A STUDY OF THE HISTOPATHOLOGIC CHANGES IN THE ADRENAL CORTEX OF RATS DURING THE PRODUCTION AND INHIBITION OF LIVER CANCERS

by

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A THESIS

Presented to the Department of Pathology
and the Graduate Division of the University of Oregon Medical School
in partial fulfillment
of the requirements for the degree of
Master of Science

June 1952

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INTRODUCTION

There is much experimental evidence to indicate that the adrenal cortex is not altered by the development of neoplasms in other organs of the body. Ball et al[1] first noted adrenal gland enlargement in rats bearing experimental tumors but felt that this change was due to cachexia resulting from the tumors. Dalton[2] also reported adrenal gland enlargement with generalized lipoid depletion in tumor-bearing mice and attributes it to either the release of toxic substance from large amounts of necrotic tumor tissue, to chronic shock from emanciation, or to concurrent infection. He could demonstrate no specific effect from the tumor, only a slight generalized lipoid depletion associated with cachexia, which might represent adrenal insufficiency. Sarason[3] studied autopsy specimens of adrenal glands from humans dying with cancers and divided them into two groups. The glands from patients with cachexia were enlarged and showed varying degrees of lipoid depletion. The glands from patients dying suddenly with no appreciable weight loss were normal in size and only slightly enlarged and contained a normal amount of lipoid material.

Recent experimental evidence published by Richardson et al[4] that the steroid Methylcholanthrene inhibited liver cancer in rats fed a carcinogenic diet of 3-Methyl-4-dimethylaminoazobenzene suggests a wide field of investigation into the affects of steroids on cancers. In common with other chemotherapeutic agents known to inhibit or slow the progression of neoplasms, Methylcholanthrene itself has carcinogenic properties. Squamous cell carcinomas follow its application to the skin, and sarcomas are produced by embedding it subcutaneously or applying it to serosal surfaces. With this evidence at hand, it seemed desirable to study the histologic effects of this anticarcinogenic steroid on the adrenal cortex.

PURPOSE

These experiments were designed to study the adrenal cortical histologic changes at frequent intervals during liver carcinogenesis and carcinogenic inhibition. Groups of rats were fed 3-Methyl-4-dimethylaminoazobenzene* and administered 20-Methylcholanthrene** by various routes and, with control animals, were sacrificed at frequent intervals. The adrenal, pituitary, and body weight were recorded, and the fresh tissues were immediately fixed while still in a fresh state to avoid the microscopical changes produced by postmortem autolysis.

A general microscopical study of all the body organs was made, with special emphasis on the adrenal cortex, and representative photographs were taken in an effort to concisely and accurately define the adrenal cortical changes occuring with cancer inhibition.

MATERIAL AND METHODS

Animals

Two hundred and seventy-five healthy young adult white rats of the Sprague-Dawley strain were used, one hundred and twenty-four males and one hundred and fifty-one females, ranging in weight from one hundred and thirty grams to three hundred and sixty grams. These animals were raised in our animal room and maintained on Purina Laboratory Chow since birth.

* Hereafter referred to as m'Medab.

** Hereafter referred to as MCA.

Groups

Group 1 included forty-eight rats, twenty-one males and twenty-seven females, which were placed on a basal semi-synthetic diet to which was added 0.06% m'Medab(5) and 0.1 grams MCA per 3000 grams of food (0.003%)(6).

Group 2 consisted of forty animals, twenty males and twenty females, which were fed the basal semi-synthetic ration containing 0.06% m'Medab. In addition, each rat received an injection of 0.090 grams MCA in 3 cc. of an aqueous suspension subcutaneously.

Group 3 comprised forty rats, twenty males and twenty females, which were fed the basal diet to which 0.06% m'Medab was added. Each received an initial injection of 0.090 grams MCA in 3 cc. of an aqueous suspension intraperitoneally.

Group 4 included twenty rats, ten males and ten females, and served as a control group. They were fed the basal diet containing 0.06% m'Medab and received one 3 cc. subcutaneous injection of the aqueous suspension at the beginning of the experiment.

Group 5 consisted of twenty rats, ten males and ten females, and also acted as a control group. They received the basal diet containing 0.06% m'Medab and in addition one 3 cc. intraperitoneal injection of the aqueous suspension at the initiation of the experiment.

Group 6 was made up of twenty rats, ten males and ten females. They were fed the basal diet to which was added 0.1 grams MCA per 3000 grams of food (0.003%).

Group 7 included twenty rats, ten males and ten females, which were fed the basal diet. In addition each received one intial subcutaneous injection of 0.090 grams MCA in 3 cc. of aqueous suspension.

Group 8 comprised twenty-nine animals, twenty-four females and five males, which were fed the basal diet. Each also received one initial injection of 0.090 grams MCA intraperitoneally.

Group 9 was composed of twenty-four rats, twelve males and twelve females. These were maintained on the Purina Laboratory Chow diet.

Group 10 consisted of six rats, three males and three females, which were fed the basal diet only.

Group 11 included eight animals, three males and five females, which were maintained on Purina Laboratory Chow diet and in addition received 5 milligrams of Cortisone subcutaneously daily for eight days.

Diets

Purina Laboratory Chow is a commercial preparation manufactured in pellet form by the Ralston Purina Company of St. Louis, Missouri, for the maintainance of Laboratory animals. It consists of:

Crude Protein, not less than	23%
Crude Fat, not less than	5%
Crude Fibre, not less than	6%
Nitrogen-free extract	44%
Ash, not more than	9%
Bone meal	1%
Sodium Chloride	0.5%
Magnesium Sulfate	0.2%

Vitamins A, D, B_{12}, Riboflavin, Niacin, Thiamin, Brewers Yeast

The basal semi-synthetic diet was formulated by Griffin et al[7] and contains:

Casein	18%
Glucose Monohydrate	73%
Corn Oil	5%
Wesson Salt Mixture	4%, containing:

$NaCl$, $Ca_3(PO_4)_2$, $MgSO_4$, KCl, Fe Si, KH_2PO_4, $K_2Al_2(SO_4)_2$, $CuSO_4$, NaF, KI.

Vitamins A, D, Thiamin, Riboflavin, Calcium Pantothenate, Choline, Pyridoxine

It was supplied in daily portions of approximately 15 grams in a powder form to each animal of Groups 6, 7, 8 and 10.

The 0.06% m'Medab that was fed to animals of Groups 1, 2, 3, 4 and 5 and the 0.003% MCA that was fed to Groups 1 and 6 were weighed out on an analytical balance, mixed with the basal ration in 3000 gram lots, and rationed to the animals in a similar manner.

The animals were kept in 7-1/2 X 9 inch individual cages, with solid metal sides and 1/2 inch wire mesh bottoms, each cage equipped with its own feed can and water bottle. The weight of each animal was recorded at weekly intervals.

Injections

Past experiments[4] have shown that incision of the skin and peritoneal cavity under anesthesia for the purpose of depositing chrystalline MCA was time-consuming and produced a high morbidity and mortality in the animals. Therefore, an aqueous suspension of MCA[8] was used which

consisted of:

MCA	0.3%
AEROSOL, O.T.	0.01%
METHOCEL, 4000	0.1%
ETHYL ALCOHOL	1.0%
DISTILLED WATER	q.s.
NOAH to pH 8	

3 cc. of this aqueous suspension was injected under the skin of the back of rats in Groups 2 and 7 and intraperitoneally through the anterior abdominal wall in rats of Groups 3 and 8, using a 15 gauge needle on a ten cc. syringe. The control animals in Groups 4 and 5 received 3 cc. subcutaneous and intraperitoneal injections respectively of the aqueous suspension which contained no MCA.

Rats of Group 11 received 5 milligrams of Cortison in 0.2 cc. normal saline daily for eight days under the skin of the back using a 21 gauge needle on a Tuberculin syringe.

Killing Schedule

Animals in Groups 1, 2 and 3 were killed at 3-day intervals for the first two weeks, then weekly until the twenty-ninth week. The remaining eight animals in Group 1 were sacrificed during the forty-seventh and forty-eighth weeks.

Rats of Groups 4, 5, 6 and 7 were killed at weekly intervals until the twenty-ninth week.

Rapid adrenal cortical changes were expected to occur following the intraperitoneal injection of MCA so one Group 8 rat was sacrificed 3, 7, 11, 24, 30, 48, 60, 72 and 96 hours respectively after the initial

injection. The remaining animals were killed at weekly intervals through the seventeenth week.

Group 9 animals were killed at irregular intervals throughout the course of the experiment and were used to establish the normal weight and appearance of the adrenal gland.

Rats of Group 10 were also killed at irregular intervals and were used to determine the influence of the basal semi-synthetic diet on the adrenal cortex.

Autopsy Routine

All animals were killed by ether inhalation, weighted on a pharmaceutical balance and the terminal body weights recorded. A note was made of the general physical condition of each animal. An abdominal incision was then made, the adrenal glands quickly excised and placed in 10% formalin fixative approximately three minutes after death. The body was then decapitated, the cranium opened, the brain removed and the remaining head with the pituitary still in its bed was placed in 10% formalin approximately five minutes after death. The liver, spleen with pancreas attached, ovaries, visible lymph nodes, and a segment of the sternum were placed in Vandegrifts fixative(9) which in our hands has proven to give the best cellular detail(10). The stomach, large and small bowel, kidneys, testes, lungs, heart, trachea with thyroid attached, salivary glands, and any abnormal abdominal lesions were removed and placed in 10% formalin. The skin of the back was incised on all animals receiving subcutaneous injections and all lesions were removed and fixed in 10% formalin. A detailed description of these tissues was recorded.

After a minimum of three days representative blocks were cut

from these tissues for histologic study. The pituitary glands were shelled out of the skull and weighed on an analytical balance. The adrenal glands were similarly weighed and both organs were submitted in their entirety for sectioning.

Histologic sections were stained with Hematoxylin and Eosin and all the aforementioned tissues were microscopically studied. Frozen sections of one adrenal gland from each of the animals in Group 8 killed during the first four days of the experiment and from each of the rats in Group 9 were stained for fat with Sudan III. This was done to study the lipoid distribution in normal glands and those subjected to acute abdominal insult. Detailed microscopic descriptions of all sections were recorded.

Colored or black and white photomicrographs under low and high power were taken of all representative changes in the adrenal glands and other organs.

OBSERVATIONS

Weights

Since the animals used in the experiment varied so greatly in weight it was decided that the most accurate determination of alterations in adrenal and pituitary gland weights could be made by expressing those weights as percentage of the total body weights of the animals.

Group 9 (Laboratory Chow diet) was killed to establish normal values for pituitary and adrenal weights of animals in the stock cages. The twelve male rats were all large vigorous adult animals weighing more

than 200 grams and the percentage of adrenal gland weights was found to vary from 0.016% to 0.0275% of the total body weights. The twelve female rats included six fully-grown animals weighing over 200 grams and six younger animals weighing 180 grams or less. It was found that the adrenal gland weights were closely comparable within each of these weight categories but differed appreciably between the two groups. Thus the younger females' adrenal glands tended to be somewhat heavier, varying from 0.038% to 0.046% of the total body weight. On the other hand, the larger older females' adrenal glands tended to weigh less, varying from 0.023% to 0.033% of the total body weight. Since the animals used in this experiment were fully-grown and the great majority weighing over 200 grams it was decided that the lower range of 0.023% to 0.033% would be considered as the normal percentage of adrenal weight to total body weight for the females. These figures are in close agreement with normal values established by other investigators(11). The proportions are not considered to be absolute values, since no great effort was made to remove all the excess periadrenal tissue, but it is felt that the error introduced is relatively constant throughout all the animals in the experiment and these figures are accurate enough for comparison between groups.

The percentage of pituitary gland weight to body weight was found to be highly variable. This in part can be explained by the small size of the glands and the difficulty of removal in toto without extraneous tissue clinging to them. The male glands of Group 9 varied widely in a range of 0.0017% to 0.0054% of body weight, the female glands varying from 0.0032% to 0.0066%. Both sexes were rather evenly distributed throughout these ranges without respect to age or weight of the animals.

The percentage of pituitary gland to adrenal gland weight was calculated for each animal and it was found to range from 10% to 24% in males and from 13% to 24% in the females with the younger females all varying from 13% to 15%. Thus, there was no significant differences between sexes in the percentage of pituitary to adrenal weight so the range of 10% to 24% was assumed to be normal for both sexes (Chart No. 1).

Rats in Group 10 (Basal Diet) were studied to determine the difference that diet alone would make on the organs. The small number of animals precludes any definite conclusions, but the range in percentage of adrenal to body weights did not differ appreciably from that of the animals on the Purina Laboratory Chow, varying from 0.012% to 0.026% in the males, and from 0.019% to 0.024% in the females. The percentage of pituitary weights to body weights and therefore the proportion of pituitary to adrenal weights also fell into the range designated as normal for the purpose of this experiment. Many experiments have reported the influence of high and low protein diets on the weight of the adrenal gland[12, 13, 14] but the protein content in the two diets used in this experiment did not differ sufficiently to cause this change.

Throughout the experiment great variations were found to occur in the proportion of adrenal and pituitary to body weights, depending on the health of the animals, and to the type of drug they were receiving. It was noted that the glands of the animals spontaneously dying underwent rapid postmortem changes which increased the weights of the glands. So it was decided to omit these glands from the weight curves, only considering immediately fixed glands obtained from freshly killed animals.

In Group 1 (m'Medab + MCA diet) the adrenal glands in general showed an increase in weight with a gradual return to normal in 2 to 4

weeks. After the twentieth week the adrenal glands again show a tendency to gain in weight. This is considered to be a valid assumption since the majority of these animals were in good physical condition and any body weight loss occurred very slowly.

The pituitary glands showed a decrease in the relative proportion to body weights which was maintained throughout the duration of the experiment, though with wide variations and only the general trend can be considered.

Therefore, the proportion of pituitary weight to adrenal gland weight decreased at the onset of the experiment and remained low for the duration (Chart No. 2).

Group 2 (n'Medab diet plus MCA subcutaneously) displayed essentially the same trend in adrenal and pituitary weight changes as did Group 1, though to a lesser degree. No terminal rise in the adrenal weights was noted, but this may be due to the fact that this group was not allowed to go farther than 29 weeks. Chart No. 3 shows an initial small rise in the adrenal weights lasting for about 4 weeks, with a return and maintenance of normal values for the remainder of the experiment. It appears that the females adrenal glands tended to remain heavier for a longer time, till about 10 weeks, and then to fall slightly below normal for the remainder of the experiment.

The pituitary gland weights showed an immediate and permanent decrease. Those of the females showed a tendency to increase in weight late in the experiment which cannot be attributed to a decrease in body weight since this was not great.

The proportion of pituitary weights to adrenal weights reflected these changes by promptly falling below normal and remaining so until the

25th week when the rise in the pituitary weights resulted in a return to normal (Chart No. 3).

Group 3 (m'Medab diet plus MCA intraperitoneally) showed a similar early increase in adrenal gland weights, but in this series the weights tended to remain elevated throughout the experiment. The pituitary weights in common with the other series immediately decreased below normal which was maintained until the twentieth week, followed by a tendency to return toward normal. As a result the percentage of pituitary weight to adrenal weight decreased immediately and remained below normal throughout the experiment (Chart No. 4).

Group 4 (m'Medab diet plus aqueous suspension subcutaneously) adrenal gland weights showed a transient early increase, then progressively diminished in weight reaching subnormal levels by the eighth week. The pituitary glands showed the usual immediate decrease in weight which was maintained during the entire experiment, although there seemed to be some tendency to increase toward normal after the tenth week. The resultant pituitary to adrenal gland proportion decreased primarily but after the tenth week showed a definite tendency to climb back to normal (Chart No. 5).

Group 5 (m'Medab diet plus aqueous suspension intraperitoneally) adrenal and pituitary weight changes resembled those of the preceeding group closely. There was a primary increase in adrenal weights with a return to normal in 6 to 8 weeks, followed by a slow and progressive decrease in adrenal weights during the remaining experiment. The pituitary weights likewise showed an immediate fall below normal with only a tendency to increase toward normal beyond the twentieth week. The resulting pituitary to adrenal gland relationship fell below normal immediately and gradually climbed back to normal after the twentieth week (Chart No. 6).

Group 6 (Basal plus MCA diet) adrenal weights showed the familiar early rise to above normal with a return to the normal range at about the eighth to tenth week. The normal adrenal weight was generally maintained throughout the rest of the experiment. The pituitary weights also fell below normal immediately but after the eighteenth week there was a definite tendency for the glands to gain in weight toward normal. Therefore, the pituitary to adrenal gland ratio fell below normal immediately with only a slight tendency to return to normal after the sixteenth week (Chart No. 7).

In Group 7 (Basal diet plus MCA subcutaneously) the early rise in adrenal gland weights was not so apparent as the other series, and in the main remained within normal limits throughout the experiment. The pituitary weights again dropped below normal early and remained subnormal during the entire experiment. The resulting pituitary weights in proportion to adrenal weights were maintained constantly below normal during the entire experiment (Chart No. 8).

Group 8 (Basal diet plus MCA intraperitoneally) suffered a high mortality rate and only females survived beyond the second week so no comparison can be made between the sexes. But the females showed the same early increase in adrenal gland weights which returned after about three weeks and remained normal during the rest of the study (Chart No. 9).

Group 11 (Laboratory Chow + Cortisone subcutaneously) animals were all killed at once after eight days and showed a slight loss in adrenal gland weights at that time. The percentage of pituitary weights to body weights was highly variable but it is felt that these also showed a decrease below normal. No significant alteration from normal occurred in the relationship of pituitary to adrenal gland weights (Chart No. 10).

Autopsy Findings

An effort was made to kill animals as soon as they appeared to be failing in health, but this practice was not entirely successful in preventing deaths, since many of the animals, especially those receiving intraperitoneal injections of MCA, sickened and died in a matter of hours. These dead animals were autopsied in the described manner and the findings were included in the following discussion with the exception of the adrenal glands which underwent postmortem changes in a very short time rendering them unfit for study. The gross findings in this experiment fell into certain broad categories and they will be discussed as such.

Groups 1 (m'Medab plus MCA diet) and 6 (Basal plus MCA diet) presented similar autopsy findings. Spontaneous deaths were quite unusual in Group 1, occurring in only 2 animals (5%), but more common in Group 6, including 4 animals (20%). None of the livers or any other abdominal organs in either of the groups showed any gross abnormalities.

Groups 2 (m'Medab diet + MCA subcutaneously) showed similar findings on gross examination and will be discussed together. After the thirteenth week the livers of Group 2 began to show evidence of cirrhosis by enlarging, becoming paler and mottled yellow-brown in contrast to the normal beefy-red. The surfaces gradually became roughened, later irregularly lobulated, affecting all lobes to a variable extent. No cirrhosis occurred in Group 7. Five animals died spontaneously in Group 2 (12%) while 4 died in Group 7 (20%).

For the first month after subcutaneous injection nothing could be palpated in the skin and on excision there was found a small collection of MCA powder in the subcutaneous tissue. This was surrounded

by a thin fibrous capsule and was loosely attached to the underlying fascia and muscle. During the second month a soft 1 cm. freely-moveable mass could be palpated under the skin. On excision this proved to be a mass of MCA powder which was surrounded by a thick fibrous capsule with numerous trabeculations extending throughout the MCA deposit. During the following weeks this mass gradually enlarged and became firmer in consistancy, though remaining quite discrete and usually unattached from the underlying muscle. The MCA nidus gradually diminished in size and finally disappeared, though occasionally in the late sarcomas a small collection of unaltered powder could be found in their center. It was impossible to tell with certainty by gross inspection when these granulomatous masses underwent malignant degeneration. They eventually reached two to five cm. in diameter and on cut section the frankly malignant lesions were firm and pearly-grey in color and made up of very dense fibrous tissue arranged in whorls and trabeculations, the whole mass interspersed with areas of hemorrhage and necrosis. The fibrosarcomas remained localized discretely under the skin and only loosely attached to the underlying fascia, though a few of the advanced tumors grossly invaded the musculature and bony thorax. Occasionally the larger tumors would ulcerate the overlying skin.

Group 3 (m'Medab diet plus MCA intraperitoneally) and 6 (Basal diet plus MCA intraperitoneally) likewise will be considered together. These series suffered a high morbidity and mortality which resulted in a rather limited number of animals available for adrenal gland study. Nineteen animals (47%) of Group 3 and twelve (41%) of Group 6 died from the intense chemical peritonitis or complications produced by the dense adhesions that formed. Three hours after intraperitoneal injection of

MCA, the powder was found spread evenly and diffusely over the parietal peritoneum and in small collections among the loops of bowel, but most had become adherent over the surface of all lobes of the liver. Even this early there was a thin fibrin deposition over the surface of the liver. The stomach, duodenum, spleen, and diaphragm had become loosely adherent to the liver surface. During the ensuing weeks there was a steadily increasing deposition of fibrin and fibrous tissue with contiguous formation and contraction of adhesions until in the more severe cases all the abdominal viscera were inextricably enveloped by a mass of dense fibrous tissue entirely filling the abdominal cavity. After thirteen weeks small hard masses were found over the surface of the liver and diaphragm. These were circumscribed, chalky-white in color, and cut with a gritty sensation. As these masses grew to several cm. in diameter they softened and became more fleshy in consistency with areas of hemorrhage and necrosis. Rarely similar tumor originated from the intestinal serosa. Usually they appeared in the region of the liver and diaphragm, spreading by direct extension down the paravertebral gutters to involve the spleen, stomach, kidneys, and adrenal glands secondarily. Cirrhosis was noted occasionally on gross inspection in animals of Group 3.

Groups 4 and 5 received m'Medab in the diet and the aqueous suspension subcutaneously and intraperitoneally, respectively, and will be considered together. No lesion could be found in any animals that was directly attributable to the aqueous suspension. Spontaneous deaths were minimal in these groups. Only one (5%) died in Group 4, and two (10%) in Group 5.

The livers in both groups began to show changes of minimal cirrhosis by eight weeks. From this period on all the livers showed

progressively more severe cirrhosis. At the termination of the experiment the livers were almost doubled in size, the surfaces very lobulated with many pedunculated masses along the edges of the lobes. In addition three livers from animals of Group 4 contained tumors on gross examination. These consisted of numerous pale grey hard irregularly circumscribed sessile nodules ranging from 2 mm. to 2 cm. in diameter. One liver also contained a large 5 cm. pedunculated soft hemorrhagic and necrotic tumor hanging down into the pelvis. The abdominal surface of this animals diaphragm was covered with pinhead-sized hard nodules representing transperitoneal metastases. Group 5 contained no liver tumors visible to the naked eye.

No significant pathology was seen on gross examination of the animals in Groups 9 (Laboratory Chow diet), 10 (Basal diet), and 11 (Laboratory Chow diet plus Cortisone subcutaneously). There were no spontaneous deaths in these groups.

No insight into the microscopic appearance of the adrenal glands could be gained on gross examination of the killed animals, regardless of group. All appeared pale pink to ivory-white in color, smooth, firm, and any estimation of weight proved to unreliable. The adrenal glands of animals dying spontaneously showed a variable amount of enlargement, depending on the amount of postmortem change that had occurred. Many were two or three times enlarged and all became a dirty motted grey in color, very soft and friable.

Many of the animals, regardless of grouping displayed a focal chronic pneumonia on gross examination which appeared as pinhead to one mm. firm areas of consolidation over the surfaces of the lungs with contraction of the overlying pleura. In a few of the animals this pneu-

monia was more serious with variable-sized abscesses scattered through the lung parenchyma. These consisted of thick fibrous walls and contained a thick dry greenish-yellow cheesy material.

The pituitary glands of all animals, regardless of grouping, appeared similar on gross examination and no estimation of variability in weight could be made.

The testes of many males receiving the basal diet (Groups 1, 2, 3, 4, 5, 6, 7, 8 and 10) gradually diminished in size and softened in consistency. Late in the course of the experiment they appeared translucent, quite cystic in consistency, and one-third to one-half of normal size.

Microscopic Changes

Group 1 animals (m'Medab plus MCA diet) commenced to show early cirrhosis of liver at three weeks, consisting of a minimal increase in the perilobular connective tissue. Throughout the remainder of the experiment twenty-four animals (52%) showed this minor change. In addition seven livers (15%) contained microscopic bile duct cysts after the twenty-sixth week. Four livers (9%) contained focal areas of fatty metaplasia after the eleventh week. These two findings are a common accompaniment and considered to be part of the process of cirrhosis. In addition, one liver (2%) contained several microscopic benign hepatomas after the forty-eighth week. Another liver (2%) contained minimal bile duct proliferation at the twenty-ninth week. It is interesting that one liver (2%) contained a malignant hepatoma. It has been previously reported (4) that m'Medab and MCA administered orally give complete inhibition of liver carcinogenesis. Of several hundred animals

treated to a similar diet in our laboratory this is the only one to show a malignant liver change.

The adrenal glands of forty-five animals were found to be fit for microscopic study. During the first two weeks the glands became hyperemic. There was a loss of lipoid vacuoles from the glomerulosa and fasciculata zones. There was evidence of hyperplasia of the reticularis zone by an increase in cellularity and encroachment on the large blood spaces of this layer. No mitotic figures were seen but binucleated cells were common. After the third week the glomerulosa cells began to enlarge and there was a simultaneous gradual loss of vacuoles and granules from the cytoplasm of the cells (Fig. 1). Soon after the process was initiated in the glomerulosa it began in the fasciculata. The fasciculata cells began to lose their rich granularity, at first in individual cells scattered among the normal cells (Figs. 2 and 3). This was accompanied by a loss of the large lipoid vacuoles to a variable extent. The reticularis zone retained its lipoid vacuoles and cytoplasmic granularity. By the twenty-fifth week these changes had progressed to a general loss of vacuolarity and granularity affecting all the cells of the glomerulosa and fasciculata, though many vacuoles could still be found (Figs. 4 and 5). The animals living until the 47th and 50th weeks presented this change in its most advanced form. Both glomerulosa and fasciculata cells were enlarged, with extreme depletion of cytoplasmic granules and lipoid droplets, until the cytoplasm had become very pale and vesicular with only a fine fibrillary network present (Fig. 6). At this late stage the reticularis showed a slight loss of granularity and vacuoles. The transitional zone disappeared from adrenal glands demonstrating this extreme stage of depletion,

but remained unaltered in other glands.

Group 2 (m'Medab diet plus MCA subcutaneously) animals showed many liver changes. After 3 weeks thirty-one animals (91%) presented varying degrees of liver cirrhosis. Cystic dilitation of bile ducts was found in 13 animals (13%). Fatty metaplasia, in focal areas or generalized throughout the liver parenchyma was seen in 15 animals (36%). This was agin noted to be an accompaniment of cirrhosis. Bile duct proliferation occurred in 8 animals (20%) and benign bile duct adenomas in one animal (3%). Eleven animals (28%) had benign hepatomas. Malignant liver cancers were limited to malignant hepatomas which occurred in six animals of this series (29%). Malignant hepatomas first appeared at 17-1/2 weeks and at first consisted of multiple microscopic clusters of neoplastic cells scattered throughout the livers. Later these enlarged to encompass several low power fields. No metastases were found to arise from these cancers.

The skin lesions consisted at first of a mild edema of the subcutaneous tissue with a slight mononuclear cell infiltration. After several weeks there was a fibroblastic proliferation into the area and many very large multinucleated giant cells appeared. The entire lesion took on the appearance of a granuloma and was relatively avascular. At the twentieth week these granulomas underwent malignant change, fibrosarcomas arising from multiple sites in the granulomas. One of these tumors also contained rhabdomyosarcomatous elements. Late in the course of the experiment these tumors were seen to be invading by direct extension into the spinal musculature, but no distant metastases were found. Twelve of the animals (72%) living after the twentieth week developed sarcomas.

Thirty-four pairs of adrenal glands were suitable for histologic study and the adrenal cortical changes roughly paralleled those in the previous group. Changes in the first three weeks were limited to generalized hyperemia and hyperplasia of the reticularis with minimal lipoid vacuolar depletion of the fasciculata zone. (Fig. 1). After the fourth week there was a gradually progressive depletion of the large lipoid vacuoles from the enlarging glomerulosa and fasciculata cells, later a depletion of the cytoplasmic granules in these two layers (Figs. 1, 2, and 3). But the process did not appear to advance as far as it did in the first group, even at the twenty-ninth week. The transitional zone remained relatively unaltered through the entire course of the experiment.

Group 3 (m'Medab diet plus MCA intraperitoneally) animals developed very severe chemical peritonitis. Four days after the intial injection the abdominal viscera was covered by a thin exudate consisting of fibrin and mononuclear inflamatory cells. Large multinucleated giant cells were found in abundance. Fibroblasts and capillary tufts soon were seen proliferating in the exudate. The granulomatous reaction gradually progressed until the organs were finally bound to each other by dense fibrous bands which were highly vascular and thickly invaded by chronic inflammatory and giant cells. After the thirteenth week malignant changes could be found arising from multiple sites throughout the peritoneum but most consistantly from the surface of the liver. They were primarily fibrosarcomas, but most also contained angiosarcomatous, liposarcomatous, leiomyosarcomatous, and rhabdomyosarcomatous elements. These tumors spread rapidly by direct extension throughout the peritoneal cavity, and late in the experiment could be found in-

vading the bowel, spleen, kidneys, adrenals, and even the pelvic organs occasionally (Fig. 7)..

Microscopic cirrhosis of the liver was seen first at sixteen weeks. It occurred in thirteen of the animals (65%) after this time and consisted of only minimal to moderate perilobular connective tissue proliferation. In addition 2 animals (10%) had bile duct cysts, 2 (10%) showed focal areas of fatty metaplasia and one (5%) showed a small amount of biliary proliferation. No malignant liver tumors were found in this series.

An additional finding of interest was seen in the kidneys. It was characterized by rounded, focal, nodular hyaline areas in the glomerular tufts affecting occasional glomerulae in a haphazard fashion. These lesions closely resembled those seen in diabetic glomerulosclerosis in the human. It occurred in 5 animals (12%) in this series. The renal arterioles and tubules remained unaltered (Fig. 8)..

The adrenal glands of twenty-one animals were studied and they behaved in a manner similar to the previous groups. There was early hyperemia of the cortices with some hyperplasia of the reticularis cells and slight loss of lipoid vacuolarity from the fasciculata and glomerulosa zones. After about the sixth week the glomerulosa cells enlarged and underwent a depletion of the cytoplasmic granularity (Fig. 1). The cells of this layer became pale and vesicular in appearance. The fasciculata cells similarly enlarged and gradually lost their cytoplasmic granules, first in individual cells and finally the entire layer was involved (Figs. 3, 4, and 5). The reticularis zone remained relatively unaffected except for perhaps a slight loss of vacuolarity. The transitional zone remained unaltered throughout the experiment.

Group 4, receiving m'Medab in the diet and the aqueous suspension subcutaneously, acted as a control group. No skin lesion was found any of the animals that could be ascribed to the aqueous suspension.

The livers began to show microscopical evidence of cirrhosis after 4 weeks. Thereafter, all of the remaining eighteen animals displayed various degrees of cirrhosis proportional to the length of time on the carcinogenic diet. In the late stages this was very severe and the liver consisted of thick sheets of connective tissue with isolated hepatic lobules imbedded in the meshes. The other common accompaniments of cirrhosis were also present in marked degrees, including: bile duct cysts in 8 animals (40%), benign hepatomas in 13 animals (65%), fatty metaplasia in 14 animals (70%), and bile duct proliferation in 13 animals (65%). In addition, benign biliary adenomas (cholangiomas) occurred in one animal (5%).

Malignant liver tumors appeared at 16 weeks and thereafter occurred in all of the remaining eleven animals. All of these animals had malignant hepatomas, three had hepatic cell adenocarcinomas (27%), and five had bile duct adenocarcinomas (45%). One animal also had an interstitual fibrosarcoma arising from the cirrhotic perilobular fibrous tissue.

Metastases occurred in three animals (27%). One malignant hepatoma produced grossly visible transperitoneal implants on the diaphragm and also pulmonary metastases seen as venous thrombi. A biliary adenocarcinoma in another animal also spread as venous thrombi to the lungs. A second biliary adenocarcinoma was found to have spread via the lymphatics to the periaortic lymph nodes in the region of the coeliac axis.

The adrenal gland changes in the eighteen animals studied were limited to hyperemia, minimal lipoid vacuolar depletion, and hyperplasia of the reticularis zone for the first six weeks. Fig. 1. Thereafter, there was a progressive lipoid depletion of the glomerulosa and fasciculata zones. This occurred first as a loss of vacuolarity in the glomerulosa followed by a similar loss in the fasciculata. Then there was a gradual depletion of the cytoplasmic granularity with swelling of the cells of the glomerulosa and fasciculate layers, at first occurring in individual cells, but finally diffusely involving all cells of both layers. This depletion process became very advanced in this series and the animals beyond twenty weeks were in an advanced state of lipoidal depletion, the cells appearing large, pale, and vesicular with a fibrillary network in their cytoplasm. The reticularis remained relatively unaffected by this sequence of events until the terminal stage, when it too showed cellular enlargement with marked vacuolar and granular depletion, but never to the extent of the other layers. Likewise, the transitional zone remained unaltered until the extreme stage of depletion when it was occasionally seen to be absent (Figs. 1 through 6).

No pathologic evidence was found in Group 5 (m'Medab diet plus aqueous suspension intraperitoneally) to indicate that the aqueous suspension exerted any harmful affect. Benign liver changes were common in this group also: Cirrhosis appeared at 3 weeks and was found in all eighteen of the rats after that time; bile duct cysts were seen in 6 rats (32%), benign hepatomas in 9 (47%), fatty metaplasia in 12 (63%), bile duct proliferation in 9 (47%), and biliary adenomas in 2 (11%).

Malignant liver tumors appeared at fourteen weeks and were found thereafter in 6 (50%) of the remaining animals. All of these tumorous livers contained malignant hepatomas. In addition one also

contained a biliary adenocarcinoma. No metastases were found in this group.

Adrenal cortical changes closely paralleled those of the preceding group, though they were less extensive. The adrenal glands of seventeen animals were suitable for study. The one animal showing a significant early increase in adrenal gland weight was found to have extreme hyperemia of that gland associated with hypertrophy of the fasciculata cells . . . . After 4 weeks the glands began to show focal areas of lipoid vacuolar and granular depletion in the glomerulosa and fasciculata zones with swelling of these cells. After the fifteenth week this process became generalized, effecting all the cells of both these layers. The terminal animals showed very marked loss of vacuoles and cytoplasmic granules, the cells being very large and pale-staining, containing only a fine fibrillary network in their cytoplasm (Figs. 1 through 6).

Group 6 received only MCA in the basal diet. The liver changes were insignificant, only 2 animals (11%) showing microscopical fatty metaplasia. No MCA-induced sarcomas developed in this group.

The animals killed in the first 5 weeks showed the familiar evidence of reticularis hyperplasia and a slight loss of the lipoid vacuolarity in the glomerulosa and fasciculata zones, but these findings disappeared promptly. . . . For the remainder of the experiment the adrenal glands appeared relatively normal in the healthy animals. Rats killed because of a poor or moribund condition showed evidences of lipoid granular depletion and loss of the lipoid vacuolarity in the glomerular and fascicular zones, with swelling of these cells (Figs. 1 through 6).

Significant pathology in Group 7 (Basic diet plus MCA subcutaneously) was limited to the site of the MCA injection. The earliest animals killed showed a diffuse mononuclear inflammatory cell infiltration of moderate intensity into the area injected. This was soon followed by fibroblastic proliferation and the appearance of many multinucleated giant cells, the whole granulomatous zone remaining relatively avascular. After the twentieth week multiple foci of malignant degeneration began to appear which soon fused into large sarcomatous masses. Nine animals showed subcutaneous sarcomas which represented 90% of the animals living beyond the twentieth week. All the tumors were predominantly fibrosarcomas, but three also had liposarcomatous elements and two others contained areas of rhabdomyosarcomas.

The adrenal glands of seventeen animals were fit for study and only two animals showed the early changes in the adrenal cortex of hyperemia, hyperplasia of the reticular zone with some lipoid vacuole depletion of the fascicular and glomerular layers. (Fig. 1). Thereafter, the glands in general remained relatively normal microscopically except for an occasional animal which showed the familiar depletion of the fasciculata and glomerulosa zones of their lipoid vacuoles and cytoplasmic granularity, with swelling of the fasciculata cells (Figs. 1 through 6). The transitional zone remained relatively normal throughout the course of the experiment.

Group 8 received MCA intraperitoneally and was fed the basal diet. Only one animal (5%) had microscopical evidence of minimal cirrhosis, and no malignant liver tumors occurred.

As early as 3 hours after the injection the internal organs were covered with a thin film of fibrin sparsely interspersed with

granulocytic cells. This gradually organized in later animals to become a dense layer of fibroblasts infiltrated by many chronic inflammatory cells and multinucleated giant cells, with rich vascularity. Thick adhesions bound the internal viscera tightly to each other. After the thirteenth week multiple sarcomas began to appear in this granulomatous peritoneal reaction. Only four animals lived beyond the thirteenth week but they all contained fibrosarcomas. These tumors spread rapidly throughout the peritoneal cavity by transperitoneal extension and were seen to involve the liver, spleen, stomach, large and small bowel, kidneys, and adrenal glands (Fig. 7). Twenty-four hours after the intraperitoneal injection, the adrenal glands became quite hyperemic, and the lipoid vacuoles began to disappear from the fasciculata and glomerulosa zones. This was accompanied by hyperplasia of the reticular layer. Throughout the remainder of the experiment almost all of the adrenal glands showed various degrees of lipoid vacuolar and cytoplasmic granular depletion of the fascicular and glomerular zones with swelling of these cells. In many of these animals this process reached an extreme degree, with cells appearing very pale with only a fine fibrillary network in the cytoplasm (Figs. 1 through 6). Fat stains were made on sections of adrenal glands from animals killed in the first four days of the experiment. Seven hours after the intraperitoneal injection of MCA a loss of lipoid vacuolarity was detected in the reticular zone. After eleven hours the reticular cells were almost devoid of lipoid vacuoles and the fasciculata cells showed vacuole depletion. At twenty-four and thirty-eight hours both the reticularis and fasciculata zones were almost entirely depleted of their fat vacuoles. By sixty hours after the initial injection the lipoid vacuoles had been

restored to the reticular and fascicular cells in normal concentration. The glomerulosa zone remained apparently unaltered, retaining its rich supply of lipoid vacuoles. The transitional zone remained fat-free in all the sections. Seventeen pairs of adrenal glands were suitable for study in this group.

Group 9 was composed of rats chosen at random from the stock cages on the Laboratory Chow diet to study the normal apperance of the adrenal glands. No significant pathological findings were seen in any of the organs except for a minimal amount of focal chronic pneumonia in a few rats.

It was immediately obvious that there was a definite variation in the microscopical appearance of these glands in the male and in the female. The male glands contain a glomerulosa zone which is made up of small densely-packed cells with a moderate amount of granular cytoplasm. All the cells of this layer contained many small lipoid vacuoles in their cytoplasm. The transitional zone was four to five cells thick, made up of very small darkly-nucleated cells with a minimum of homogenous cytoplasm apparently devoid of granules or lipoid vacuoles. The fasciculata cells were somewhat larger, being about equal in size to the glomerulosa cells, and were alligned as fascicules in orderly fashion extending from the transitional zone to the reticularis zone. The nuclei were pale, containing diffuse chromatin somewhat condensed peripherally and usually only one central nucleolus. The cytoplasm was rather plentiful and was evenly granular but displaced by a variable number of small lipoid droplets which gave the cytoplasm a faint honey-combed appearance. The reticularis cells were smaller and contained darker nuclei. They were arranged in irregular clusters and cords separated by large blood sinuses. The cytoplasm was sparse and homogenous in character, with few apparent lipoid droplets.

The cells of the female adrenal cortex appeared to be significantly larger than those of the male. The glomerulosa cells were more loosely distributed, the nuclei paler, and the cytoplasm was less granular than those of the male. Many large cells were present which contained little cytoplasmic granularity and were engorged with large lipoid vacuoles. Many fat cells were present in the glomerulosa. The transitional zone resembled that of the male. The fasciculata cells were much larger in the female, though also arranged in regular fascicules. The cytoplasm was more profuse, the granularity less pronounced and each cell contained many large and small lipoid vacuoles. Individual fascicular cells were so vacuole-laden that they resembled fat cells. The reticularis of the female resembled that of the male except the cells were somewhat larger, paler, and with fewer lipoid vacuoles (Figs. 9 through 14).

This sex different was not absolute, in that some glands of each sex tended to resemble glands of the opposite sex, but in general this description held true.

Group 10 was examined to determine if there was any difference in the glands of animals of the basic diet. It was found that the glands of this group exactly resembled those of Group 9 (Figs. 9 through 14).

Group 11 was included to observe the changes that occurred in adrenal glands after the administration of Cortisone for eight days. The glands of the 3 male animals were very uniform in appearance. They all showed lipoid vacuole storage in the fasciculata zone, whose cells were all extremely engorged with vacuoles of all sizes, until the normal cytoplasmic granularity was almost entirely replaced. The reticularis, glomerulosa and transitional zones remained relatively unchanged (Fig. 15).

The five females showed similar changes of less severity (Fig. 16).

Other interesting microscopic changes occurred in the adrenal glands which were not confined to the glands of any particular series and did not seem to be influenced by any diet regime of route of injection. Fatty metaplasia was a common occurrence in the glomerulosa and fasciculata of all glands that had undergone extensive granular and lipoid vacuolar depletion of these layers.

Accessory cortical nodules of glomerulosa cells were a common and normal occurrence in all rats regardless of sex. These were subcapsular collections of glomerulosa cells partially separated from the remaining glomerular layer by a band of connective tissue extending inward from the capsule (Fig. 17). These are reported to occur frequently in human adrenal glands also(15). They are most common in children and are thought to become incorporated into the zona glomerulosa during adult life.

Hemorrhage with cystic degeneration in the adrenal cortex was a rare occurrence, present in two animals late in the course of the experiment and associated with malignant tumors and extreme granular and vacuolar depletion of the gland. This phenomenon also occured in one animal four days after the intraperitoneal injection of MCA (Fig. 18).

Focal areas of necrosis in the fascicular zone was found in three animals in different series distributed throughout the course of the experiment (Fig. 19).

Peculiar round homogenous pink-staining intracytoplasmic bodies were seen in the fasciculata cells of six animals without respect to series or duration of the experiment (Fig. 20). The significance of these formations is unknown and have been referred to as "colloid forma-

tion" by Selye who felt that they might represent storage of cortical hormones(16). Miller[17] refers to these bodies as "liposomes" and reports their presence in normal mouse adrenal glands.

Capsular thickening was a common but not invariable accompaniment of extreme vacuolar and granular depletion of the fascicular and glomerular zones. In a few cases the capsule was seen to be actively proliferating into the substance of the glomerulosa zone.

Focal adenomas of fasciculata cells occurred in five rats whose adrenal cortices were undergoing extreme granular and vacuolar depletion. This phenomenon appeared as circumscribed collections of tremendously enlarged fascicular cells with granular cytoplasm and containing a variable number of lipoid vacuoles (Fig. 21).

Postmortem change was microscopically evident in the adrenal glands in a matter of minutes after the death of the animal. This consists of swelling of the cells in all layers, generalized hyperemia, loss of vacuolarity, and replacement of the cytoplasmic granularity with a homogenous pink-staining material. For this reason these glands were considered unfit for study of adrenal cortical changes in this experiment and were discarded.

Myeloid metaplasia was a common occurrence in the liver of all rats including those fed Laboratory Chow. Its incidence was 25%. It also was seen in all spleens in variable amounts. This phenomenon is considered to be a normal occurrence in the livers and spleens of rats.

Focal chronic pneumonia was microscopically evident in 34% of all animals in this experiment. This is a common occurrence in our rat colony. It did not appear to affect the health of the animals except in a few cases in which large abscesses were present. These were asso-

ciated with large areas of exudate and consolidation which caused the death of the animals.

The pituitary glands were exceedingly uniform in all the rats used in this experiment. One gets the impression that there is an occasional alteration of the normal acidophil - basophil cell relationship, but this change did not correlate with any of the adrenal gland changes or any of the drug regimes. It is felt that a paraffin section stained with Hemotoxylin and Eosin is not an accurate method of studying cytological changes in the pituitary gland.

An occasional epithelium-lined cyst filled with homogenous pink staining material was seen in the pars intermedialis.

Testicular atrophy occurred in 22% of all males fed the Basal diet. This consists of a loss of spermatozoa from the lumen of the seminiferous tubules. Subsequently the germinal epithelium degenerates and desquamates into the lumen of the tubules. Large multinuclear giant cells then appear in the tubules. Lastly, the Sertoli cells disappear from the walls of the tubules leaving them collapsed and empty. The Leydig cell clusters appear to be unaffected by this process (Fig. 22). There was no significant alteration noted in the ovaries of any of the females in this experiment.

DISCUSSION

The adrenal glands of all series underwent transient increases in weight early in the course of the experiment. This was produced by hyperplasia of the reticular zone, hyperemia of the entire gland, and some depletion of the lipoid vacuoles in the fascicular and glomerular

zones. This was interpreted as representing stimulation of the gland with hypersecretion and depletion of its hormonal stores. This was easy to understand in those groups receiving subcutaneous and intraperitoneal injections which might act as a stress to the animal. But it was difficult to explain in those animals which were only placed on a different diet. Perhaps just a change in diet and the handling necessary to place these animals in new and strange surroundings with the fear that they perhaps feel might constitute a stimulus to the adrenal glands. The results of this experiment show that the adrenal glands in rats are very labile and may be undergoing almost continual morphologic changes. The early increase in weight of the glands and the associated histologic changes after various types of stress have been noted by many experimenters(18, 19, 20, 21, 22).

After the initial rise in adrenal gland weights and their return to normal the adrenal weights tend to fall into three separate patterns. Control animals receiving the liver carcinogen orally and just the aqueous suspension by injection (Groups 4 and 5) represent animals in which liver carcinogenesis was allowed to proceed unchecked, since the aqueous suspension was found to be innocuous. The adrenal glands became progressively lighter throughout the course of the experiment. Histologically, these glands showed a progressive loss of vacuolarity and intracytoplasmic granularity of the glomerulosa, fasciculata, and finally the reticularis zones. Finally the cells appeared swollen, pale, with only a fine fibrillary network in the cytoplasm. These changes correlated more closely with the state of health of the animals and the pathological lesions they contained rather than to the length of time they were maintained on the carcinogenic diet. These adrenal gland

changes have been noted by other authors in animals undergoing experimental carcinogenesis[2, 23]. Sarason[3] has studied the glands of thirteen humans dying of cancers and reports similar changes. Many explainations have been proposed for this change. Dalton[2] suggested that it might represent a response of the gland to release of toxic substances from large amounts of necrotic tumor tissue. Selye[16] proposed that it might represent exhaustion of the gland from continuous stress exerted on the body by the neoplasm. Sarason[3] suggests that it might represent a response to infection of the neoplastic tissue. But all authors agree that it accompanies the general cachexia of the animal and could be a result of an initial stimulation of the adrenal cortex being maintained and leading to eventual cortical exhaustion. Begg[24] theorises that the clinical state of malignant cachexia may be due to hypofunction of the adrenal cortex.

Another type of adrenal weight curve and microscopic finding occurred in animals fed the Basal diet and receiving MCA in the food or subcutaneously (Groups 6 and 7). After the initial increase in adrenal weights there was a prompt return to normal which was maintained throughout the remaining experiment. Histologic study of the glands showed that there was a minimal amount of lipoid depletion in the cortex. This is understandable since the majority of these animals remained in relatively good health. Even the animals that developed subcutaneous sarcomas in Group 7 remained in fair health since the tumors remained rather localized to the subcutaneous tissue and did not invade any vital structures.

Group 1 (Wesdab + MCA diet) represented a third type of adrenal response. After the initial increase in weight of the adrenal glands,

they promptly returned to normal until the seventeenth week when a gradual increase in weight occurred up to the forty-eighth week. Microscopically, the glands showed marked lipoid depletion of all layers after the seventeenth week comparable to animals suffering from marked cachexia, yet these animals had maintained their body weight and the great majority of them appeared in quite good health when killed. Only one tumor occurred in these animals, demonstrating almost complete inhibition of liver cancers, and cirrhosis was never more than moderate.

Groups 2 and 3 (m'Medab diet plus MCA subcutaneously and intraperitoneally) also demonstrated inhibition of liver cancer, but these animals also exhibited cirrhosis or sarcomas and many suffered from marked cachexia, so that the lipoid depletion seen in the adrenal glands could be due to these changes rather than the tumor inhibition.

An occasional moribund animal was found to have very heavy adrenal glands. Microscopic examination revealed these glands to be loaded with lipoid droplets, filling cells of all layers. This change resembles that seen in adrenal glands after the administration of Cortisone, except it was generalised and not confined to the fasciculata layer as it was in Group 11. Vogt[20] has noted the same phenomenon in the adrenal cortex during various stresses and he believes that if a stress is severe enough or sufficiently prolonged the usual lipoid depletion may be replaced by lipoid storage. There was no apparent correlation with any of the drug regimes. These glands account for the few exceedingly heavy adrenal glands on the graphs depicting percentage of adrenal gland weights to body weights.

It was of interest to note that there was a sex variation in normal rat adrenal glands, reflected both in the weights of the

glands and their histologic appearance. Throughout the experiment the female glands tended to remain heavier than the males, although the weight changes are in the same direction and of approximately the same magnitude. After the male glands had undergone some swelling and intracytoplasmic granular depletion in the fascicular and glomerular zones they more closely resembled female glands and thereafter the sex difference between the adrenal glands became indistinguishable.

A recent report in the literature indicates that there is a sex difference in the susceptability of rats to m'Medab-induced liver cancers, males tending to develop tumors more readily than females(25). This is supported by the greater frequency of liver cancers in human males than in females(26). Results from this experiment were analyzed with this possibility in mind and it was found that there was no significant sex variation in the development of benign or malignant liver changes, MCA sarcomas, or in the inhibition of liver cancers. It has already been pointed out that equivalent microscopic changes occur in both the male and female adrenal glands and with depletion the glands eventually become indistinguishable. This lack of sex variation may be due to the high doses of drugs used in this experiment or to their continued use in the diet.

Some authors refer to the extreme lipoid depletion and loss in weight of the adrenal glands seen in cachectic animals as "atrophy"(16). The term has been avoided in this paper since it is felt that this change does not represent atrophy in the true pathological sense of the word. By definition, atrophy is "a decrease in size due to fewer elements or smaller elements or both."(26). The appearance of these glands did not fill these criteria. On the contrary, the cells were

seen to swell in size as lipoid depletion progressed. And in no case was a decrease in the number of cells demonstrated. If one may be excused for using a physiological term for a pathological entity, perhaps "exhaustion" might be a more accurate description of these glands. The only true atrophy seen in adrenal glands by this investigator occurred following hypophysectomy, though there is no doubt that it occurs after other conditions. The cells of all layers become very small, with very small dark nuclei and sparse cytoplasm devoid of any granularity or vacuolarity.

Selye reports[16] that hyperplasia occurs in the adrenal cortex following any non-specific stress, with the appearance of many mitotic figures, binucleated cells, and associated with hypertrophy of the cells. This experiment failed to reveal any mitotic figures in any of the layers during conditions which certainly could be classified as stress. Hyperplasia occurred in the reticular zone in all animals at the onset of the experiment, with evidence of increased cellularity and thickening of this layer with enchroachment and partial obliteration of the normally widely-dilated blood sinuses. Many binucleated cells were seen, yet mitotic figures were absent.

The incidence of testicular atrophy in male rats fed the Basal semisynthetic diet was very high, and in one case was evident eleven hours after being placed on the diet. In contrast, no alteration in ovarian appearance was noted. This atrophy could not be correlated with any adrenal cortical changes or with any of the drug regimes. It is known[27] that vitamin E is necessary for functional integrity of the testes and that atrophy occurs in its absence. Lack of vitamin E may account for the testicular atrophy occurring in this

experiment. Perhaps the ovaries do not depend on vitamin E for their function.

The phenomenon referred to as nephrosclerosis occurred only in animals that received MCA intraperitoneally and thus must have resulted from a toxic action of MCA given by this route on the kidney, since MCA administered by any other route did not produce this change. It occurred as early as one week after the administration of the drug. It is not felt that this influenced the mortality of the animals because it was only a focal phenomenon and many glomerulae were spared and apparently normal.

There was a significant difference in the induction times of subcutaneous and intraperitoneal sarcomas. The subcutaneous tumors were first seen at twenty weeks while the intraperitoneal sarcomas appeared at thirteen weeks. This correlated well with the increased severity of the peritoneal response and the greater vascularity of the peritoneal granulomas compared to the subcutaneous reaction. It is felt that the induction time of MCA-induced sarcomas is inversely proportional to the degree of tissue response to the irritant.

CONCLUSIONS

The adrenal glands of Sprague-Dawley rats were studied during the induction of liver cancer by 3-Methyl-4-dimethylaminoazobenzene and during the inhibition of liver carcinogenesis by 20-Methylcholanthrene.

The adrenal glands of all animals underwent a transient early increase in weight with histologic changes thought to represent stimulation of the glands with hypersecretion and depletion of their hormonal

stores. Thereafter, the glands fell into three separate patterns:

1. The adrenal glands of rats developing liver cancers progressively decreased in weight. This was manifested histologically by depletion of lipoid vacuolarity and cytoplasmic granularity with cellular hypertrophy, occurring mainly in the glomerular and fascicular zones. This change was thought to represent adrenal cortical exhaustion from long-continued stimulation and might have contributed to the marked cachexia that occurred in these animals.

2. The adrenal glands of rats receiving MCA subcutaneously microscopically showed only a minimal amount of lipoid depletion and cellular hypertrophy. These animals developed subcutaneous fibrosarcomas which remained rather localized, did not invade vital structures, and produced little emaciation.

3. The adrenal glands of rats receiving both carcinogens orally gradually increased in weight late in the experiment. Histologically these glands showed marked lipoid depletion and cellular hypertrophy indistinguishable from that seen in cachectic animals with liver cancers. Yet these rats developed no tumors and remained in good health throughout the experiment. This suggests that cancer inhibition might constitute a chronic stimulation leading to adrenal cortical exhaustion.

There was found to be a sex variation in normal rat adrenal glands, both in weight and histological appearance. After lipoidal depletion and cellular hypertrophy the glands of the two sexes became microscopically indistinguishable, but the female glands remained heavier than those of the males throughout the experiment.

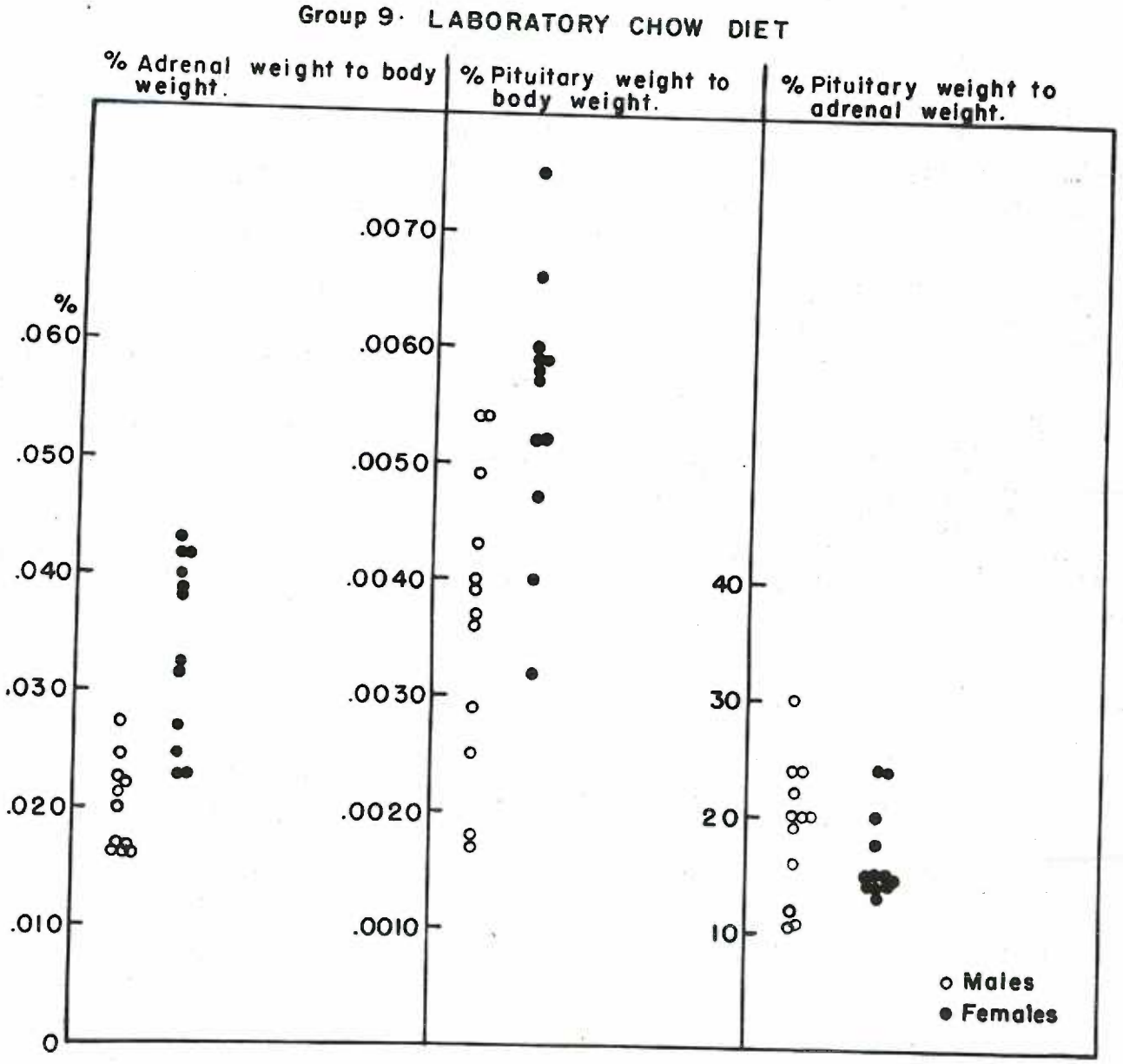
Study of adrenal glands which have undergone even minimal postmortem change is a fruitless occupation in that no insight can be gained

as to their morphological or functional state during life.

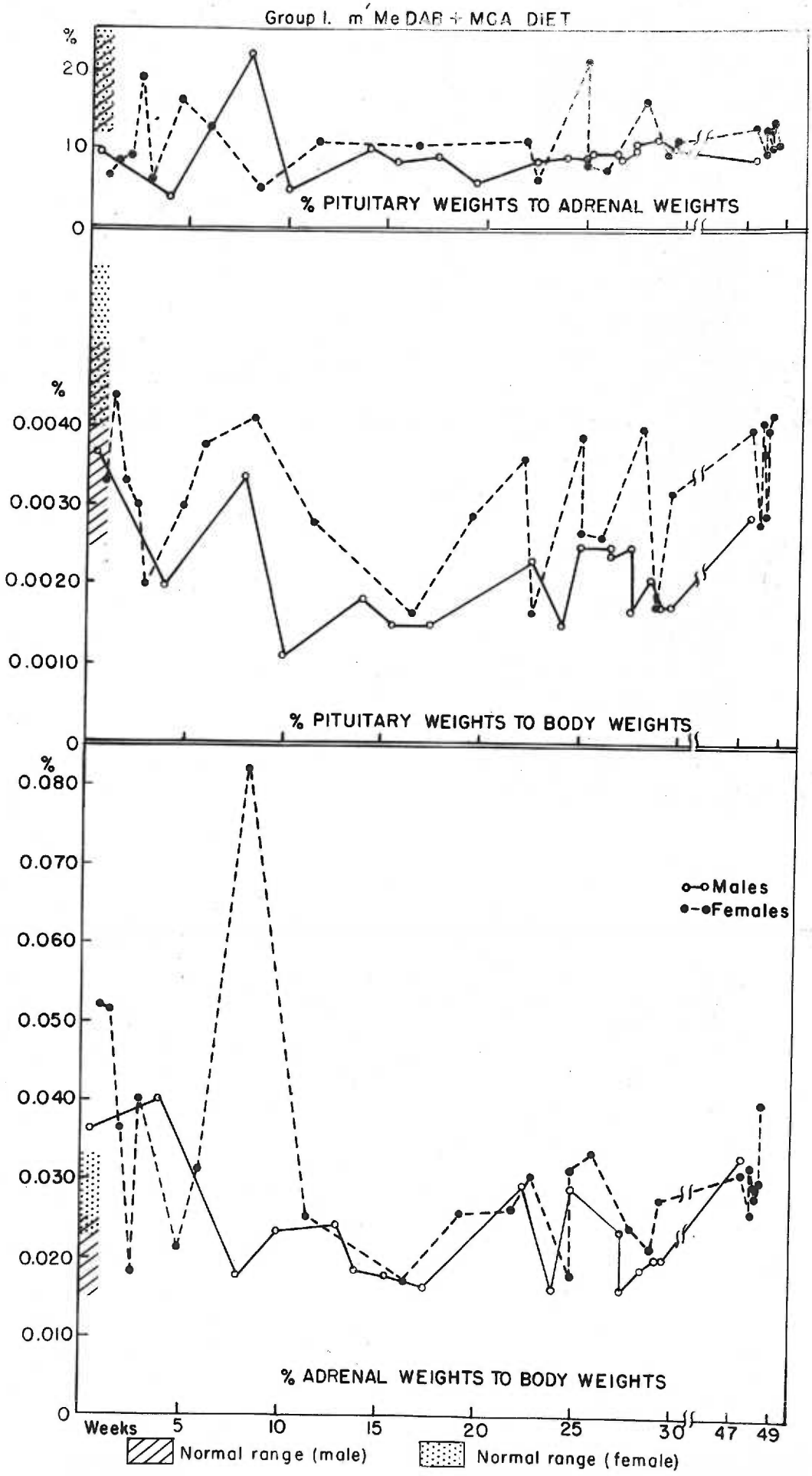
Immediate fixation of the fresh tissue is essential.

Routine Hematoxyline and Eosin stained sections give a very poor insight into the functional state of the adrenal cortex. Fat stains show that radical changes in the lipoid content of the glands is attended by only minimal changes in the routinely stained sections. Therefore, it is recommended that fat stains be included in any histologic study of the adrenal cortex.

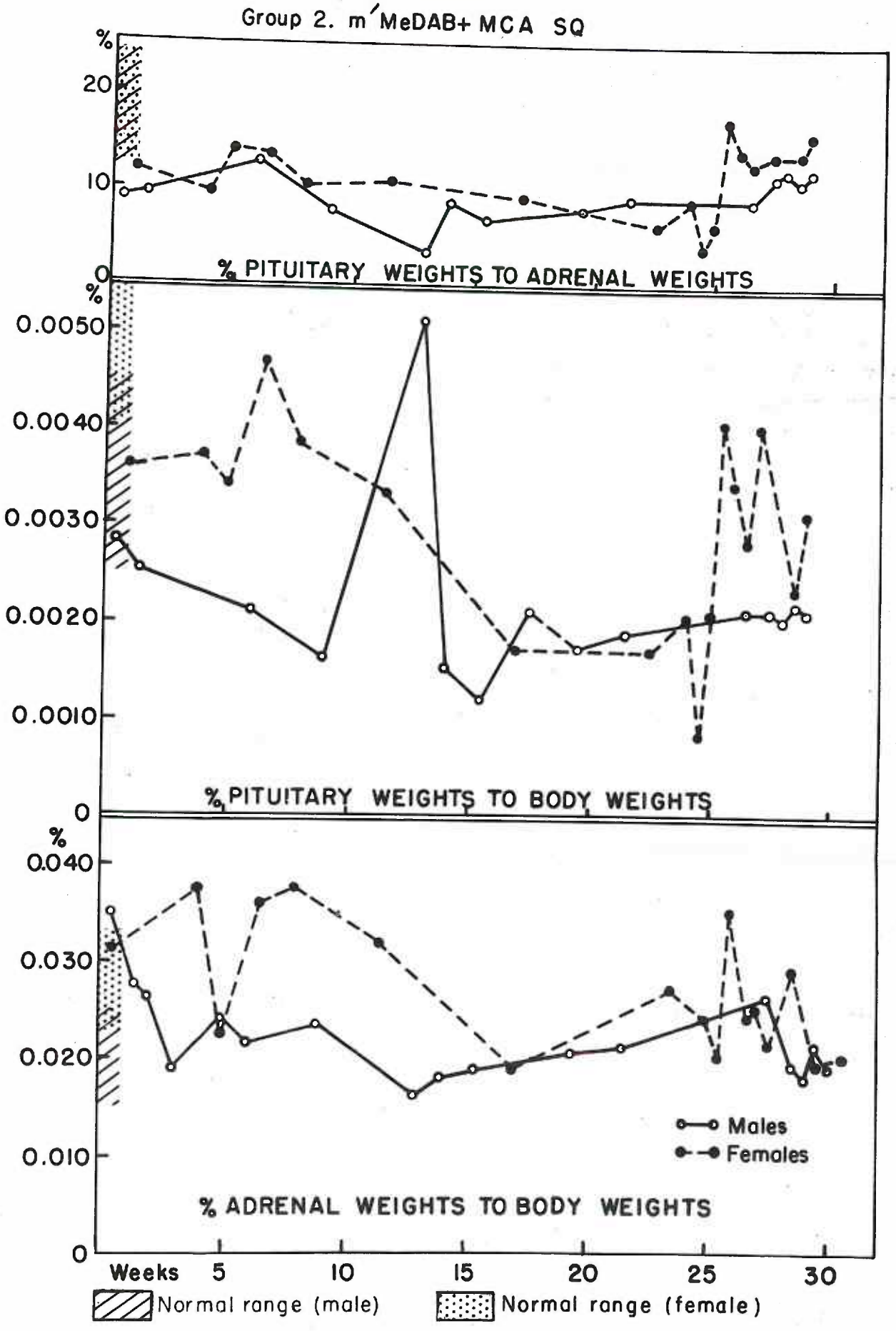
Graph I. Group 9. (Laboratory chow diet)


Group 9· LABORATORY CHOW DIET
% Adrenal weight to body weight.
% Pituitary weight to body weight.
% Pituitary weight to adrenal weight.
%
.060
.050
.040
.030
.020
.010
0
.0070
.0060
.0050
.0040
.0030
.0020
.0010
40
30
20
10
Males
Females

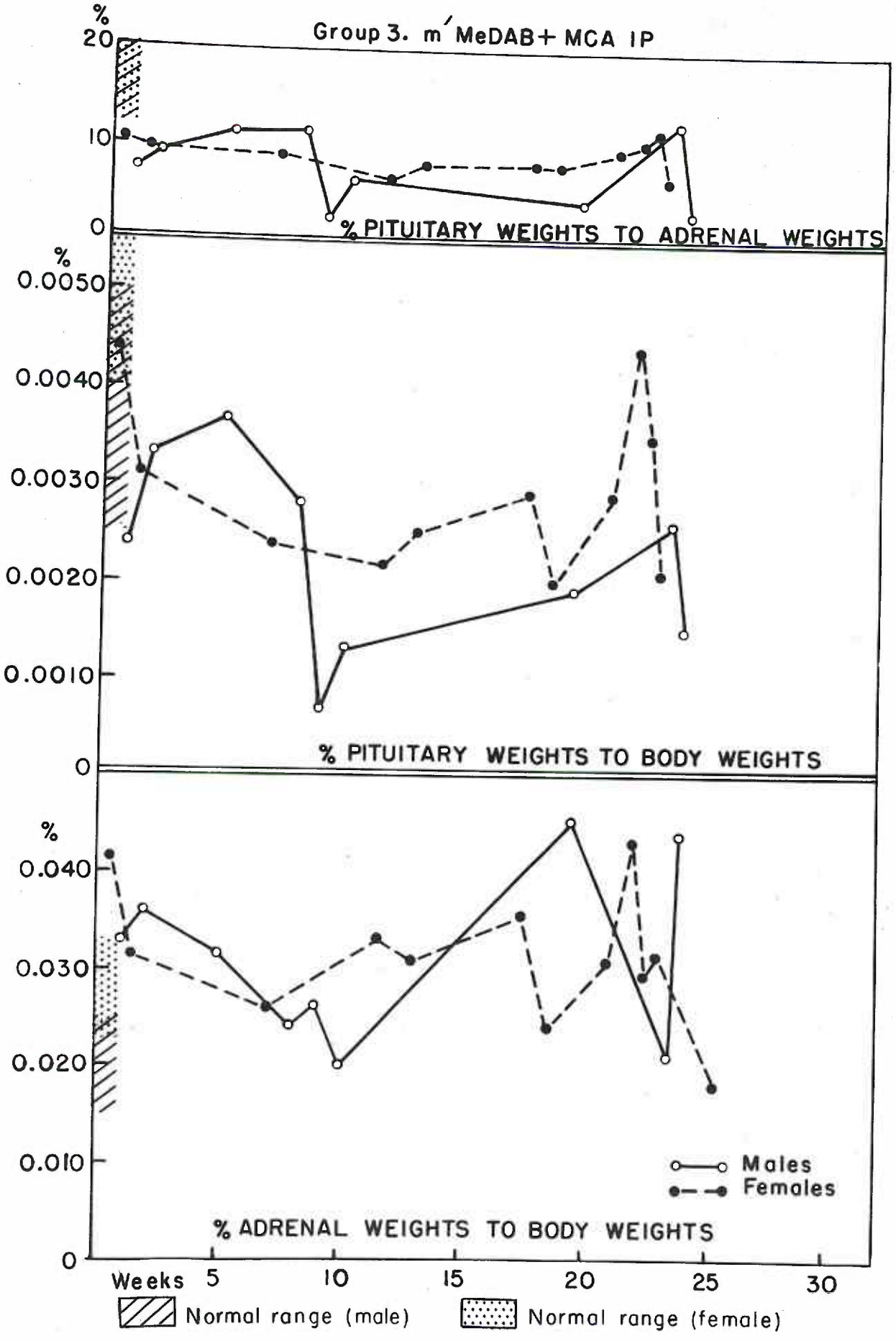
Graph II. Group 1. (m'Medab plus MCA diet)


Group I. m'Me DAB + MCA DIET
%
20
10
0
% PITUITARY WEIGHTS TO ADRENAL WEIGHTS
%
0.0040
0.0030
0.0020
0.0010
0
% PITUITARY WEIGHTS TO BODY WEIGHTS
%
0.080
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0
Males
Females
% ADRENAL WEIGHTS TO BODY WEIGHTS
Weeks
5
10
15
20
25
30
47
49
Normal range (male)
Normal range (female)

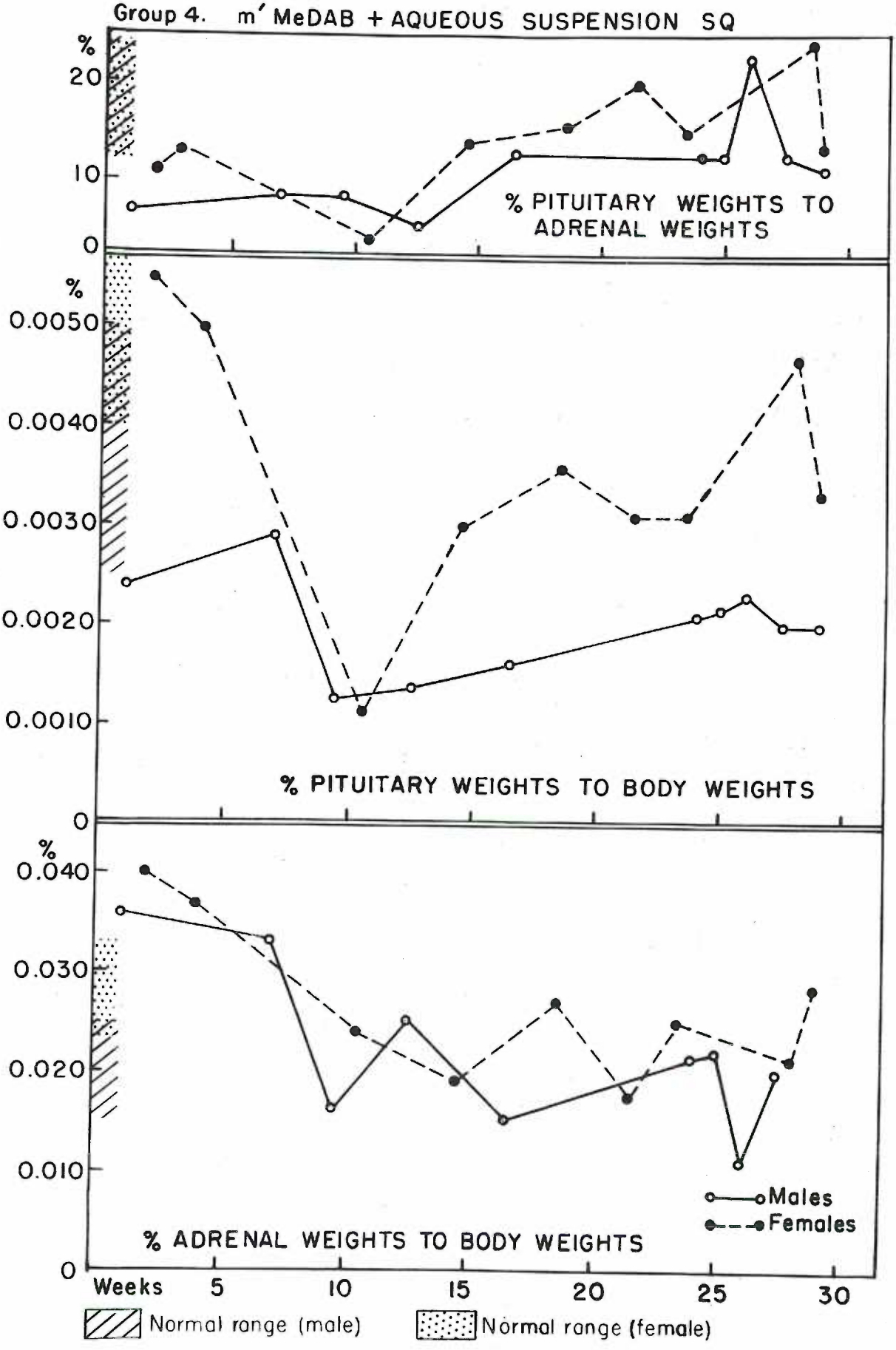
Graph III. Group 2. (m'Medab diet plus MCA subcutaneously)


Group 2. m'MeDAB+ MCA SQ
%
20
10
0
% PITUITARY WEIGHTS TO ADRENAL WEIGHTS
%
0.0050
0.0040
0.0030
0.0020
0.0010
0
% PITUITARY WEIGHTS TO BODY WEIGHTS
%
0.040
0.030
0.020
0.010
0
Males
Females
% ADRENAL WEIGHTS TO BODY WEIGHTS
Weeks 5 10 15 20 25 30
Normal range (male)
Normal range (female)

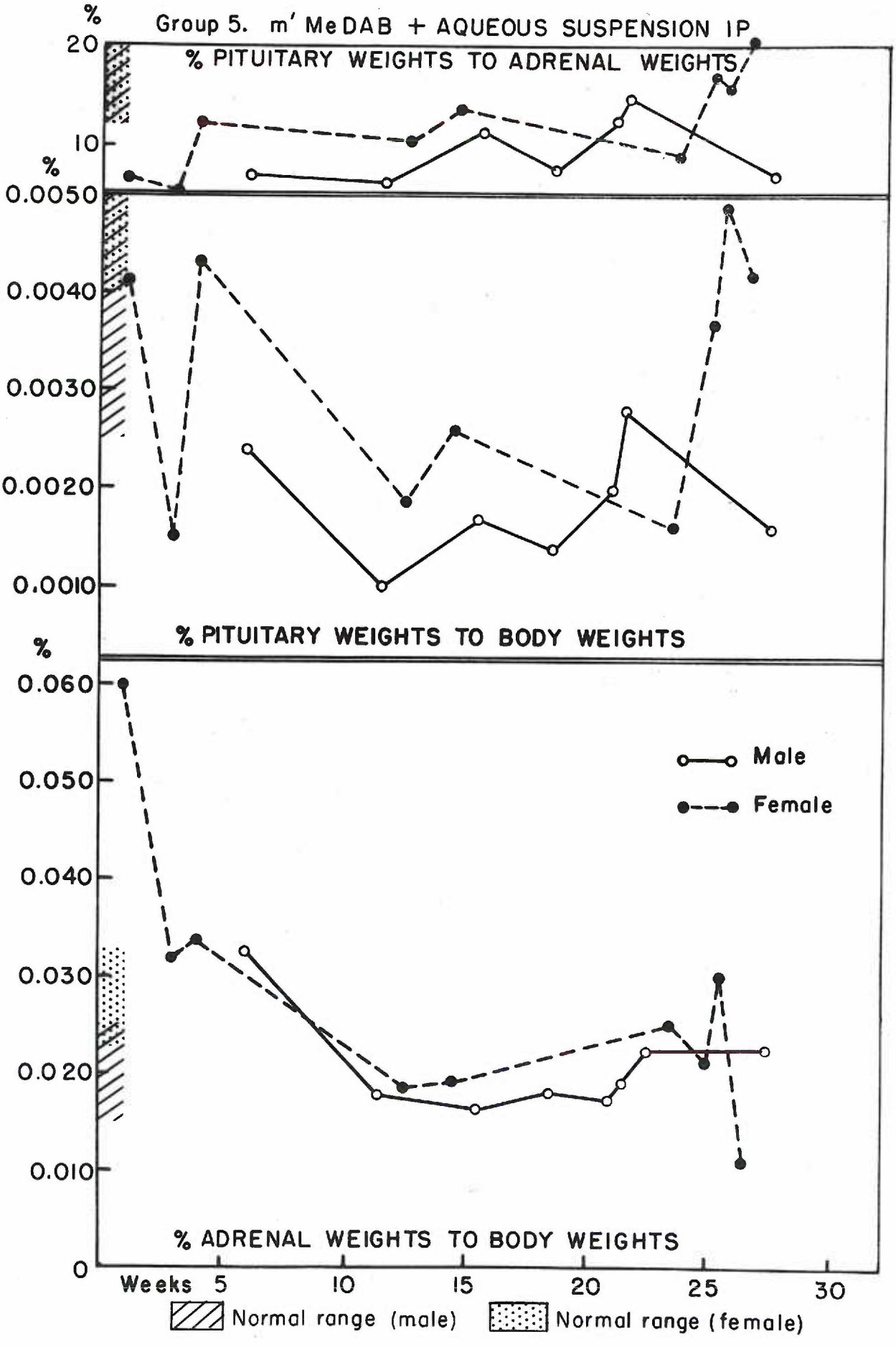
Graph IV. Group 3. (m'Madab diet plus MCA intraperitoneally)


Group 3. m´MeDAB+ MCA IP
%
20
10
0
% PITUITARY WEIGHTS TO ADRENAL WEIGHTS
%
0.0050
0.0040
0.0030
0.0020
0.0010
0
% PITUITARY WEIGHTS TO BODY WEIGHTS
%
0.040
0.030
0.020
0.010
0
Males
Females
% ADRENAL WEIGHTS TO BODY WEIGHTS
Weeks 5 10 15 20 25 30
Normal range (male)
Normal range (female)

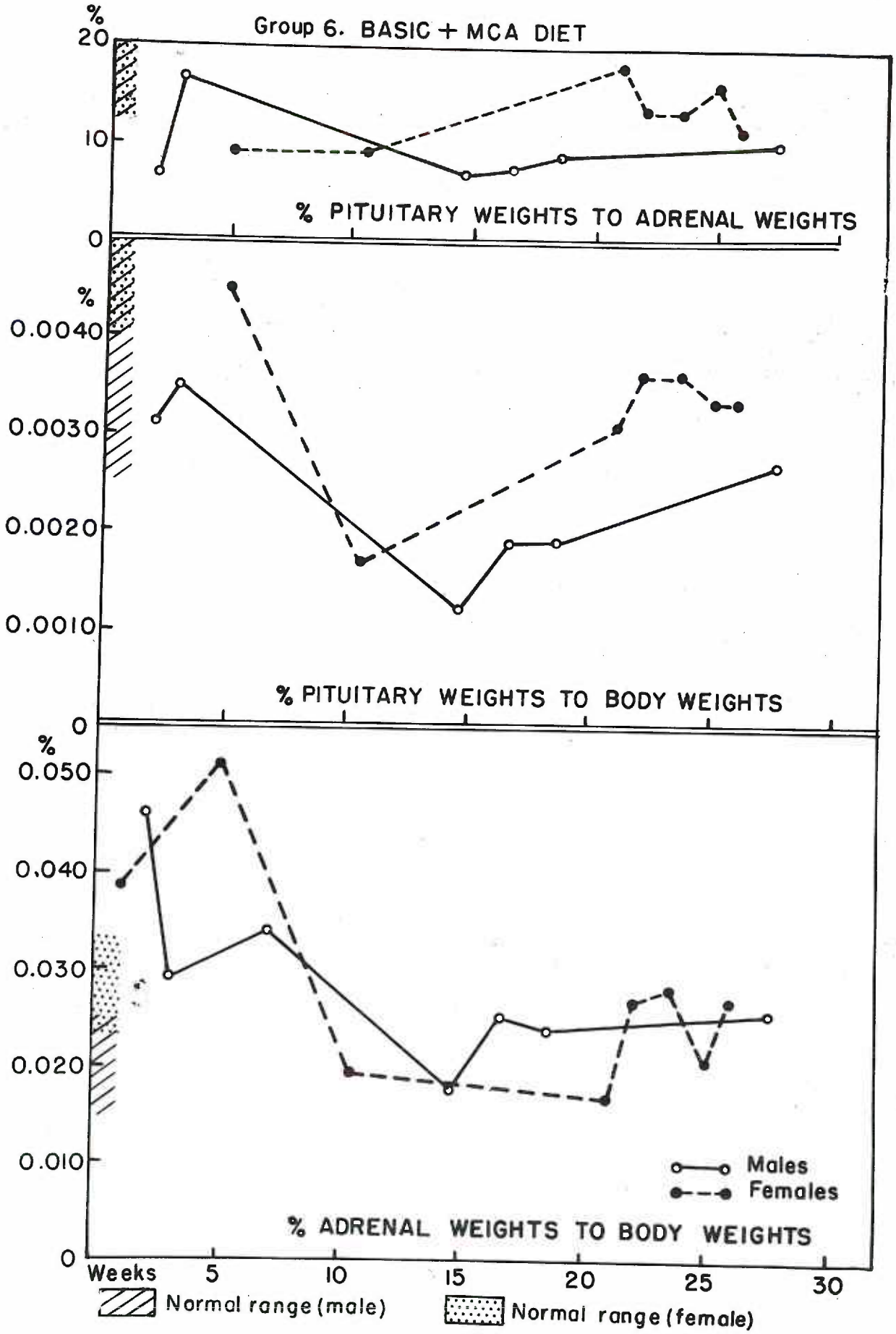
Graph V. Group 4. (m'Medab diet plus aqueous suspension subcutaneously)


Group 4. m' MeDAB + AQUEOUS SUSPENSION SQ
%
20
10
0
% PITUITARY WEIGHTS TO ADRENAL WEIGHTS
%
0.0050
0.0040
0.0030
0.0020
0.0010
0
% PITUITARY WEIGHTS TO BODY WEIGHTS
%
0.040
0.030
0.020
0.010
0
Males
Females
% ADRENAL WEIGHTS TO BODY WEIGHTS
Weeks
5
10
15
20
25
30
Normal range (male)
Normal range (female)

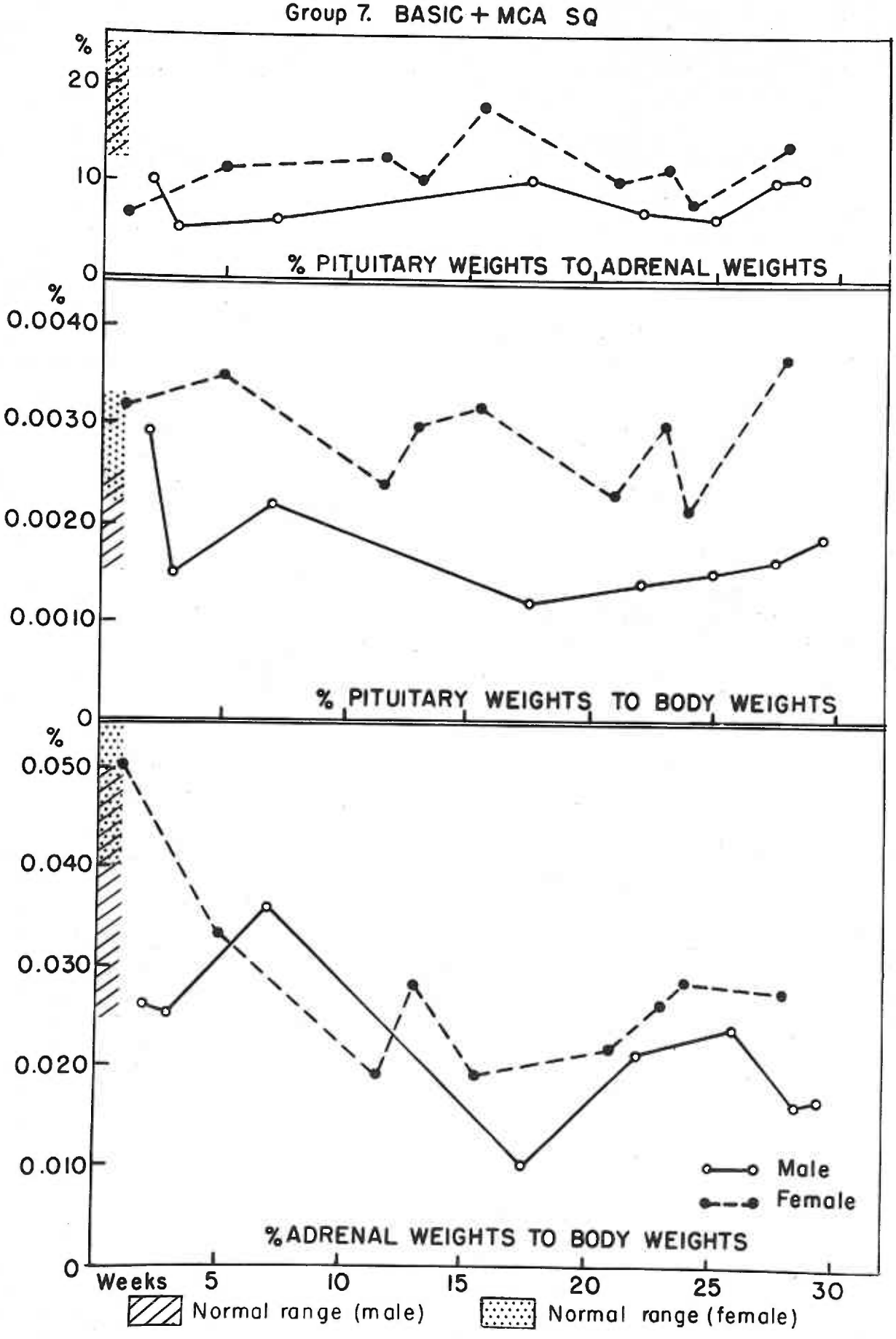
Graph VI. Group 5. (m'Medab diet plus aqueous suspension intraperitoneally)


Group 5. m' MeDAB + AQUEOUS SUSPENSION IP
% PITUITARY WEIGHTS TO ADRENAL WEIGHTS
%
20
10
%
0.0050
0.0040
0.0030
0.0020
0.0010
% PITUITARY WEIGHTS TO BODY WEIGHTS
%
0.060
0.050
0.040
0.030
0.020
0.010
0
Male
Female
% ADRENAL WEIGHTS TO BODY WEIGHTS
Weeks 5 10 15 20 25 30
Normal range (male)
Normal range (female)

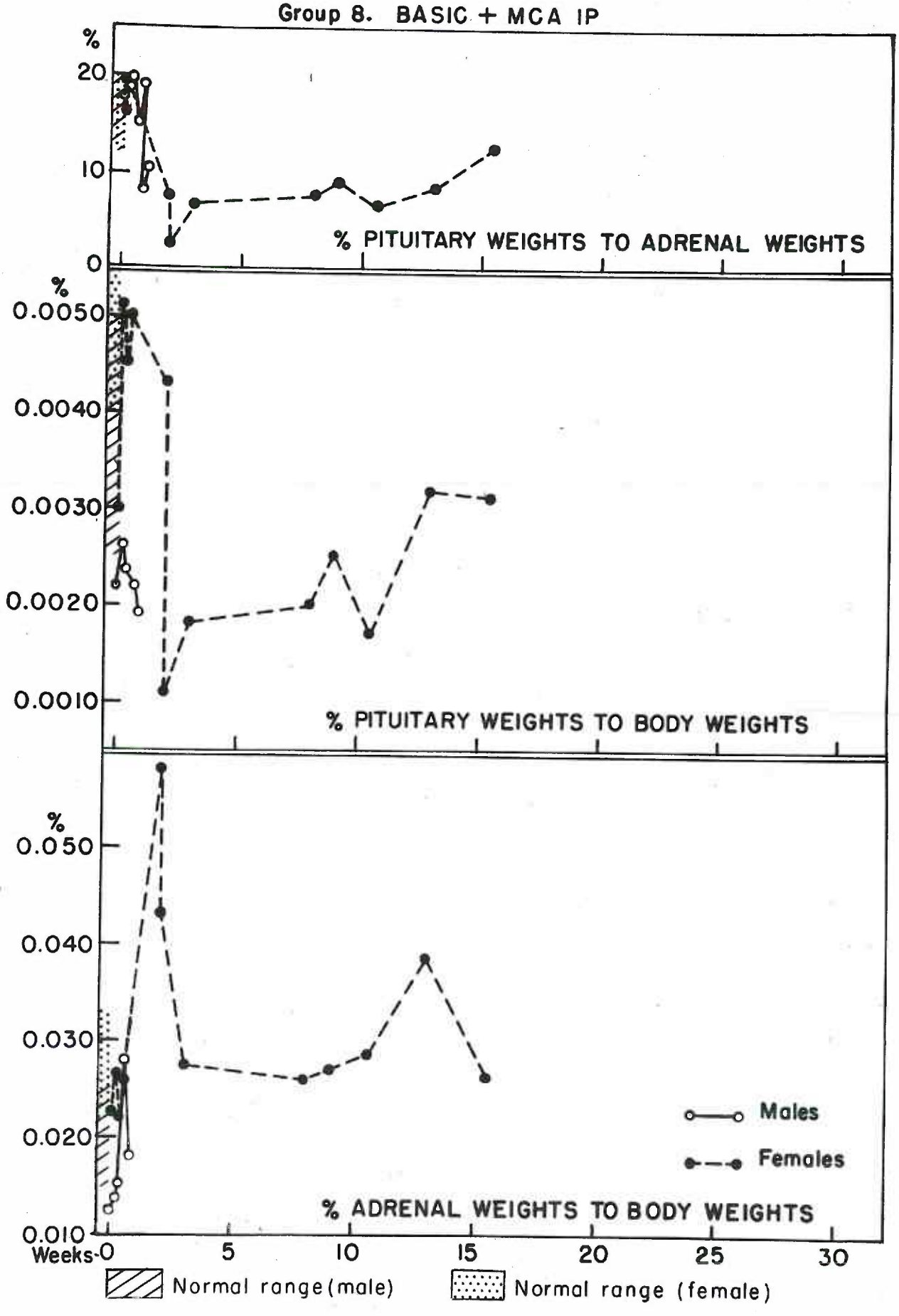
Graph VII. Group 6. (Basal plus MGA diet)


Group 6. BASIC + MCA DIET
%
20
10
0
% PITUITARY WEIGHTS TO ADRENAL WEIGHTS
%
0.0040
0.0030
0.0020
0.0010
0
% PITUITARY WEIGHTS TO BODY WEIGHTS
%
0.050
0.040
0.030
0.020
0.010
0
Males
Females
% ADRENAL WEIGHTS TO BODY WEIGHTS
Weeks
5
10
15
20
25
30
Normal range (male)
Normal range (female)

Graph VIII. Group 7. (Basal diet plus MCA subcutaneously)


Group 7. BASIC + MCA SQ
%
20
10
0
% PITUITARY WEIGHTS TO ADRENAL WEIGHTS
%
0.0040
0.0030
0.0020
0.0010
0
% PITUITARY WEIGHTS TO BODY WEIGHTS
%
0.050
0.040
0.030
0.020
0.010
0
Male
Female
%ADRENAL WEIGHTS TO BODY WEIGHTS
Weeks 5 10 15 20 25 30
Normal range (male)
Normal range (female)

Graph IX. Group 8. (Basal diet plus MCA intraperitoneally)


Group 8. BASIC + MCA IP
%
20
10
0
% PITUITARY WEIGHTS TO ADRENAL WEIGHTS
%
0.0050
0.0040
0.0030
0.0020
0.0010
% PITUITARY WEIGHTS TO BODY WEIGHTS
%
0.050
0.040
0.030
0.020
0.010
% ADRENAL WEIGHTS TO BODY WEIGHTS
Males
Females
Weeks 0
5
10
15
20
25
30
Normal range (male)
Normal range (female)

Graph X. Group 11. (Laboratory chow diet plus Cortisone subcutaneously)

Group II. LABORATORY CHOW DIET, CORTISONE SQ

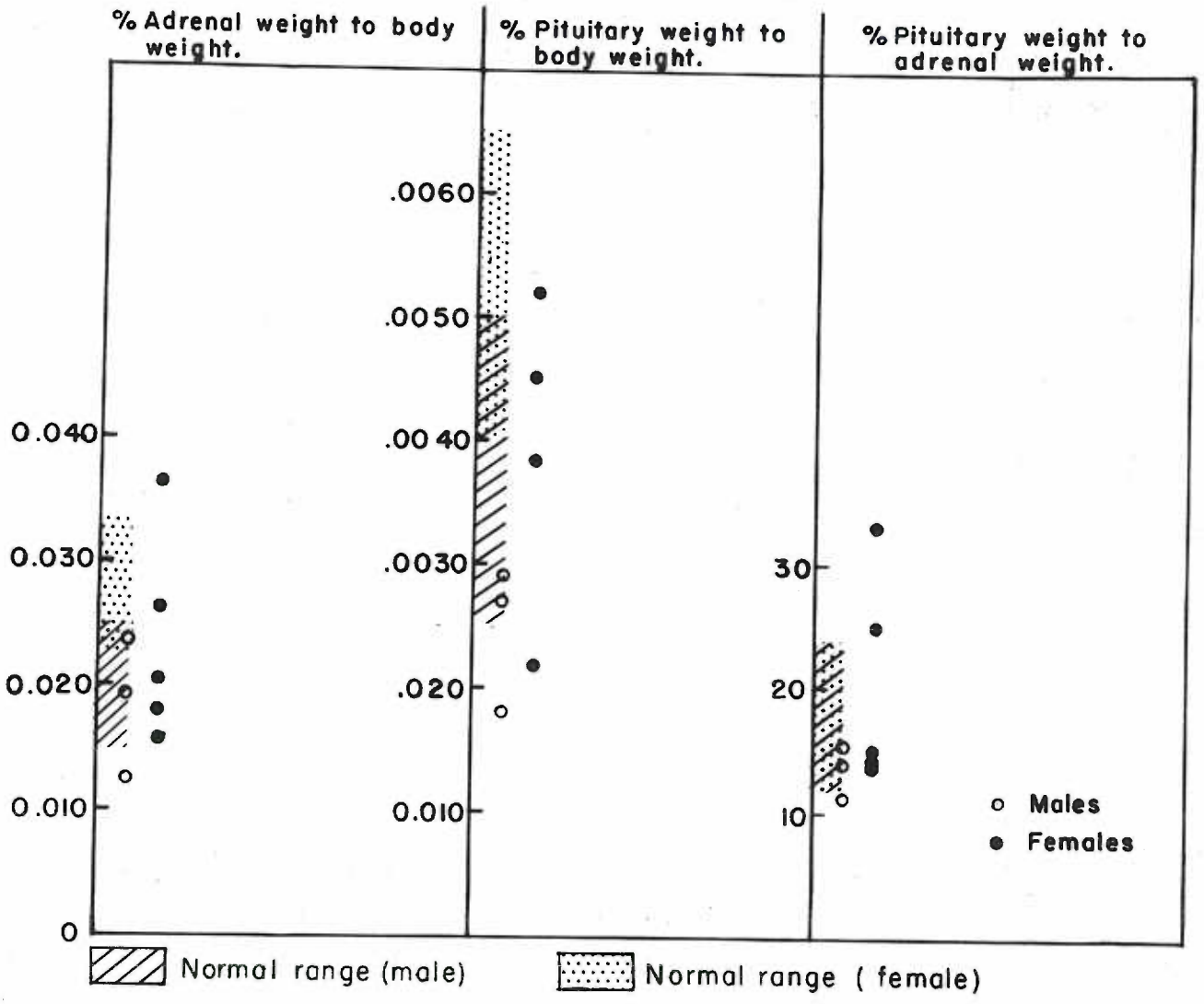
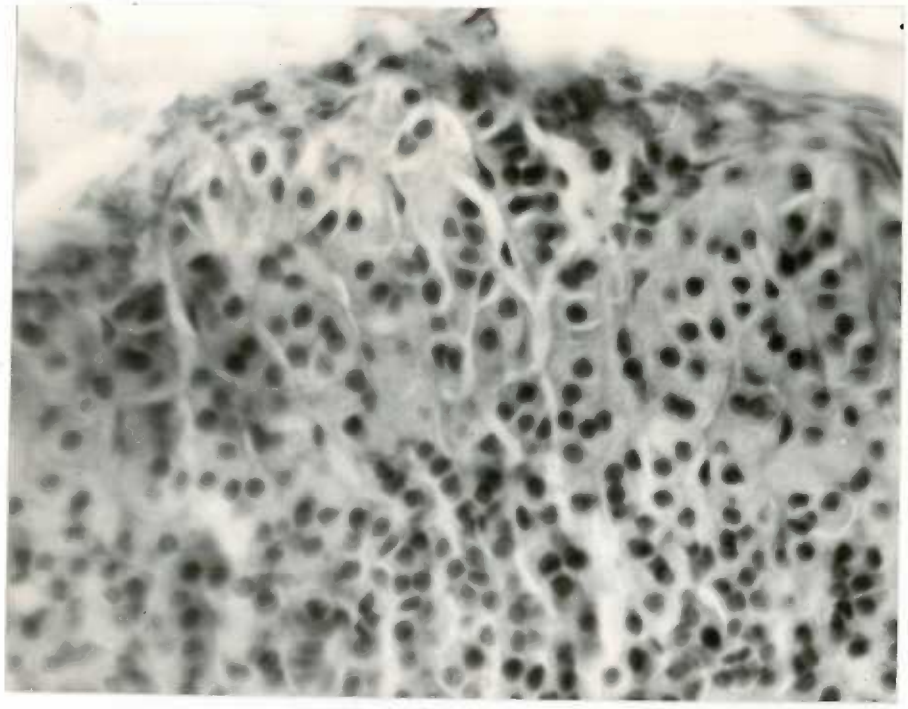


Figure 1. Hypertrophy, cytoplasmic granular depletion of the zona glomerulosa. 475x

The process is confined to this zone, the fascicular cells remain normal in cell size and cytoplasmic content.

Figure 2. Focal hypertrophy, cytoplasmic granular and vacuolar depletion in cells of the zona glomerulosa and fasciculata. 200x

The process is somewhat more advanced in the zona glomerulosa. The transitional zone is unaltered. Compare with the normal adrenal cortex in Figures 11 and 12.



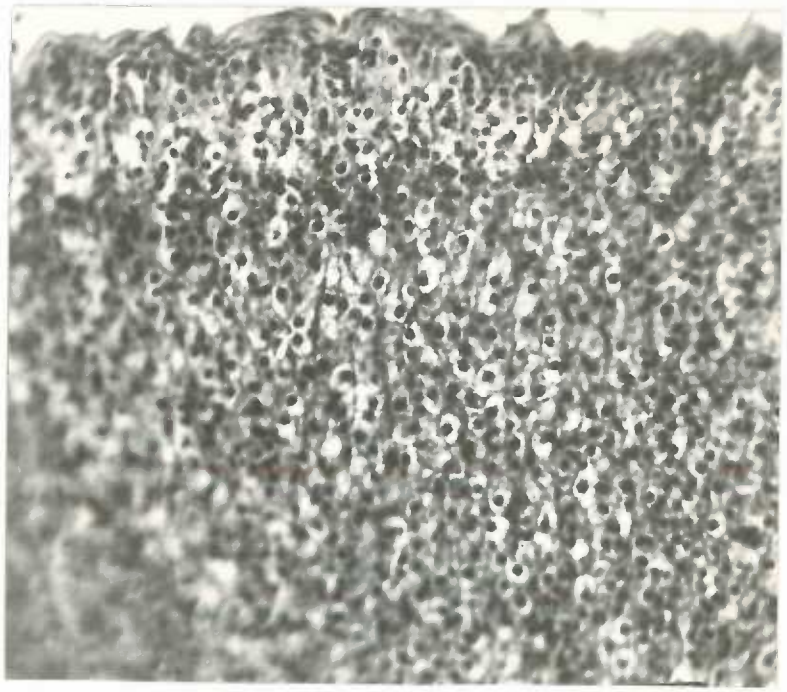


Figure 3. Focal hypertrophy, cytoplasmic granular and vacuolar depletion in cells of the zona fasciculata. 475x

Many unaffected cells are seen which are smaller in size and contain a normal amount of cytoplasmic granularity and vacuolarity. Notice the unaltered transitional zone at the upper edge of the picture. Compre with the normal adrenal cortex in Figures 13 and 14.

Figure 4. Generalised hypertrophy, cytoplasmic granular and vacuolar depletion in cells of the zona glomerulosa and fasciculata. 100x

The process is more advanced in the zone glomerulosa, while the transitional zone remains unaffected. Compare with the normal adrenal cortex in Figures 9 and 10.

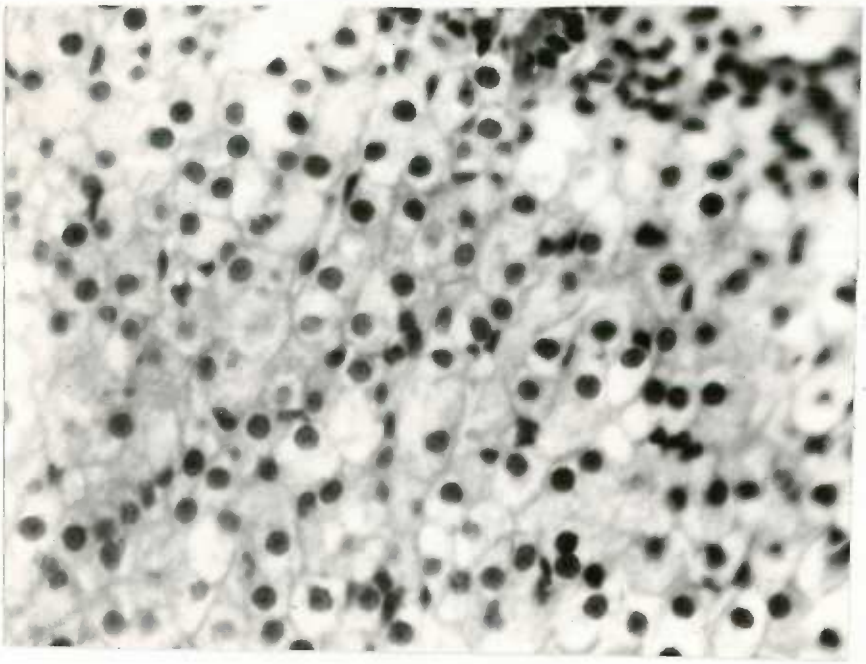
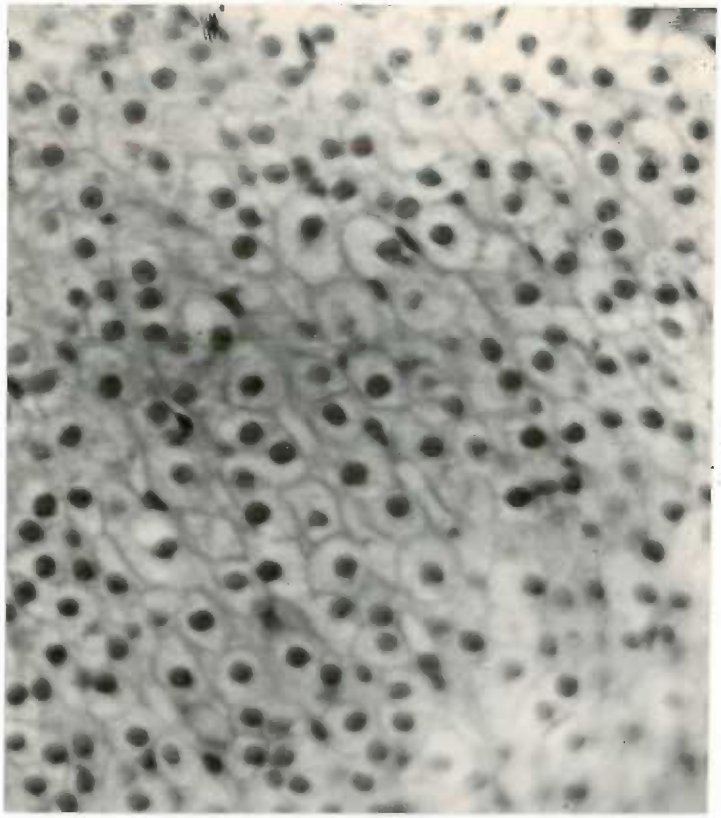


Figure 5. Generalised hypertrophy, cytoplasmic granular and vacuolar depletion in cells of the zona fasciculata. 475x

All cells are affected though the severity of the change varies between cells. Compare with the normal adrenal cortex in Figures 13 and 14.

Figure 6. Extreme generalised hypertrophy, cytoplasmic granular and vacuolar depletion in cells of the zona fasciculata. 475x

The cytoplasm of the cells contains only a very fine fibrillary network giving the cells a faint honeycomb appearance. Compare with the normal adrenal cortex in Figure 13 and 14.



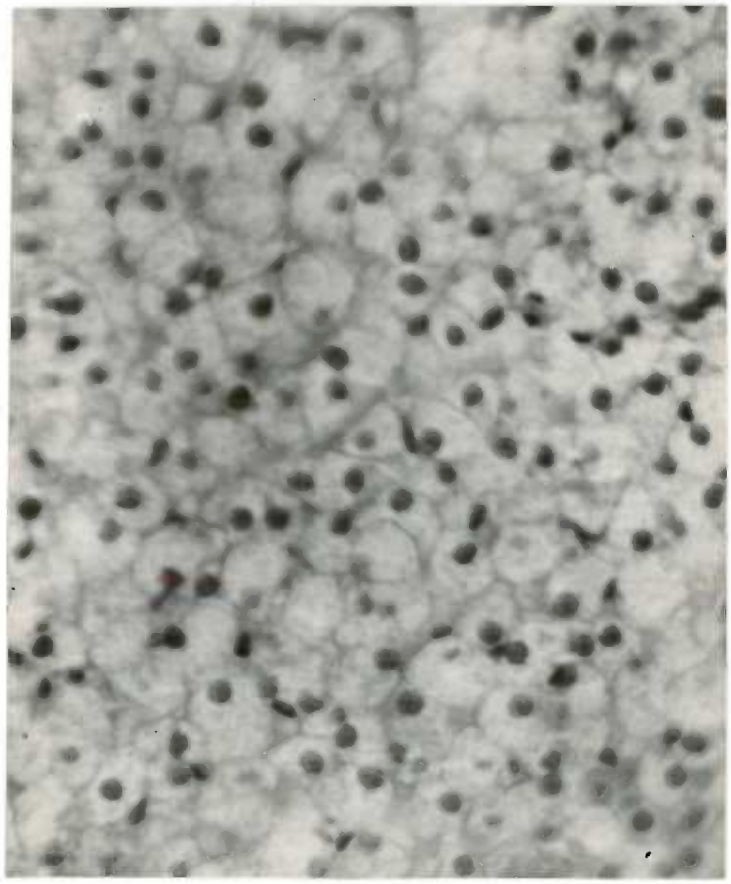
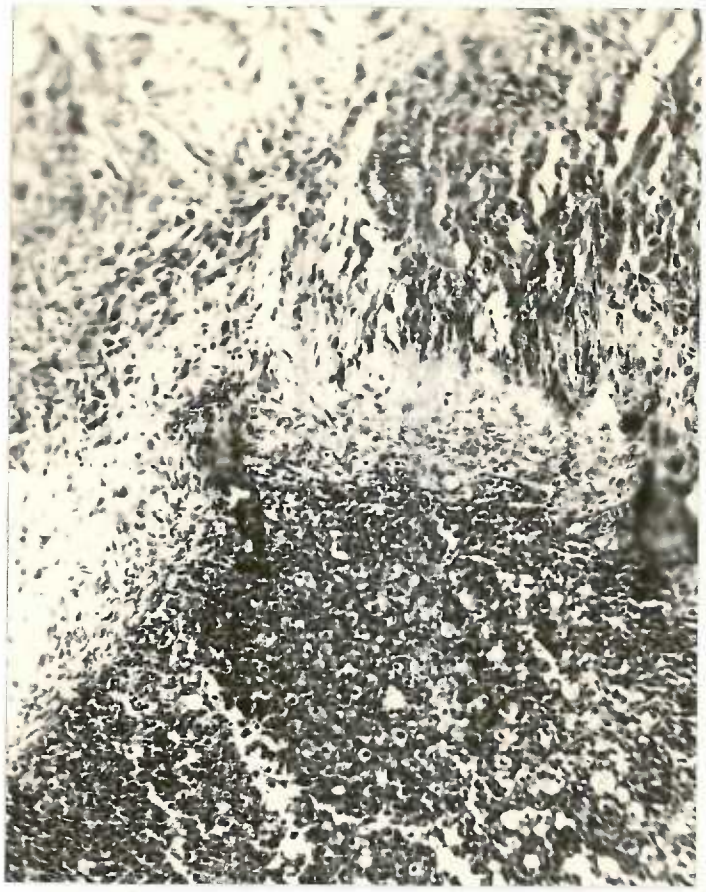


Figure 7. Intraperitoneal fibrosarcoma invading the capsule of an adrenal cortex undergoing focal hypertrophy, cytoplasmic granular and vacuolar depletion in cells of the zona fasciculata. 100x

Figure 8. Nephrosclerosis. 475x
Capillary tufts of the glomerulus are undergoing focal hyaline degeneration while the surrounding tubules are unaffected.



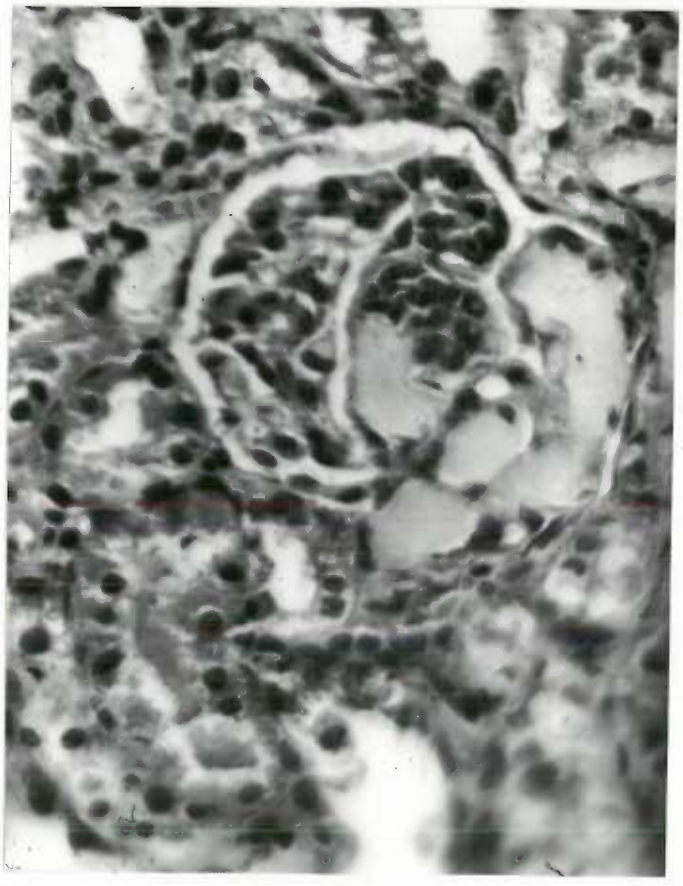
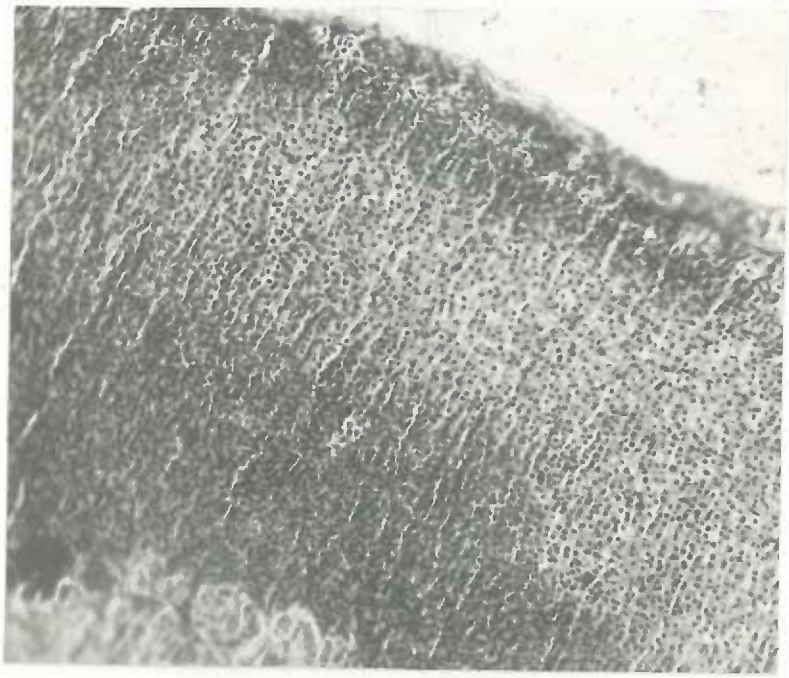


Figure 9. Adrenal cortex of a normal male rat. 100x

Comparison with Figure 10 reveals that the male cortex is thinner and the cells are darker-staining.

Figure 10. Adrenal cortex of a normal female rat. 100x

Comparison with Figure 9 shows the female cells to be somewhat larger than those of the male.



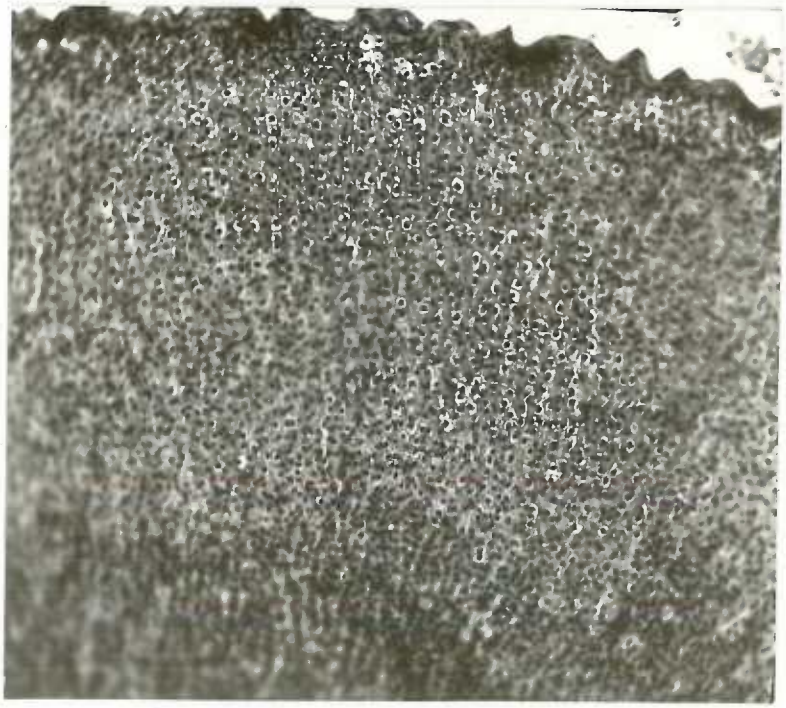
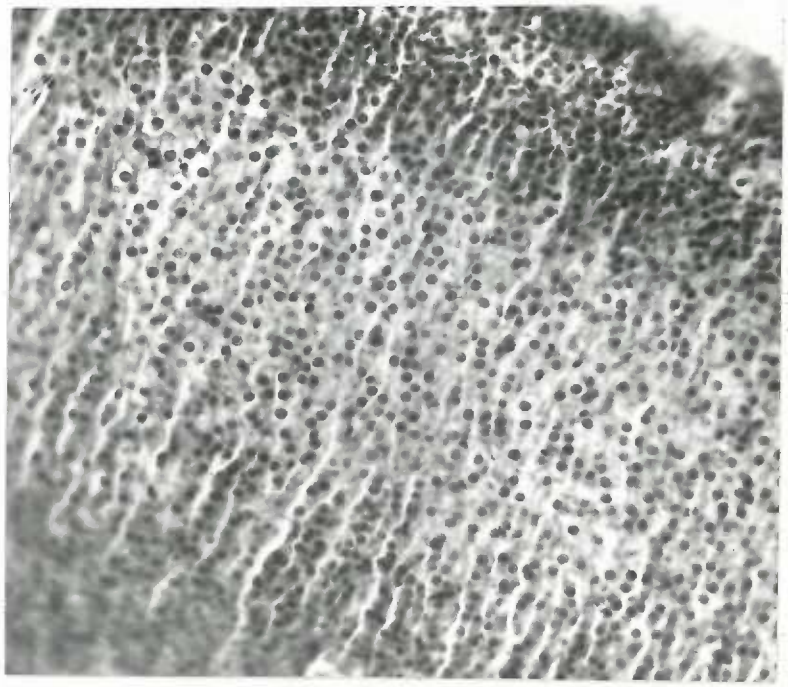


Figure 11. Adrenal cortex of a normal male rat. 200x

Compare with Figure 12.

Figure 12. Adrenal cortex of a normal female rat. 200x

The larger cells in the female gland are demonstrated, with their granule-poor cytoplasm. Compare with Figure 11.



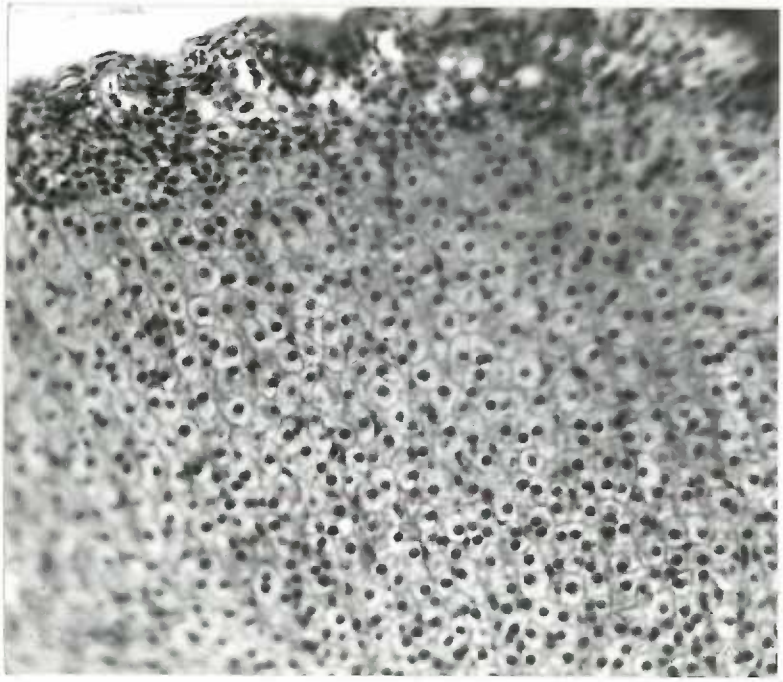
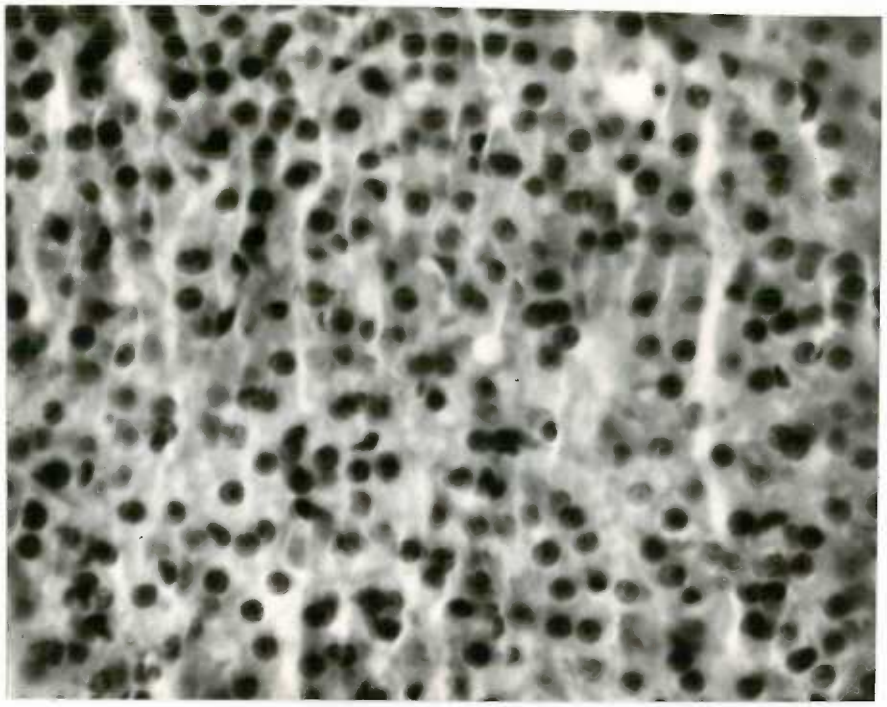


Figure 13. Adrenal cortex of a normal male rat. 475x

The small cells with densely-granular cytoplasm are demonstrated. Compare with Figure 14.

Figure 14. Adrenal cortex of a normal female rat. 475x

The vacuole-rich cytoplasm of the fascicular cells is demonstrated. Compare with Figure 13.



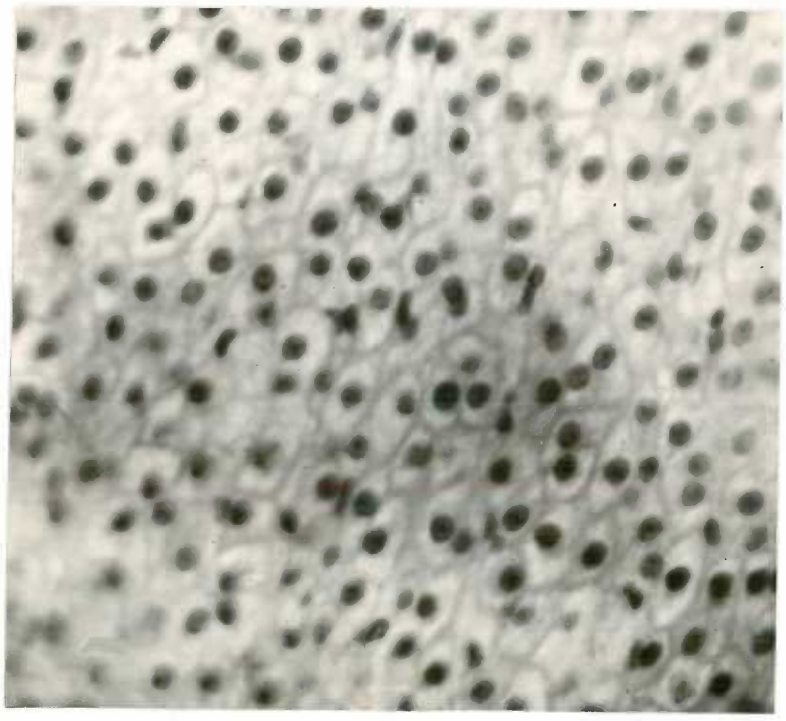
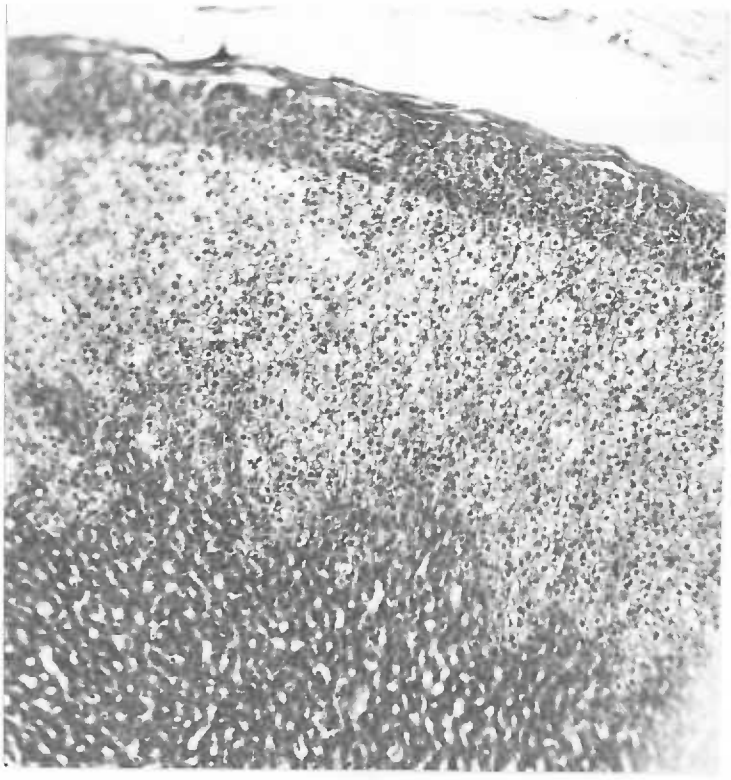


Figure 15. Adrenal cortex of a male rat after eight daily subcutaneous injections of 5 mgm. of Cortisone Acetate. 100x

The fascicular cells are engorged with cytoplasmic vacuoles, whereas the glomerulosa and reticularis cells are unaltered. Compare with Figure 16.

Figure 16. Adrenal cortex of a female rat after eight daily subcutaneous injections of 5 mgm of Cortisone Acetate. 100x

Vacuolar storage is not as marked as in the male, Figure 15.



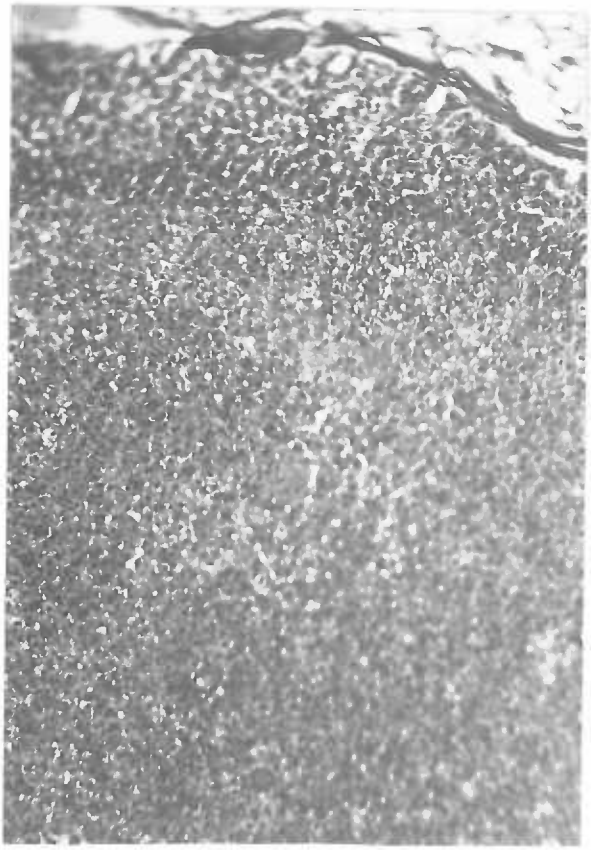


Figure 17. Accessory adrenal cortical nodule. 100x

A small nodule is seen in the zona glomerulosa at the top of the photograph immediately under the capsule of the gland.

Figure 18. Focal hemorrhage and cystic degeneration of the zona fasciculata. 100x

Intact reticulum cells form septa between the cysts.

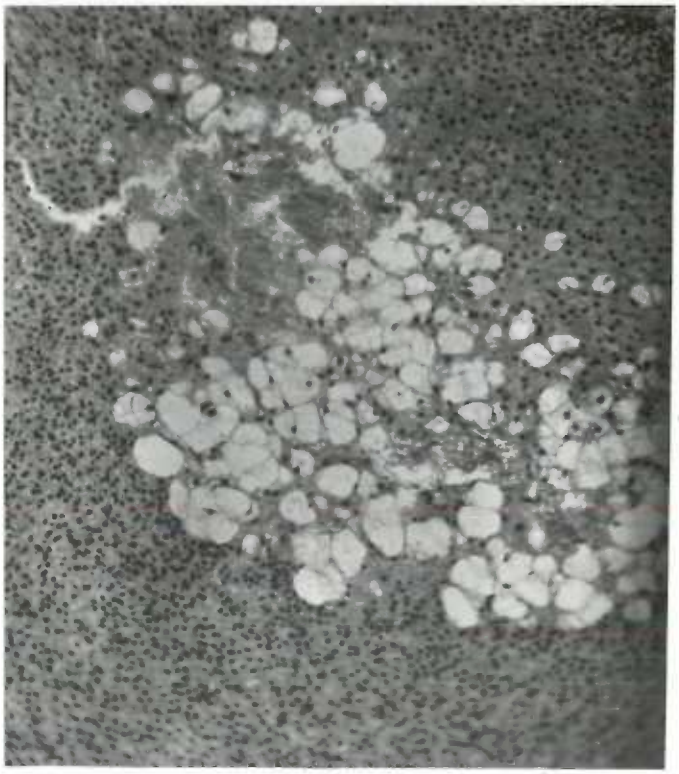
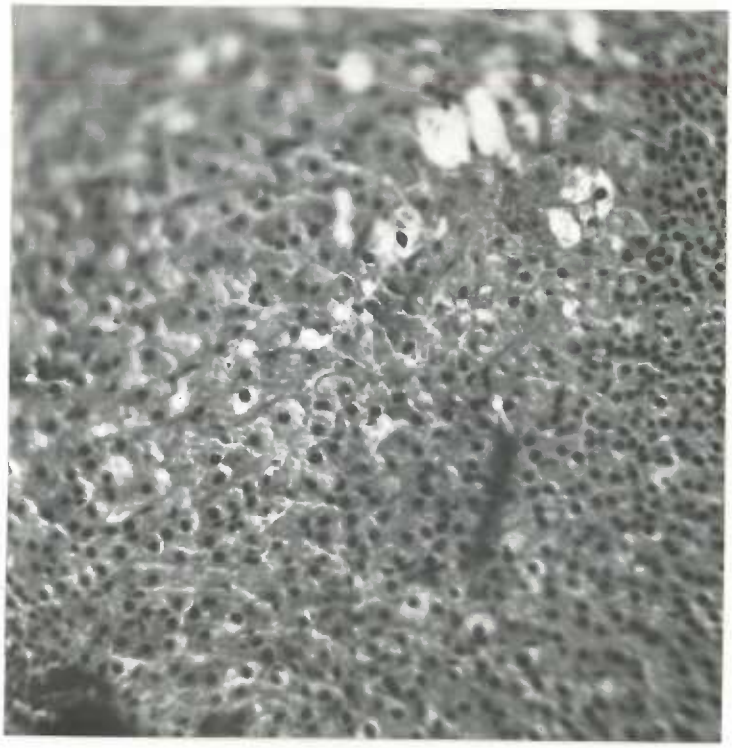


Figure 79. Focal necrosis of the fasciculata zone. 475x

The upper left-hand part of the photograph shows necrotic cells with areas of cystic degeneration.

Figure 20. Colloid Formation. 475x

These are the small round bodies in the cytoplasm of the fasciculata cells indicated by arrows.



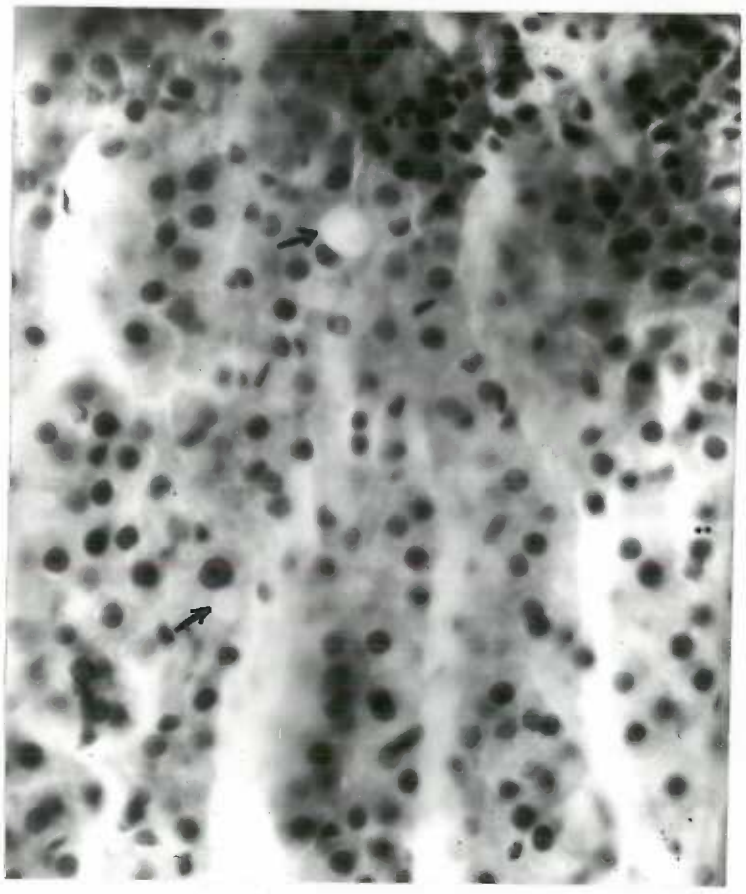
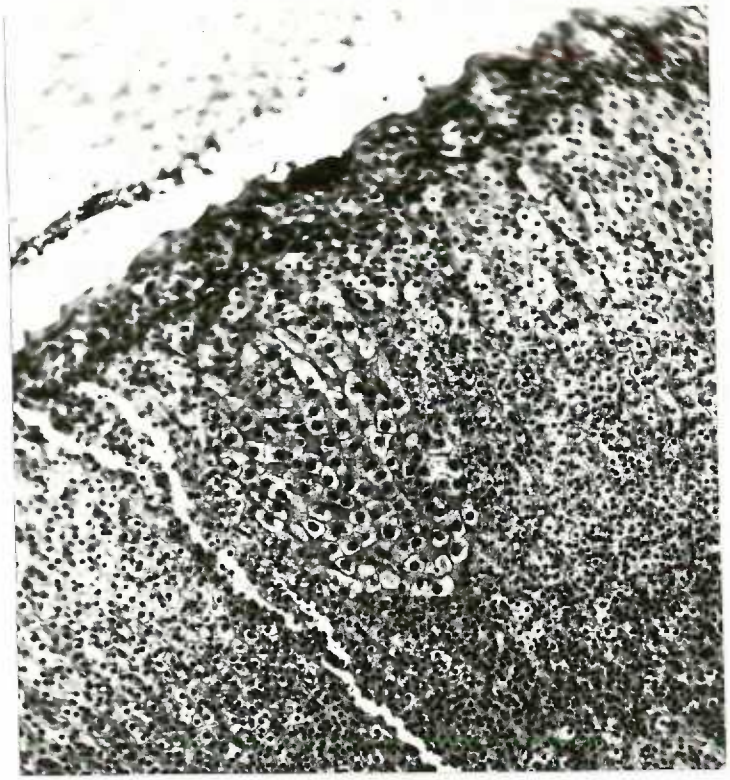


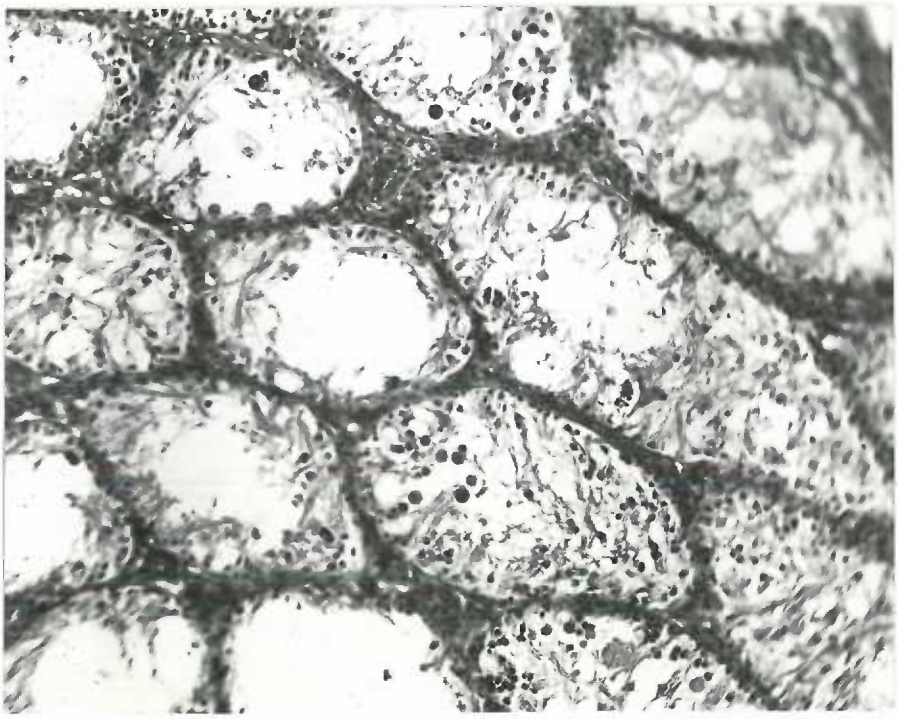
Figure 21. Adenoma of the zona fasciculata. 100x

A circumscribed collection of very large cells are seen in the zona fasciculata, the remaining cells of the layer show cytoplasmic granular depletion and hypertrophy.

Figure 22. Testicular atrophy. 100x

The seminiferous tubules are devoid of spermatozoa, the germinal cells have desquamated into the tubular lumens and degenerated. The Leydig cells are not affected.





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