pituitary hormone production."

Prior to and subsequent to Selye's work, extensive literature has accumulated concerned with the subjection of pregnant animals to numerous agents. These include, vitamin deficiencies, radiations, chemical agents and anoxia. The emphasis in these studies has been placed upon resultant malformations in the offspring of experimental animals. References, other than to the production and description of those malformations, are limited. It is not within the scope of this paper to review extensively the literature on the subject, but certain work will be referred to in order to illustrate the trend of the experiments and to clarify the more pertinent references utilized in the discussion which follows.

The general subject of fetal malformations has been reviewed extensively by Warkany ('47). He reports the

occurrence of skeletal deformities in the offspring of rats maintained on a vitamin A deficient diet, a riboflavin deficient diet, or a diet high in calcium, low in potassium and deficient in vitamin D. It was also reported that general starvation resulted in resorption of the embryos if initiated in early pregnancy, and in stillbirths or reduction in the weight of offspring if initiated in the latter part of pregnancy. In addition to the results from nutritional deficiencies, abnormalities of various kinds have been reported in the young of mothers treated with androgens, estrogens, X-ray and of those infected with rubella and toxoplasma. In all these reported experiments the investigators made few, if any, observations upon the reaction of the mothers to the different treatments.

Fraser ('51), investigating the effect of cortisone upon pregnant mice, found that injections of 1.2 - 2.5 mg. of the hormone on four consecutive days at any time between the ninth and seventeenth days of pregnancy produced such defects as hare lip in the young. The incidence of malformation was found to be dependent upon two factors; dosage and susceptibility of the strain of mice. An excessive dosage resulted in abortion. With low dosages, no malformations were recorded. In addition to malformations, there was reduced embryo weight and a more frequent occurrence of stillbirths. Upon analysis of his results, Fraser presented a hypothesis that all types of maternal insult reported in the literature might be regarded as various forms of stress exerting their effects by causing

the mothers' adrenal glands to produce excessive amounts of cortical compounds. The compounds thus produced are possibly the active principles in the production of abnormalities. This interpretation by Fraser of the extensive study and experimentation on malformations is believed to be the first of its kind.

Other investigators have also observed the effects of cortical compounds upon pregnant animals. Some of these are cited in order to illustrate further that variations in dosage result in variations of response.

Administration of cortical extracts has been reported to induce labor and terminate pregnancy in the rat (Britton '33). Contrary to this, extracts with a moderate excess of hormone have been administered without interfering with pregnancy or parturition (Grollman '34).

Cortical hormone administration also has given varying results depending upon the dosage. Hall ('38) reported that the administration of cortical hormones throughout pregnancy did not affect litter size or weight; whereas Seifter ('51) reported that heavy overdosage of cortisone resulted in an increased fetal mortality. He attributed this effect to a reaction on the ground substance of the placenta, although no histological observations of the placenta were made.

Another group of investigations is cited to illustrate how some agents of a more nonspecific nature have been found to influence pregnancy.

Chih ('48) reported embryos of normal size but smaller

litters from rats exposed to high temperatures.

Ingalls ('50) demonstrated that maternal anoxia in mice resulted either in fetal death or, in a smaller percentage of the cases, congenital deformity.

No observations upon the response of the mother were made in these two investigations.

Haskins ('48) observed severe abnormalities in six animals from nine litters of mothers injected with nitrogen mustard, a mitotic inhibitor. Injections made earlier than the thirteenth or fourteenth day of pregnancy resulted in fetal death. He noted that many of the placentae, especially those of the more severely affected embryos, had brown, necrotic borders. Whether this increased placental necrosis was primary to fetal defectiveness or merely parallel to it could not be determined. Histological studies were not included in the experiment cited.

Gross abnormalities also have been reported to occur in the offspring of rats treated with trypan blue (Gillman '48). These abnormalities occurred in only a small percentage of the offspring, the highest incidence being 19%. Observations made upon the mothers of these offspring indicated that the principal pathological alterations were severe anemia, gross enlargement of the adrenals, hyalinization of the glomeruli, and fatty livers. The authors conjectured that the malformations were the result of subtle changes in metabolism which, in this early stage, resulted in a modification of the chorion at a "chemical" level causing perversion of embryonic growth.

Histological studies were not made of the placentae, but they were sectioned grossly and it was noted that trypan blue particles were absent from them.

Attempts have been made to duplicate in experimental animals the toxemic or eclamptic conditions sometimes encountered in pregnant women.

Armstrong ('43) reported a syndrome, similar to the toxemias of pregnancy, in rats maintained on a modified pork diet. She reported that 30% of the animals which were apparently healthy up to the last day of pregnancy became sick. Pregnancy in these animals ended in protracted labor, convulsions, and death of the mother. Starting on the eighteenth day, the mothers exhibited a marked retention of body fluids. At autopsy marked fatty degeneration of the liver and destruction of the kidney tubule epithelium were observed. The fetuses were invariably well formed, of normal weight, but dead. Observations showed the placental weights and the fetal-placenta weight ratios to be below average.

Symeonidis ('49) reported an eclampsia-like syndrome in rats injected with progesterone. Injections of .20 mg. of progesterone during the fifteenth to eighteenth days of pregnancy resulted in death, abortion or resorption of the fetuses, and obvious illness of the mother. The mothers exhibited edema, hypertension and albuminuria. Histologically, lesions of the kidney, liver and placentae were found.

From a review of the literature, it is apparent that experiments on pregnant rats have been concerned mainly

with resultant malformations of the offspring or with the total effects upon pregnancy, and that observations upon the placentae have been few and of a general nature. Because of this scarcity of knowledge concerning the placentae of pregnant rats under experimental conditions, and the importance of the placenta as a fetal-maternal link, an investigation of the effect of a toxic agent upon the placentae was judged to be of great interest. Further, if the action of the various agents as stressors has played a role in causing the phenomena reported in the literature, as suggested by Fraser, it was thought that additional observations considering this action upon pregnancy, maternal organs, offspring and placentae would be of value.

It is the purpose of this paper to describe the effects of formaldehyde, a nonbiological active stressor agent, chronically administered to pregnant rats. An attempt has been made to determine whether the animals' own defense mechanisms might in some way be involved in the results mentioned above.

METHODS AND MATERIALS

Ninety-one albino rats of the Sprague-Dawley strain, varying between three and five months in age, were used in these experiments. Thirty-seven of these animals died as the result of experimental treatment. Those surviving were divided into four groups: fifteen nonpregnant and twelve pregnant controls, fourteen nonpregnant and thirteen pregnant injected animals. Experimental animals were given subcutaneous injections of .20 c.c. of 10% formalin three times a day for ten days (from the sixth day of pregnancy through the fifteenth for the pregnant injected series), and similar injections of .30 c.c. for four additional days (from the sixteenth to the twentieth days of pregnancy). Each animal received its last injection on the morning of the day on which it was killed. Pregnant animals were killed on the morning of the twentieth day of pregnancy. The day that spermatozoa were found in the vaginal smear was considered as day one. Thus the embryos were nineteen days old at the termination of the experiment. The normal nonpregnant controls were killed after at least two normal estrous cycles had been recorded. Daily vaginal smear and weight records were maintained.

The animals were killed with illuminating gas and

autopsied immediately. In the pregnant animals, the embryos and resorption areas were counted; then the entire uterus and concepti were removed, weighed, and pinned to a paraffin block. The two uterine cornua were split longitudinally along the antimesometrial side. A placenta, generally one near the center of one of the cornua, with a living embryo attached, was cut so that a center portion perpendicular to the long axis was obtained. This section and a small portion of the liver were fixed immediately in cold PAF (120 c.c. 95% alcohol, 15 gm. picric acid, 15 c.c. formaldehyde, 15 c.c. water) for glycogen study. A center cut was then taken from another placenta and fixed in 10% calcium-formol for lipid study. Following this, the embryos were removed and stored temporarily in .85% saline solution until the rest of the tissues were weighed and fixed. Finally, the remainder of the uterine cornua and placenta was fixed in Bouin's solution.

The adrenal glands and ovaries were subsequently removed, cleaned of fat, and weighed. The right adrenal was fixed in 10% calcium-formol; the left adrenal and ovaries were fixed in Bouin's solution. A portion of the kidney was the last tissue to be removed and was also fixed in Bouin's solution.

After all the tissues and organs had been fixed and weighed, the embryos were weighed. They were prepared by severing the umbilical cord at the belly, and gently blotting in order to remove the excess saline solution and tissue fluids. Some of the embryos were selected for clearing by the Lipman technique and were fixed in 95% alcohol.

The uterine horns that had been fixed in Bouin's solution were carried through graded alcohols to 70% where a center cut was taken from one of the placentae and prepared for histological study. The remaining placentae were prepared for weighing.

The fetal placenta, composed chiefly of the labyrinth and small cellular trophoblast, which projects from the uterine wall and resembles a "button", was peeled away from the maternal tissue, being permitted to separate along a normal line of cleavage. When it was removed, there remained an impression of the line of attachment on the maternal side. Under a dissecting lens the maternal portion was carefully trimmed around the border of the impression and cleared for mesentery attachment. The maternal and fetal portions were then weighed separately in 70% alcohol. The weight of each component and the total weight were recorded.

The right adrenal gland and placenta which had been fixed in formalin were embedded in 20% gelatin, sectioned at approximately 20 micra on a freezing microtome, mounted, and "stained" for lipids with Sudan Black dissolved in propylene glycol.

The left adrenal, ovaries, placenta, and representative kidney sections which had been fixed in Bouin's solution were embedded in paraffin, sectioned at 6 micra, mounted, and stained with Lillie's haematoxylin and eosin.

The placenta and liver, fixed in PAF, were washed in 95% alcohol, embedded in paraffin, sectioned at 6 micra,

mounted, and "stained" for glycogen according to Lillie's periodic acid, leucofuchsin technique.

In all cases placenta sections containing a large portion of the central radial artery were selected for study in order that a legitimate comparison might be made.

Embryos selected for skeletal study were prepared and stained with alizarine Red S according to the technique of Lipman.

RESULTS

The results of the experiment have been divided into three categories: first, the effects upon the mother; second, the effects upon the offspring; and third, the effects upon the placenta.

A. Effects upon the Maternal Animal.

1. General observations. In general, both pregnant and nonpregnant injected animals showed a progressive loss of normal vigor and spontaneous activity. Accurate measurement of food intake was not made; but, a normal consumption of greens, which were given twice a week, was observed and taken as an indication that the animals were feeding in a normal manner. Water intake was also believed to be normal.

The mortality rate of the experimental animals was high. Of thirty-six pregnant injected animals, eighteen died after varying numbers of injections and five resorbed their young at some time during the injection period. The five which resorbed their young were alive at the termination of the experiment. Thirteen pregnant injected animals lived, and observations on these were recorded in detail. Of twenty-eight nonpregnant injected animals, fourteen died after varying numbers of injections. Data were recorded for the

remaining fourteen. The combined mortality for all injected animals was 50%. This percentage, however, does not give an accurate picture of treatment and response as various groups or shipments of animals reacted differently. For example, twenty-two animals from one shipment were injected, of which sixteen lived; whereas in another shipment, only four of nineteen injected animals survived. In a group of ten animals obtained from the Zoological Laboratories and injected during pregnancy, all ten survived and bore some living young. The reason for this variability of response is not clear.

One of the outstanding reactions of the majority of the injected animals in both groups was the gradual development of severe edema. In general, this edema increased progressively up to the eleventh or twelfth day. Among individual animals there was a variability in response as shown by the daily weight curves (Figs. 1a, 1b). The pregnant animals, as a group, showed a more severe edema with less variability among the individuals. The weight curves for the experimental groups and pregnant control group are shown in Fig. 2. It can be seen that on the thirteenth or fourteenth day there was a tendency toward a decrease or a stabilization in the weight of the experimental animals. Although this seeming decrease or stabilization in weight continued to the twentieth day in the nonpregnant injected series, it lasted for approximately two days in the pregnant injected series, after which there was a progressive rise to the twentieth day.

Also shown on the graph is a line representing the

combination of the weight increase of the nonpregnant injected series (weight increase due primarily to edema) and the weight increase of the pregnant control series (weight increase due to normal changes of pregnancy and growing concepti). A comparison of this line with that representing the pregnant injected series shows a striking similarity. This suggests that in the pregnant injected series the rise in weight following the plateau was due, at least in part, to the growing concepti and was not entirely due to any difference in physiological response of the pregnant animals to the injections.

In these tests the water retention appeared to involve chiefly the subcutaneous tissues, causing a swelling of the body which was especially evident in the forelegs, chest, and face. It was noted further that the abdominal cavity contained an abnormal amount of fluid, in such quantity as to pour copiously from the incision at autopsy.

A small number of the pregnant injected animals was permitted to go to term. Such animals showed protracted labor, prolonged gestation time, stillbirth, or inability to deliver their young. Two of these animals died. Those unable to deliver their young were autopsied. The uterus contained full term, dead fetuses exhibiting varying degrees of maceration.

2. Maternal organs.

(a) Adrenal Glands. It can be seen from Tables 1a, 1b, 1c, 1d that the most striking change in maternal organs occurred in the adrenal glands. There was a

statistically significant weight increase in the adrenals of the pregnant injected animals (based on milligrams per hundred gram body weight) as compared with the other groups. On this basis, the average weight of the adrenals of the pregnant injected animals was 23.6% more than that of the nonpregnant injected animals, 57.8% more than that of the pregnant control animals, and 41.3% more than that of the nonpregnant control group. These changes were even more striking when the comparisons were made using the actual adrenal weights; the pregnant injected series showing a 33.7% increase over the nonpregnant injected series, a 70.4% increase over the pregnant control series, and a 86.8% increase over the nonpregnant control group. From these tables it can also be noted that the increase in weight of the pregnant injected animals' adrenals was greater than the sum of the increase in weight of the adrenals of the injected nonpregnant animals and the pregnant controls.

Histologically, the adrenals of the pregnant injected series exhibited a greater hypertrophy and hyperplasia than the nonpregnant injected group. No consistent or specific changes could be observed in the pregnant injected animals that did not occur also in the nonpregnant injected group. These groups seemed to differ only in their degree of response. There was in both groups a disruption of the normal distinct zonation of the adrenal cortex (Fig. 3). This seemed more evident in the pregnant injected series, but was not characteristic of that group.

In the injected series, both pregnant and nonpregnant,

the cells of the zona glomerulosa were enlarged, and in many individuals the entire zone seemed diminished or at least indistinguishable. In some cases, however, the zone appeared somewhat widened. In all of the injected animals the cells of the zona fasciculata were enlarged and more vacuolated, and in many the regular arrangement into cords was disrupted. The zona reticularis showed a hypertrophy of its cells, which resulted in less densely packed nuclei and a loss of distinctness as a separate zone. Three of the animals developed a pronounced fatty metaplasia, and nine others showed evidence of its development to varying extents in the cells of the zona glomerulosa and possibly in the outer portion of the fasciculata. The two most severe cases are shown in Figures 3 and 4.

Lipid distribution in the adrenals could not be determined accurately from the preparations made. It could be ascertained, however, that lipids were being stored in the injected series. As in the histological studies, no definite characteristic or consistent change was distinguishable in the pregnant injected animals. All the adrenals of the injected series demonstrated storage of lipids, and large globules of lipid were found in the areas of fatty metaplasia. One difference between the normal and injected pregnant animals was seen in the amount of lipid in the zona glomerulosa; normal pregnant animals had an increased lipid concentration (Figs. 5, 6) which was not evident in the injected pregnant animals.

(b) Ovaries. A slight decrease in ovarian weight was observed in the nonpregnant injected

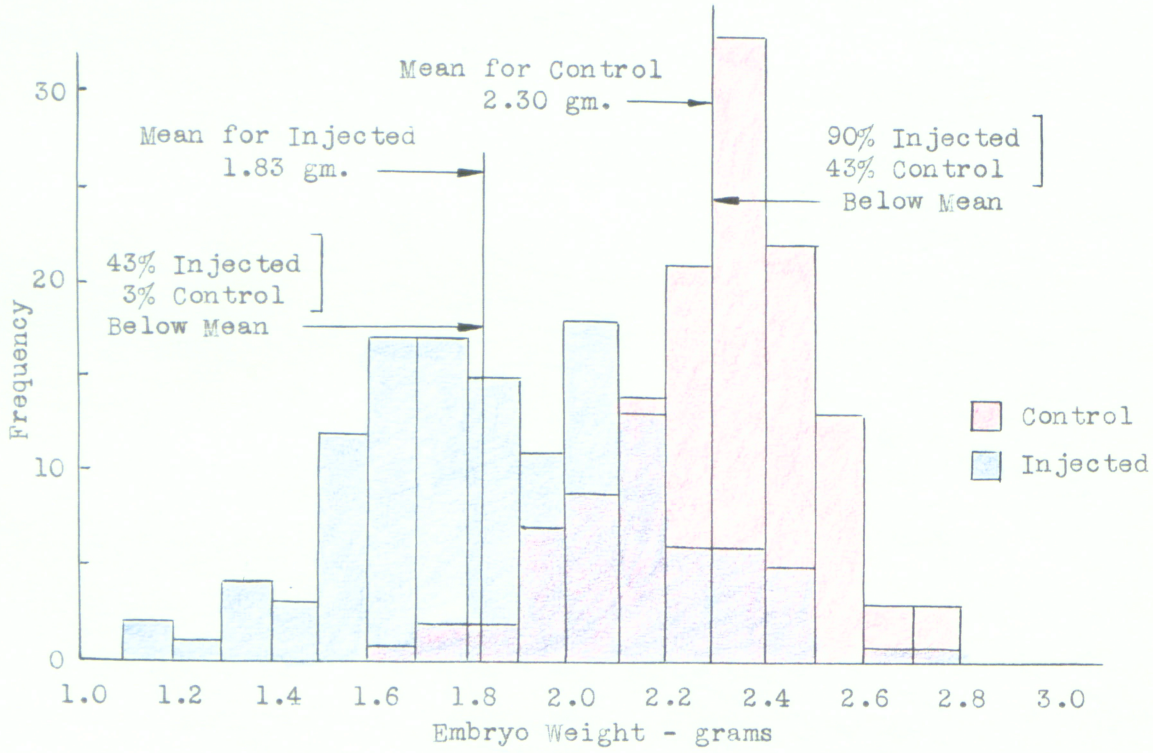
animals as compared with nonpregnant controls (Tables 1a, 1b). This, however, was demonstrated in only a few animals. In the pregnant series, formalin injection had apparently little or no effect upon ovarian weight. The ovaries of all pregnant animals were observed grossly, and the corpora lutea counted. The ovaries of the injected and noninjected groups showed no significant weight difference (Tables 1c, 1d), or change of gross appearance (Fig. 7). Histologically the corpora lutea of the pregnant injected series showed no obvious alteration in structure. The only other histological observation made on the ovaries was to determine the presence or absence of "wheel cell" formation in the stroma. Wheel cells were seen definitely in four of the nonpregnant injected animals but none (with the exception of one animal which showed evidence that these cells might have been forming) was observed in the pregnant injected series.

In the thirteen pregnant injected animals, 177 corpora lutea were counted as compared with 143 in the fourteen animals of the control series. In the injected group, 134 of the 177 corpora lutea were accounted for by living embryos, and 23 by observed resorption sites. Twenty corpora lutea were unaccounted for. In the control group, 130 of the 143 corpora lutea were accounted for by living embryos, one by an observed resorption site, and 7 by a sterile uterine horn in one animal. Five were unaccounted for. Thus, in the control series, 13 of 143 (9.1%) of the ova failed to develop or the embryos were resorbed; whereas in the injected series,

43 of 177 (24.2%) of the ova failed to develop fully.

B. Effects upon Embryo.

The offspring of injected animals showed a statistically significant decrease in average weight (Fig. 8, Tables 1c, 1d). These embryos, in comparison with those of the control group, also showed considerably more variation in size among individuals from different animals and even among individuals of the same litter. The extreme weights of the fetuses in the experimental group were 1.16 and 2.70 grams, as compared with 1.69 and 2.75 grams for the controls.



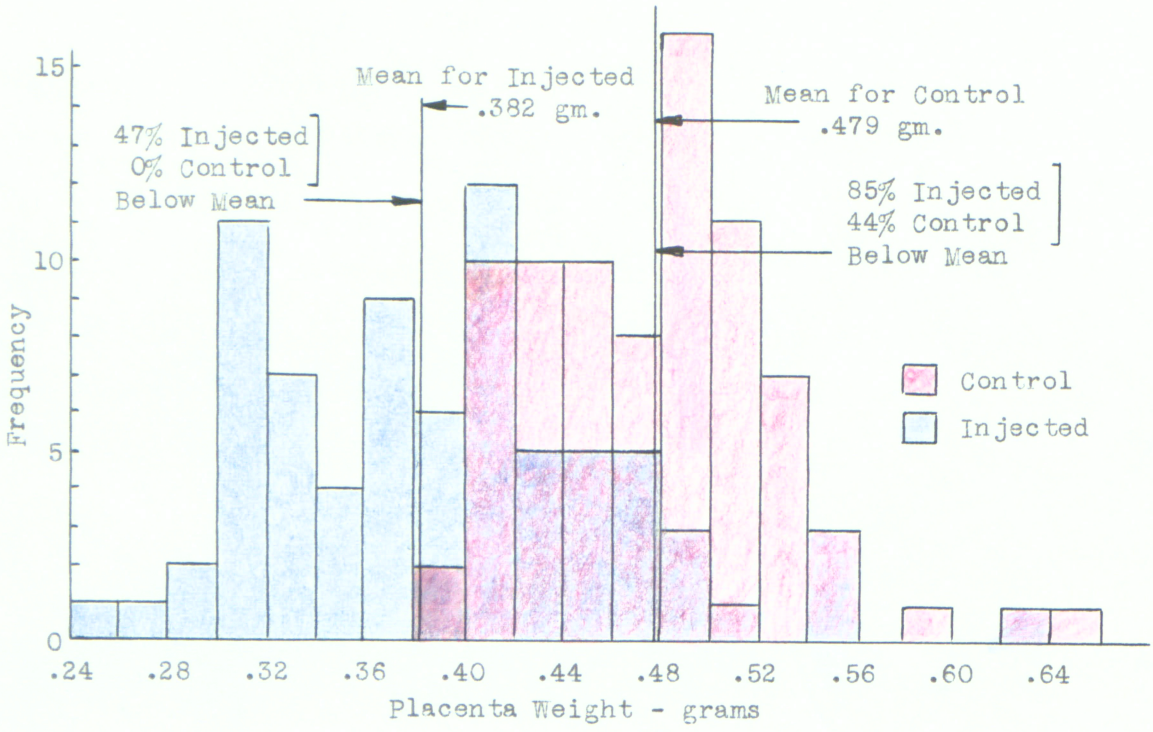
Frequency Distribution of Fetuses by Weight

It is of significance that in the experimental animals 82 of 132, or 62.0%, of the embryos weighed less than 2.0 grams, as compared with 12 of 130, or 9.2%, in the control group. Never-

theless, some of the experimental animals bore litters similar in weight to those of normal animals. Retardation of bone development was observed in the cleared specimens of embryos of injected animals but there were no malformations in skeletal structures (Fig. 9). This retardation was manifested chiefly by an attenuated degree of development (smaller size of bones) rather than by a delayed appearance of ossification centers. No metacarpal or metatarsal bones were ossified in the more severely affected embryos (Fig. 9). The sternebrae were not ossified in twelve; others showed only the beginning of ossification in the supraoccipital bones. Of 34 control embryos, 94% had more than 3 sternebrae ossified; whereas in 61 experimental embryos, only 36% had 3 or more sternebrae ossified. All the controls had three ossified metacarpals and metatarsals, whereas seven from the injected series had less than three.

C. Effects upon the Placenta.

1. Weight. After the placentae had been removed for sectioning, there remained 76 in the injected series and 80 in the control series. Analysis of the weights of these (Tables 1c, 1d) showed that the average fetal and maternal portions and the total weight were, in general, lower in the injected series than in the control series. In the injected group 85% of the individual placenta weights were below the average placenta weight of the control series. None of the control placentae weighed less than the mean weight of the injected series.



Frequency Distribution of Placentae by Weight

It was also noted that the average weight of the placentae of three of the injected animals was similar to those of the control animals.

In analysing these data, placental weights were not correlated with embryo weights, but there was an indication of a correlation. To establish this possible correlation, a small group of animals was tested but the results were not conclusive. This matter will be investigated further at a later time.

2. Histological observations. In studying the placentae histologically, the descriptions and terminology used by Bridgman ('48) and Mossman ('37) were followed. The sections studied were cross sections through

the center of the placenta. The shape of these sections, as indicated by the following diagram, resembled a "half moon" with the flatter surface representing the area from which the umbilical vessels arise, and the opposite convex surface, the wall of the uterus.

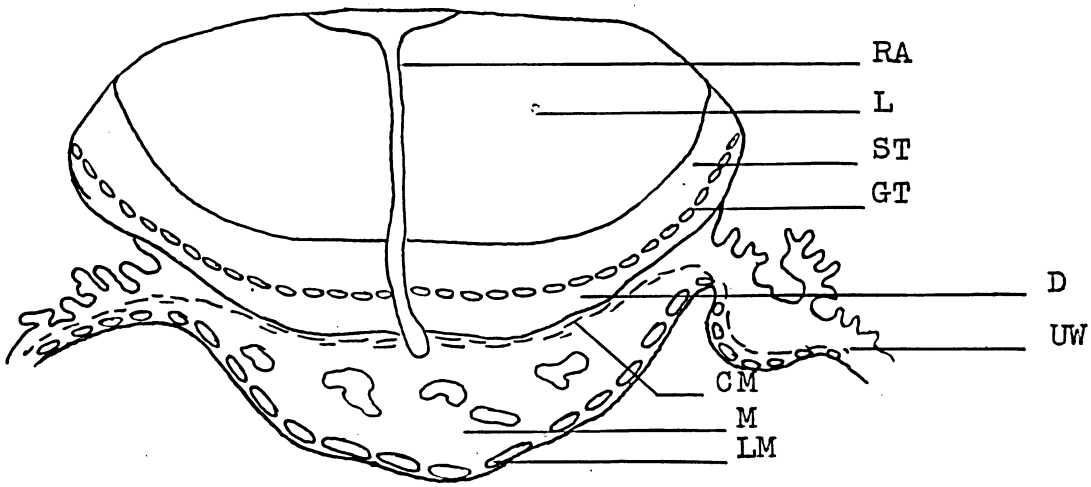


Diagram of the chorioallantoic placenta of the rat. (RA) radial artery, (L) labyrinth, (ST) small cell trophoblast, (GT) giant cell trophoblast, (D) decidua, (UW) uterine wall, (CM) circular muscle, (M) metrial gland, (LM) longitudinal muscle.

In such a section the following areas or zones can be identified:

a. The labyrinth. A large zone making up the bulk of the placenta, and from the center of which the umbilical vessels arise.

b. The small celled trophoblast. A narrower zone which can be differentiated below the labyrinth.

c. The giant celled trophoblast. A very narrow zone (usually a single layer of large cells)

which lies outside the small celled trophoblast.

d. The decidual zone. The endometrium of pregnancy.

e. The metrial gland. The remaining area lying between a thin layer of interrupted circular smooth muscle and the outermost longitudinal muscle.

a. Labyrinth. The labyrinthine portion of the placenta in experimental animals differed but slightly from that observed in normal placentae. The most obvious change was a decreased amount of blood in the maternal spaces, a condition prevailing in at least seven of the placentae studied (Figs. 10, 11).

Six of the placentae from the experimental group demonstrated changes indicative of destruction or degeneration (Figs. 10, 11). Typically, these labyrinthine areas were more or less filamentous in appearance, showing a disruption of the syncytium in several places, and a vesiculation of some of the nuclei. A diminution of labyrinthine tissue was suggested by the smaller size of the labyrinth and the varying degrees of reduction in the amount of cytoplasm. There was no evidence of extensive pycnosis. Invasions by small trophoblastic cells were noted in two or three specimins although infarcts were not observed. The syncytium appeared almost as a solid mass in one of these, with the maternal blood sinuses being reduced to very narrow channels between the cytoplasmic strands of the labyrinth.

The nuclei and cytoplasm appeared normal, however.

Precise observations upon glycogen distribution could not be made in the sections available for study. Although three of the placentae from injected animals showed a clear cut reduction in the amount of glycogen, but slight variations were observed in the remainder, none being markedly different from the average normal pattern.

The lipid content and distribution in the injected series was not observed to vary from the normal pattern.

b. Small cellular trophoblast.

This layer was normal in appearance in most of the experimental animals, but in two of them was reduced in width, being almost absent in certain areas. In places it was completely traversed by cells from the underlying giant-cell layer (Fig. 13). In four animals the cells of this layer gave an appearance of having undergone destructive processes, inasmuch as the elements had lost the distinct form evident in the control animals.

The fetal glycogen cells, which are dispersed in groups throughout the small cellular trophoblastic layer, varied slightly from the normal in their distribution, being absent from the layer in half the experimental animals, and in but two of the normal animals. The glycogen distribution appeared to be normal within the cells themselves.

Apparently the lipid distribution in the small cellular trophoblast of the experimental group was the same as in normal animals. In two cases, cells containing many lipid

granules were seen lying within the layer, but these could be traced either to the giant-cell or to the labyrinthine layers.

c. Giant-cell layer. It was in the giant-cell layer that the most pronounced and consistent changes were observed, a characteristic enlargement being noted in all the experimental placentae (Figs. 12, 13). This enlargement was most evident in the central areas. Some peripheral cells and cells located along the basal margin showed evidence of degeneration to a degree greater than in the normal. This resulted in some of the experimental placentae having a slightly narrower zone at the periphery. The central portions, however, in all experimental placentae observed were wider than in the normal placentae. There was also a tendency for the cells of the giant-cell zone to occur in masses several cells thick in scattered areas (Fig. 13). Some of these widened areas, particularly in three of the animals (Fig. 13), traversed the entire width of the small cellular trophoblast and reached the labyrinth. This condition was not noted in the control placentae where the giant-cell layer was uniformly one or two cells wide throughout its extent.

Glycogen usually was absent, but when present, was seen sparsely distributed as fine granules in the normal giant-cell layer. This was true for the experimental animals in general, although in three of them a number of cells of this layer had several large glycogen granules in their cytoplasm.

The most characteristic difference was noted in the lipid distribution in the giant-cell layer, there being an increase in the lipid content of this layer (Figs. 14, 15) in all the placentae of experimental animals studied. This did not appear in every cell in every placenta, but the majority of cells demonstrated a definite increase of sudanophilic lipid over the normal. The distribution of lipid in the zone varied, being greater among the superficial cells in some, and among the more basally located cells in others.

d. Decidua. The decidua in the experimental animals varied from normal in the amount of tissue having undergone symplasma formation, the extent of karyorrhexis, and the number of intact decidual cells which remained. Five placentae in the experimental group exhibited extensive karyorrhexis and symplasma formation with very little intact decidual tissue. Five others exhibited a more extensive symplasma formation than normal, but showed little karyorrhexis, and had a very narrow intact decidual zone (Fig. 13). The rest of the experimental animals were not distinctly different from the normal in this respect. Changes in glycogen and lipid distribution were insignificant.

e. Metrial gland. The general appearance of the metrial gland in the experimental group gave the impression of a diminished amount of tissue. The cellular elements of this area in eight of the animals showed an increased vacuolization and a decrease in the number of undifferentiated cells.

Maternal glycogen cells were observed in only six of the experimental animals, whereas a relatively large number was present in all but three of the control series (Figs. 16, 17). Glycogen distribution in the rest of the metrial gland was noticeably diminished in about half of the experimental group. Occasionally bundles near the center of the peripheral longitudinal muscle layer were observed to be devoid of glycogen. This condition was not encountered in any of the control group. Changes in lipid distribution were not apparent in this layer.

In summary, the giant-cell zone demonstrated a consistent and well defined alteration in response to the treatment. The labyrinth, small cellular trophoblastic layers, decidua, and metrial gland were affected by the treatment, but the alterations in these areas were too inconsistent to enable one to draw any definite conclusions.

DISCUSSION

The results presented here indicate that the animals utilized in this experiment reacted in a variable manner to the injections of formaldehyde. Because of this variability of response and the relatively small number of animals used, clear-cut conclusions are difficult to make. The author is of the opinion, however, that the results do indicate certain trends in response which have not been presented before with the same connotation proposed in this dissertation.

In his studies on malformations, Fraser ('51) recognized the possibility that various types of maternal insult (vitamin deficiencies, chemical and hormone injections, etc.) inflicted upon animals were but different forms of stress. It seems logical, then, to assume that the many reactions of maternal animals and offspring reported in the literature might be manifestations of the effects of nonspecific stress.

In contrast to the administration of cortical compounds, the present experiment was conducted by use of an agent the chronic administration of which was known to stimulate the animals' own defense mechanisms. This procedure was believed to eliminate variabilities due to dosage, so that any response of the animal was an exhibition of its individual ability to adjust to the agent.

The results indicate that in response to the stressor agent, formaldehyde, the animals reacted in the following generalized manner:

1. There is a definite effect upon the offspring (at least insofar as 19 day old embryos are concerned). This is manifested by retardation in growth, as indicated by a reduction in weight and a retardation in skeletal development, but no malformation. The severity of this condition is markedly variable.

2. There is an increase in fetal mortality.

3. There is an involvement of the placenta as shown by variations in size and weight. Histological alterations in the labyrinth, small cellular trophoblast, decidua, and metrial gland are subtle and variable. In general, the changes that were observed showed a tendency toward diminution of glycogen, together with degenerative processes in the tissues of those areas. The effect on the giant-celled trophoblast, was constant as shown by hypertrophy of that zone and increased lipid content. Those animals with reduced embryo weights showed the more severe placental changes.

4. The adrenal glands of pregnant animals react more in response to the formaldehyde injections, as shown by increase in size and weight, than do those of nonpregnant animals. No distinctive histological alterations or distinctive changes in lipid distribution were observed in one group that were not observed in the other. The only difference in response was a matter of degree or intensity.

5. The ovaries, although reacting to formaldehyde injections with varying degrees of intensity in the nonpregnant animals, apparently are not affected during pregnancy. At any rate there was no evidence of any reactions on the twentieth day of gestation, the time of autopsy.

Role of Various Agents as Stressors.

According to the interpretation of Selye, a stressor agent is one capable of eliciting a condition of stress in an organism. This is manifested in many organ responses, but adrenal enlargement is the most characteristic. In the concept of stress, the organism responds in a stereotyped manner to a wide variety of agents such as infection, intoxication, trauma, heat, cold, muscle fatigue and radiation. Pregnancy itself is a mild form of stress. The specific effects of these various agents are quite different, but one common feature is typical of them all, i.e., the organism passes into a state of general systemic stress. The stereotyped response superimposed upon all specific effects represents the somatic manifestations of the general-adaptation syndrome. It is in the light of the above that Fraser considers as stressor agents the various agents used in experiments during pregnancy. It must be noted, however, that the majority of treatments used (vitamin deficiencies, inanition, anoxia, hormone administration) are biologically active with very important specific physiological effects. Their specific activities and nonspecific actions as stressor

agents when administered chronically tend to merge, and thus to mask one another.

Only a few of the stressor agents used in all work cited above are without important biological activity. Among the agents lacking a specific biological activity are trypan blue and nitrogen mustard. Of these, nitrogen mustard has a possible specific role, as it is a strong mitotic inhibitor. The response to cortical compounds varies with the dosage as pointed out previously. It would, therefore, appear that a non-biologically active toxic substance would be best for use as a stressor agent. For this reason formaldehyde was selected as such a compound.

The use of formaldehyde as a stressor substance has introduced two problems which at this time cannot be solved completely. The first of these is that the formaldehyde is exerting its effects as a toxic agent directly on the placenta by circulating in small amounts in the blood stream. This effect is being isolated now by attempting a series of experiments on adrenalectomized animals.

The second problem introduced is that, because of severe tissue damage, tissue fragments or formaldehyde protein compounds may be circulating in the blood, in which case they might exert an effect similar to the action of trypan blue. Absence of severe alterations in the kidney and liver in animals in the experiment suggests, however, that this second problem is not a factor.

Variable Responses of Animals to Formaldehyde.

The responses of animals to the formaldehyde injections show little uniformity.

1. One evidence of non-uniform response to the injections is the variable number of embryos resorbed. The over-all rate of resorption is greatly increased, but it does not necessarily follow that this increased rate will be evident in each injected pregnant animal, since in some animals all fetuses were alive at the termination of the experiment.

Increase in fetal mortality has been reported by a majority of the authors studying the effects of various agents on pregnant animals. In the early work of Selye ('39, '47), resorption and abortion of fetuses under the condition of maternal stress were attributed to a shift in production of various anterior pituitary hormones. Reduction in gonadotropic hormone production, with its subsequent gonadal effects, brings about the termination of pregnancy.

Electro-shock in the latter half of gestation (Rosvold '49) prolongs gestation, makes labor difficult, diminishes milk production in lactation, and has an adverse effect on maternal behavior. Calhoun ('49) demonstrated that fear can diminish the number of litters, and has a detrimental effect on lactation and the ability of mothers to wean their young. Selye ('51) states that experiments comparable to these two have not been conducted to test whether those responses are specific. It may be pointed out that in other experiments

difficult delivery, prolonged gestation or protracted labor, stillbirth, and resorption are common. Mason attributes these results, in his vitamin A deficient animals, to general lethargic conditions and decreased tonus of abdominal muscles. In six of the animals which were permitted to go to term in the present experiment, death of the mother, stillbirth, protracted labor, and lack of maternal instinct (eating placentae, etc.) were all observed.

In none of the experiments cited above was the ovaries carefully examined. The ovaries of nonpregnant, stressed animals have been studied extensively (Selye '39). It is of interest, in this respect, to consider some of the observations made in the present investigation. The gross appearance of the ovaries and their corpora lutea was essentially normal in all animals, whether they were resorbing fetuses or not. A slight difference in size of corpora lutea could have gone unobserved; but any such alteration in mass would be insignificant, since Kelsey ('50) has shown that two corpora lutea are all that are necessary to maintain pregnancy in the rat from the eighth day to term. Histologically the corpora lutea appeared normal.

It has been shown by Selye ('33) that the corpora lutea of pregnancy were not affected by hypophysectomy after mid-pregnancy. This fact indicated that the placentae had replaced the activity of the pituitary in the maintenance of pregnancy. Selye showed, however, that whereas the ovary was essentially unaltered by hypophysectomy at that time,

an anterior pituitary insufficiency is reflected by the appearance of some of the thecal cells of the ovary. These cells in the nonpregnant hypophysectomized animal undergo marked alteration in their structure. The nuclei round up and lose their nucleoli, and the chromatin agglomerates into clumps to give the nucleus the "cart wheel" appearance typically seen in plasma cells. Signs of these changes were observed by Selye in pregnant hypophysectomized rats.

Selye ('39) observed that these "wheel cells" appeared in the atrophied ovaries of animals subjected to prolonged stress (formaldehyde injections for 40 days). This phenomenon was instrumental in the formulation of the "shift in anterior pituitary hormone" hypothesis. Swingle ('51) observed that the situation as described by Selye might not be entirely complete. He observed that pseudopregnancy, in which deciduomata formation can be initiated, may be induced by three day injections of formaldehyde (o.25 - 0.50 c.c. per day). From this observation, and after investigating several other compounds, Swingle concluded that apparently vigorous nonspecific stress will bring about luteotropin release in the rat. The diestrus of the rats under such circumstances is due to a true pseudopregnant condition in contrast to the interpretation by Selye that the diestrus during stress is due to ovarian atrophy and cessation of function.

The observation of a definite indication of "wheel cell" formation in four animals of the nonpregnant injected series in the present experiment shows that it is possible within

the time treated to get anterior pituitary deficiency effects. Only a hint of such formation was noted in the pregnant injected group.

The relationship of pituitary-ovarian activity as it pertains to the work reported herein is by no means clear. It can be postulated, in the light of the above discussion, that pregnancy advances to a sufficient stage despite the injections, so that it can be maintained independent of a fully functioning pituitary gland. It is believed that the resorption of the young cannot be attributed indisputably to misfunction of the pituitary-ovary complex. If the cause for resorption does not occur at this level, then one must consider other effects such as hormonal or mechanical involvement of the placentae, effect on adrenal activity, increased requirements or decreased sensitivity of target organs, increased destruction of hormones before being utilized, or even possible physical impositions.

2. Another variable condition noted was the amount of edema which appeared after several injections of formaldehyde. Some animals went through the entire experiment with far less edema than others, (Figs. 1a, 1b) for which the author is unable to account. The edema was more severe and consistent in the pregnant than in the nonpregnant injected animals. The edema was not due to kidney damage, as the kidneys of even the most severely affected animals were normal in histological appearance (Fig. 18). It may be attributed tentatively to increased adrenal cortical activity. The adrenal response

apparently is greater in pregnant animals, and the severity of the edema possibly may be associated with its increase. Schuurnans ('51) observed that cortisone and desoxycorticosterone often have a similar effect in producing edema. He assumes that the retention of water during pregnancy is due to the hypertrophy of the adrenal cortex. The increased steroids from the adrenal cortex act by increasing the capillary permeability to plasma albumins, causing an edema in the subcutaneous tissue. Tobain ('49) has found excessive urinary cortisteroids in women with severe edema of pregnancy. He attributes the renal retention of Na and water to these increased products. Whether the electrolyte balance was upset in the animals of the present experiment was not determined.

Further elaboration upon the possible causes of the edema in these animals cannot be made because of the limited amount of experimental evidence obtained; nevertheless, it seems significant that a more severe amount of edema developed in pregnant injected animals than in the nonpregnant injected animals.

In Fig. 2 a curve has been plotted representing the combined weight changes of nonpregnant injected animals and normal pregnant animals. It is interesting to note the similarity of this curve to that of the pregnant injected animals. After the thirteenth or fourteenth days, the weights of nonpregnant injected animals tend to decrease or become stabilized. This stabilization is maintained for two or

three days in the pregnant injected series as well, but then the weight rises progressively to the termination of the experiment. The curve for the combined weights of the pregnant controls and nonpregnant injected animals by its close parallelism to the curve for the weight of the pregnant injected series, suggests that the rise in weight after about the fourteenth day is due to the growing concepti, rather than to any further increase in edema. The fact that the total increase for the pregnant injected series is less than that for the combined weight increases of the pregnant control and nonpregnant injected series could be explained by the lower weights of the concepti of the pregnant injected animals. Whether the increase is due solely to the growing concepti cannot be determined, but they do make a large contribution.

3. The offspring of the injected mothers showed a wide variability from embryo to embryo with respect to weight and bone development. When these offspring were considered as a group, the data revealed a definite retardation in growth. With respect to bone development, the results reported may be misleading, inasmuch as in the injected group cleared for study only 14 of 61 embryos weighed 2.0 grams or more, whereas in the control series only 5 of 34 weighed less than 2.0 grams. An examination of embryos from injected animals revealed that 64% had fewer than three sternebrae. If all the embryos from the injected series had been studied, this percentage would probably have been lower (the lowest value possible, however, would have been 30%). The percentage

of animals in the control series having less than three sternebrae was 5.9%. It is not likely that this percentage would vary to any great extent if all the embryos had been studied. In spite of this possible discrepancy, there is a significant alteration in growth. In only 7 of the 61 animals was there a retardation of as much as one day as indicated by the retarded ossification of the metacarpals and metatarsals. Normally, one metacarpal and metatarsal are ossified on the 18th day, and two more of each on the 19th day. Thus relatively few were retarded to any great extent, as judged by the appearance of ossification centers. Nevertheless, a majority demonstrated retardation in growth as indicated by the smallness of bones of the head, long bones, and ribs.

Lichtenstein ('51) reports, along with slight reduction in the number of offspring, reduced weight and bone abnormalities in the young of pregnant animals treated with protamine zinc insulin. Change in bone development was demonstrated mainly by a reduction in the number of sternebrae. All his control animals had six sternebrae at term, whereas in the experimental series, 33% had fewer than four. The malformations included wavy ribs and irregularities in the ends of a few of the femurs in seven fetuses in nine litters. He suggests these changes are due to a direct effect of the insulin, or perhaps to a toxic effect. Lichtenstein's experiment is cited because, with the exception of the malformations which were very few, a similarity to the findings reported here can be noted. Whether the changes would still be present

at term in the animals of the present experiment is not known.

In the opinion of the author the significance of the results obtained is the indication that a nonbiologically active substance can have an effect which might be otherwise interpreted as a specific effect. Thus, it might be that in other experiments the reduction in embryo size, and possibly other effects, could be due to a stressor action of the agents employed. Where or how this is brought about is a question. Mason ('35) and Gillman ('48) suggest interference at the placenta. Subtle "chemical" or histological alterations interfere with the regulation of the nutrition of the embryo and are manifested later in a perversion of embryonic growth and differentiation. It is possible that changes in the placenta may have an effect upon the embryo, although the evidence for this is not entirely conclusive.

4. Reference to variation in placental size under experimental conditions was found in but two instances in the literature. Mason ('35) observed variation in placental size in his vitamin A deficient animals; and Armstrong ('43) reported a reduction in placental weight (.342 gm. as compared to a normal of .481 gm.) for her toxic animals. Armstrong also reported that there was no reduction in the weight of the experimental offspring, although there is a possibility that post-mortem swelling masked reduction in embryo weights inasmuch as the embryos examined were dead. Mason merely noted that the placentae of more severely retarded embryos were more affected.

In the work reported here the results indicate a reduction in placental size, not only in the fetal portion, but in the maternal portion as well. Also, the placentae of animals with smaller embryos were smaller. As an attempt to correlate placental and embryonic weights was unsuccessful, it cannot be said, at present, that small embryo weights are always associated with small placentae.

The reduction of placental weight found in this experiment seems to be significant for two reasons. First, very few investigators have made any attempt to record placental weights of experimental animals. It was found in this investigation, and in one other reported in the literature, that under experimental conditions alterations in placental size can occur. It seems of importance, then, to take this fact into consideration in subsequent experiments. Second, reduced placental weights were precipitated by nutritional deficiencies in the two instances reported in the literature, whereas the same response observed in the present investigation was caused by a nonbiologically active agent.

Histological alterations in the rat placenta have been noted in some of the experiments cited in the literature. Lesions were quite extensive in the investigations of Symeonidis. However, it is difficult to determine from his descriptions and illustrations to which cell areas he is referring. In general, it seems that there was extensive necrosis and degeneration in the labyrinth, small cellular trophoblast, and possibly in the giant-cell layer. Dilation

of blood vessels and periplacental hemorrhages were also noted.

In the experiments of Armstrong, the maternal sinuses of the experimental animals' placentae were devoid of blood. The fetal blood sinuses showed hemolysis of the blood and thrombi. These observations on the vascularity of the placentae were the only histological observations reported in her work.

Focal necrosis in the maternal decidua, hemorrhage, and leucocytic infiltrations of the maternal trophoblastic junctional zone were observed by Mason in the placentae of vitamin A deficient animals.

The most extensive studies on placental alterations are those of Huggett and Pritchard ('45a, '45b, '47). Some of their more pertinent findings are briefly presented here. They found that estrogen and gonadotropins administered in sufficient quantities on or before the twelfth day caused fetal death. Ovariectomy also resulted in fetal death. In animals so treated, and also in animals in which the embryos were destroyed by crushing, the placentae grew and differentiated to the seventeenth day. After the fifteenth day there was no interference with growth by the administration of hormones. The most affected part of the placenta was the decidua basalis, which showed an extensive necrosis. This was considered to be the cause of death. Vitamin A deficiency and other factors besides endocrine products caused death in the same manner. Placentae from animals in their experiments eventually underwent destruction, the labyrinth first, then

the reticularis (small-cell trophoblast), and finally, after an initial hypertrophy, the giant-cell layer. The placentae, up to the time of their destruction, continued to function normally. From the experiments of Huggett and Pritchard, it can be seen that the sequence of susceptibility was first the decidua, then the embryo, and finally the placenta.

It seems of importance to note that placental changes were found in the experimental animals of Mason, Armstrong, Huggett and Pritchard and in the present experiment. Had the placentae in the experiments cited in the extensive literature concerned with various treatments of maternal animals been studied, more alterations in structure might have been found. Furthermore, if more alterations had been found, they might have had an important bearing on the results and interpretation of those and similar experiments. In the present work histological studies were made on sampled material only. If all of the experimental placentae instead of sample placentae had been observed, the amount of response might have been different from that reported. But, since the sample placentae for the control series, which presented a constant picture, was taken in the same manner as for the experimental group, the results are thought to be significant. It seems clear that the labyrinth and small-cell trophoblast are only mildly affected. Degenerative changes are only indicated, and the extensive destruction of these areas reported by Symeonidis in his experiments were not observed. Anemia of the placenta was encountered frequently, but the

hemolysis and thrombi noted by Armstrong were not evident. The decidua was apparently the most affected area, showing a greater tendency toward destruction of tissue than in the normal. The reduction of decidual tissue in the junctional region suggests a more progressed aging. Hemorrhage and leucocytic infiltrations were not believed to be involved. Generalized decrease in glycogen content indicated a functional disturbance. A discussion of alterations of the metrial gland and their significance cannot be made, as the cytological details having a bearing on the subject were not studied.

The findings in this experiment suggest that destructive alterations in the placentae had been initiated, which may be the point at which embryonic development is influenced. These and similar results from the experiments of others indicate that histological and histochemical studies of the placentae are necessary for the evaluation of the results of experiments on mother-fetal relationships.

The Response of the Adrenal Glands.

The adrenal glands of the pregnant injected animals reacted to a greater extent, insofar as hypertrophy and weights are concerned, than did those of the nonpregnant injected animals. The possible role of the adrenal glands in causing edema has been pointed out. Two other matters of interest might be noted.

The first concerns the fatty metaplasia of the glomerular zone and outer fasicular layer in both injected series, and is

of interest because cells of these layers, in at least two of the animals, attained a signet ring stage comparable to fat cells. Accumulation of lipid as extensive as this has been found only in animals treated with testoids (Selye '50b). Large fat vacuoles have been reported to appear during the resistance stage in animals subjected to cold, but no signet ring cells have been reported. In the findings reported here the accumulation of large vacuoles in the cells of the fasicular and glomerular zones was the rule and the signet ring stage the exception. Although in a few of the animals the occurrence of fatty metaplasia is more extensive than has been recorded in the literature, it is felt that the occurrence can be attributed to a response to the formaldehyde. The functional significance of this type of reaction is not known, but it is assumed by Selye to be indicative of increased storage and increased production of cortical substances. It must be noted that, from the histological and histochemical sections studied, none of the changes observed was restricted to the injected pregnant animals, but occurred equally in both injected series.

Secondly, Selye reports the occurrence of "lumina" formation due to cellular cytolysis in the cortices of animals subjected to severe stress. This formation is also found in animals treated with corticotropin and folliculoids, and is seen occasionally in normal late pregnancy and lactation. This "lumina" formation in pregnancy was assumed to be due to an increased folliculoid production, causing

cellular dissolution due to over-stimulation. It is of interest to note that, under conditions of combined pregnancy and external stress, "lumina" formation was not observed in any of the adrenals.

The Response of the Giant-Cell Layer.

The response of the giant-cell layer in injected animals and its possible significance are worthy of special attention. Apparently the increase in the width of the giant-cell layer as observed here has been noted in only one other investigation. Huggett and Pritchard ('45a) observed hypertrophy of the giant-cell zone. They interpreted the increase as a piling up of the giant-cells due to a decrease in size of the placenta. Studies other than those of a histological nature were not made, and the significance of the change was not conjectured.

In early development the giant-cell layer is characteristically phagocytic, a property demonstrable until the eighteenth day (Bridgman '48). Histochemical observations on this zone have been made on normal placentae only (Bridgman '48 and Wislocki '46).

The increased prominence and increased lipid content of this layer under the experimental conditions described here invite some speculation.

The fact that the giant-cell layer is phagocytic suggests that under the conditions of this experiment the increased lipids may be due to ingestion by the cells of particles

resulting from a fatty degeneration of surrounding tissue. But this possibility is not likely for several reasons. The appearance of lipid in metrial gland cells on the eighteenth day has been considered by Bridgman ('48) to represent a fatty degeneration. However, the lipid content of the giant cells remains constant in the normal animal from the sixth through the twentieth day, at least, so that if lipid increase is due to fatty degeneration, it does not involve the giant-cell layer.

The phagocytic action of the giant cells was also found to decrease with age. Bridgman ('48) found that trypan blue particles were not taken up to any great extent in giant cells after the eighteenth day, and these particles were not found in the tissues of the placentae in Gillman's ('48) experiments.

The lipid in the cells does not appear as fatty degenerative products but as discrete cytoplasmic inclusions.

Another possible cause for the lipid accumulation and increased size of cells is anoxia. It is known that with a reduced oxygen supply cells become larger. It is also known that in early degenerative stages brought about by a reduced oxygen supply, emulsified fatty substances in the cytoplasm precipitate out and can be made visible histochemically. This precipitate gives an apparent increase in lipid content. Possible causes of anoxia in the present experiment might include a decreased blood supply or a premature separation of the placenta, but it cannot be said conclusively that these conditions existed to the extent of causing anoxia.

The above structural alterations to which the anoxia might be attributed were not present in all treated animals, but the increase in lipid was present in all. Observations were not made as to whether similar changes in the lipid content of this area might occur in normal animals near parturition when normal cleavage or separation occurs, and no report on this was found in the literature.

In addition to the possibility that the changes observed in the giant-cell layer are manifestations of degenerative processes, the possibility that these changes are revealing functional alterations presents another matter for consideration. The placenta has, in addition to its nutritive role, an equally important role as a temporary endocrine organ.

The elaboration of several hormones has been attributed to the placenta. Progesterone (Salhanick '52), estrogen (Pearlman '48), and chorionic gonadotropins (Seegar '40) have been isolated from human placentae. These hormones per se have not been isolated from rat placentae. However, progestational (Haterius, '35) and gonadotropic (Averille '50, Kelsey '50) activities of the rat placenta have been demonstrated. Wislocki ('43) has shown in monkey and man that the lipid content of the syncytial trophoblastic elements coincides with the state of steroid hormone activity. When progestational and estrogenic activity of the placenta are at their height, the lipid content of the syncytial trophoblast is high. Wislocki observed also a decreased amount of lipid in degenerating trophoblastic elements of the monkey and man

which was associated with a fall in progesterone and estrogen levels. From these observations he advanced the concept that the syncytium and its lipid droplets have a definite relation to the normal production of steroid hormones. Therefore, estrogen, progesterone, and the corticoid hormones (or active principles similar in structure and function to these), all being steroid hormones, may possibly be associated with the placental lipids.

Localization of the source of placental hormones in the rat has not been made. Dr. Pearl Zeek, in a personal communication, states that in her opinion the giant-cells are certainly to be suspected of having an endocrine function. The fact that they have phagocytic activity can be attributed to their trophoblastic origin. Because of this origin they might also be considered to have an endocrine activity. These giant cells are structurally unlike any other phagocytic cells of the body, and there is a striking similarity between them and the syncytial trophoblast of the human placenta.

The fact that toxemias of pregnancy are believed by many to be diseases of adaptation, together with the fact that in such cases there seems to be an excessive increase in corticoids, has led some authorities (Selye '51) to consider the placenta as the source of their production.

Utilizing this information, it seems of importance to note that within a zone suspected of hormone elaboration we find changes (increased lipid) which have been considered as indicative of steroid hormonal activity. It is also of

importance to note that an experimental application of a stressor agent brings about this alteration. These speculations are presented in full knowledge of the lack of specific evidence.

Some problems which have evolved from the present experimentation are enumerated here.

1. An experiment should be conducted in which the results obtained from subcutaneous formaldehyde injections (as reported here) are compared with results using identical treatment on adrenalectomized animals, and with those obtained from injecting animals in an deinnervated area and injecting animals intravenously with nonirritating dilute formalin solution. Such comparisons should isolate any response due to the direct action of the formalin from effects due to stress.

2. An experiment should be conducted to study the effects of formaldehyde injections initiated on various days of pregnancy with autopsy near term. Then, if it seemed warranted, studies might be made on animals in which injections were initiated within the first few days of pregnancy with autopsy on various subsequent days.

3. Studies on the placenta incorporating more extensive histochemical methods might be of value.

4. A study might be made to test whether the administration of female sex hormones results in any amelioration of the effects from formaldehyde irritation.

It is hoped that results obtained from this proposed work, if carried out, will provide factual information on

which to base a rational understanding of the response of pregnant animals subjected to stress.

SUMMARY

The following response to formaldehyde injections were observed in the animals of this experiment.

I Maternal Animal.

1. The mortality rate for all injected animals was 50%, although this varied in different groups tested.

2. The pregnant animals developed a more severe and less variable edema than did the nonpregnant animals.

3. The adrenal glands of the pregnant animals showed a 23.6% increase (based on mg./100 gm. body weight) of the average weight over that of nonpregnant animals. Histological changes were not restricted to one injected group but occurred in both, differing only in degree.

4. The ovaries of the pregnant animals were apparently unaffected by the treatment.

II Embryo.

1. The fetal mortality rate, as measured by comparative counts of offspring and corpora lutea, was increased from 9.1% for the normal animals to 24.2% for the experimental animals.

2. There was a definite retardation in development as shown by:

(a) Decrease in average weight from 2.3 gm. for normal animals to 1.8 gm. for experimental animals.

(b) Retardation of bone growth as shown by the small size of the bones and the reduced number of sternebrae.

III Placenta.

1. The experimental animals had lower mean placenta weights and more variable individual placenta weights than the normal.

2. Minor histological degeneration or abnormal changes occurred in the placentae of the experimental animals.

3. There was a consistent and characteristic enlargement and increased lipid content of the giant-cell zone. The possible significance of this change is discussed.

BIBLIOGRAPHY

Armstrong, Williamina E. and P. Swanson, 1943. A Syndrome of Dietary Origin in the Pregnant Rat Resembling Toxemia of Pregnancy. Jour. Amer. Dietetic Assoc., Vol. 19, p. 756.

Averill, S.C., Ray, E.W., and W.R. Lyons. 1950. Maintenance of Pregnancy in Hypophysectomized Rats with Placental Implants. Proc. Soc. Exp. Biol. Med., Vol. 75, p. 3.

Bridgman, J. 1948. Morphological Study of Development of Placenta of Rat: Outline of Development of Placenta of White Rat. Jour. Morph., Vol. 83, p. 61.

_____1948. Morphological Study of Development of Placenta of Rat: Histological and Cytological Study of Development of Chorioallantoic Placenta of White Rat. Jour. Morph., Vol. 83, p. 195.

Britton, S.W., and R. Klein. 1933. Effects of Adrenalectomy and Adrenal Cortical Extracts or Reproduction. Amer. Jour. Physio., Vol. 105, p. 12.

Calhoun, J.B. 1949. A Method for Self-Control of Population Growth Among Mammals Living in the Wild. Science, Vol. 109, p. 333.

Chih, Yun Hsu. 1948. Influence of Temperature on the Development of Rat Embryos. Anat. Rec., Vol. 100, p. 79.

Fraser, F. Clarke, and T.D. Fainstat. 1951. Production of Congenital Defects in the Offspring of Pregnant Mice Treated with Cortisone. Pediatrics, Vol. 8, p. 527.

Garrett, S.S. 1950a. ACTH in the Etiology of Eclampsia. West. Jour. Surg. and Gyn., Vol. 58, p. 229.

_____ 1950b. "Alarm" in the Etiology of Eclamptic Toxemia. West. Jour. Surg. Obst. and Gyn., Vol. 58, p. 689.

Gillman, J., Gilbert, C., and T. Gillman. 1948. A Preliminary Report on Hydrocephalus, Spina Bifida and Other Congenital Anomalies in the Rat Produced by Trypan Blue: the Significance of these Results in the Interpertation of Congenital Malformations Following Maternal Rubella. So. Afr. Jour. Med. Sc., Vol. 13, p. 47.

Grollman, Howard. 1934. Effect of Extracts of Adrenal Cortex on Growth and Reproductive System of Normal Rats: Particular Reference to Intersexuality. Amer. Jour. Physio., Vol. 107, p. 480.

Hall, V.E., Chamberlin, P.E., and O.H. Muller. 1938. Effect of Administration of Adrenal Cortical Hormone Preparations on Fertility, Pregnancy, and Lactation in the Normal Rat. Amer. Jour. Physio., Vol. 122, p. 16.

Haskin, D. 1948. Some Effects of Nitrogen Mustard on Development of External Body Form in Fetal Rat. Anat. Rec., Vol. 102. p. 493.

Haterius, H.O. 1935. Oophorectomy and Maintenance of Pregnancy in the Rat. Proc. Soc. Exp. Biol. Med., Vol. 33, p. 101.

Huggett, A. St. G. and J.J. Pritchard. 1945a. Experimental Fetal Death. The Surviving Placenta. Proc. Roy. Soc. Med., Vol. 38, p. 26.

_____1945b. Placental Growth After Fetal Death in Rat. Jour. Physio., Vol. 104, P. 104.

Ingalls, T.H., Curley, F.J., and R.A. Prindle. 1950. Anoxia as a Cause of Fetal Death and Congenital Deffect In the Mouse. Amer. Jour. Diseases of Child., Vol. 80, p. 34.

Kelsey, R.C. and R.K. Meyer. 1950. Amount of Luteal Tissue Required for the Maintenance of Pregnancy in the Rat. Proc. Soc. Exp. Biol. and Med., Vol. 75, p. 736.

Lichtenstein, H., Guest, G.M., and J. Warkany. 1951. Abnormalities in Offspring of White Rats Given Protamine Zinc Insulin During Pregnancy. Proc. Soc. Exp. Biol. and Med., Vol. 78, p. 398.

Mason, Karl E., 1935. Foetal Death, Prolonged Gestation, and Difficult Parturition as a Result of Vitamin A Deficiency. Amer. Jour. Anat., Vol. 57, p. 303.

Mossman, Harland W. 1937. Comparative Morphogenesis of the Fetal Membranes and Accessory Uterine Structures. Contributions to Embryology; Carnegie Inst., Vol. 26, p. 128.

Parviainen, K. Sovia, and C.A. Ehrnrooth. 1950. The Toxemia of Late Pregnancy and the Theory as to the Syndrome of General Adaptation. Acta. Obst. et. Gynec. Scandivavica, Vol. 29, p. 3.

Pearlman, W.H. 1948. In G. Pincus and K.V. Thimann (Eds.), The Hormones. Academic Press, New York.

Pritchard, J.J., and A. St. G. Huggett. 1947. Experimental Fetal Death in the Rat. Histological Changes in the Membranes. Jour. Anat., Vol. 81, p. 212.

Rosvold, H.E. 1949. The Effects of Electroconvulsive Shocks on Gestation and Maternal Behavior. Jour. Comp. Physiol. Psychol., Vol. 42, p. 207.

Salhanick, Hilton A., Noall, M.W., Zarrow, M.X., and L.T. Samuels. 1952. The Isolation of Progesterone from Human Placentae. Science, Vol. 115, p. 708.

Schuurnans, R. 1951. Cortisone and Toxemia of Pregnancy. Brit. Med. Journal, May, 1951, p. 1016.

Seegar, G.E. and G.O. Gey. 1940. The Production of Hormones by Human Placental Cells in Continuous Tissue Culture. Proc. Amer. Physio. Soc. 459.

Seifter, J., Christian, J.J., and W.E. Ehrich. 1951. Effect of Cortisone and Other Steroids on the Hibernating Gland of Pregnant White Rats. Fed. Proc., Vol. 10, p. 334.

Selye, Hans. 1939. The Effect of Adaptation to Various Damaging Agents on the Female Sex Organs in the Rat. Endoc., Vol. 25, p. 615.

_____ 1946. The General Adaptation Syndrome and the Diseases of Adaptation. Jour. Clin. Endoc., Vol. 6, p. 117.

Selye, Hans. 1947. Endocrinology. ACTA, Inc., Montreal, Canada.

_____1950a. Stress. (The Physiology and Pathology of Exposure to Systemic Stress). ACTA Endocrinologica, Inc., Montreal, Canada.

Selye, Hans. 1951. Annual Report on Stress. ACTA, Inc. Montreal, Canada.

Selye, Hans., Collip, J.B., and D.L. Thompson, 1933. On The Effect of the Anterior Pituitary-like Hormone on the Ovary of the Hypophysectomized Rat. Endoc., Vol. 17, p. 494.

Selye, Hans, and Helen Stone. 1950b. On the Experimental Morphology of the Adrenal Cortex. Charles C. Thomas, Publisher, Springfield, Illinois.

Swingle, W.W., Seay, P., Perlmutt, J., Collins, E.J., Barlow, G., and E.J. Fedor. 1951. An Experimental Study of Pseudopregnancy in Rat. Amer. Jour. Physio., Vol. 167, p. 586.

Symeonidis, Alexander. 1949. Eclampsia-like Condition in Pregnant Rats Injected with Progesterone. Jour. Nat. Cancer Inst., Vol. 10, p. 711.

Tobain, L. Jr. 1949. Cortical Steroid Excretion in Edema of Pregnancy, Pre-eclampsia and Essential Hypertension. Jour. Clin. Endo., Vol. 9, p. 319.

Warkany, Josef. 1947. Etiology of Congenital Malformations. Advances in Pediatrics, Vol. II, Interscience Pub., Inc., New York.

Wislocki, G.B., and H.S. Bennett. 1943. The Histology and Cytology of the Human and Monkey Placenta. Amer. Jour. Anat., Vol. 73, p. 335.

Wislocki, G.B., Deane, H.W., and E.W. Dempsey. 1946. The Histochemistry of the Rodents Placenta. Amer. Jour. Anat., Vol. 78, p. 281.

Table 1a

NONPREGNANT CONTROL SERIES

Rat Number	Total Weight, gm.	Adrenal Weight, gm.	Adrenal/ 100 gm.	Ovary Weight, gm.	Ovary/ 100 gm.
310 *	232	64.3	27.7	66.8	28.7
312 *	240	68.4	28.5	72.3	30.1
291 *	234	65.5	27.9	73.5	31.4
290 *	216	55.1	25.5	63.1	29.2
279 *	208	52.3	25.1	67.5	32.4
301 w	260	54.7	21.0	71.2	27.3
306 w	212	55.5	26.1	55.2	26.0
293 w	218	51.2	23.4	60.7	27.8
300 w	246	60.5	24.5	72.2	29.3
299 w	218	55.9	25.6	64.0	29.3
297 n	234	72.1	30.8	67.1	28.6
302 n	220	56.6	25.7	70.6	32.0
305 n	230	59.6	25.9	63.2	27.4
307 n	228	61.1	26.7	64.0	28.0
277 n	220	55.2	25.0	71.4	32.4
Average	227	59.2	25.9	66.8	29.3
Standard Dev.			2.28		1.98

Note: Test for significant difference was calculated on all data according to the "t" method. Snedecor, "Statistical Methods."

* Estrous
w Diestrous
n Proestrous

Table 1b

NONPREGNANT INJECTED SERIES

Rat Number	Total Weight, gm.	Adrenal Weight, gm.	Adrenal/ 100 gm.	Ovary Weight, gm.	Ovary/ 100 gm.
207	280	92.4	33.0	47.1	16.8
200	260	88.9	34.1	27.2	10.4
226	250	61.2	24.4	38.0	15.2
230	278	88.1	31.6	66.2	23.8
232	290	70.5	24.3	47.1	16.2
234	304	76.4	25.1	81.2	26.7
239	316	101.3	32.0	55.9	17.6
240	268	90.3	33.6	70.3	26.2
242	286	90.8	31.7	63.8	22.3
245	282	94.5	33.5	51.7	18.3
247	290	74.0	25.5	62.3	21.4
228	274	79.9	29.1	83.2	30.3
271	262	87.9	33.5	71.1	27.1
282	260	62.0	23.8	36.4	14.0
Average	278	82.7	29.6	57.2	20.2
Standard Dev.			4.10		5.84

Note: Test for significant difference was calculated on all data according to the "t" method. Snedecor, "Statistical Methods."

Table 1c

PREGNANT CONTROL SERIES

Rat Number	Total Weight, gm.	Reprod. Tract Weight, gm.	Total Body Weight, gm.	Adrenal Weight, gm.	Adrenal/ 100 gm.	Ovary Weight, gm.	Ovary/ 100 gm.	Total Placenta Weight, gm.	Embryo Weight, gm.	No. of Embryos	No. of Corpora Lutea
251	340	62	278	57.9	20.8	104.9	37.7	.4711	2.43	12	12
252	356	30	326	73.7	22.6	146.4	44.9	.4719	2.54	5	16
259	352	56	296	59.1	19.9	96.3	32.5	.5422	2.43	11	11
264	322	59	263	61.4	23.3	116.3	44.2	.4346	2.21	12	12
276	304	45	259	67.6	26.1	83.8	32.3	.4755	2.12	10	10
286	300	42	258	53.4	20.6	77.0	29.8	.5274	2.31	9	9
288	320	54	266	63.1	23.7	89.9	33.7	.5009	2.33	11	12
289	320	52	268	58.8	21.9	96.4	35.9	.5098	2.33	10	10
311	298	46	252	57.0	22.6	88.7	35.1	.4559	2.09	10	10
304	358	70	288	80.4	27.9	114.2	39.6	.4570	2.42	14	14
294	350	58	292	69.4	23.7	100.7	34.4	.4355	2.37	12	12
308	360	65	295	77.3	26.2	107.3	36.3	.4589	2.09	14	15
Average	331	53	278	64.9	23.2	101.8	36.3	.4788	2.30	130*	143*
Standard Dev.					2.46		4.61	.0306	.148		

Note: Test for significant difference was calculated on all data according to the "t" method. Snedecor, "Statistical Methods."

* Total Number

Table 1d

PREGNANT INJECTED SERIES

Rat Number	Total Weight, gm.	Reprod. Tract Weight, gm.	Total Body Weight, gm.	Adrenal Weight, gm.	Adrenal/ 100 gm.	Ovary Weight, gm.	Ovary/ 100 gm.	Total Placenta Weight, gm.	Embyro Weight, gm.	No. of Embryos	No. of Corpora Lutea
206	390	44	346	100.9	29.1	92.7	26.7	.3832	1.98	11	11
224	362	22	340	92.2	27.1	122.1	35.9	.3038	1.55	6	15
229	400	80	320	102.2	31.9	100.1	31.2	.4582	2.24	17	17
231	330	26	304	128.0	42.1	83.1	27.3	.3208	1.73	7	13
235	296	36	260	84.6	32.5	87.4	33.6	.3584	1.81	10	12
238	356	38	318	94.6	29.7	102.4	32.2	.3123	1.57	12	13
241	368	52	316	142.7	45.1	119.7	37.8	.4225	1.99	13	16
243	322	46	276	125.7	45.5	87.8	31.8	.3772	1.92	12	14
250	346	38	308	114.9	37.3	99.0	32.1	.3517	1.54	11	11
262	310	31	279	103.0	36.9	98.6	35.3	.3328	1.66	9	12
275	314	23	291	123.9	42.5	104.0	35.7	.4715	1.85	5	15
295	360	52	308	137.1	44.5	106.8	34.6	.4629	2.15	12	14
303	314	38	276	88.2	31.9	101.5	36.7	.4106	1.88	9	14
Average	343	40	303	110.6	36.6	100.4	33.1	.3819	1.83	134*	177*
Standard Dev.					6.67		3.26	.0588	.223		

Note: Test for significant difference was calculated on all data according to the "t" method. Snedecor, "Statistical Methods."

* Total Number

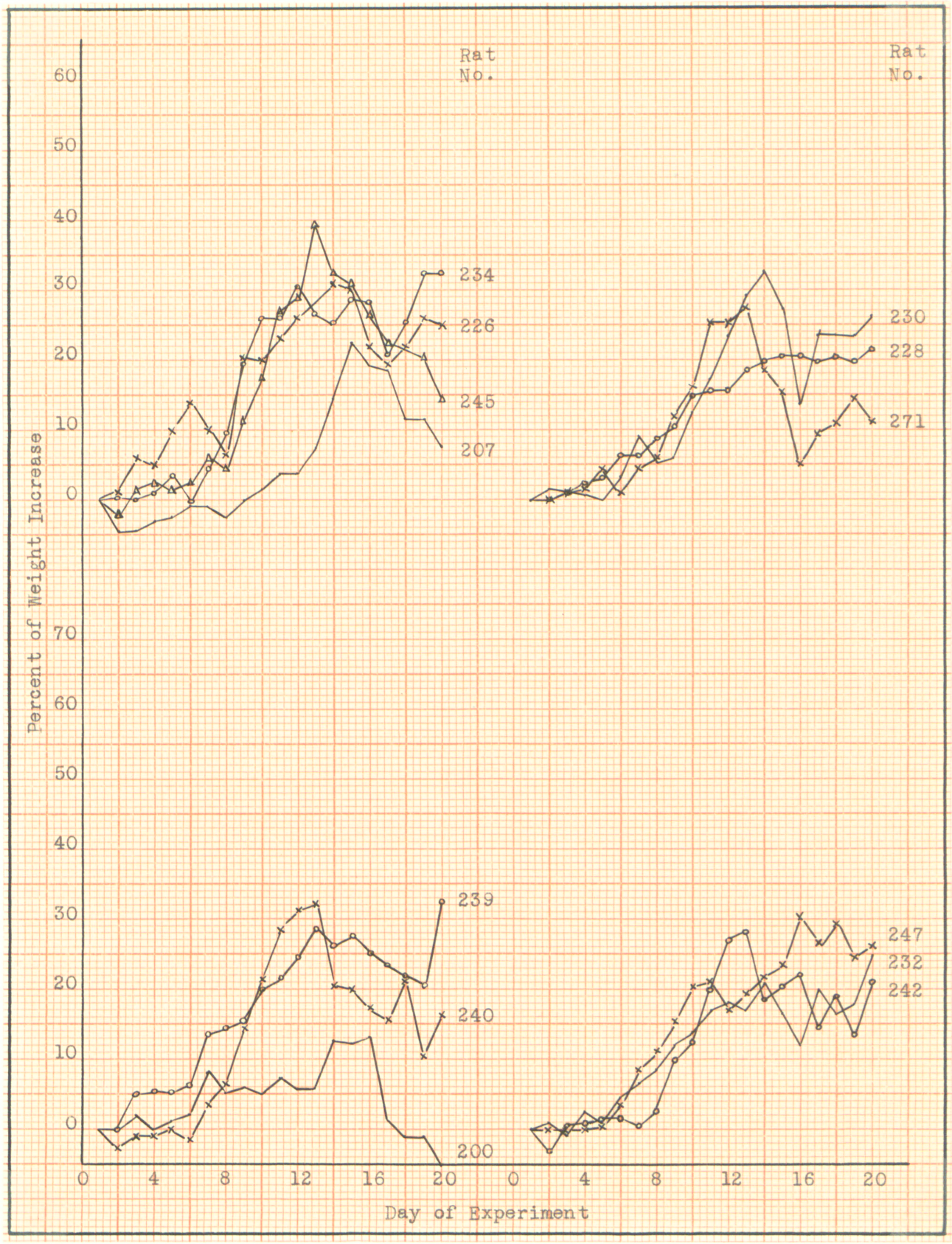


Fig. 1a. Individual daily percent of weight increase for nonpregnant experimental group.
Note: One animal omitted.

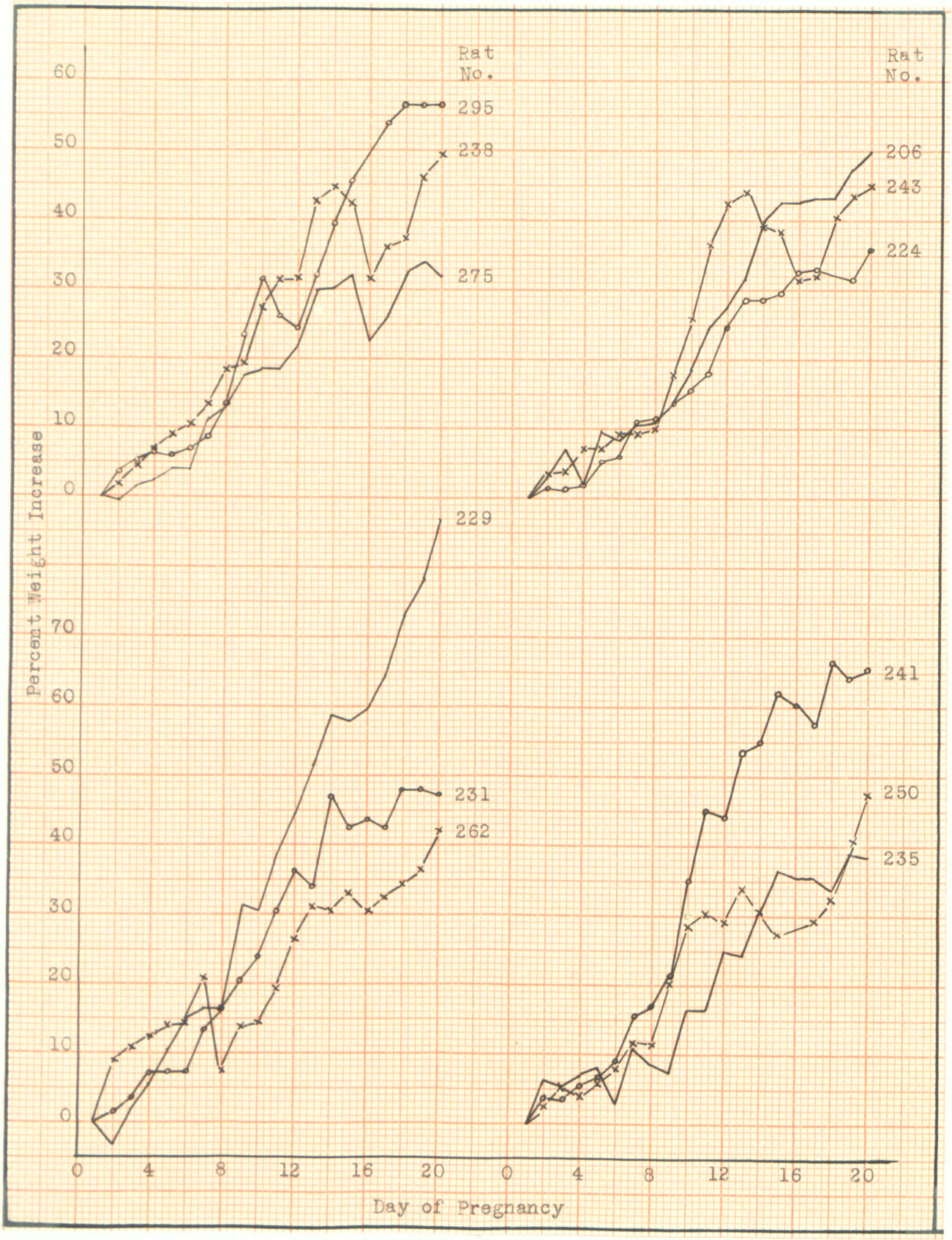


Fig. 1b. Individual daily percent of weight increase for pregnant experimental group.
Note: One animal omitted.

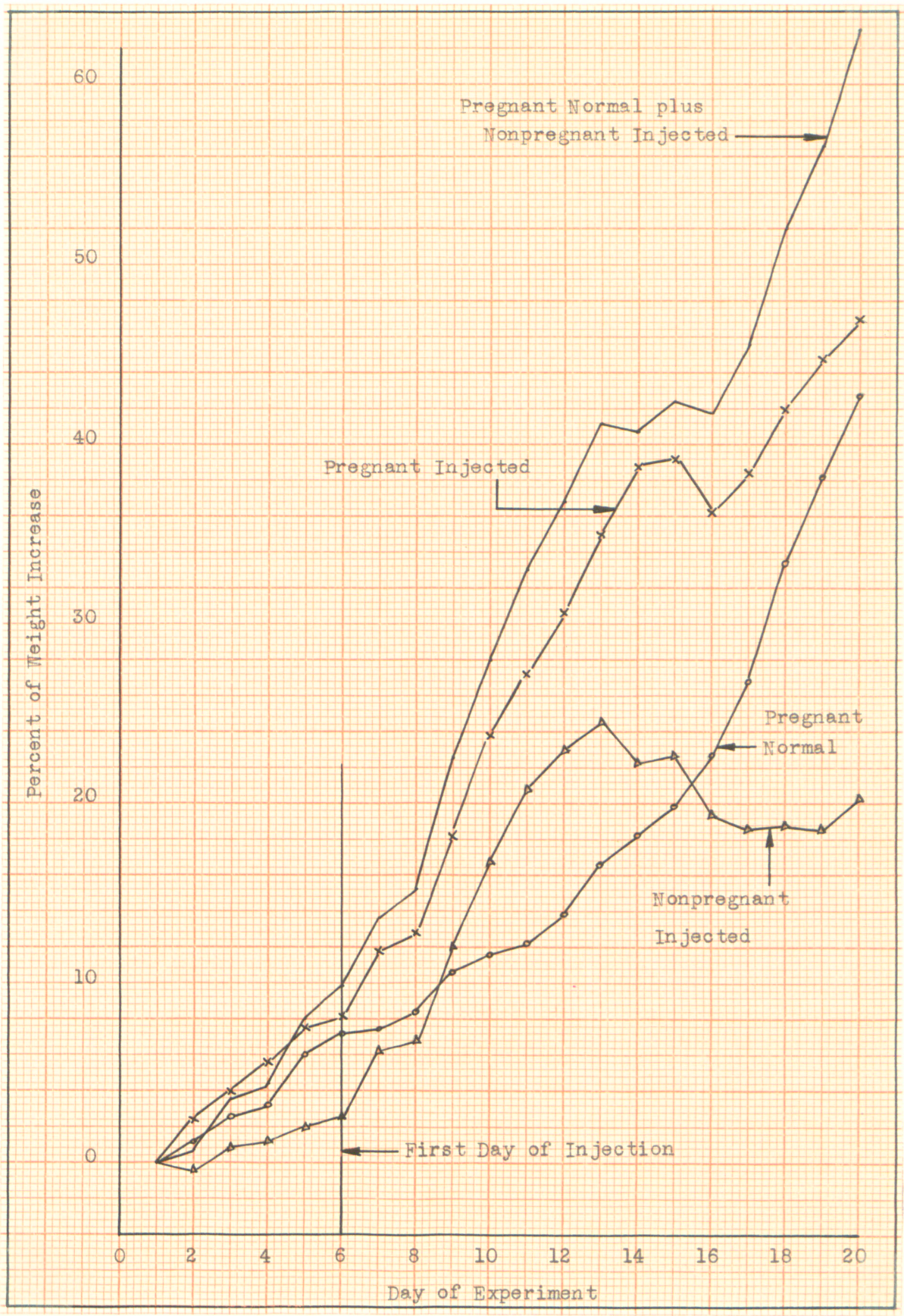
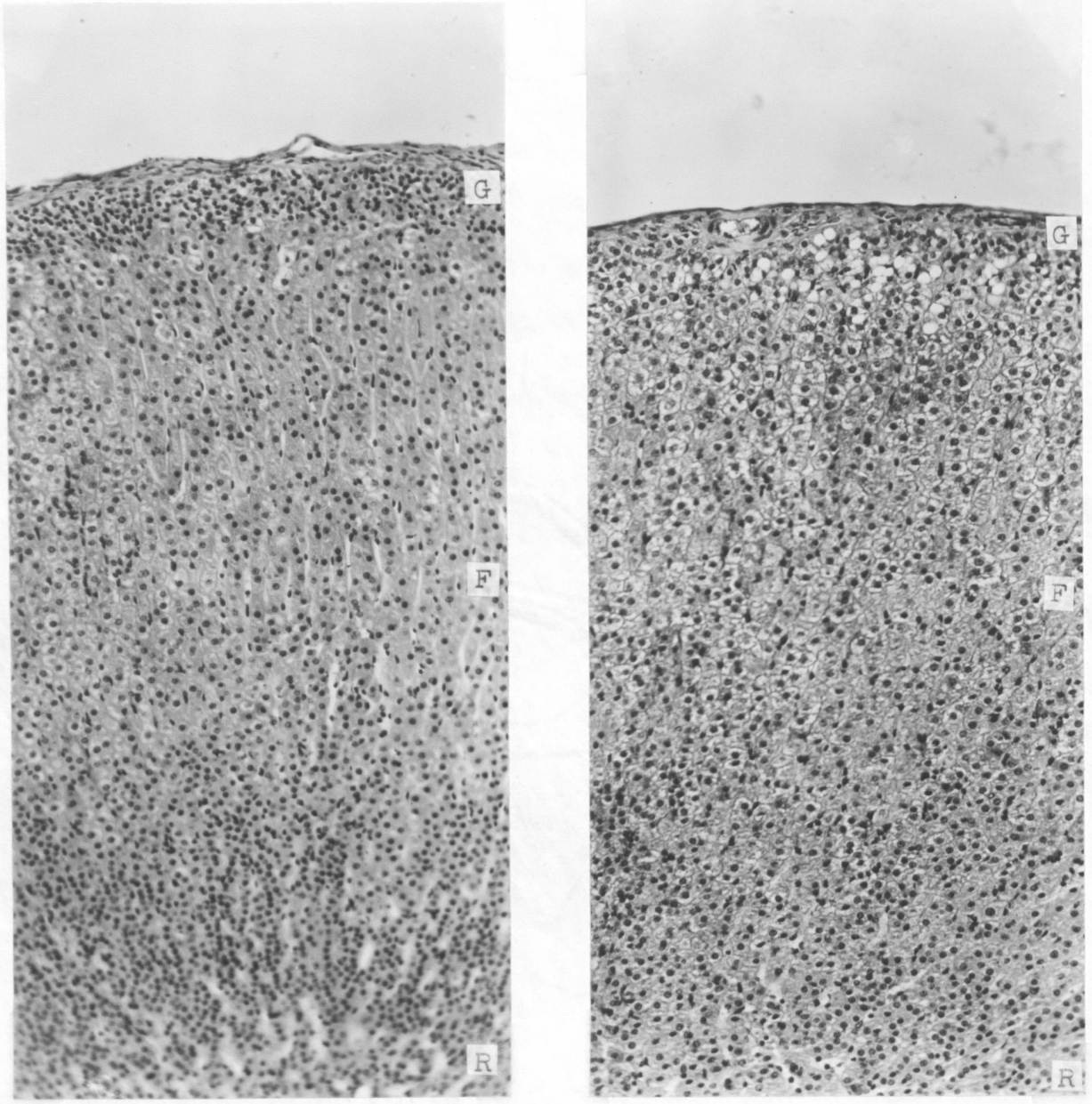


Fig. 2. Daily percent of weight increase based on group averages.



Normal

Pregnant Injected

Fig. 3. Normal adrenal gland on left. Note arrangement into zones and regular arrangement of cells within the zones. Experimental adrenal on right. Indistinct zonation and irregular arrangement of cells. Fatty metaplasia in outer fasciculata and glomerulosa. (G) glomerulosa, (F) fasciculata, (R) reticularis. Low power magnification.

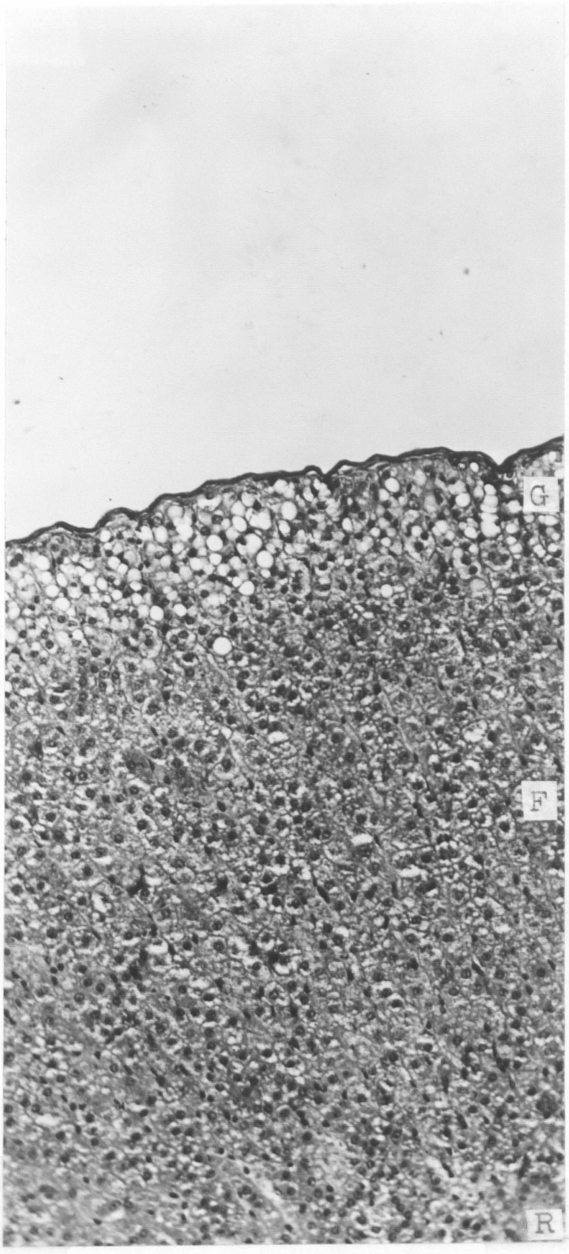


Fig. 4. Adrenal gland from nonpregnant injected animal (207) showing extreme fatty metaplasia of glomerulosa. Compare with Fig. 3. Low power magnification.

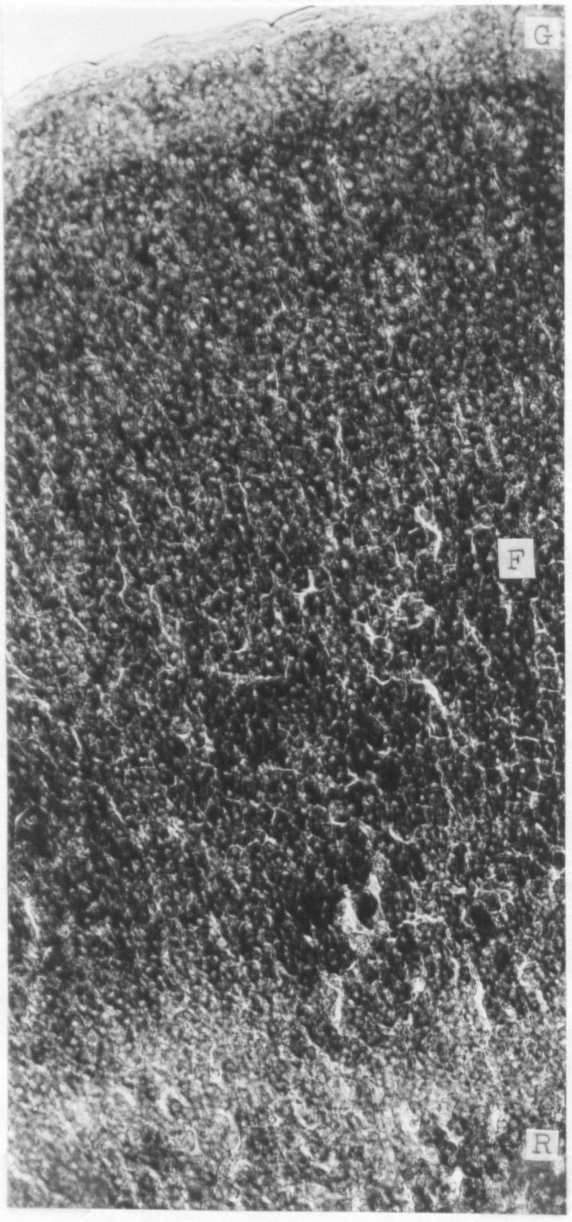


Fig. 5.

Normal adrenal "stained" with Sudan black. Note slight Sudanophilic reaction of glomerulosa.

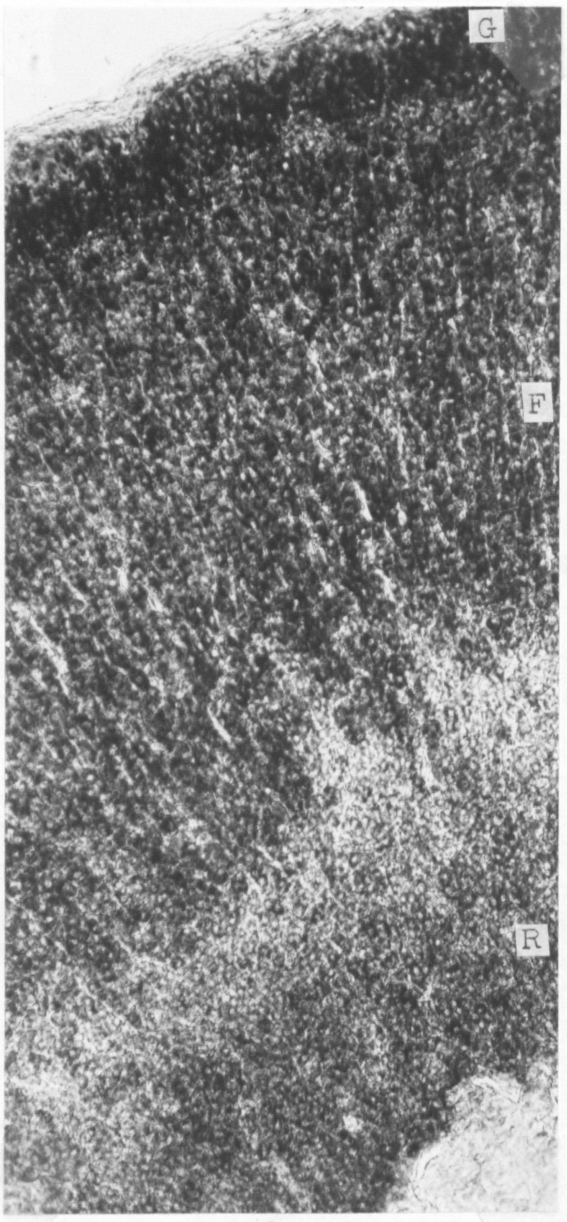


Fig. 6.

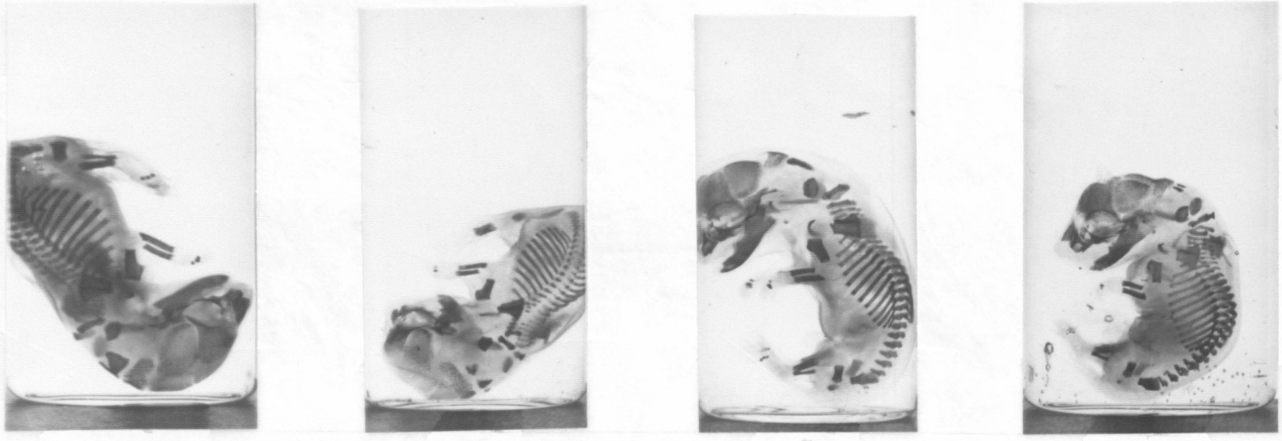
Adrenal gland from pregnant control "stained" with Sudan Black showing increased lipid in glomerulosa.

(G) glomerulosa, (F) fasciculata, (R) reticularis. Low power magnification.

Fig. 7. Gross view of ovaries of injected animal (left) and normal pregnant animal (right) showing no essential difference in size of corpora lutea.

Fig. 8. Embryos showing gross variation in size. Left embryo from injected mother (approximately 1.6 gm.), right embryo from normal mother (approximately 2.3 gm.).

(a) (b) (c)



(d) (e) (f) (g)

Fig. 9. Cleared embryos showing extent of bone development. Normal a and d, injected b, c, e, f, and g, exact weights of each not known. Embryos b and c from same mother show extreme variation of development within same litter. Note absence of metacarpals and metatarsals and only beginning development of supraoccipital bone in c. Embryos f and g from same mother show variation in development not as extreme as b and c. In embryo e note supraoccipital bone not yet fused.

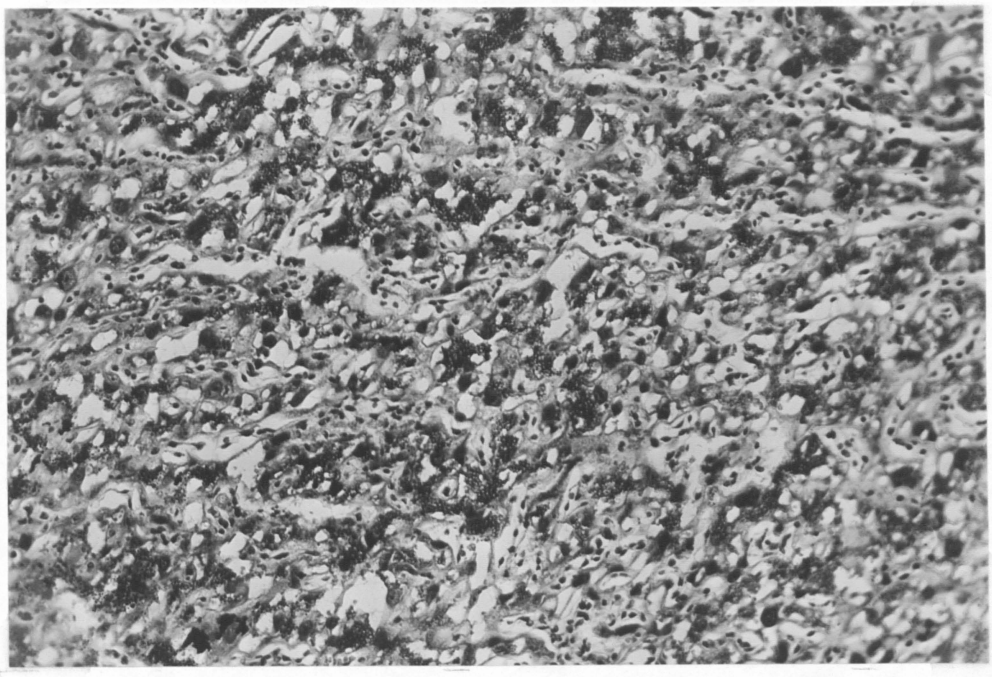


Fig. 10. Normal placental labyrinth showing characteristic appearance. Note blood in sinuses and well formed anastomosing cords of cells.

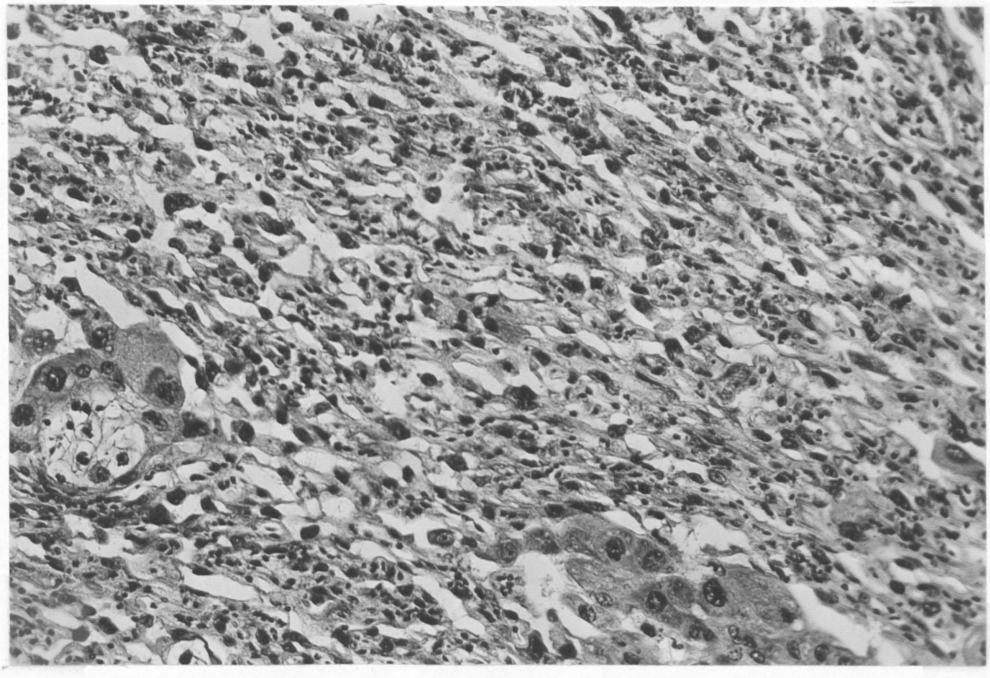


Fig. 11. Experimental placental labyrinth (higher magnification than Fig. 10). Note absence of blood in sinuses, filamentous appearance of syncytium which seems broken in several places. Also note invasion of small cell trophoblast elements.

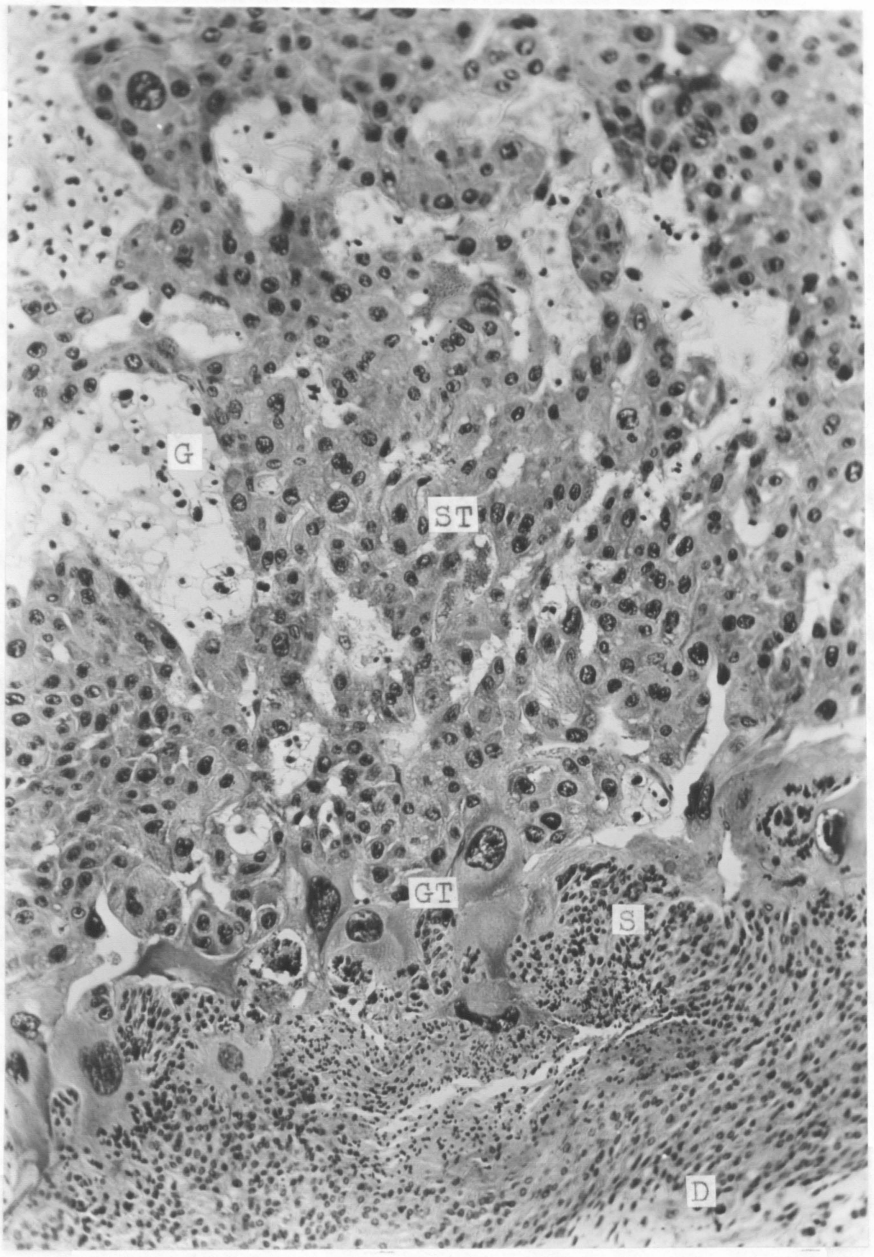


Fig. 12. Maternal-fetal junction area of normal placenta. Low power magnification. (ST) small cell trophoblast, (G) fetal glycogen cells, (GT) giant cell trophoblast, (S) symplasma formation, (D) decidua.

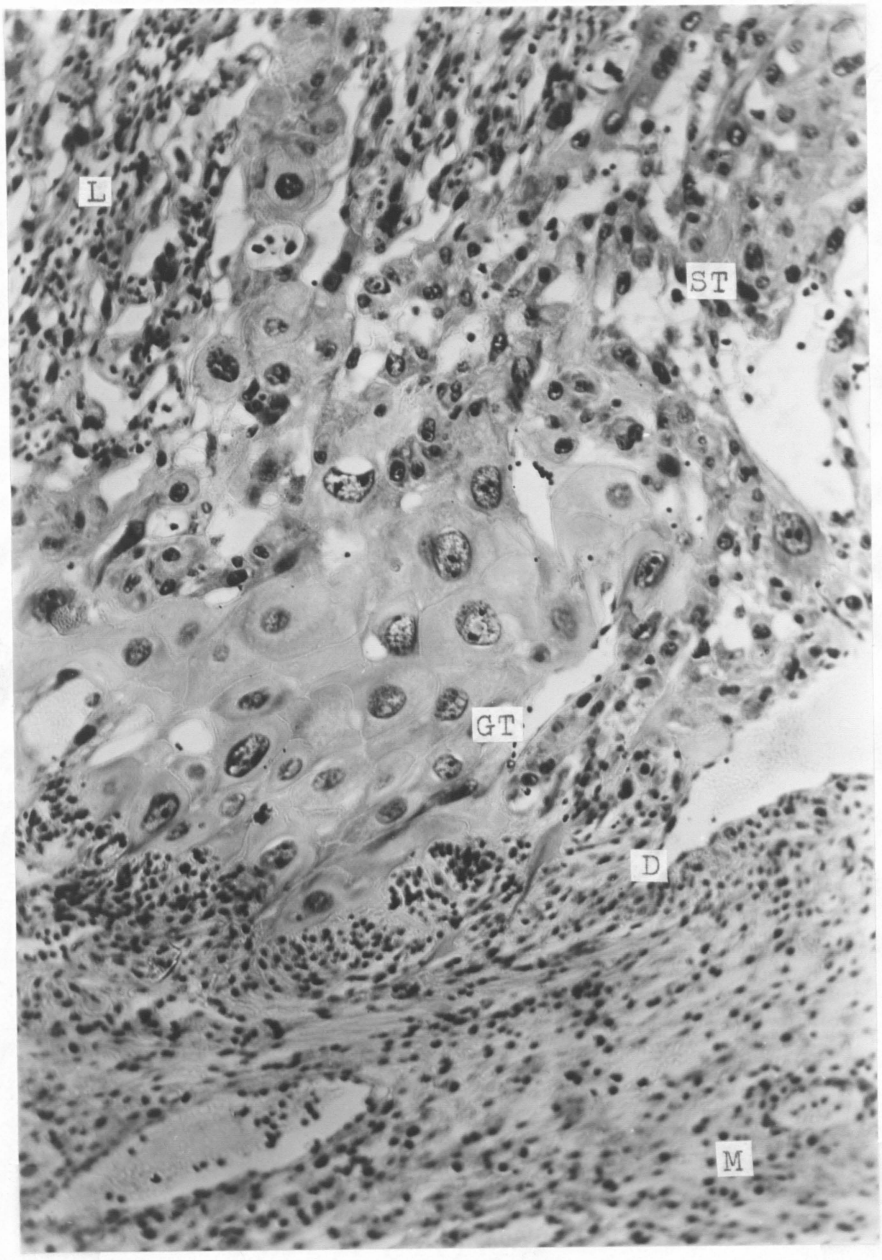
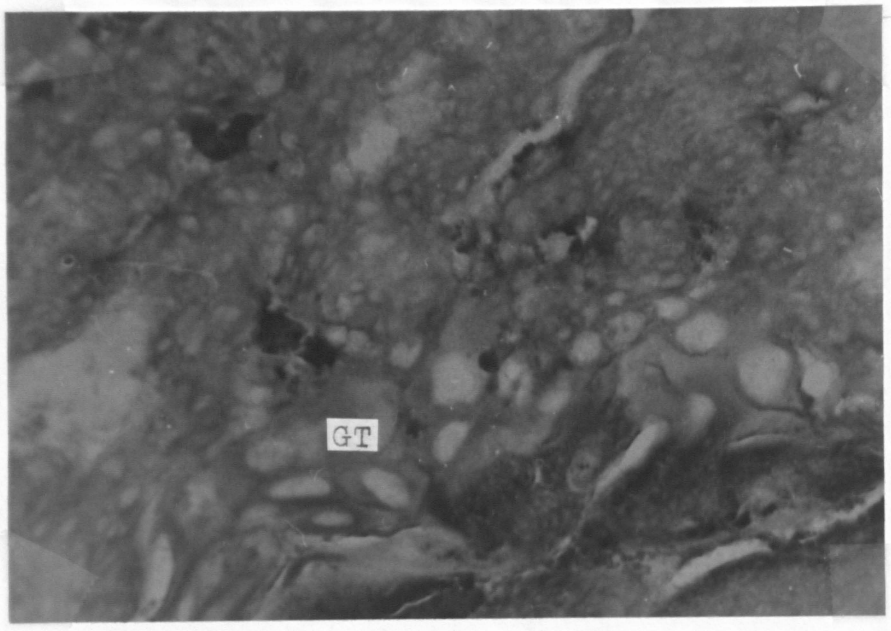
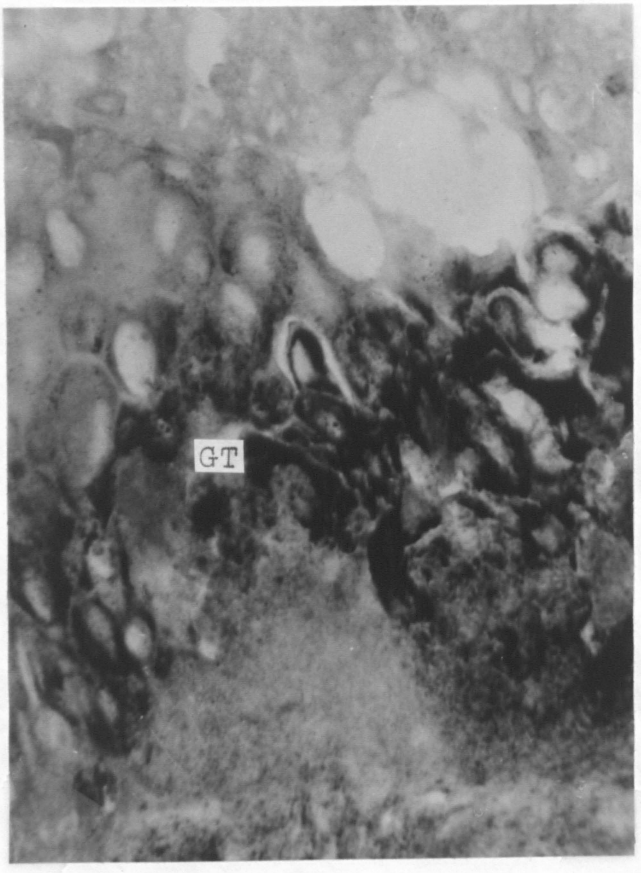


Fig. 13. Maternal-fetal junction area of experimental placenta. Higher power magnification than Fig. 12. Note decrease in width of small cell trophoblast, large group of giant cells traversing width of small cell trophoblast, and decreased width of decidua. (L) Labyrinth, (ST) small cell trophoblast, (GT) giant cell trophoblast, (D) decidua, (M) metrial gland.



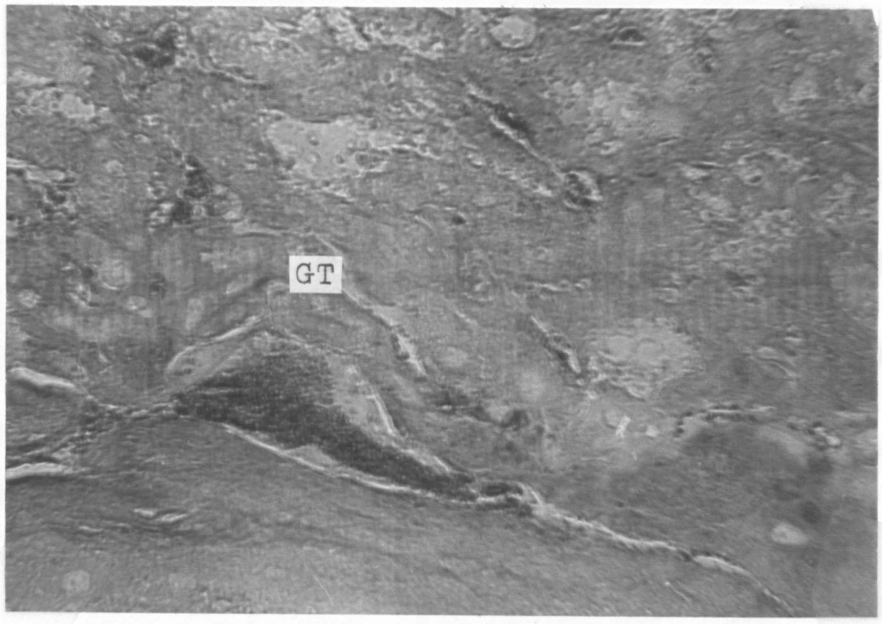
(a)



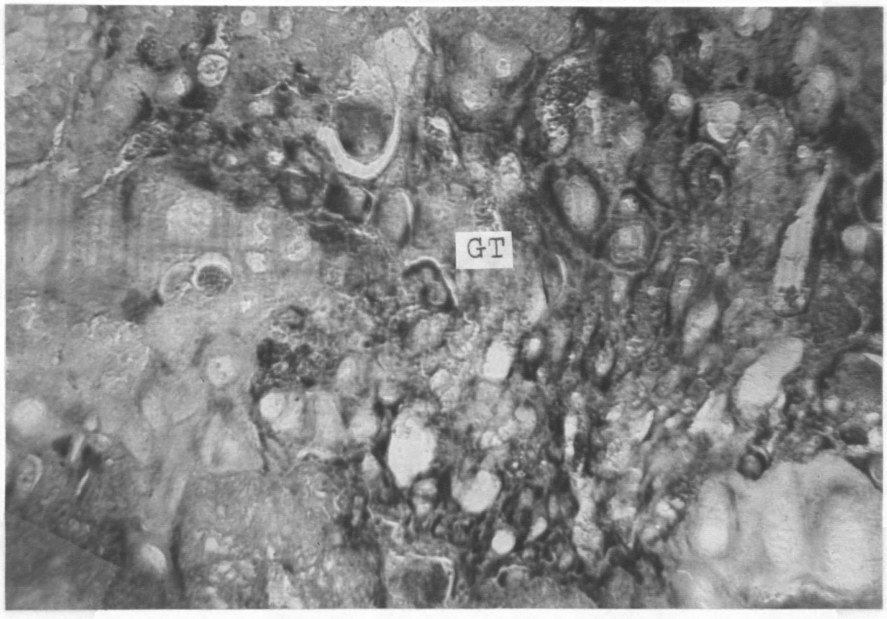
(b)

Fig. 14.

Normal (a) and experimental (b) showing increased lipid content of cells of enlarged giant cell trophoblast (GT).

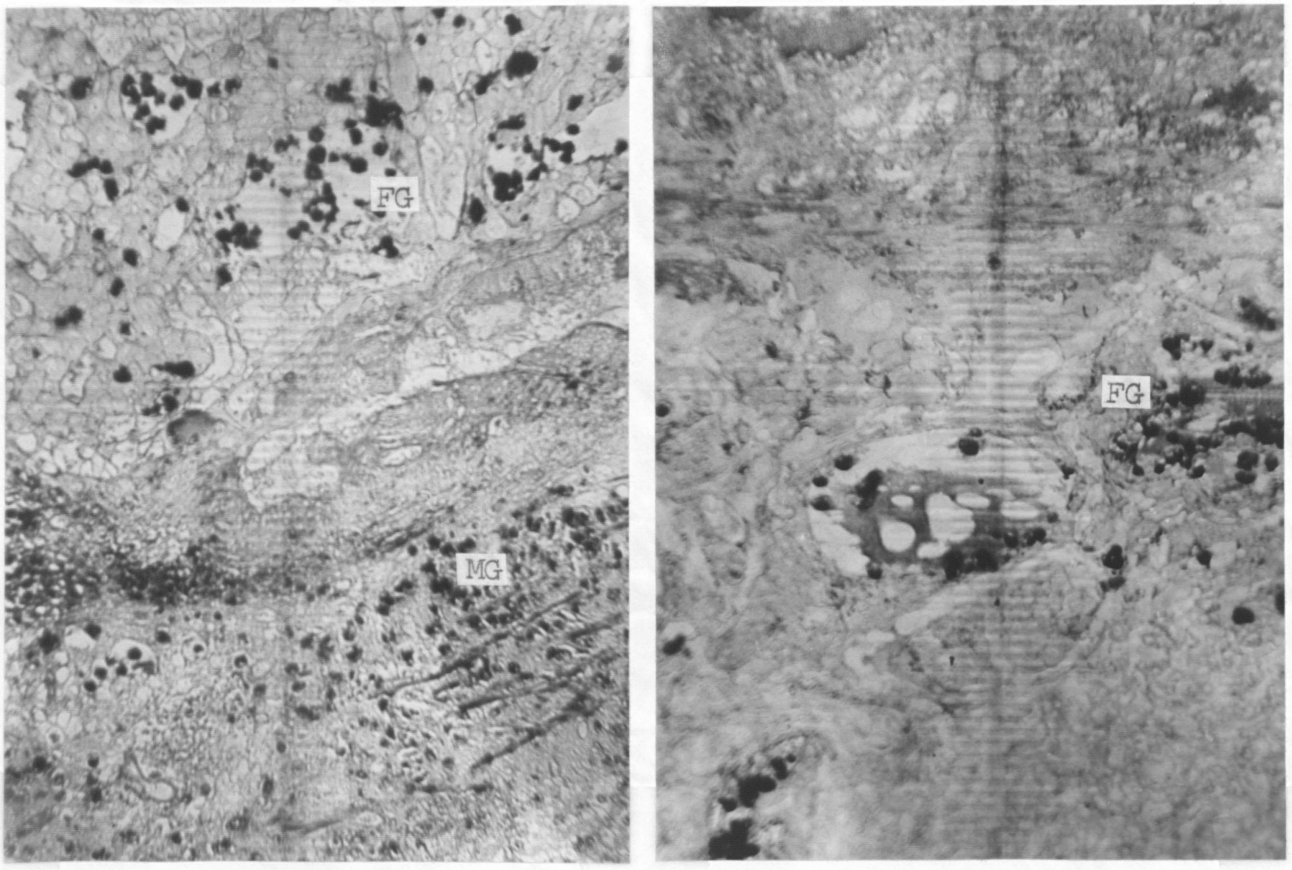


(a)



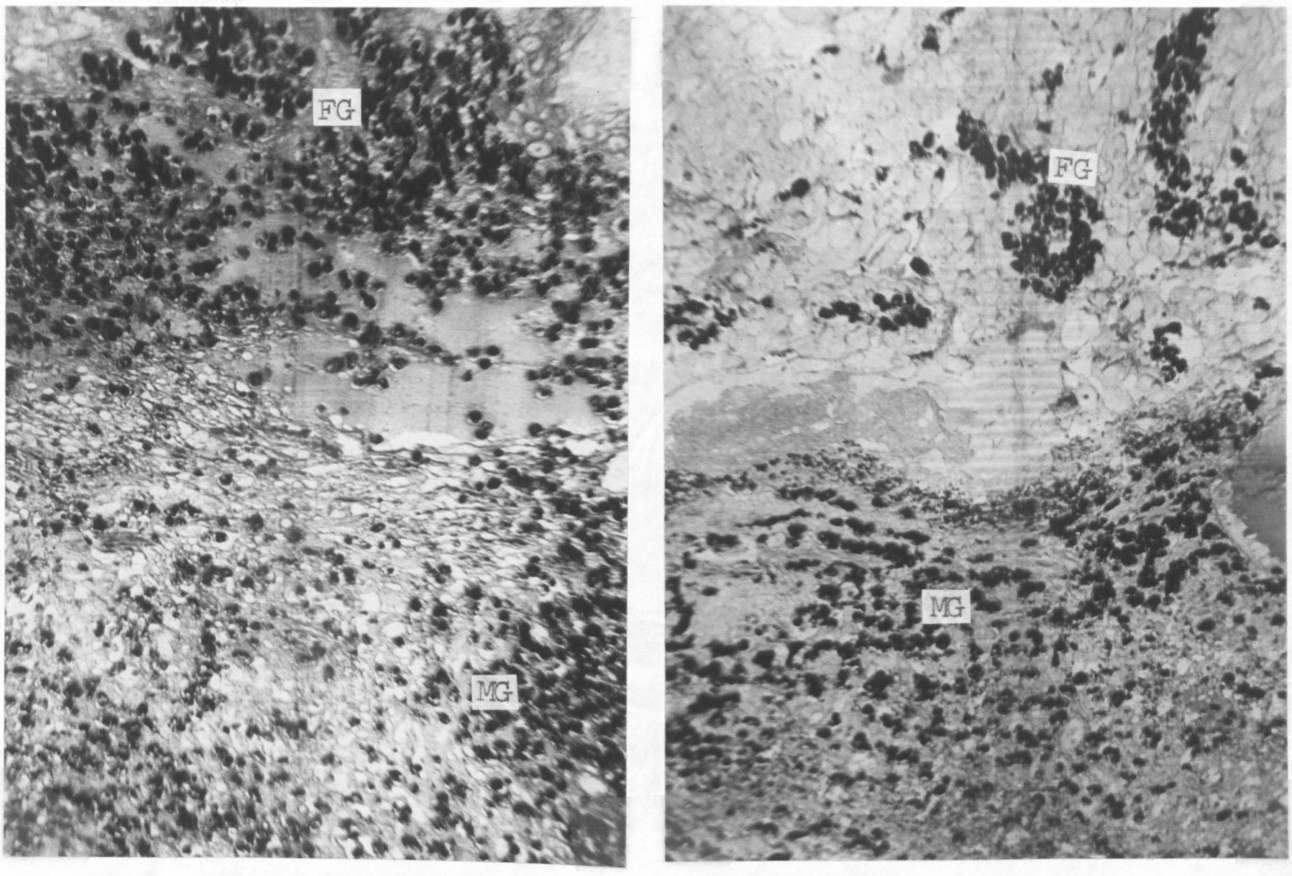
(b)

Fig. 15. Normal (a) and experimental (b) showing increased lipid content of cells of enlarged giant cell trophoblast (GT). Note three extremely enlarged cells at lower right of (b).



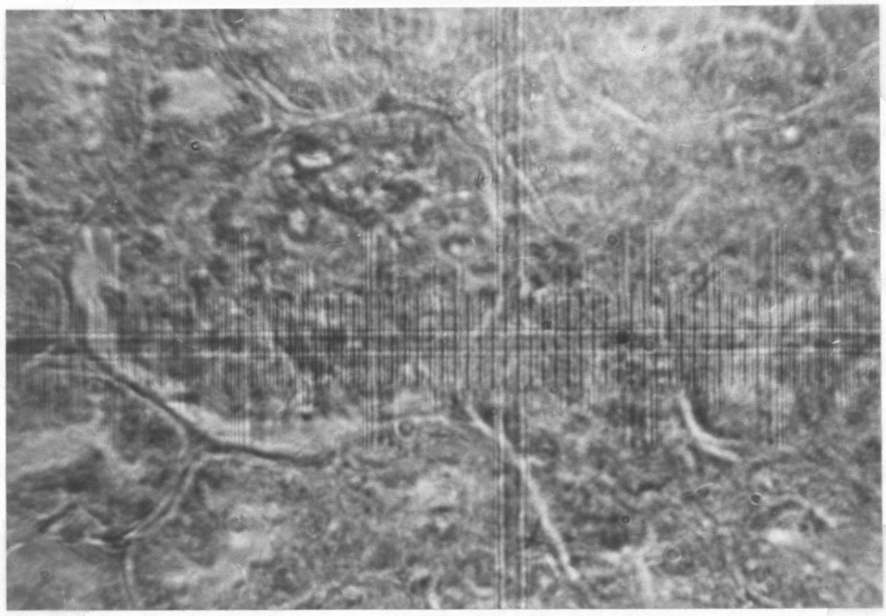
(a) (b)

Fig. 16. Maternal-fetal junction area of placenta [normal (a), experimental (b)] showing the minimal amount of glycogen cells for each group. Photo (a) is typical of only four normal animals. Photo (b) is representative of nine experimental animals. (FG) glycogen cells in small cell trophoblast layer, (MG) glycogen cells in maternal layers.

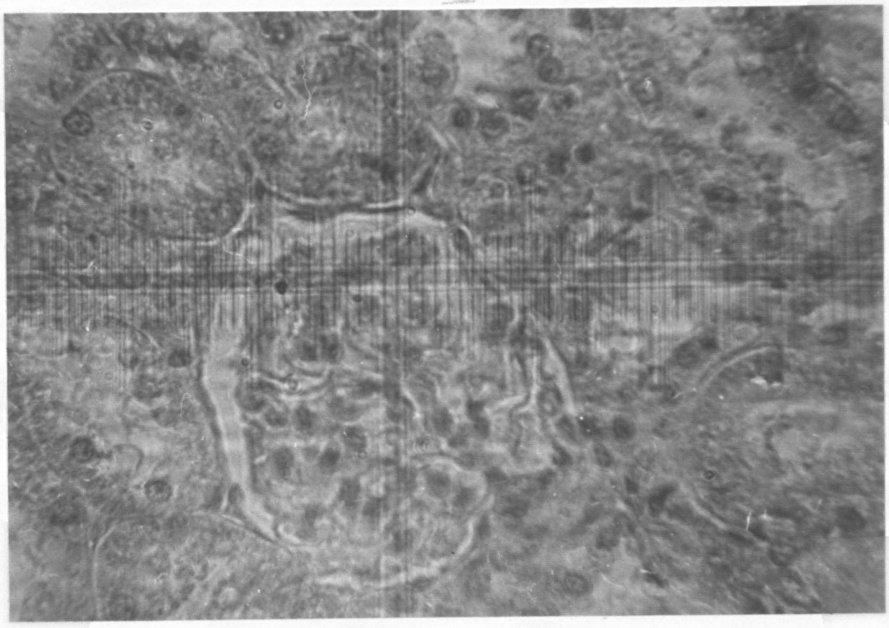


(a) (b)

Fig. 17. Maternal-fetal junction area of placenta [normal (a), experimental (b)] showing the maximum amount of glycogen cells found for each group. Note that Figs. 16 and 17 show only the condition of the glycogen cells, not the total glycogen content. (FG) glycogen cells in small cell trophoblast layer, (MG) glycogen cells in maternal layers.



(a)



(b)

Fig. 18. Kidney, glomerulus and tubules [normal (a), experimental (b)] showing that the experimental is essentially unaltered by the treatment. High power magnification.