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*On The  
Experimental Morphology  
of the Adrenal Cortex*

HANS SELYE, M.D., PH.D.  
and HELEN STONE

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OF THE  
ADRENAL CORTEX

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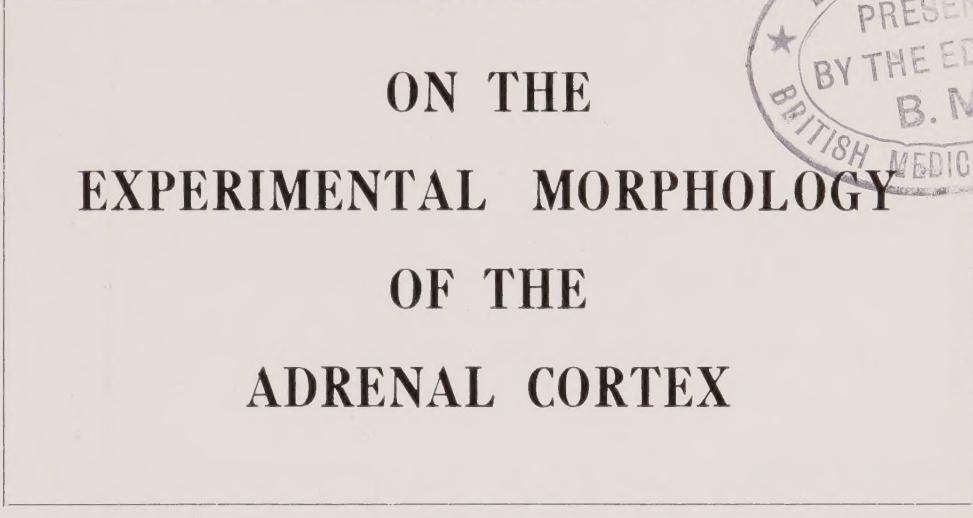
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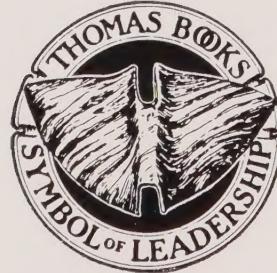
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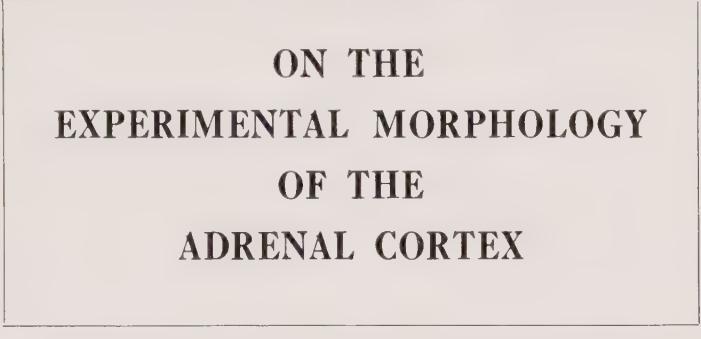
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**ON THE  
EXPERIMENTAL MORPHOLOGY  
OF THE  
ADRENAL CORTEX**



\* 1 \*

## Introduction

OUR WORK on the general-adaptation-syndrome and the diseases of adaptation (Selye, 1946) led us to undertake a large series of systematic investigations concerning the effect of various hormone preparations upon the adrenal, the kidney, the cardiovascular apparatus, the liver, the spleen and the thymico-lymphatic apparatus. For reasons which are discussed below, special attention was given to the effect of those hormones which exert a marked influence upon nitrogen metabolism, particularly anterior-pituitary preparations, thyroxine, testoids and corticoids. Because of the volume and diversity of the material, it did not appear advisable to publish our findings in a single communication, or even in the same journal, but cross references will permit the interested reader to make intercomparisons between the changes observed in the different organs of the same experimental animals.

The present communication is devoted solely to the changes produced in the adrenal cortex. It will describe the manifold morphologic alterations that can be elicited by various combinations of the above-mentioned hormones under diverse experimental conditions. Some of the fundamental experiments will be reported in great detail, but for purposes of comparison, we shall also adduce material from numerous other unpublished experiments performed in this laboratory. This will permit us to give a fairly balanced and complete synopsis of the most important facts concerning the experimental morphology of

the adrenal cortex, on the basis of personal observations made under comparable laboratory conditions.

To accomplish this, rather time-taking and costly large scale experiments had to be undertaken, but we considered them to be necessary at the present time when physiologists, biochemists, pathologic anatomists and clinicians alike are devoting so much of their attention to the rôle of the adrenal cortex in the defense of the organism against various types of systemic or local stress.

Before proceeding to the description of our observations, it may be well to outline the principal problems which directed our planning of these experiments.

Firstly, we felt the need of establishing *morphologic criteria, which would permit the appraisal of adrenocortical function* and the recognition of individual corticotrophic and anti-corticotrophic agents by the appearance of the suprarenal cortex. The technics now in routine use by pathologic anatomists give us but little information as they are generally limited to the establishment of adrenal weight and lipid content, or the registration of such obvious histologic changes as atrophy, hypertrophy, hyperplasia and the presence of neoplastic tissue.

In previous years, we emphasized — and perhaps overemphasized — the fact that, in response to a great variety of non-specific systemic stresses, the adrenal cortex responds in essentially the same manner. First, there is hypertrophy, hyperplasia and discharge of secretion granules (total lipids, cholesterol, hormonal steroids, ascorbic acid) a response which corresponds to the "alarm reaction" stage of the general-adaptation-syndrome; later, during the "stage of resistance" the same stimulus results in an increased storage of secretory granules, while the total size of the adrenal cortex (though always above normal) may be either smaller or larger than during the

alarm reaction, depending upon the intensity of the systemic stress to which the organism is exposed (Selye, 1946; Selye, 1947). As we shall see from the observations described in this paper, it is becoming increasingly more evident that, in addition to this non-specific response to stress, the adrenal cortex can respond in a very specific, almost "pathognomonic" manner to certain humoral agents. Indeed, even the above-mentioned non-specific response is largely dependent upon experimental conditions, especially dietary factors. A more intimate study of these specific factors, which can influence adreno-cortical structures and function, promised to furnish a basis for the analysis of lesions occurring in the adrenals of man under the influence of spontaneous diseases.

Secondly, we wished to adduce further evidence, from these experiments, for or against our concept which postulates the *existence of several, qualitatively different, corticotrophic agents* (Selye, 1944; Selye, 1947). Some prominent investigators, especially biochemists (Li, 1947; Sayers and Sayers, 1948), did not subscribe to this interpretation since, in their opinion, a single, chemically pure, hypophyseal corticotrophin exerts all the known corticotrophic actions. As we shall see from the data presented in this paper, there is no doubt that various agents can produce qualitatively quite distinct morphologic changes in the adrenals. Yet, this finding is compatible with both of the above-mentioned views. It is possible that a single corticotrophic hormone is secreted by the pituitary, but that, depending upon other humoral factors, it may exert various, qualitatively different, effects upon the adrenal cortex.

Thirdly, through these experiments we hoped to furnish a basis for the development of therapeutic *procedures permitting the normalization of derailments in cortical*

*function*, for instance, those which are supposed to exist during the so-called “diseases of adaptation.” As we shall see from the experiments described below, a good deal of the evidence already at hand suggests that the adrenal cortex can not only be “stimulated” or “inhibited” by various stimuli, but that the use of specific stimulators enables us to arrive at what might be called the “*pharmacology of the adrenal cortex.*”

# ✖ 2 ✖

## Materials

### Experimental Animals

ADULT WISTAR albino rats were used for all the principal experiments. Their initial body weight was usually between 120-150 gm. but as their growth rate was considerably influenced by the treatment, both initial and final body weights are indicated in the tables below. Since the diet greatly influences corticotrophic hormone actions (Selye, 1947), it is necessary to state that our animals were given either "Purina fox chow" or modifications of the synthetic diet described in Chart I. In the latter case, only the protein and NaCl content were varied in the different experiments, as indicated in the text.

### Surgical Procedures

Unilateral nephrectomy was performed through the costo-lumbar approach under ether anesthesia. The left kidney was exteriorized, ligated at its pedicle and after its removal, skin and muscles were sutured in a single layer.

Castration was performed through a suprapubic incision under ether anesthesia. A single ligature was placed around the two spermatic cords and after removal of both testes, skin and muscle were sutured in a single layer.

## CHART I

## COMPOSITION OF THE BASIC SYNTHETIC DIET

(parts per cent)

Casein.....	20.0
Cornstarch.....	72.0
Fat (Mazola) .....	1.0
Cod Liver Oil.....	1.0
Celluflour.....	1.0
Mineral Mixture (see below).....	4.0
Supplements (see below).....	0.54

*MINERAL MIXTURE*

4 gm. per 100 gm. of diet

NaCl.....	23.4	gm.
MgSO <sub>4</sub> . 7H <sub>2</sub> O.....	24.6	gm.
Na <sub>2</sub> HPO <sub>4</sub> .....	14.2	gm.
K <sub>2</sub> HPO <sub>4</sub> .....	69.6	gm.
CaHPO <sub>4</sub> . 2H <sub>2</sub> O.....	69.8	gm.
Ca. Lactate. 5H <sub>2</sub> O.....	15.4	gm.
Ferric citrate.....	1.2	gm.
KI.....	0.16	gm.

*SUPPLEMENTS*

0.54 gm. per 100 gm. of diet

Thiamine chloride.....	0.8	mg.
Riboflavin.....	0.8	mg.
Pyridoxine.....	0.8	mg.
Ca. pantothenate.....	1.5	mg.
Nicotinic acid.....	1.5	mg.
Choline chloride.....	400.0	mg.
Inositol.....	100.0	mg.
p-amino-benzoic acid.....	30.0	mg.

## Hormone Preparations

LYOPHILIZED ANTERIOR PITUITARY (LAP) was prepared from fresh beef adeno-hypophyses, by suspending 40 mg. of dry powder per cc. of 1% saline solution. This was administered in an amount equivalent to 15 mg. subcutaneously twice daily.

ANTERIOR PITUITARY EXTRACT (APE) was prepared from fresh beef adeno-hypophyses by mixing 50 gm. of the tissue with 250 cc. of physiological saline. 0.4 cc. of this was injected subcutaneously twice daily.

PURIFIED CORTICOTROPHIC HORMONE preparations were used only in a few experiments. These are described in the text.

METHYL-TESTOSTERONE was administered in the form of compressed pellets weighing approximately 50 mg. One or more of these were implanted subcutaneously as indicated in the experimental part.

SODIUM THYROXINATE was given as a microcrystalline suspension containing 500  $\gamma$  per cc. of distilled water. This was injected subcutaneously in doses ranging between 50 and 200  $\gamma$  once daily as indicated in the text. Thyroxine was used in many of our experiments in combination with LAP or APE since our earlier observations revealed that thyroid hormone greatly potentiates the corticotrophic effect of anterior pituitary preparations (Selye et al., 1945; Selye, 1947).

DESOXYCORTICOSTERONE ACETATE (DCA) was administered subcutaneously in the form of compressed pellets weighing approximately 45 mg.

## Organ Weights

The organs were fixed in Suza solution immediately upon dissection. They were weighed on an analytical balance after complete fixation. The weights are given in the tables together with the standard errors calculated according to the following equation:

$$SE = \sqrt{\frac{\sum (X - \bar{X})^2}{N(N - 1)}}$$

Where  $X$  is the observed value,  $\bar{X}$ , the mean, and  $N$ , the number of animals.

## Histologic Technic

Unless otherwise stated, the adrenals were sectioned after embedding in paraffin and stained with the hematoxylin-eosin technic. Special stains were used in some instances (e.g., for the detection of lipids, hyalin, fibrin, "plasmalogens"), but all adrenals whose weights are mentioned in this communication, have been studied at least on H and E sections for purposes of general orientation.

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## Experimental Experiment I

OUR FIRST experiment was designed to re-examine the relationship between the dietary protein concentration and the corticotrophic effect of LAP. For this purpose, seven groups each consisting of 10 male albino rats, were unilaterally nephrectomized (a procedure previously shown to increase sensitivity to some LAP actions). They all received the basic synthetic diet, but the casein content was 20% in the controls (group 1) and 5%, 10%, 15%, 20%, 25% and 50% in the LAP injected groups 2 to 7 respectively. These variations in casein concentration were effected by correspondingly adjusting the starch content of the food. With the exception of the controls (group 1) all animals received 15 mg. of LAP twice daily subcutaneously. All animals received tap water as drinking fluid and the experiment was terminated after 25 days of treatment. Thus the only variable in the treatment was the protein content of the food.

The principal data are summarized in Table I.

It is evident that a fairly close direct proportionality exists between the corticotrophic effect of LAP and the protein content of the ingested food. It is noteworthy that this was so although under the conditions of this experiment the LAP did not cause any pronounced somatic growth. Thus, at the end of the experiment, the animals kept on a 50% protein diet (group 7) were approxi-

TABLE I  
EFFECT OF LAP ON ADRENAL WEIGHT AS INFLUENCED BY  
DIETARY PROTEIN

GROUP	HORMONE TREATMENT	DIET	$\sigma$ Unilateral Nephrectomy 25 days		
			Body weight (gm.)		Adrenal (mg.)
			Initial	Final	
I	Control	20% Protein	124±4	209±9	30±1
II	LAP	5% Protein	125±4	123±6	41±1
III	LAP	10% Protein	124±4	160±6	54±3
IV	LAP	15% Protein	126±6	190±10	65±3
V	LAP	20% Protein	126±4	201±8	70±4
VI	LAP	25% Protein	124±5	185±6	79±4
VII	LAP	50% Protein	126±4	204±8	83±3

mately as heavy as the controls, yet their adrenals were almost three times as large as those of the untreated rats (group 1) and more than twice as large as those of the rats receiving the same amount of LAP on a 5% protein diet (group 2). These results are especially striking if we consider that the entire adrenal-weight increase caused by LAP is due to cortical enlargement.

We shall now describe the changes in adrenal weight observed in six experiments in which various protein-metabolism regulating hormones and hormone combinations (thyroxine, methyl-testosterone, LAP, ALE, and DCA) were administered under different experimental conditions. The corresponding histologic changes seen in these animals will subsequently be surveyed in conjunction with additional experimental material gathered from the slide collection of this Institute.

## Experiment II

Fifteen groups, each consisting of 12 unilaterally nephrectomized, female albino rats were used in this experiment. The first series of five groups was kept on an 18% protein synthetic diet, the second on a 50% protein synthetic diet and the third on a 50% protein synthetic diet supplemented with 4% NaCl. Tap water was given to all animals as a drinking fluid.

In this, as in several subsequent experiments, unilateral nephrectomy was performed and NaCl or a high-protein diet was administered to some groups of animals as our earlier work (Selye, 1946; Selye, 1947) had shown that the corticotrophic action of anterior-pituitary preparations is increased by high intakes of dietary protein, while the activity of corticoid hormones is augmented by high-sodium diets and unilateral nephrectomy.

The five groups of each series were treated as follows: Group 1 remained untreated, group 2 received 50  $\gamma$  of thyroxine per day subcutaneously from the 20th to the 60th day of the experiment. Group 3 received two 50 mg. pellets of methyl-testosterone subcutaneously, on the day of the unilateral nephrectomy, when the experiment began. Group 4 received both thyroxine and methyl-testosterone in the manner in which these compounds were administered to groups 2 and 3 respectively. Group 5 received thyroxine and methyl-testosterone in the same manner as group 4 and, in addition, two LAP injections of 15 mg. each daily from the 40th to the 60th day of the experiment.

It should be said that very chronic overdosage with thyroxine and LAP is poorly tolerated by the animals. That is why it was necessary to commence treatment with

TABLE II  
EFFECT OF THYROXINE, LAP AND METHYL-TESTOSTERONE ON THE  
ADRENALS OF FEMALE UNILATERALLY NEPHRECTOMIZED RATS  
AS INFLUENCED BY DIETARY PROTEIN AND NaCl.  
60 DAY EXPERIMENTS

GROUP	HORMONE TREATMENT	SERIES I		SERIES II		SERIES III	
		♀ Unilateral Nephrectomy 60 days—18% Protein		♀ Unilateral Nephrectomy 60 days—50% Protein		♀ Unilateral Nephrectomy 60 days—50% Protein + 4% NaCl	
		Body Weight (gm.)	Adrenal (mg.)	Body Weight (gm.)	Adrenal (mg.)	Body Weight (gm.)	Adrenal (mg.)
		Initial	Final	Initial	Final	Initial	Final
I	Controls	138 ± 6	187 ± 9	145 ± 7	196 ± 11	41 ± 2	141 ± 7
II	Thyroxine	136 ± 7	175 ± 6	142 ± 9	185 ± 6	54 ± 2	144 ± 7
III	Methyl-Testosterone	135 ± 7	189 ± 13	28 ± 1	191 ± 7	30 ± 1	142 ± 8
IV	Thyroxine + Methyl-Testosterone	136 ± 7	192 ± 7	31 ± 1	140 ± 6	195 ± 7	36 ± 1
V	Thyroxine+ Methyl-Testosterone+ LAP	139 ± 7	225 ± 13	90 ± 6	140 ± 7	221 ± 7	105 ± 8

these compounds during the second half of the experiment to permit a 60 day treatment period of the same animals with the less toxic, but more slowly acting methyl-testosterone.

The principal data are summarized in Table II.

It is immediately evident upon perusal of the table that under these experimental conditions neither the protein nor the salt supplements of the *diet* exerted any consistent or pronounced influence upon the adrenal weight changes occasioned by the various hormones and hormone combinations. Our previous work (Selye, 1946; Selye, 1947; Hay et al., 1948; Moya et al., 1948; Prado et al., 1947) — as well as the first experiment of this communication — had clearly shown that on diets containing 15% protein or less, the adrenal stimulation by LAP is greatly diminished. Apparently, the adrenal enlargement caused by thyroxine alone (groups 2) or thyroxine in combination with LAP (groups 5) is not thus inhibited, at least at the protein concentrations used in this series.

THYROXINE (groups 2) caused a significant, though moderate, increase in adrenal weight which was quite manifest on all three diets.

METHYL-TESTOSTERONE (groups 3), produced a marked involution of the adrenal on all three diets. This confirms our previous observations concerning the effect of this steroid (Selye, 1947).

Combined treatment with *thyroxine and methyl-testosterone* (groups 4) revealed that the testoid hormone almost completely annulled the corticotrophic effect of thyroxine. Indeed the adrenal weight on all three diets was significantly below the normal control level, the atrophy caused by methyl-testosterone being nearly uninfluenced by thyroxine.

Simultaneous treatment with *thyroxine, methyl-testos-*

*terone and LAP* (groups 5), showed on the other hand, that the corticotrophic effect of the thyroxine plus LAP is not prevented by methyl-testosterone. It would be incorrect, however, to imply from this that the intense corticotrophic action of the thyroxine plus LAP treatment is uninfluenced by simultaneous treatment with a testoid substance. As we shall see later, the histologically characteristic manifestations of methyl-testosterone action were not prevented either by thyroxine alone (groups 4) or by thyroxine plus LAP (groups 5).

Since in this experiment there was no group treated with LAP alone, two major questions remained unanswered, namely: 1. Was the failure of the low-protein diet to inhibit the corticotrophic action of LAP due to the fact that it still contained 18%, that is, a comparatively high protein concentration or did the protein-catabolism caused by thyroxine provide additional endogenous protein-catabolites, which permit the corticotrophic action of LAP to fully manifest itself? 2. Is methyl-testosterone entirely unable to inhibit the corticotrophic action of LAP or is there a partial inhibition, as compared with the effect of LAP (or LAP plus thyroxine), although the adrenals are still very significantly above the control level? These questions could only be answered by comparison with animals receiving LAP alone. For this reason, and in order to obtain various additional pertinent data, a rather extensive third experimental series was performed in which a group treated with LAP alone was included.

### Experiment III

Forty-two groups, each consisting of six female albino rats were used in the third experiment. Series I, II and III

were killed on the 57th, series IV, V and VI on the 75th day of the experiment. All animals received the same basic synthetic diet; series I and IV with 18%, series II, III, V and VI with 50% protein. In addition, in series III and VI an NaCl supplement was given, in an amount corresponding to 4% of the diet. In all series groups 1 acted as intact untreated controls. Groups 2 as unilaterally nephrectomized, untreated controls. Groups 3 to 7 were all unilaterally nephrectomized; they were treated with thyroxine in groups 3, LAP in groups 4, methyl-testosterone in groups 5, thyroxine and methyl-testosterone in groups 6, thyroxine, methyl-testosterone and LAP in groups 7.

Thyroxine was administered throughout in daily doses of 50  $\gamma$  subcutaneously, beginning on the first day of the experiment; LAP in a dose of 15 mg. twice daily subcutaneously, beginning on the 35th day of the experiment; methyl-testosterone in the form of two 50 mg. pellets, one implanted subcutaneously on the first day, the second on the 35th day of the experiment.

This arrangement permitted us to verify the data of the first experiment on a large group of animals under slightly different conditions and at two lengths of treatment. Furthermore, it gave us the necessary additional groups of animals treated with LAP alone.

The pertinent data are summarized in Table III.

Perusal of the table indicates that the *diet* again had little effect upon the corticotrophic or anti-corticotrophic actions of the various hormones and hormone combinations, with the possible exception of the animals receiving LAP alone (groups 4) in the 75 day series. Here, the adrenal enlargement was somewhat more pronounced on the 50% protein diet (with or without NaCl supplement). Hence, it may be concluded that 18% protein

TABLE III  
EFFECT OF THYROXINE, LAP AND METHYL-TESTOSTERONE ON THE  
ADRENALS OF FEMALE UNILATERALLY NEPHRECTOMIZED RATS  
AS INFLUENCED BY DIETARY PROTEIN AND NaCl.  
57 DAY AND 75 DAY EXPERIMENTS

SERIES		I		II		III		IV		V		IV			
HORMONE TREATMENT		$\frac{\text{♀}}{\text{♂}} - 57 \text{ days}$ 18% Protein		$\frac{\text{♀}}{\text{♂}} - 57 \text{ days}$ 50% Protein		$\frac{\text{♀}}{\text{♂}} - 57 \text{ days}$ 50% Protein + 4% NaCl		$\frac{\text{♀}}{\text{♂}} - 75 \text{ days}$ 18% Protein		$\frac{\text{♀}}{\text{♂}} - 75 \text{ days}$ 50% Protein		$\frac{\text{♀}}{\text{♂}} - 75 \text{ days}$ 50% Protein + 4% NaCl			
GROUP		Body Weight (gm.)	Adrenal (mg.)	Body Weight (gm.)	Adrenal (mg.)	Body Weight (gm.)	Adren. (mg.)	Body Weight (gm.)	Adren. (mg.)	Body Weight (gm.)	Adren. (mg.)	Body Weight (gm.)	Adren. (mg.)		
I	Normal Controls	149 ± 4	47 ± 2	148 ± 3	198 ± 4	59 ± 3	148 ± 3	184 ± 7	51 ± 4	151 ± 5	202 ± 7	49 ± 2	157 ± 3	196 ± 5	
	Unilateral Nephrectomy Controls	148 ± 2	206 ± 6	49 ± 4	148 ± 4	202 ± 6	55 ± 3	151 ± 5	196 ± 10	49 ± 3	152 ± 5	195 ± 2	48 ± 4	152 ± 5	190 ± 9
II	Unilateral Nephrectomy	148 ± 4	201 ± 6	63 ± 4	151 ± 4	203 ± 5	72 ± 3	153 ± 3	192 ± 7	67 ± 3	152 ± 3	191 ± 6	59 ± 6	151 ± 5	187 ± 3
	Thyroxine +												62 ± 5	148 ± 5	200 ± 5
III	Unilateral Nephrectomy	148 ± 4	201 ± 6	63 ± 4	151 ± 4	203 ± 5	72 ± 3	153 ± 3	192 ± 7	67 ± 3	152 ± 3	191 ± 6	59 ± 6	151 ± 5	187 ± 3
	Thyroxine -												62 ± 5	148 ± 5	200 ± 5

IV	Unilateral Nephrectomy + LAP	$145 \pm 3$	$228 \pm 15$	$102 \pm 11$	$148 \pm 4$	$227 \pm 6$	$107 \pm 14$	$148 \pm 3$	$231 \pm 11$	$121 \pm 12$	$156 \pm 5$	$211 \pm 19$	$121 \pm 8$	$159 \pm 7$	$179 \pm 10$	$164 \pm 21$	$153 \pm 5$	$220 \pm 12$	$158 \pm 21$
V	Unilateral Nephrectomy + Methyl- Testosterone	$146 \pm 4$	$218 \pm 4$	$31 \pm 1$	$148 \pm 4$	$210 \pm 4$	$33 \pm 1$	$149 \pm 5$	$210 \pm 8$	$37 \pm 2$	$154 \pm 6$	$209 \pm 8$	$34 \pm 2$	$154 \pm 6$	$213 \pm 6$	$37 \pm 1$	$151 \pm 4$	$208 \pm 6$	$40 \pm 3$
VI	Unilateral Nephrectomy + Thyroxine + Methyl- Testosterone	$151 \pm 2$	$212 \pm 5$	$37 \pm 1$	$151 \pm 3$	$213 \pm 9$	$46 \pm 2$	$151 \pm 4$	$205 \pm 8$	$41 \pm 2$	$152 \pm 2$	$212 \pm 5$	$37 \pm 7$	$150 \pm 4$	$204 \pm 6$	$46 \pm 2$	$151 \pm 6$	$211 \pm 9$	$44 \pm 3$
VII	Unilateral Nephrectomy + Thyroxine + Methyl- Testosterone + LAP	$149 \pm 2$	$242 \pm 10$	$109 \pm 2$	$148 \pm 4$	$266 \pm 6$	$113 \pm 7$	$149 \pm 6$	$242 \pm 12$	$104 \pm 8$	$156 \pm 3$	$204 \pm 9$	$147 \pm 7$	$150 \pm 5$	$217 \pm 15$	$105 \pm 16$	$154 \pm 6$	$221 \pm 11$	$158 \pm 23$

represents a nearly optimal concentration, which does not markedly inhibit the response of the adrenal cortex to these hormonal stimuli. (Compare also with Table II.)

PARTIAL NEPHRECTOMY (groups 2) failed to influence the weight of the adrenals significantly as compared to the normal control values (groups 1).

THYROXINE (groups 3) elicited a moderate, but highly significant, adrenal enlargement in all six series.

LAP (groups 4) produced a very pronounced and highly significant adrenal enlargement in all six series. As stated above, there was some tendency for this enlargement to be particularly marked in the 50% protein series (irrespective of NaCl intake) of the more chronic 75 day experiments.

METHYL-TESTOSTERONE (groups 5) given alone, again exerted its pronounced consistent, and highly significant anti-corticotropic action in all six series.

METHYL-TESTOSTERONE IN COMBINATION WITH THYROXINE (groups 6) proved highly effective in counteracting the adrenal cortical hypertrophy normally produced by the latter. This effect was manifest in all six series, but the inhibition was especially pronounced in the animals receiving the 18% protein diet (series I and IV).

LAP IN COMBINATION WITH THYROXINE AND METHYL-TESTOSTERONE (groups 7) caused a very pronounced and highly significant adrenal enlargement in all six series. This effect remained uninfluenced by the protein and NaCl concentration of the diet and was not significantly greater than that obtained by LAP alone (groups 4). It may be concluded that methyl-testosterone does not inhibit the intense corticotrophic effect of the LAP-thyroxine combination, although it exerts a marked anti-corticotropic effect in untreated and merely thyroxine treated rats. Since our previous observations (Selye et al., 1945)

showed that thyroxine greatly potentiates the corticotrophic action of LAP it was deemed of interest to include for comparison an additional group of rats receiving only thyroxine plus anterior-pituitary extracts without methyl-testosterone. It was also considered of importance to establish whether castrate males would react essentially in the same manner as females and hence an additional experiment was performed in which these latter factors were examined under slightly different experimental conditions.

## Experiment IV

Sixteen groups, each consisting of eight castrate male rats, were used in the fourth experiment. Series I was not nephrectomized, series II, was unilaterally nephrectomized. All animals received a "Purina fox chow" diet, but series I was given tap water while the nephrectomized animals of series II received 1% NaCl as a drinking fluid. Since the toxicity of mineralo-corticoids is greatly increased by unilateral nephrectomy and NaCl supplements, we considered it of interest to include series II in order to obtain further evidence concerning the inactivity of such sensitizing measures as regards corticotrophic and anti-corticotrophic actions.

Group 1 acted as untreated controls. Groups 2 were treated with thyroxine, groups 3 with APE, groups 4 with methyl-testosterone, groups 5 with thyroxine and APE, groups 6 with thyroxine and methyl-testosterone, groups 7 with APE and methyl-testosterone and groups 8 with thyroxine, methyl-testosterone and APE.

Thyroxine was administered throughout, once a day, in a dose of 200  $\gamma$  subcutaneously, APE twice daily in a dose of 0.4 cc., subcutaneously and methyl-testosterone in

the form of a single 50 mg. pellet. All hormone treatments were commenced on the day on which series II was unilaterally nephrectomized and the experiment was terminated 21 days later.

This arrangement allowed us to verify the data of experiments II and III, again under slightly different conditions, notably a shorter period of treatment in castrate males; at the same time it permitted comparison with the previously missing groups receiving thyroxine and APE for comparison with groups 8.

Perusal of Table IV indicates that *castrate males* react essentially in the same manner as females, that — providing the dose of thyroxine is raised — 21 days of treatment suffices to induce the adrenal responses previously described and that *partial nephrectomy and NaCl treatment* fails to influence any of the corticotrophic or anti-corticotrophic effects under consideration.

THYROXINE (groups 2) elicited a highly significant adrenal enlargement; this was even more pronounced in the animals receiving *APE* (groups 3).

METHYL-TESTOSTERONE (groups 4) given alone, elicited its usual intense anti-corticotrophic effect.

THYROXINE PLUS APE (groups 5) resulted in a more pronounced adrenal enlargement than APE alone (groups 3), which confirms our earlier observations (Selye et al., 1945).

THYROXINE PLUS METHYL-TESTOSTERONE (groups 6) again resulted in an inhibition of the usual corticotrophic action of thyroxine, but, since the dose of thyroxine used was larger in this than in the previous series, the inhibition was correspondingly less intense and the adrenal weight was not below the control level of the untreated animals (groups 1).

APE PLUS METHYL-TESTOSTERONE (groups 7) pro-

TABLE IV

EFFECT OF THYROXINE, APE AND METHYL-TESTOSTERONE ON THE  
ADRENALS OF UNILATERALLY NEPHRECTOMIZED CASTRATE  
MALE RATS AS INFLUENCED BY DIETARY SODIUM.  
21 DAY EXPERIMENTS

GROUP	HORMONE TREATMENT	I		II		Adrenal (mg.)	
		$\sigma^7/c$ Not Nephrectomized 21 days—Purina		$\sigma^7/c$ Unilateral Nephrectomy 21 days—Purina+NaCl			
		Body Weight (gm.)	Adrenal (mg.)	Body Weight (gm.)	Adrenal (mg.)		
Initial	Final	Initial	Final	Initial	Final		
I	Controls	111±7	175±6	36±1	114±6	40±1	
II	Thyroxine	113±5	168±8	43±3	113±5	52±3	
III	Anterior Pituitary Extract	113±5	222±6	82±3	111±7	94±4	
IV	Methyl-Testosterone	111±5	172±5	29±1	111±5	33±2	
V	Thyroxine+ Anterior Pituitary Extract	113±4	202±5	140±16	112±4	121±6	
VI	Thyroxine+ Methyl-Testosterone	114±6	180±5	38±2	114±5	40±2	
VII	Anterior Pituitary Extract+ Methyl-Testosterone	113±7	227±8	72±5	114±6	92±7	
VIII	Thyroxine+ Methyl-Testosterone+ Anterior Pituitary Extract	111±8	230±8	111±8	114±8	121±13	

duced adrenals of essentially the same size as those in the animals treated with APE alone (groups 3). This shows that the testoid compound fails to counteract the adrenal-enlarging action of exogenous anterior-pituitary extracts. Apparently the adrenal atrophy produced by methyl-testosterone in untreated, or thyroxine treated, animals is primarily due to an inhibition of pituitary corticotrophin secretion and not to an inactivation of the circulating corticotrophic substance.

APE IN COMBINATION WITH THYROXINE AND METHYL-TESTOSTERONE (groups 8) exerted essentially the same corticotrophic effect (as judged by adrenal weight only, as we shall see in the histologic section!), as thyroxine plus APE (groups 5). This confirms the inability of the testoid to interfere with the adrenal stimulating action of anterior-pituitary extracts, even if the latter is potentiated by thyroxine.

The data of experiments II, III and IV were quite consistent, but in view of the rather fundamental importance of the conclusions to be drawn, we decided to repeat the work once more, again under somewhat different conditions, in order to ascertain that these hormones and hormone combinations regularly produce the same effects.

## Experiment V

Thirty-two groups, each consisting of eight female or castrate male albino rats, were used in the fifth experiment. Series I and II were killed on the 42nd, series III and IV on the 63rd day of the experiment. Series I and III were not nephrectomized, series II and IV were unilaterally nephrectomized.

Qualitatively the hormone treatment was the same in all groups as in the corresponding groups of experiment

TABLE V

EFFECT OF THYROXINE, APE AND METHYL-TESTOSTERONE ON THE  
ADRENALS OF FEMALE AND CASTRATE MALE NOT NEPHRECTO-  
MIZED AND UNILATERALLY NEPHRECTOMIZED RATS.  
42 DAY AND 63 DAY EXPERIMENTS

GROUP	HORMONE TREATMENT	SERIES		I		II		III		IV	
		♀ Not Nephrectomized 42 days—Purina		♀ Unilateral Nephrectomy 42 days—Purina		♂/c Not Nephrectomized 63 days—Purina		♂/e Unilateral Nephrectomy 63 days—Purina		Body Weight (gm.)	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final	Adrenal (mg.)	Adrenal (mg.)
I	Controls	119±8	176±7	52±3	119±12	172±9	55±3	120±5	252±9	40±3	45±3
	Thyroxine	119±8	188±4	77±4	119±7	175±6	68±4	122±7	211±9	54±4	60±3
III	Anterior Pituitary Extract	119±10	243±8	67±4	120±10	242±6	74±4	122±4	295±11	66±3	122±5
	Methyl-Testosterone	120±10	196±12	34±2	119±10	190±10	37±4	122±5	226±8	39±1	273±16
V	Thyroxine+ Anterior Pituitary Extract	120±9	248±7	110±8	120±11	251±6	125±9	121±8	260±8	122±12	121±8
	Thyroxine+ Methyl-Testosterone	120±8	184±9	43±2	120±12	177±6	44±1	122±8	185±11	42±1	123±7
VII	Anterior Pituitary Extract+ Methyl-Testosterone	119±11	243±7	63±1	120±8	252±5	72±2	122±5	303±10	62±4	121±8
	Thyroxine+ Methyl-Testosterone+ Anterior Pituitary Extract	119±10	285±10	114±10	120±9	241±9	124±8	121±8	311±15	115±9	123±7

IV, but thyroxine was given in a dose of 75 γ per day subcutaneously. Treatment with all hormone preparations was begun on the first day of the experiment.

This arrangement permitted a direct comparison between the corticotrophic effect of thyroxine and anterior-pituitary extract, under experimental conditions which resulted in essentially the same degree of cortical enlargement through these two different stimuli.

Perusal of Table V indicates that even when thyroxine and anterior pituitary extract are given in doses so chosen as to result in the same degree of cortical enlargement, the effect of simultaneous methyl-testosterone administration upon this corticotrophic effect is essentially different in the two cases. Here again, *females and male castrates* reacted similarly and *unilateral nephrectomy* did not significantly influence the adrenal response to the hormones used.

THYROXINE alone (groups 2) and *APE* alone (groups 3) exerted a distinct corticotrophic action of essentially the same degree, while *methyl-testosterone* alone (groups 4) caused a significant decrease in adrenal weight in all series.

However, while the corticotrophic action of thyroxine was abolished when *thyroxine plus methyl-testosterone* (groups 6) were given simultaneously, no such inhibitory action was observed in the groups receiving *APE plus methyl-testosterone* (groups 7) at the same time.

The corticotrophic action of *APE* was augmented by the thyroid hormone, as seen in the animals receiving *thyroxine plus APE* (groups 5), but methyl-testosterone again failed to exert any inhibitory action (as judged by the adrenal weights) in the groups receiving *thyroxine, methyl-testosterone and APE* (groups 8). It may therefore be concluded that thyroxine can stimulate the an-

terior lobe of the pituitary to produce corticotrophic substances similar to those present in the APE. This stimulating effect of the thyroid hormone is abolished by methyl-testosterone although the latter cannot similarly counteract exogenous APE. It is difficult to explain, however, why methyl-testosterone, which is so effective in antagonizing the corticotrophic action of thyroxine (cf., groups 2 and 4) fails to counteract the APE synergizing action of thyroxine (cf., groups 3 and 5) when given in combination with both these preparations (cf., groups 5 and 8).

It appeared of interest to compare the effects of methyl-testosterone, a testoid compound, with that of desoxycorticosterone acetate (DCA), a mineralo-corticoid substance. In previous publications (Selye, 1940a; Selye, 1940b) we already had occasion to point out that both testosterone and DCA cause adrenal cortical involution, but the effect of the former tends to be more pronounced than that of the latter. At first sight this was somewhat surprising and seemingly contrary to the principle of "compensatory atrophy," which is applicable to so many endocrine glands (Selye, 1947). It must be remembered, however, that the adrenal cortex normally produces both cortoid and testoid compounds, so that both could cause "compensatory atrophy." Furthermore — due to its greater toxicity — DCA is more likely to act as a stressor and this counteracts its anti-corticotrophic effect.

Additional studies revealed that both these types of steroids also tend to inhibit the adreno-cortical enlargement caused by non-specific stresses during the general-adaptation-syndrome; here again, testoids proved to be more effective than DCA. For instance, in animals in which a general-adaptation-syndrome is produced by re-

peated formaldehyde injections, testosterone tends to inhibit the cortical enlargement more effectively than DCA (Selye, 1940b).

The reason for this is still not entirely clear, but perhaps the protein anabolic properties of the testoids are also involved. Since endogenous or exogenous protein-catabolites increase the ability of the pituitary to produce corticotrophin under stress, protein-anabolic testoids may inhibit hypophyseal corticotrophin secretion by diminishing the available supply of endogenous protein-catabolites. Be this as it may, it is now clearly established that under conditions of great systemic stress, the adrenal cortex enlarges even if enormous doses of DCA are administered (Selye, 1948a). Indeed, in nephrectomized-NaCl rats in which DCA exerts highly toxic effects, this corticoid itself can cause pronounced adreno-cortical enlargement, through the non-specific stress mechanism.

Because of these interesting relationships between the anti-corticotropic actions of DCA and those of the testoids, another experiment was performed along essentially similar lines, but using DCA instead of methyltestosterone.

## Experiment VI

Eight groups each consisting of ten female albino rats, were unilaterally nephrectomized and maintained on "Purina fox chow" with 1% NaCl as drinking fluid. The experiment was terminated after 21 days of treatment.

Group 1 acted as untreated controls, group 2 was treated with thyroxine, group 3 with anterior-pituitary extract (APE), group 4 with desoxycorticosterone acetate (DCA), group 5 with thyroxine and APE, group 6 with

thyroxine and DCA, group 7 with APE and DCA and group 8 with thyroxine, APE and DCA.

Thyroxine was administered throughout in daily doses of 200  $\gamma$  subcutaneously; APE in a dose of 0.4 cc. twice daily, subcutaneously; DCA in the form of a 50 mg. pellet implanted subcutaneously on the day of the partial nephrectomy.

Unilateral nephrectomy was performed and NaCl supplements were given to accentuate the toxic effects of DCA. That under these conditions the hormones actually did damage the animals was shown by the inhibition of growth noted in the groups receiving this steroid. The apparently high final body weight in the group treated with thyroxine and DCA (group 6) was merely due to the development of a pronounced edema. Histologic studies also confirmed the existence of cardiovascular damage and nephrosclerosis, such as are normally seen in rats heavily overdosed with DCA. These toxic manifestations counteracted the usual "compensatory atrophy" effect of DCA so that the final adrenal weight was within normal range.

Perusal of Table VI indicates that *thyroxine* (group 2) and *APE* (group 3) cause pronounced adrenal enlargement, *DCA* (group 4) led to no change in the gross weight of the gland (perhaps because the animals developed severe nephrosclerosis and the resulting "stress effect" outweighed the "compensatory atrophy effect" of the steroid), *thyroxine plus APE* (group 5) caused a more pronounced enlargement than could be expected by mere summation of the separate actions of the two hormones, hence we may well speak of a true potentiation, *thyroxine plus DCA* (group 6) treatment resulted in adrenals approximately as heavy as those of the group receiving thyroxine alone (group 2), *APE plus DCA*

TABLE VI

EFFECT OF THYROXINE, APE AND DCA UPON THE ADRENALS OF  
UNILATERALLY NEPHRECTOMIZED NaCl-TREATED RATS.  
21 DAY EXPERIMENT

GROUP	HORMONE TREATMENT	♀ Unilateral Nephrectomy 21 days—Purina+NaCl		
		Body Weight (gm.)		Adrenal (mg.)
		Initial	Final	
I	Controls	121±4	159±6	44±3
II	Thyroxine	121±4	164±6	60±3
III	Anterior Pituitary Extract	121±6	224±7	83±8
IV	Desoxycorticosterone Acetate	121±4	147±9	47±5
V	Thyroxine+ Anterior Pituitary Extract	120±4	193±11	128±8
VI	Thyroxine+ DCA	121±4	202±13	62±5
VII	Anterior Pituitary Extract+ DCA	121±5	153±8	89±5
VIII	Thyroxine+ Anterior Pituitary Extract+ DCA	120±4	192±8	121±8

(group 7) revealed no inhibition of the APE action and finally, *thyroxine, APE and DCA* (group 8) administration produced adrenals essentially similar in weight to those of the group treated only with thyroxine and APE (group 5).

This experiment thus confirms our earlier data, which showed that the ability of DCA to prevent the corticotrophic effect of non-specific stress or thyroxine is considerably less than that of methyl-testosterone.

Since we had previously found that DCA is non-toxic on NaCl-free diets (Selye et al., 1949) it appeared of interest to verify whether its anti-corticotropic action is really more manifest under such nutritional conditions which preclude the production of cardiovascular and renal disease. At the same time such an experiment promised to throw some light upon the much discussed histologic changes seen in the adrenals of rats receiving Na-deficient diets.

## Experiment VII

Six groups of 10 female hooded rats each were unilaterally nephrectomized. Groups 1-4 were given the synthetic diet (see Chart I), except that in the mineral mixture, the NaCl was eliminated and the  $\text{Na}_2\text{HPO}_4$  replaced by an equivalent amount of  $\text{K}_2\text{HPO}_4$ , in order to make the diet Na- and Cl-free. 3.5 gm. instead of the usual (4 gm./100 gm. of diet) of mineral mixture was added to the diet in these groups. Distilled water was given *ad lib.* Group 1 received no other treatment. Group 2 was implanted, at the time of nephrectomy, with two 40 mg. pellets of DCA. Group 3, though also given the Na- and Cl-free diet, received 1% NaCl as drinking fluid. Group 4 was kept on the Na- and Cl-free diet with 1% NaCl to drink, and in addition was implanted with two 40 mg. pellets of DCA. Group 5 was given our basic synthetic diet with an addition of 4% NaCl as well as 1% NaCl as in the drinking water. Group 6 in addition to the high NaCl intake of group 5 was implanted with two 40 mg. pellets of DCA as in Groups 2 and 4.

Treatment was continued for 40 days. At this time the moribund condition of Group 6 made it necessary to terminate the experiment.

TABLE VII

EFFECT OF DCA ON THE ADRENAL AS INFLUENCED BY DIETARY NaCl.  
40 DAY EXPERIMENT

GROUP	HORMONE TREATMENT	DIET	♀ Unilateral Nephrectomy 40 days		
			Body weight (gm.)		Adrenal (mg.)
			Initial	Final	
I	Control	Na and Cl-free Distilled water to drink.	159±2	174±5	34±1
II	DCA	Na and Cl-free Distilled water to drink.	157±3	175±4	28±1
III	Control	Na and Cl-free 1% NaCl to drink	162±2	179±4	32±1
IV	DCA	Na and Cl-free 1% NaCl to drink	160±3	178±5	36±1
V	Control	4% NaCl 1% NaCl to drink	160±3	167±3	31±2
VI	DCA	4% NaCl 1% NaCl to drink	162±3	137±5	32±2

Table VII summarizes our results.

It will be noted that marked adrenal involution occurred only in the rats (group 2) receiving DCA without NaCl supplements and in fact these proved to be the only DCA treated animals which failed to develop hypertension, nephrosclerosis and cardiovascular lesions. The rather interesting histologic changes observed in the adrenals of these groups will be discussed later.

It may incidentally be mentioned that the rise in the plasma Na/Cl ratio so characteristic of DCA intoxica-

tion was most pronounced in group 4 ( $\text{Na}$  181 m.eq/L;  $\text{Cl}$  95.5 m.eq/L) while in the corresponding controls (group 3) the blood concentrations of sodium (145.9 m.eq/L) and chloride (102.2 m.eq/L) were within the range found in our normal rats kept on the stock diet. This hypochloremic alkalosis is difficult to explain since the blood  $\text{CO}_2$ -combining power was 38.5 and 37.6 vol. % respectively in groups 4 and 3, so that presumably the excess  $\text{Na}$  in group 4 must have been bound to some anion other than  $\text{Cl}$  or  $\text{HCO}_3$ . The changes in the electrolyte metabolism of these animals will be the subject of a special communication (Schaffenburg, 1949). We mention them here merely to point out that the highest plasma  $\text{Na}/\text{Cl}$  ratio was noted in the group having the largest adrenal weight, although this was not the group that received the highest concentration of  $\text{NaCl}$  in the diet. Conversely, the lowest adrenal weight (group 2) was associated with the lowest  $\text{Na}/\text{Cl}$  ratio (plasma  $\text{Na} = 133.5$  m.eq/L;  $\text{Cl} = 98.8$  m.eq/L; plasma  $\text{CO}_2$ -combining power = 28.5 vol. %).

## ✖ 4 ✖

# Histologic Findings and Discussion

SPECIAL attention was given to the histologic and cyto-logic changes in the adrenal cortex which accompanied the changes in gross weight discussed above. However, in order to enlarge the scope of this study, we also include in this report many observations made in a large number of additional experiments, which were performed at our Institute during the last ten years. These data illustrate the effects, upon the adrenal, of various non-specific stress situations, diets, drugs, hormones, removal of endocrine glands, etc. This procedure helped us to survey most of the typical structural changes that can be experimentally produced in this gland; accordingly it enabled us to review the present-day status of the experimental morphology of the adrenal cortex on the basis of personal experience gathered under uniform laboratory conditions.

In connection with the present purely morphologic study, it would not be profitable to discuss all these experiments in detail. Furthermore, a full description of the extensive material from which we gathered our data would require an unwarranted amount of space. Hence, this part of our report is not written in the manner customary in experimental endocrinology. It is more comparable to the account of a pathologic anatomist who endeavours to characterize a certain structural change on the basis of tissues obtained from many patients, their case

histories being given in an abridged form, mentioning merely the pertinent data.

The principal outcome of our studies was to show that in addition to the usual non-specific response to simple stimulation and inhibition, the adrenal cortex is capable of responding to certain stimuli, with various highly specific reaction forms. Some of these have not yet been described, others were only occasionally mentioned as incidental findings in man. As it was hitherto not possible to reproduce them experimentally, the pathogenesis and physiologic significance of many among these structural changes could not be subjected to systematic study up to now.

The very existence of such specific responses is difficult to reconcile with the current theory that the adrenal cortex responds only to a single corticotrophin and that the manifold experimental conditions, which can cause structural changes in the suprarenal cortex, do so through the intermediary of the anterior pituitary whose corticotrophin production they merely increase or decrease. The evidence at hand appears to be in better accord with our previously expressed view that, either the pituitary produces a number of different corticotrophins, or — and this is even more probable — "that the same pituitary trophic principle exerts different actions depending upon the simultaneous presence of other hormones (e.g., steroids)" (Selye, 1947).

The reader interested in the manifold functional implications of qualitatively different corticotrophic actions is referred to a recent, more extensive treatise, in which these have received special attention (Selye, 1950).

The following is a list of those qualitatively different morphologic changes which we were able to distinguish in our experimental material:

1. Atrophy.
2. Hypertrophy.
3. Hyperplasia.
4. Capsular adenomas.
5. Lipid granule storage or discharge.
6. Cholesterol granule storage or discharge.
7. Plasmal granule storage or discharge.
8. Ascorbic acid granule storage or discharge.
9. Fatty metaplasia.
10. Colloid formation.
11. Fibrinoid degeneration.
12. Cytolysis.
13. "Chromidiosis."
14. Lymphoid and myeloid metaplasia.
15. Formation of lumina within the cortical parenchyme.
16. Holocrine secretion.
17. Hyperemia.
18. Hemorrhagic infarction.
19. Focal necrosis.
20. "Toxic involution."

It must be emphasized that this is not a complete list of all the changes that can be experimentally produced in the adrenal cortex; it merely comprises those concerning which we could gather sufficient personal experience to attempt their interpretation. Although several of these responses may occur simultaneously in the same adrenal, we discuss them separately in order to emphasize that they are relatively independent of each other. Thus they represent qualitatively distinguishable reaction types of the adrenal cortex, exhibited under different experimental conditions.

## Atrophy

It is generally assumed that adreno-cortical atrophy ensues when hypophyseal corticotrophin production becomes inadequate. It had already been emphasized, however, that there are various, qualitatively different, types of cortical atrophy (Selye, 1940a; Selye, 1947). Among these, the almost specific involution of the reticularis or "x-zone," which can be induced in the mouse adrenal by treatment with testoid compounds, has received the greatest attention (Martin, 1930; Burrows, 1936; Selye, 1939; Selye, 1940a). In the rat adrenal, such a selective effect upon the x-zone is not obvious because the reticularis does not assume the character of a clearly delimited region. Nevertheless, it is evident from our earlier observations (McEuen et al., 1937a; McEuen et al., 1937b; Selye, 1947) that testosterone considerably decreases the size of the adrenal cortex in the rat also, and here again, this involution is accompanied by rather specific histologic changes. Thus, under the influence of chronic testosterone (or methyl-testosterone) treatment, there is pronounced sclerosis of the capsule and many of the cortical cells throughout the fasciculata undergo "fatty metaplasia," a phenomenon which we shall discuss in greater detail below. (Figures 3, 4 and 5).

HYPOPHYSECTOMY results in a fairly even involution of all cortical layers, although the reticularis is most, the glomerulosa least rapidly affected (Collip et al., 1933; Selye, 1947), (Figure 2).

Since only certain, partially purified anterior-lobe extracts (poor in other hormonal activities) restored these cortices to normal, we concluded, in 1933, that the corticotrophic principle is distinct from other pituitary hormones (Collip et al., 1933).

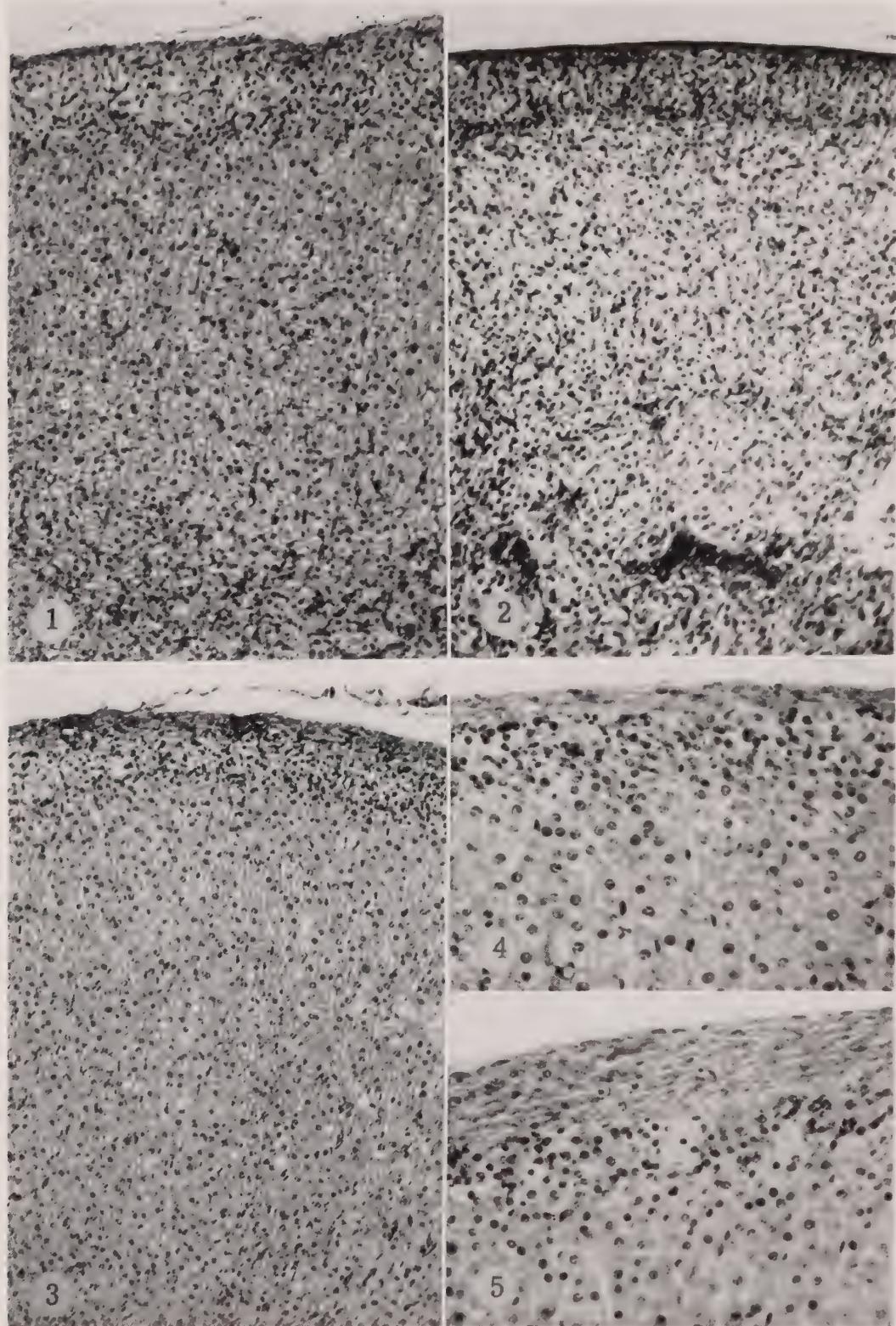


PLATE I. ATROPHY

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Recently, considerable emphasis has been laid in the literature upon the comparatively good maintenance of the glomerulosa after hypophysectomy in the rat (Deane and Greep, 1946). Indeed it has been suggested that this zone actually becomes wider after ablation of the anterior lobe (Deane et al., 1948). We find, however, that some involution of the glomerulosa occurs even as soon as two weeks after hypophysectomy (Figure 2); later this atrophy becomes very striking (Selye, 1947). Furthermore, adequate doses of hypophyseal corticotrophin cause marked hypertrophy of the glomerulosa cells in the rat adrenal (Figure 61). Thus, we cannot subscribe to the view, that, unlike the other layers of the cortex, the glomerulosa of the rat adrenal is independent of hypophyseal stimulation.

It is most likely that the glomerulosa acts as a matrix for the adrenal cortical tissue, somewhat like the sperma-

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#### PLATE I. ATROPHY

*Figure 1.* Adrenal cortex of a control rat showing normal glomerulosa, fasciculata and reticularis. The medulla is not within this visual field (magnification  $\times 100$ ).

*Figure 2.* Atrophic adrenal cortex of a rat two weeks after hypophysectomy. Note considerable atrophy of the cortex. The involution is most pronounced in the fasciculata and reticularis, but the cells of all layers are subnormal in size. The fasciculata is rich in light vacuoles (corresponding to lipid granules). The area below the arrow is medullary tissue (magnification  $\times 100$ ).

*Figure 3.* Adrenal cortical atrophy following testosterone treatment. Note that the cells of all layers are somewhat subnormally developed, the capsule is sclerotic and many cells in the fasciculata contain large vacuoles corresponding to lipid deposition in signet ring cells (magnification  $\times 100$ ).

*Figure 4.* Capsule and outer cortex of the adrenal in a normal control rat under higher magnification (magnification  $\times 200$ ).

*Figure 5.* Capsule and outer cortex of a testosterone treated rat. Note especially the marked proliferation of the connective tissue in the capsule (magnification  $\times 200$ ).

togonia and ovogonia act for the mature sperm and egg cells respectively. It is known that hypophysectomy affects the mature germinal cells of both sexes much more rapidly than the large reserve of immature cells. Nevertheless, the process of maturation depends upon the action of hypophyseal gonadotrophin on the immature elements. Probably the behavior of the glomerulosa is essentially similar to that of the other matrix tissues. It must be kept in mind that no gland under pituitary control (adrenal cortex, gonads, thyroid) is entirely dependent for its maintenance upon hypophyseal stimulation and that generally, the least differentiated ("embryonic") cell types are most independent of central trophic stimuli, probably because it is, especially, the maturation and function of the cells that is stimulated by the hypophysis. In many lower vertebrates, or embryonic mammals, the entire somatic growth is independent of the hypophysis and we have found that even rats continue to grow after hypophysectomy during the first weeks of life (Selye, 1947).

It has been claimed that in the rat the glomerulosa is the source of the mineralo-corticoids, especially because after hypophysectomy, electrolyte metabolism tends to be maintained, while hypoglycemia occurs quite readily. It should be pointed out, however, that even after complete adrenalectomy, fatal hypoglycemia ensues more easily than severe derangements in mineral metabolism. Furthermore, the simultaneous posterior-lobe deficiency complicates the derangement in mineral metabolism because of the failure to produce "antidiuretic hormone." It is also noteworthy that in the rat, crude corticotrophin-containing, pituitary extracts are very effective in causing nephrosclerosis, cardiovascular changes and hypertension such as are produced by mineralo-corticoids (e.g., DCA), (Selye, 1946).

Other findings (see "Hypertrophy," below) confirm

the existence of some relationship between glomerulosa and Na metabolism, but the above observations do not support the view that this effect is independent of pituitary control. The claim that sodium deficiency stimulates the glomerulosa even after hypophysectomy (Deane et al., 1948), was based on very few observations and requires confirmation. Perhaps Na-deficiency — as so many other agents, which will be reviewed below — influences the glomerulosa by a dual action: both directly and through the hypophysis.

It is rather characteristic of the cortical involution produced by hypophysectomy that, during the first few weeks after the operation, the individual cells are small, but comparatively rich in lipid granules. Perhaps this is due to a defect in the mechanism responsible for their discharge. It is also quite typical that frequently the nucleus in these involuting cells tends to be eccentrically located in close attachment to the cell membrane (Figure 7).

Overdosage with *corticoids*, especially DCA, causes essentially similar changes as hypophysectomy, but, the nucleus usually retains its central position in the cell. Often the cell limits become particularly sharp, due to condensation of cytoplasm in the vicinity of the cell membrane (Figures 9 and 59).

Some investigators reported that DCA causes a rather selective lipid depletion and involution of the glomerulosa (Sarason, 1943; Deane et al., 1948) but this was not evident in our material.

Differences in the diet, or the strain of the animals used, may explain such variations in the responses observed in different laboratories.

As shown by the animals in group 5 of experiment VII of this paper, diets very rich in *NaCl* cause selective atrophy of the glomerulosa (Figure 58). This action

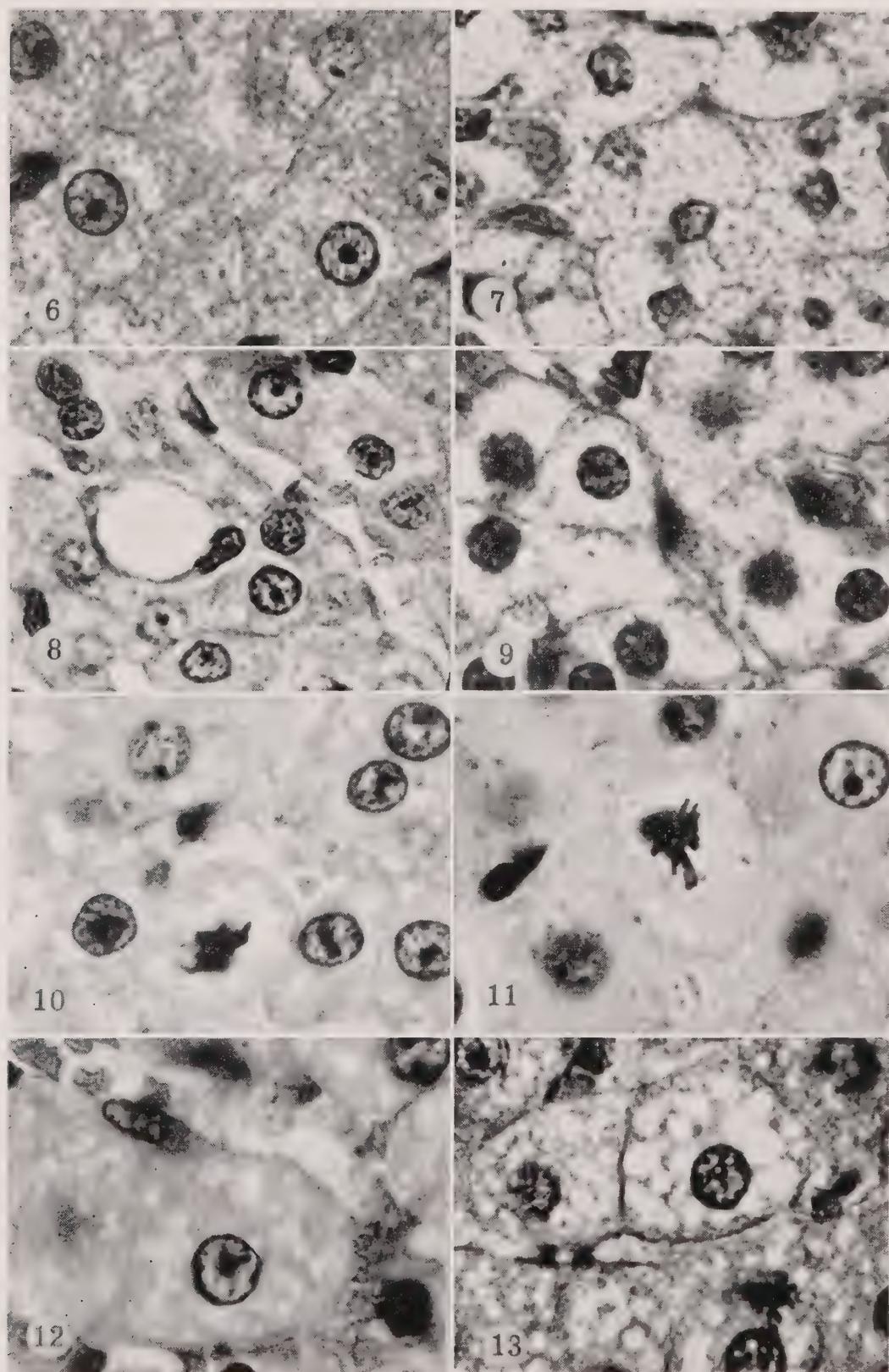


PLATE II. ATROPHY, HYPERTROPHY AND HYPERPLASIA

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## ← PLATE II. ATROPHY, HYPERPLASIA AND HYPERPLASIA

*Figure 6.* Adrenal cortex of normal control rat. All pictures on this plate show representative cells from the outer fasciculata of rats having approximately the same body weight (magnification  $\times 1000$ ).

*Figure 7.* Cortical atrophy caused by hypophysectomy. Note greatly reduced size and frequently excentric position of nucleus. The cytoplasm still contains many lipid vacuoles. Compare with other types of atrophy illustrated in Figs. 8 and 9 (magnification  $\times 1000$ ).

*Figure 8.* Cortical atrophy produced by testosterone. Note that here cytoplasm and nucleus are also poorly developed. There are few small lipid droplets, but "fatty metaplasia" has occurred in some of the cells. One such typical "fat cell" is in the center of the field. Note great distension of cytoplasm by a lipid vacuole which compresses the nucleus and pushes it to the side so that the cell assumes the typical signet ring appearance of a common fat cell (magnification  $\times 1000$ ).

*Figure 9.* Cortical atrophy produced by DCA. Note especially prominent cell contours. Both cytoplasm and nucleus are subnormal in size, but small lipid vacuoles are clearly visible (magnification  $\times 1000$ ).

*Figure 10.* Cortical hypertrophy and hyperplasia produced by APE. Note large round cell in mitosis, with distinct spindle. The other cells are rich in cytoplasm, but the nucleus is not considerably enlarged. Binucleated cell in right upper corner of this field (magnification  $\times 1000$ ).

*Figure 11.* Cortical hyperplasia and hypertrophy produced by APE. Another typical mitosis is seen in the rounded, central cell of this field. The nucleus in the right upper corner is vesicular. Most cells have discharged their cytoplasmic lipid granules and scattered basophilic droplets are visible in the cytoplasm. Figures 10 and 11 are rather typical of acute adrenal over-stimulation (magnification  $\times 1000$ ).

*Figure 12.* Hypertrophy with beginning lipid storage in the adrenal of an APE-treated rat. Note great increase in cytoplasmic mass; nucleus is vesicular but not very much enlarged. The cytoplasm contains some medium sized lipid vacuoles (magnification  $\times 1000$ ).

*Figure 13.* Hypertrophy and marked lipid-granule storage in the adrenal of an APE and testosterone treated rat. Note the increase in cytoplasmic mass and the presence of numerous very coarse lipid granules. In the same adrenal many cells have undergone complete metaplasia into typical fat cells (magnification  $\times 1000$ ).

of sodium will be discussed in more detail below in connection with the glomerulosa hypertrophy elicited by Na-deficient food.

## Hypertrophy

It has become customary to use the term "adrenal cortical hypertrophy" as synonymous with enlargement. This may give rise to misunderstandings. The designation "hypertrophy" should be reserved — in agreement with generally accepted morphologic nomenclature — for the enlargement of individual cells; an increase in their number is designated as hyperplasia. In the case of the adrenal cortex, hypertrophy and hyperplasia usually occur simultaneously, but this is not always the case.

Generalized hypertrophy and hyperplasia are both non-specific responses of the adrenal cortex to stimulation. They always occur — especially in this fasciculata — during the general-adaptation-syndrome elicited by exposure to various types of stress as well as following administration of hypophyseal corticotrophin. In either case, the volume of the cytoplasmic mass is more markedly increased, in the hypertrophic cell, than that of the nucleus. Under certain conditions (see below), the cytoplasm of the enlarged cell discharges its lipid granules, while under other circumstances it may actually store an increased amount of lipids. The nucleus tends to become vesicular and usually contains a single very prominent nucleolus. Quite frequently, numerous basophilic granules ("chromidia") are seen in the cytoplasm of hypertrophic adrenal cortical cells, but these will be discussed separately (Figures 10-13).

Selective hyperplasia was regularly noted in the glo-

merulosa of our rats kept on NaCl-deficient diets (Figures 55-57). This confirms earlier reports suggesting a specific response of this zone in rats on the low sodium intake (Deane et al., 1948; Nichols, 1948). In our animals this change was often accompanied by the disappearance of a distinct boundary between glomerulosa and fasciculata and by focal necrosis of glomerulosa cells. These responses were not prevented by DCA even in toxic doses and, for reasons mentioned above, we doubt its independence of hypophyseal corticotrophin (Figure 60).

## Hyperplasia

As stated above, hyperplasia is also a non-specific reaction-form of the adrenal cortex. In our material it occurred quite regularly under the influence of various stresses during the *general-adaptation-syndrome*, as well as after administration of purified *corticotrophin*. It is characterized by the presence — especially in the outer fasciculata region — of many mitotic figures. Amitotic divisions of cells have never been identified with certainty in the adrenal cortex. As judged by a study of our material, hyperplasia is always associated with hypertrophy, but in the case of chronic stimulation, the cells may remain large a long time after the peak of the mitotic wave had subsided.

During a general-adaptation-syndrome, mitotic proliferation is most pronounced in the first (alarm-reaction) phase and, in the case of chronic treatment with pituitary corticotrophin, during the first few days of treatment. However, during the very first hours of the alarm-reaction, there may be a slight decrease in adrenal weight due to the discharge of secretion granules which precedes hyper-

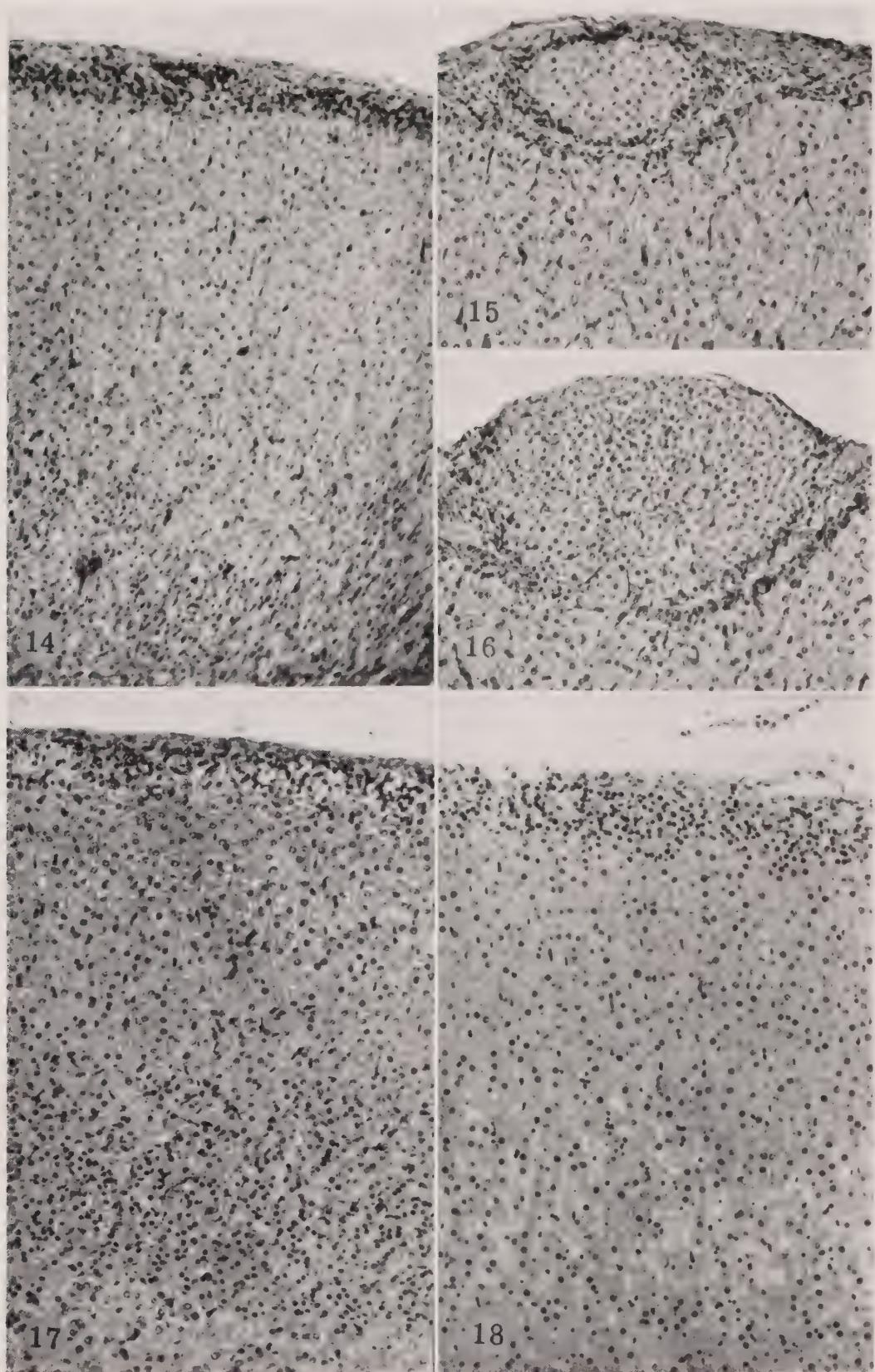


PLATE III. ADENOMA FORMATION AND HYPERSTROPHY →

trophy and hyperplasia. Hypertrophy of cortical cells, on the other hand, persists during later stages of the adaptation syndrome and, in the event of corticotrophin injections, as long as treatment is continued (Figures 10 and 11).

Administration of *protein-rich diets*, or treatment with *thyroxine*, greatly enhances the production of cortical hypertrophy and hyperplasia by non-specific stress or by crude corticotrophin-containing hypophyseal extracts. As judged by the increase in adrenal weight, the potentiating effect of dietary protein and of thyroxine was especially evident in the experiments described in the first part of the present communication. The mechanism of this sensitization is not yet clear. Should protein catabolites (e.g., amino acids) be involved in this phenomenon, then dietary protein and thyroxine may act in essentially

← PLATE III. ADENOMA FORMATION AND HYPERSTROPHY

*Figure 14.* Adrenal cortex of a control rat showing a glomerulosa islet partially separated from the remaining cortex by capsular connective tissue (magnification x 100).

*Figures 15 and 16.* Two stages in the formation of capsular adenomas, which presumably arise from glomerulosa islets such as that shown in Fig. 14. These particular tumors were found in rats simultaneously treated with APE and testosterone, but similar adenomas occur after chronic treatment with APE alone or chronic exposure to non-specific stress. Apparently they are a rather common manifestation of non-specific cortical stimulation (magnification x 100).

*Figure 17.* Cortical hypertrophy caused by exposure to cold. In comparison with Fig. 14, note enlargement of individual cells and appearance of numerous vacuoles in outer fasciculata region. Many of these vacuoles contain droplets of what appears to be degenerating cytoplasmic material (magnification x 100).

*Figure 18.* Cortical hypertrophy during general-adaptation-syndrome caused by exposure to cold. Note considerable enlargement of cytoplasmic mass as well as great irregularity of nuclei throughout the fasciculata. In this case, vacuoles are especially plentiful in the inner fasciculata and most of them contain fairly large lipid granules while droplets of degenerating cytoplasm are scarce (magnification x 100).

the same manner since the thyroid hormone increases protein turnover.

The influence of these sensitizing agents upon pure corticotrophin has not yet been adequately examined. Preliminary experiments suggest, however, that the protein intake does not alter the action of pure corticotrophin (Moya et al., 1948). Perhaps the protein catabolites merely augment corticotrophin production by the pituitary, such as occurs during stress or under the influence of crude anterior-lobe preparations.

Testoids (e.g., methyl-testosterone) and, to a lesser extent DCA, tend to inhibit the cortical hyperplasia and hypertrophy normally produced by exposure to non-specific stress; presumably because they impede the endogenous formation of corticotrophin by the anterior lobe. Neither of these steroids interferes with the corticotrophic action of pituitary extracts as shown by the experiments described in the first part of the present communication. In addition to this effect upon the hypophysis, both testoids and corticoids also appear to have a direct action upon the adrenal, as we shall see below.

### Capsular Adenomas

Adenomas of the adrenal cortex appear quite frequently in (or just below) the capsule of the gland in man. Many investigators believe them to be especially common in patients suffering from malignant hypertension, nephrosclerosis and other "diseases of adaptation" (Selye, 1950).

In the rat, small cell-groups of the glomerulosa are often partially detached from the rest of the gland by connective tissue strands. Such glomerulosa islets consist of very small cells with darkly staining nuclei and little

cytoplasm. Under the influence of exposure to non-specific stress, during the general-adaptation-syndrome, and after treatment with corticotrophic anterior-lobe extracts, these cell-groups tend to undergo hypertrophy and hyperplasia, thus giving rise to more or less voluminous adenomas (Figures 14, 15 and 16). It is probable that the formation of these growths is merely part of the non-specific response of cortical tissue to stimulation by corticotrophin.

In this connection it should be mentioned that adrenal cortical carcinomas have also been produced experimentally, for instance, by gonadectomy during the first days of life. This subject has been discussed elsewhere (Selye, 1947) and since malignant cortical tumors did not occur in our own experimental material, we shall not describe them here.

## Lipid Granule Storage and Discharge

There is a great deal of confusion in the literature concerning the functional significance of sudanophilic and osmiophilic lipid granules in the adrenal cortex.

Some investigators believe that accumulation of lipid granules within the cortical cells is a sign of decreased hormone production. In support of this view, it is emphasized that soon after hypophysectomy, the cortical cells are rich in lipids, while during the alarm reaction, the lipid granules are discharged; obviously, in the former case the cortex is inactive, in the latter, hyperactive. Those, however, who advocate the opposite theory have pointed out that several months after hypophysectomy the adrenal cortex is poor in lipids and that after chronic treatment with ACTH, as well as in the resistant phase of the general-adaptation-syndrome, the cortex is large and its cells

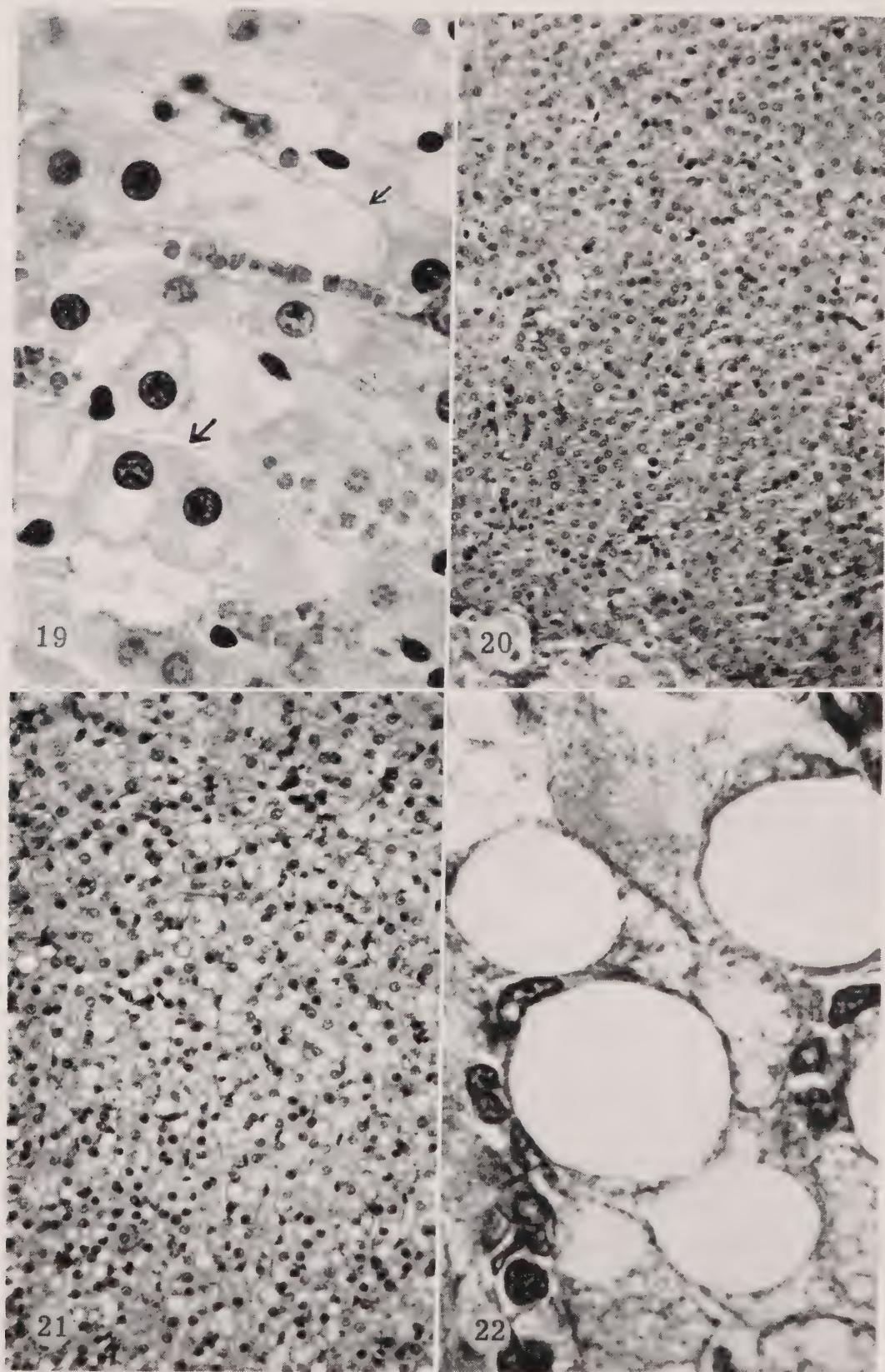


PLATE IV. FATTY METAPLASIA

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contain numerous lipid granules. Yet in the former case, corticoid secretion is practically at a standstill, while during the latter conditions it is greatly augmented.

Perusal of our own experimental material suggests that both these theories are partially correct and not incompatible with each other. It appears that *a few days after hypophysectomy* the lipid content of the cortical cells is high, presumably because the gland is not affected by any corticotrophic stimulus which would cause the discharge of granules. The total width of the cortex is subnormal, however, due to the atrophy occasioned by lack of corticotrophic stimulation. *Several months after hypophysectomy*, this atrophy becomes so severe that the lipid granules disappear from the cytoplasm together with most other cellular constituents. In other words, immediately after removal of the anterior lobe, the cortical cell can still store, although it does not secrete.

Later the atrophy is so pronounced that the cell neither stores nor secretes any significant amount of corticoids —



#### PLATE IV. FATTY METAPLASIA

*Figure 19.* This is a higher magnification of one field from the inner fasciculata of the adrenal shown in Fig. 18. Note cytolysis in one almost completely disintegrated cell (upper arrow) and one containing a large lipid granule (lower arrow) (magnification x 600).

*Figure 20.* Fatty metaplasia of cortical cells in a rat treated with testosterone. Note several signet-ring cells throughout the fasciculata (magnification x 200).

*Figure 21.* Fatty metaplasia in the cortex of a rat treated with APE and testosterone. Note that here, large signet-ring cells are more numerous than in Fig. 20 (magnification x 200).

*Figure 22.* Fatty metaplasia in the adrenal cortex of a rat treated with APE, methyl-testosterone and thyroxine. Thyroxine further exaggerates the tendency towards fatty metaplasia of adrenal cortical cells. At this magnification, the typical signet-ring shape of these elements and their similarity to common fat cells is particularly striking (magnification x 1000).

or of the lipids in which these steroid hormones are presumably dissolved.

During *acute exposure to non-specific stress* (alarm reaction) the demand for cortical hormones is excessive. The adrenal cortex undergoes hypertrophy and hyperplasia; its width is increased, but it discharges its lipid (and presumably hormone) stores into the blood to meet the urgent demands. Hence only few, if any, secretion granules remain stored in the cytoplasm. Similar intense degranulation with hypertrophy and hyperplasia is produced by folliculoid hormones. These, unlike most stimuli, tend to retain their lipid-discharging effect even in the event of chronic treatment. Neither non-specific stress nor

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## CHART II



### PROBABLE FUNCTIONAL SIGNIFICANCE OF THE SIZE AND LIPID CONTENT OF THE ADRENAL CORTEX UNDER VARIOUS CONDITIONS

It will be noted that under the experimental conditions which we studied, the adrenal medulla does not undergo any significant change in size (inner circle), while the cortex (space between two circles) is subnormal after hypophysectomy and enlarged during the alarm reaction, the stage of resistance and treatment with folliculoid hormones or ACTH. — Depletion of lipid granules (shaded area) can occur both in inactive ("A") and hyperactive ("D") adrenal cortices; but when the cortex is simultaneously enlarged ("D") this is usually indicative of acute hyperactivity under conditions precluding adequate hormone storage, while in combination with atrophy ("A"), it suggests inability of both storage and secretion. Intense lipid storage is likewise compatible with both hypo- and hypersecretion but when it is accompanied by cortical enlargement ("E"), it suggests that the gland is capable of adequate storage in spite of increased secretion, while in conjunction with atrophy ("B"), it implies that the cortex is hypoactive, although it can still retain its stores. — It must be kept in mind, of course, that all these changes take some time to develop. The large and lipid-rich adrenal cortex of an animal during the stage of resistance retains its size and lipid granules immediately after hypophysectomy, although it is already inactive. Yet, we feel that this diagram helps to clarify the correlations between the structure and function of the cortex since it proved to be in accord with observations, if this time element was not neglected.

EXAMPLES OF CONDITIONS CAUSING APPARENTANCE OF ADRENAL	PRESUMPTIVE ACTIVITY	ILLUSTRATED CHANGES IN CONDITONS	STAGE OF RESISTANCE, ACTH
A	NO SECRETION DECREASED STORAGE	NORMAL SECRETION NORMAL STORAGE	HYPER- SECRETION NO STORAGE
B	NO SECRETION NORMAL STORAGE	NO SECRETION NORMAL STORAGE	ALARM REACTION, FOLLICULOIDS
C	LONG AFTER HYPOPHYSECTOMY	SHORTHLY AFTER HYPOPHYSECTOMY	
D			
E			

CHART II

PROBABLE FUNCTIONAL SIGNIFICANCE OF THE SIZE AND LIPID CONTENT OF THE  
ADRENAL CORTEX UNDER VARIOUS CONDITIONS

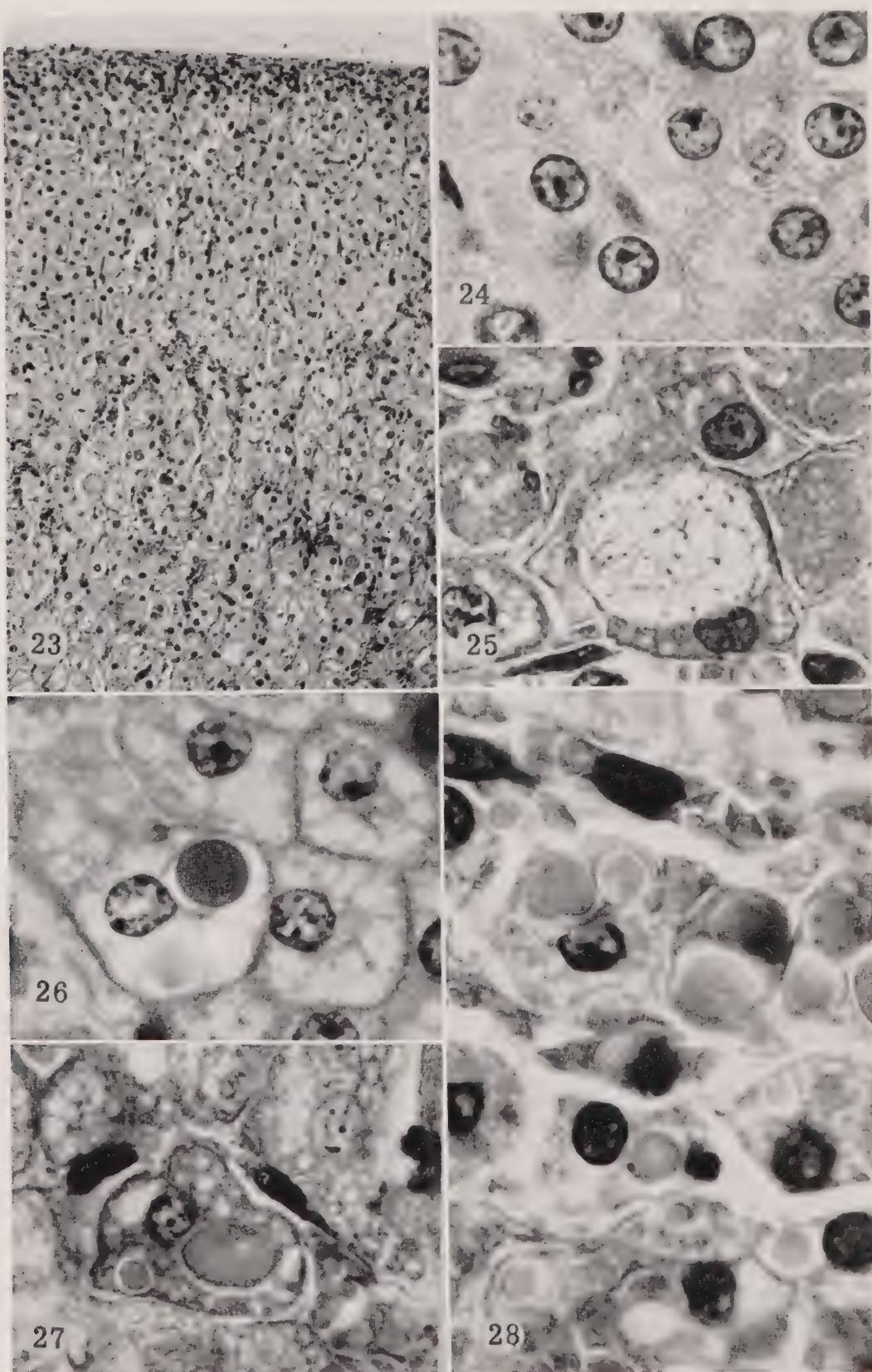


PLATE V. FIBRINOID DEGENERATION AND COLLOID FORMATION →

## ← PLATE V. FIBRINOID DEGENERATION AND COLLOID FORMATION

*Figure 23.* Colloid formation in the cortex of a rat treated with APE, methyl-testosterone and thyroxine. Note pronounced hypertrophy of cells in all parts of the fasciculata in comparison with Fig. 14 which represents a normal adrenal cortex at the same magnification. Many of the cells in the lower part of the field are distended by large, homogeneous, eosinophilic bodies (here gray). These are colloid granules similar to those shown under high magnification in Figs. 26, 27 and 28 (magnification x 100).

*Figure 24.* Adrenal cortex of normal control rat. This and all subsequent pictures on this plate show representative cells from the fasciculata of rats having approximately the same body weight (magnification x 1000).

*Figure 25.* Fibrinoid degeneration of a cortical cell in a rat treated with APE and methyl-testosterone. Note that within the large vacuole of this signet-ring cell, filaments have developed. At their intersections there are darkly staining nodules. This whole reticulum is clearly visible on this hematoxylin-eosin slide, but it becomes even more prominent on sections stained with Mallory's hematoxylin (magnification x 1000).

*Figure 26.* Colloid droplets in cortical cells of a rat treated with APE, methyl-testosterone and thyroxine. Note that central cell contains one dark, and one light colloid body, as well as a lipid vacuole. The other cells in this field contain only lipid vacuoles (magnification x 1000).

*Figure 27.* Colloid formation in the cortex of a rat treated with APE and methyl-testosterone (magnification x 1000).

*Figure 28.* Intense colloid storage in cortex of rat treated with methyl-testosterone and DCA. Note absence of the large fat vacuoles so characteristic of the adrenals of rats receiving testosterone alone. The colloid bodies formed under the influence of different treatments as illustrated in Figs. 26, 27 and 28 are similar in appearance (magnification x 1000).

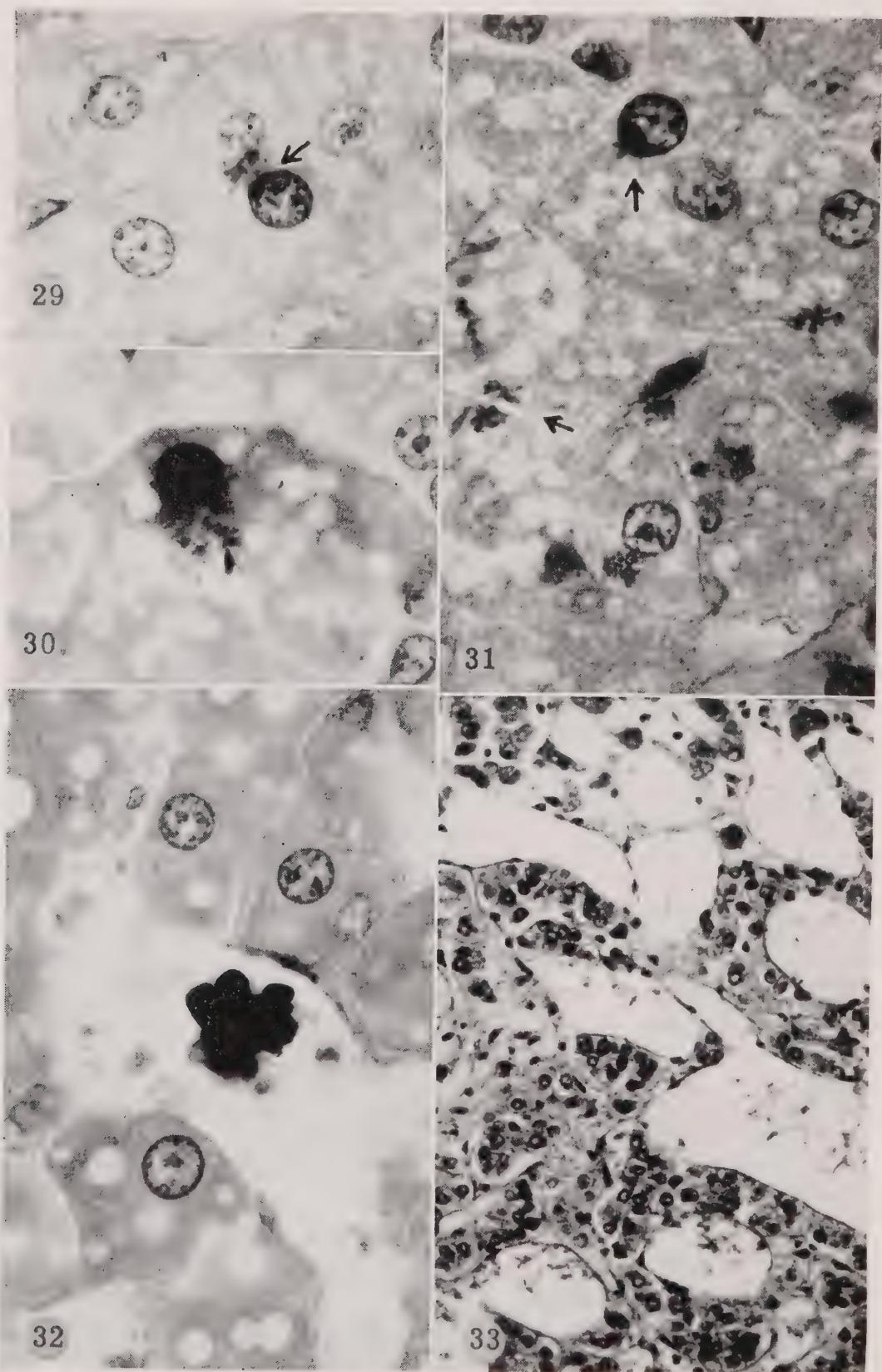


PLATE VI. "CHROMIDIA"

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folliculoids cause a discharge of adrenal cortical lipids in the absence of the pituitary and presumably their effect is dependent upon a simultaneously increased corticotrophin secretion.

Following *chronic exposure to non-specific stress* (stage of resistance of the general-adaptation-syndrome) or treatment with *corticotrophin*, the adrenal cortex is enlarged and its cells store numerous intensely sudanophilic lipid granules. Under these conditions there is every



#### PLATE VI. "CHROMIDIA"

*Figure 29.* Formation of chromidia in the outer fasciculata of a rat treated with APE. Note typical "chromatin cushion" applied to the nuclear membrane at the point indicated by the arrow. Filamentous basophilic rays connect this point with basophilic granules ("chromidia") within the cytoplasm of this cell (magnification  $\times 1000$ ).

*Figure 30.* Intense chromidiosis in the cortex of an APE-treated rat. Note that here the process is so pronounced that the nuclear structure is masked by the newly formed basophilic material (magnification  $\times 1000$ ).

*Figure 31.* Intense cytoplasmic chromatin formation in the cortex of a rat treated with APE. The upper arrow points to a nucleus with a large chromatin cushion from which basophilic material appears to emanate. The two lower arrows point to "snowflake-like" chromidia in their typical position at the point of contact between adjacent cortical cells (magnification  $\times 1000$ ).

*Figure 32.* Large basophilic body in a sinusoid at the cortico-medullary borderline of a rat treated with APE. Note that the body apparently consists of several agglutinated erythrocytes whose surface is impregnated with basophilic material. The endothelium just above this body is likewise deeply stained with hematoxylin. Such structures may occur normally but are particularly common in the large venous sinuses of the adrenal medulla in rats with pronounced chromidiosis (magnification  $\times 1000$ ).

*Figure 33.* Adrenal medulla of a rat treated with APE. Note intense basophilia of the endothelial lining, especially in the lower part of the field. The cortex of this adrenal exhibited pronounced chromidiosis (magnification  $\times 200$ ).

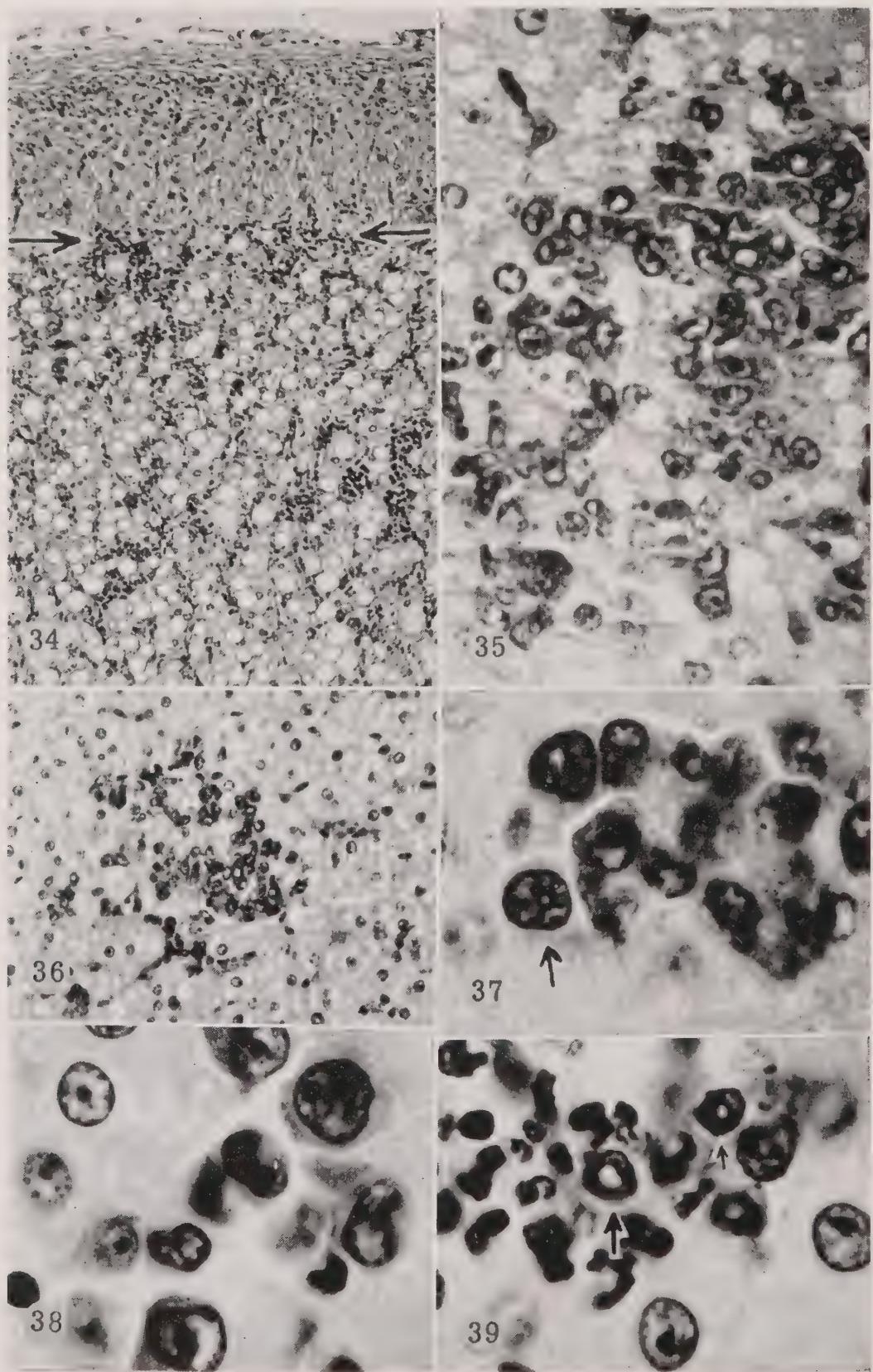


PLATE VII. MYELOID METAPLASIA

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functional indication of increased corticoid production, but apparently the gland has acquired the ability to store secretion droplets in spite of increased discharge into the blood stream.

Since it is rather important to understand the correlation between the histologic structure of the adrenal cortex and its functional activity, it may be well to illustrate these observations and their interpretation in a schematic diagram (Chart II).

Thus it appears that if both the width and the lipid



#### PLATE VII. MYELOID METAPLASIA

*Figure 34.* Myeloid metaplasia of the inner adrenal cortex in a rat treated with LAP, methyl-testosterone and thyroxine. Note sharp demarcation between the newly formed myeloid tissue (below level of arrows) and the hypertrophic, but otherwise normal, outer fasciculata and reticularis cells between capsule and arrows (magnification x 100).

*Figure 35.* Beginning myeloid transformation in the cortex of a LAP-treated rat. Note that within this circumscribed area the cytoplasm of most cells became diffusely basophilic and the nuclei showed various transitional types between typical cortical and bone marrow elements. At the same time the regular reticular structure of the fasciculata become disorganized and many cells tend to detach themselves from those in their immediate vicinity (magnification x 450).

*Figure 36.* Beginning myeloid transformation in the cortex of a LAP-treated rat. Note again transitional stages between normal cortical and hemopoietic cells in a circumscribed area of the inner fasciculata (magnification x 200).

*Figure 37.* Beginning myeloid transformation in the adrenal cortex of an APE-treated rat. The gradual development of cytoplasmic basophilia and nuclear transformation are clearly visible at this high magnification. The cell designated by the arrow shows the first stage of nuclear perforation, which eventually leads to the annular, "doughnut shaped" nucleus so characteristic of the mature heterophil leucocyte in the rat (magnification x 1000).

*Figures 38 and 39.* Other areas of the adrenal cortex shown in Fig. 37. Note additional transition types between cortical and myeloid elements. The arrows in Fig. 39 point out two more advanced stages in the formation of the annular nucleus.

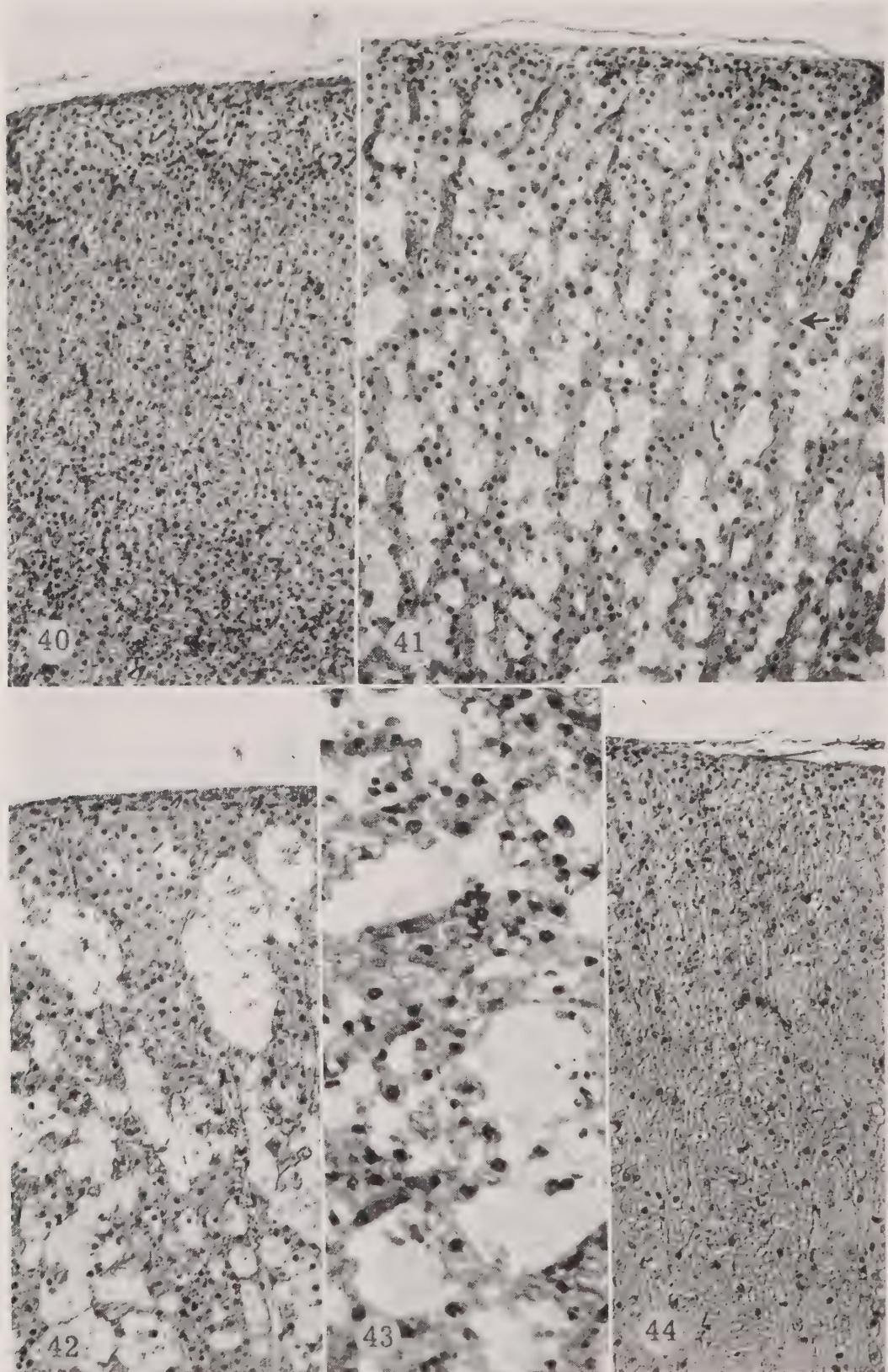


PLATE VIII. LUMINA AND INFARCTION

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content of the adrenal cortex are taken into consideration, morphologic criteria give us a fairly accurate indication of functional activity under a variety of conditions.

In our experience, neither crude anterior pituitary extracts (e.g., LAP) nor partially purified corticotrophin "ACTH 32-D" (kindly supplied by the Armour Laboratories), nor pure "ACTH" (kindly supplied by Dr. C. H. Li) caused discharge of the adrenal cortical lipids even in acute experiments. In one such investigation, up to 38 mg. of Armour's ACTH were administered in divided doses over a period of 48 hours; although the adrenal cortex greatly increased in width due to hypertrophy and hyperplasia, its cells were studded with small lipid gran-



#### PLATE VIII. LUMINA AND INFARCTION

*Figure 40.* Adrenal cortex of normal control rat (magnification x 100).

*Figure 41.* Numerous tubular structures in the cortex of a rat treated with APE, methyl-testosterone and thyroxine. Note that the hypertrophic cortical cells surround tubule-like lumina, whose cavities are filled with disintegrating cast-off cortical cells and erythrocytes. The cortical sinusoids (one of them is outlined by two arrows) contain blood cells of normal appearance. At some points (e.g., at level of lower arrow) these sinusoids appear to empty their contents into the lumina, but within the latter the blood corpuscles disintegrate (magnification x 100).

*Figure 42.* Formation of lumina in adrenal cortex of a rat treated with APE. Note similarity to tissue shown in Fig. 41 (magnification x 100).

*Figure 43.* Adrenal cortex of a rat treated with APE. Here again the formation of lumina and the discharge into them of cortical cells and blood corpuscles is particularly evident (magnification x 200).

*Figure 44.* Infarction of the entire adrenal cortex in a rat treated with APE. This picture is very reminiscent of that spontaneously occurring in the Waterhouse-Friderichsen Syndrome. It appears to arise, at least partly, due to the sudden formation of lumina with multiple massive hemorrhages into their cavities, caused by endothelial damage. Upon microscopic inspection these adrenals exhibit a bright red colour similar to that of blood (magnification x 100).

ules. This picture is indeed very different from that of the almost complete lipid discharge obtained during an alarm reaction produced by non-specific stress. Yet it is well established that non-specific stress does not discharge the cortical lipids in the hypophysectomized animal (Selye, 1946); hence corticotrophin production by the pituitary under conditions of stress must be involved in the resulting loss of lipid granules (see Chart III).

We were particularly surprised to find that pretreatment with corticotrophin actually inhibits the loss of cortical lipid granules such as is normally produced by alarming stimuli or folliculoid hormones. Hence, it was concluded that "in addition to increased corticotrophin secretion other factors must prevail during the alarm reaction to effect the usual intense discharge of cortical lipids" (Selye and Stone, 1949). Only in fasting rats did large doses of pure ACTH cause some lipid-granule discharge, perhaps because granule synthesis could not keep pace with secretion in the absence of dietary zymogen precursors.

We do not feel that the evidence available at present justifies any attempt to give a definite interpretation of these unexpected observations. It is possible of course that during the alarm-reaction the pituitary produces corticotrophic principles other than those now available in pure form, or that stress not only increases corticotrophin production, but also causes additional changes which stimulate the discharge of lipid granules from the cortex. Neither of these possibilities has been definitely proven, but experiments designed to elucidate this problem are now under way in our Institute. In any case we must conclude that increased corticotrophin production in itself cannot explain the adrenal changes characteristic of the alarm-reaction.

In this connection it should also be mentioned that the deposition of numerous comparatively small lipid granules is very common in the human adrenal. Enlargement of the adrenal cortex with storage of many small or medium-sized lipid granules has frequently been described by pathologists as being rather characteristic of hypertension, arteriosclerosis, atherosclerosis and chronic renal diseases (Gossman, 1927; Duthoit, 1933; Rinehart et al., 1941). An increase in the lipid, and especially in the cholesterol, content of the adrenals is also often noted in cases of hypertension and chronic renal disease, even if the width of the cortex is not significantly enlarged (Chauffard et al., 1912; Chauffard et al., 1914; Weltmann, 1913; Borberg, 1915; Knack, 1915; Fex, 1920; Ida, 1931; Kohno, 1928; Kohno, 1929). Hypertrophy, hyperplasia and capsular adenomas are allegedly likewise frequent in cases of arteriosclerosis (Dietrich and Siegmund, 1926).

In view of the much discussed possibility that the glomerulosa may respond to certain stimuli in a rather selective manner, we would like to mention certain experiments recently performed in this Institute in collaboration with Dr. F. Skelton. These revealed that pantothenic acid deficiency can cause an almost complete, selective discharge of sudanophilic lipid granules from all zones of the adrenal cortex except the glomerulosa (Figure 62). This may also be a specific adrenal reaction and the stimuli responsible for its development are now under investigation in our laboratory.

## Cholesterol Granule Storage or Discharge

There is no absolute parallelism between variations in sudano- and osmiophilic granules on the one hand, and

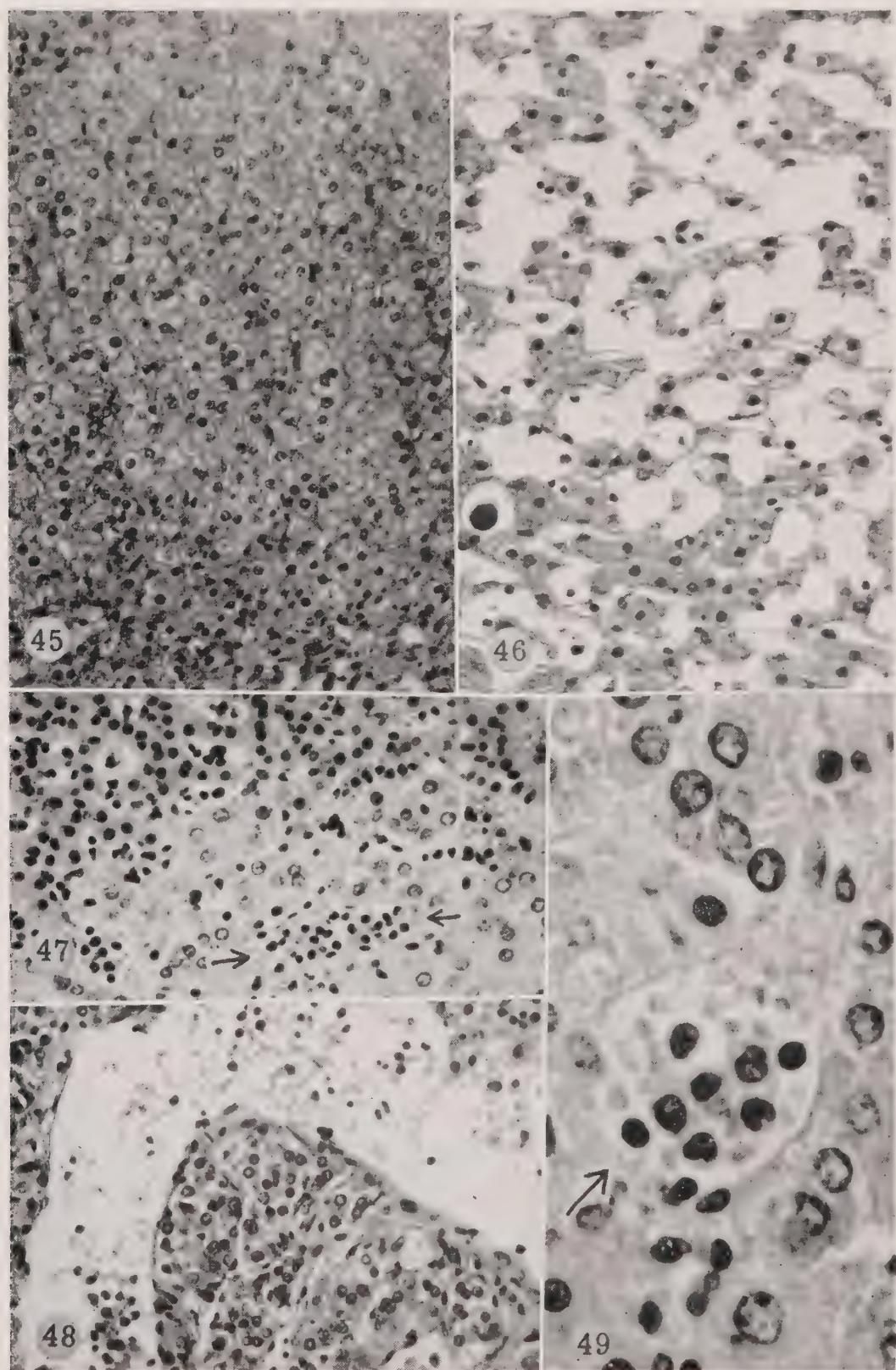


PLATE IX. HOLOCRINE SECRETION



cholesterol granules on the other. Yet, generally speaking, these changes tend to run parallel. As our own observations concerning adrenal cholesterol are very limited, the reader is referred to the excellent review of this subject recently published by Dempsey (1948).

## Plasmal Granule Storage or Discharge

The presence of aldehydes in tissues was demonstrated by their reaction with fuchsin sulfurous acid by Feulgen and Voit (1924) and further investigation showed them to consist mainly of stearal and palmital, linked in cyclic

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### PLATE IX. HOLOCRINE SECRETION

*Figure 45.* Adrenal cortex of normal control rat (magnification x 200).

*Figure 46.* Adrenal cortex of rat treated with APE. In this case the entire fasciculata assumed this spongy appearance with the formation of many lumina due to disintegration of cast-off cortical cells. It is no longer possible to differentiate between lumina and sinusoids since none of the visible channels is filled with normal blood cells. Such adrenals give the impression of a holocrine secretion of cortical cells into the lumina, which also receive blood from ruptured sinusoids. In the channels so formed, both cortical and blood cells disintegrate, their remnants being flushed down towards the medullary sinuses (magnification x 200).

*Figure 47.* Cortico-medullary borderline of the adrenal in a rat exposed to cold. Note that all the sinusoids are packed with nucleated cellular elements. Some of these resemble cortical cells such as constitute the reticularis (upper part of field), others exhibit the appearance of myeloid elements. One such cell-packed sinusoid is outlined by two arrows, another is seen to the right of the picture's number (magnification x 200).

*Figure 48.* Large medullary sinus filled with partially disintegrated erythrocytes and adrenal cortical cells (magnification x 200).

*Figure 49.* Sinusoid at the cortico-medullary borderline filled with red blood-corpuscles and diverse types of nucleated cells in various stages of disintegration. It is noteworthy that such disintegrating erythrocytes and nucleated cells are particularly plentiful in the adrenals of rats showing tubule formation in the cortex (magnification x 600).

acetal bonds to glycerol-phosphorylcholamine (Feulgen and Bersin, 1939). Their occurrence in cells can quite easily be demonstrated with Feulgen's method using Schiff's "plasmal" reagent. Such "plasmalogen" granules do not occur only in the adrenal, but also in various other tissues (e.g., ovary, myelin sheaths of nerves, liver), (Uchida, 1936; Lison, 1936; Tonutti, 1941; Albert and Leblond, 1946). It is especially noteworthy that their concentration is unusually high in the inner cortex of the kidney (Oster, 1945). We believe this region to be concerned with the production of renal pressor substances, since it undergoes especially pronounced enlargement in the "endocrine kidney" preparation (Selye and Stone, 1946; Selye, 1948a; Selye, 1948b).

Although a positive plasmal reaction is obviously not characteristic of any steroid material, certain steroid ketones may react similarly to aldehydes in this test. Allegedly any acetone-soluble, sudanophilic droplet, that is also Schiff positive, autofluorescent and birefringent, can be assumed to contain ketosteroids, since apparently no other single substance reacts positively to all these tests (Dempsey and Wislocki, 1946). It has been stated that "these reactions presumably occur in the sites of formation of adrenal hormones, which fall into the class of ketosteroids" (Deane et al., 1948). It is not within the scope of this communication to discuss the specificity of these reactions, especially since this has been done most adequately in several recent reviews (Dempsey and Wislocki, 1946; Dempsey, 1948). However, even if we assume that no single substance other than ketosteroids would react positively in all these tests, there is no reason to doubt that a granule within a cell could contain several substances, which conjointly would endow it with the ability to react positively with this whole set of reagents,

although it contained no ketosteroids. Be this as it may, it has been our experience that a positive plasmal reaction in the adrenal cortex roughly parallels the sudanophilic-lipid-granule content. Hence, it could be presumed that the plasmal reaction is in some manner related to hormone storage.

As previously stated (Albert and Leblond, 1946; Leblond, 1948) the substances reacting with Schiff's reagent do not behave like ketosteroids and are probably loosely bound aldehydes of high fatty acids ("plasmalogen"). It has also been pointed out that "the strict parallelism between the Feulgen and the DNPH (2,4-dinitrophenylhydrazine) reaction suggested that plasmalogens were also responsible for producing the DNPH reaction" (Albert and Leblond, 1946). In discussing this whole group of so-called ketosteroi<sup>d</sup> reagents it was concluded that "there is, on the whole, a marked staining in conditions of active secretion and a weak staining in resting conditions; and therefore there is a close relation between these reactions and the release of specific ketosteroids by these organs" (Leblond, 1948). We find, however, in confirmation of other investigators (Deane et al., 1948), that in the case of the adrenal cortex, very active secretory stimulation is usually accompanied by loss of plasmalogens. Hence, and in view of the general parallelism between sudanophilia and plasmalogen granules in the adrenals, we feel that both may be more characteristic of hormone storage than of hormone secretion. Their presence is probably conditioned by the same equilibrium between hormone secretion and storage, which we have discussed above in connection with adrenal lipid granules. Yet it is noteworthy that during the alarm reaction, although both lipid and plasmalogen granules are discharged, their loss from different regions of the cortex

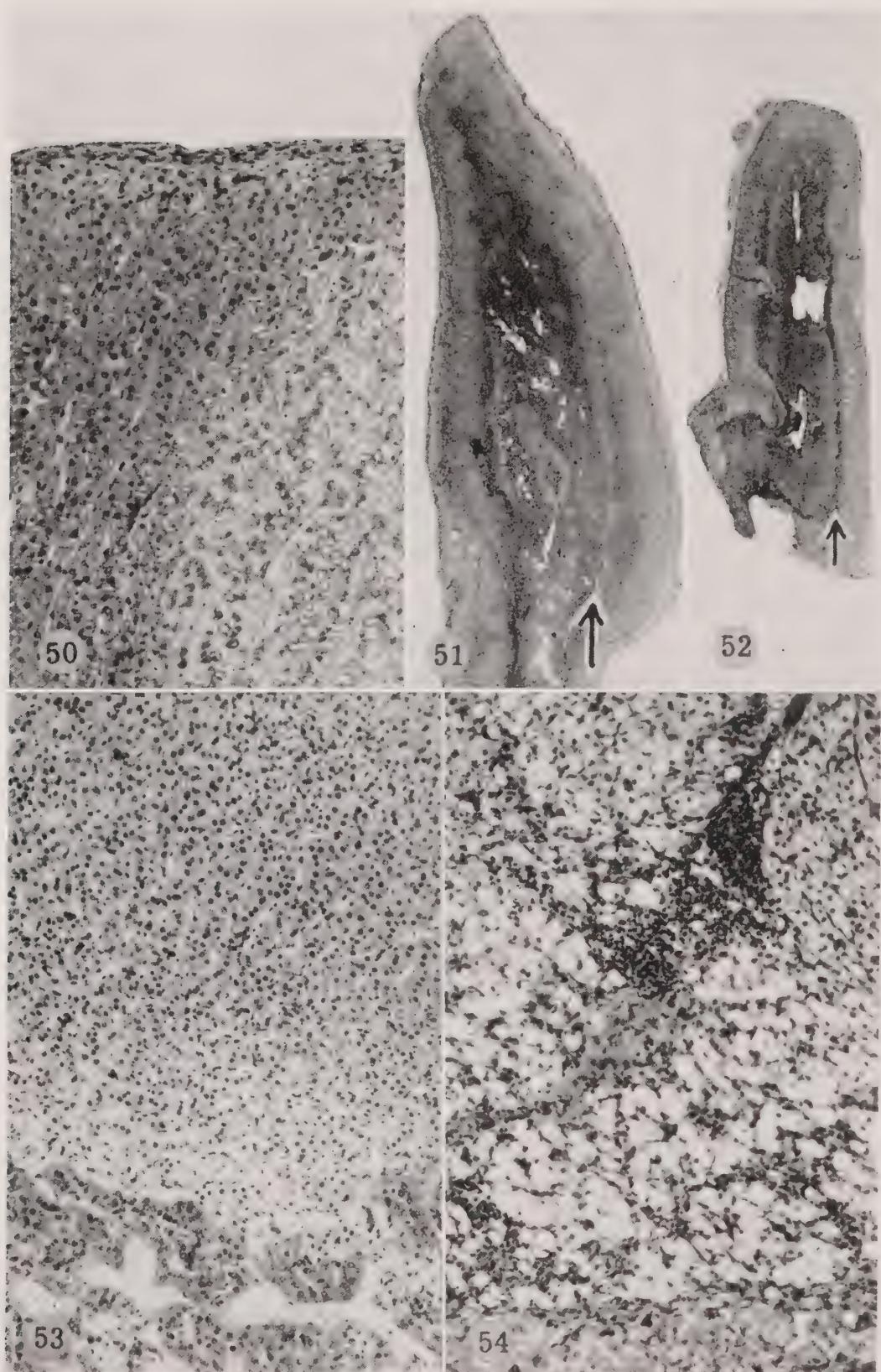


PLATE X. NECROSIS AND "TOXIC INVOLUTION"

→

does not necessarily coincide (Constantinides et al., unpublished). It has been claimed that in the course of their development, lipid granules may go through stages during which they successively acquire sudanophilia and then plasmal staining properties (Lison, 1936). All these possibilities will have to be reexamined before the significance of adrenal plasmalogens can be fully appraised.

## Ascorbic Acid Storage or Discharge

There is an extensive literature concerning the behaviour of the colorimetrically demonstrable and histologically tingeable ascorbic acid granules in the adrenal cortex. It has been amply demonstrated, for instance, that exposure to a wide variety of alarming stimuli causes the discharge of ascorbic acid granules from the adrenal cortex (Sayers and Sayers, 1948; Selye, 1947; Selye, 1946). There is much speculation regarding the possible

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← PLATE X. NECROSIS AND "TOXIC INVOLUTION"

*Figure 50.* Large area of necrosis in the adrenal cortex of an APE-treated rat. Entire lower right area of this field consists of necrotic fasciculata cells while the remainder of the tissue exhibits signs of hypertrophy. Compare with Fig. 40 (magnification x 100).

*Figure 51.* General aspect of the adrenal (cortex and medulla) of a normal control dog. The arrow points to the cortico-medullary borderline (magnification x 6).

*Figure 52.* Cross-section of the adrenal of a similar but DDD-treated dog. The arrow points to the cortico-medullary borderline. Note great decrease in the width of the adrenal cortex (magnification x 6).

*Figure 53.* Cortico-medullary borderline of a normal control dog (magnification x 100).

*Figure 54.* Cortico-medullary borderline of a DDD-treated dog showing fatty and myeloid transformation. Note the development of many fat cells and hemopoietic elements in the reticularis. The medulla (lower part of the field) is not thus affected (magnification x 100).

interaction between ascorbic acid and the corticoids or the participation of ascorbic acid in the synthesis of corticoids, yet the nature of these relationships remains essentially obscure.

In our material we find that there is a rough parallelism between the discharge of sudanophilic lipids, cholesterol, plasmalogens and ascorbic acid granules during the alarm reaction, after treatment with pituitary extracts and following hypophysectomy. Hence, in agreement with what has been said about the other tingeable granules of this group, it appears probable that the presence of ascorbic acid granules is somehow related to hormone storage in the adrenal cortex. Nevertheless, since there is no absolute proportionality between the discharge of the various tingeable granules mentioned above, there must be some slight difference in the quality of the stimuli required for the elimination of these granules from the cell. Perusal of our material suggests that in many, rather varied, experimental arrangements (exposure to stress, folliculoids, corticotrophin injections, hypophysectomy, etc.) the ascorbic-acid content of the adrenal tends to be a function of the quotient:

$$\frac{\text{sudanophilic lipids in adrenal}}{\text{corticoid hormone secretion}}$$

Our experience with the plasmal reaction is too limited to draw any definite conclusions, but it suggests that the plasmalogens could take the place of the lipid granules in this equation. If the assumption should prove to be correct that lipid and plasmalogen granules are indicative of hormone storage, then the above equation could be taken to imply that ascorbic acid is lost from the adrenal whenever corticoid secretion is excessive in proportion to the stores of corticoids (or corticoid precursors).

## Fatty Metaplasia

Under certain experimental conditions, we noted the transformation of typical adrenal cortical cells into signet-ring shaped fat cells. This uncommon transformation should be clearly distinguished from the usual accumulation of numerous smaller lipid granules as discussed above. It would be difficult to prove that the resulting signet-ring cells are in every respect identical with the characteristic elements of common adipose tissue, however, the process appears to be one of a true metaplasia of adrenal cortical into fat cells. We know of no histologic technic permitting to differentiate between the signet-ring cells experimentally produced in the adrenal cortex and the common fat cells of adipose tissue.

The earlier literature concerning this peculiar type of metaplasia has been reviewed elsewhere (Selye, 1947; Selye and Stone, 1950), hence, we shall limit ourselves to a discussion of the observations made in connection with experiments reported here.

Some degree of fatty metaplasia occurred, especially in the mid-fasciculata region of the adrenal cortex in all our animals treated with methyl-testosterone, if sufficiently large doses were administered. As far as we can judge this change is specific for testoids since we were unable to obtain it with any other agent.

It is noteworthy that hypophysectomized animals are unable to respond to testoids with this type of metaplasia, while rats simultaneously treated with testoids and anterior pituitary extracts (e.g., APE or LAP) reveal much more extensive fat cell formation than could be accounted for by the action of the testoid itself. Hence, it appears that the presence of pituitary corticotrophin is essential for this response. However, some degree of peripheral synergism exists, in the morphogenesis of this change,

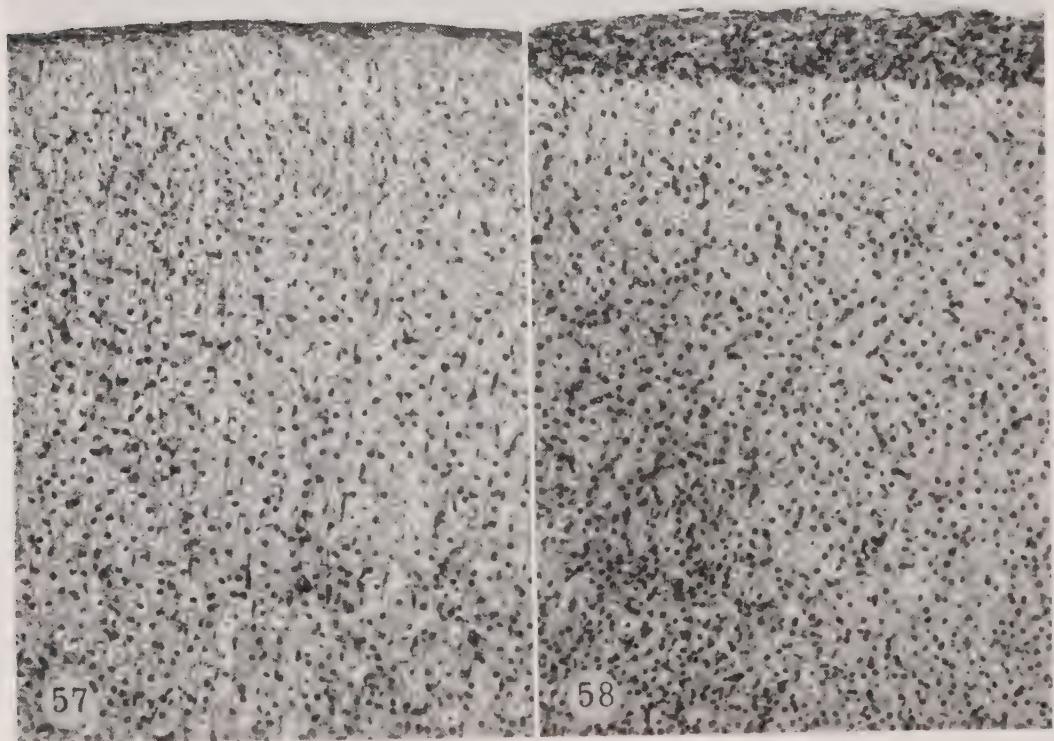
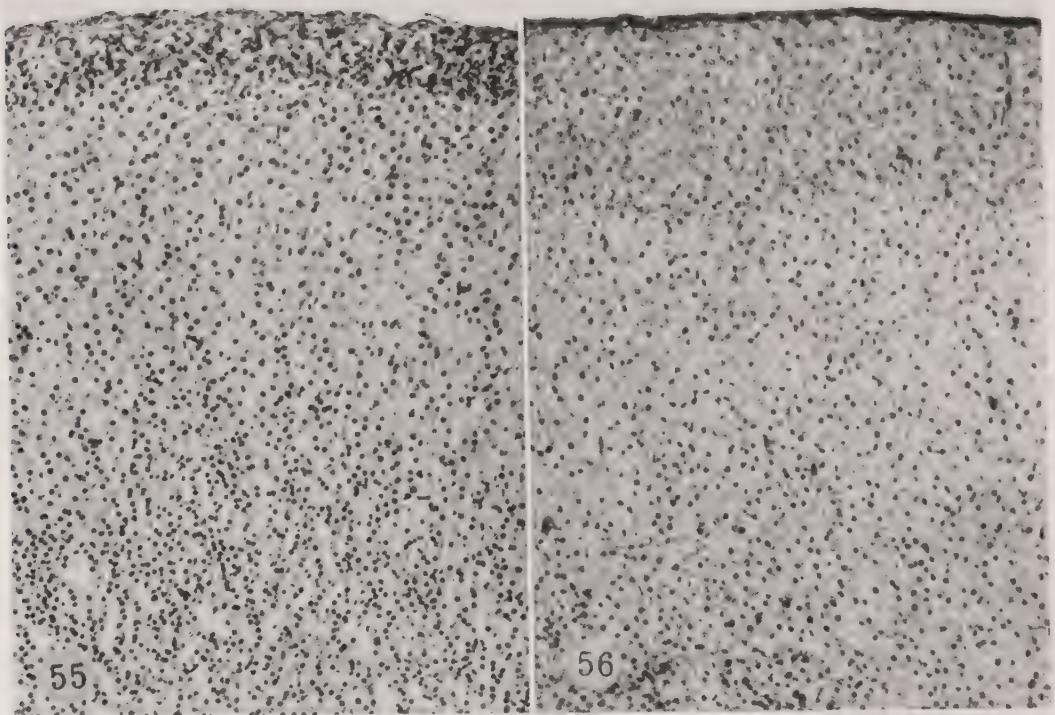


PLATE XI. GLOMERULOSA CHANGES

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between testoids and the anterior-lobe extract, since pituitary preparations alone do not cause fatty metaplasia, although they increase this action of the testoids (Figures 20 and 21).

Simultaneous treatment with thyroxine further augments the tendency for fat cell production; the most pronounced and extensive fatty metaplasia was obtained in the adrenals of animals receiving methyl-testosterone, APE and thyroxine (Figure 22).

In spite of the apparently great specificity of this change, occasionally, very large, single fat vacuoles do occur in the adrenal cortical cells of animals during the resistant phase of the adaption syndrome, for instance, when the latter is elicited by exposure to cold. In some such cases, the cells may begin to resemble adipose cells (Figures 18 and 19), but even here true signet-ring types were not observed.

The functional significance of this change is obscure, but it is perhaps reasonable to assume that the formation of an extraordinarily large vacuole, filled with sudanophilic fatty material, is indicative of increased storage. The testoids could act by inhibiting the discharge of sudanophilic materials synthetized in the adrenal cortical cell



#### PLATE XI. GLOMERULOSA CHANGES

*Figure 55.* Adrenal cortex of a control rat kept on a diet containing a normal amount of Na. Note normal relationship between glomerulosa and fasciculata cells (magnification x 100).

*Figure 56.* Great increase in the width of the glomerulosa and the size of its individual cells in a rat kept on a Na-free diet (magnification x 100).

*Figure 57.* Hypertrophy of glomerulosa cells (some of which are in the process of cytolysis) in a rat kept on the Na-free diet. Note that in this case the boundary line between glomerulosa and fasciculata became very indistinct (magnification x 100).

*Figure 58.* Thin and intensely basophilic glomerulosa in a rat receiving excessive amounts of Na (magnification x 100).

under the influence of corticotrophin (Chart III). If synthesis is greatly increased by corticotrophin (and especially by the highly corticotrophic combination of APE plus thyroxine), then such inhibition of discharge would be most likely to result in extreme storage, and "fatty metaplasia" would ensue.

## Colloid Formation

Several investigators reported that occasionally, hyaline eosinophilic colloid bodies develop in the human adrenal. The relevant literature has recently been reviewed by Velican (1948), who considers it probable that the colloid develops from disintegrating erythrocytes.

Such hyaline bodies have also been seen in a case of Addison's disease. Here they developed in conjunction with extensive "round-cell infiltrations," numerous plasma cells, as well as very unusual mononuclear giant cells with an intensely vacuolated cytoplasm (Steinbiss, 1926). We mention this comparatively rare change because some of the cellular elements, which we experimentally produced in the rat, bore a striking resemblance to them (Figures 23, 26, 27, 28; compare with Figure 24 on plate V).

It is perhaps also pertinent that hyaline granules appear in other lipid-storing endocrine cells, e.g., those of the human corpus luteum of pregnancy. This change has been discussed and illustrated in one of our earlier publications (Selye, 1947).

Perusal of our experimental material indicates that accumulation of colloid within the adrenal cortical cells sometimes occurred in animals treated only with LAP. However, this was uncommon except in groups kept on a high-protein diet (e.g., in experiment I of this report). It will be recalled that on high-protein diets the cortico-

trophic effect of LAP (as judged by adrenal weight) is much more pronounced than on protein-deficient diets.

Marked deposition of colloid bodies was consistently found in rats simultaneously treated with LAP and DCA, LAP and methyl-testosterone, and especially in those receiving LAP (or APE), methyl-testosterone and thyroxine or LAP (or APE), DCA and thyroxine. In the groups receiving methyl-testosterone in combination with a pituitary preparation, colloid-formation was accompanied by fatty metaplasia; the latter change was not seen, however, in animals treated with DCA.

It is somewhat difficult to interpret the functional significance of this change, but from a purely histologic point of view, the accumulation of colloid bodies is suggestive of storage. The tinctorial properties of the colloid (especially its eosinophilia and fuchsinophilia) are somewhat reminiscent of the colloid deposited in thyroid follicles and it is possible that the material is associated with the storage of some cortical hormone principle.

Treatment with testosterone alone or DCA alone never caused any formation of colloid bodies. However, simultaneous treatment with both these steroids inhibited the usual fatty metaplasia caused by methyl-testosterone alone; signet-ring cells were present but instead of lipid vacuoles they contained colloid bodies.

It is impossible to assess the functional implications of these changes. However, all our observations are compatible with the interpretation that corticotrophin causes colloid storage only if the stimulus for hormone production is greater than that for granule discharge. Apparently, under certain experimental conditions, steroids can inhibit the discharge of secretion granules into the blood. In this manner, both testosterone and DCA may cause an accumulation of secretion droplets within

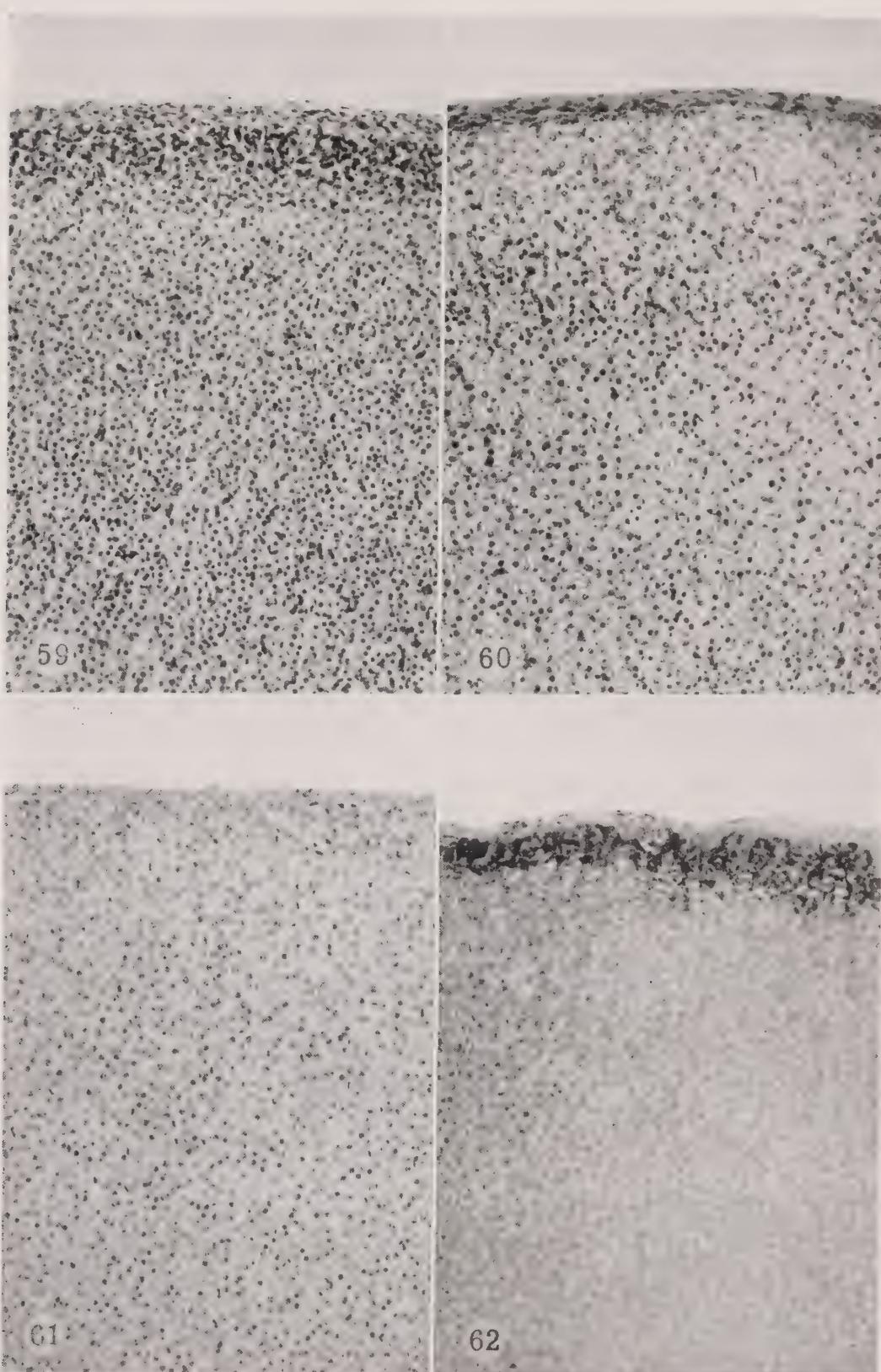


PLATE XII. GLOMERULOSA CHANGES

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the cells, but testosterone tends to induce primarily, accumulation of lipids ("fatty metaplasia"), while DCA causes the storage of colloid bodies. If both these steroids are given simultaneously, the action of DCA prevails to the extent of transforming the fat-storage type of reaction into that of colloid-storage. In agreement with this interpretation, both types of storage cells are most plentiful if the above steroids are given in conjunction with pituitary corticotrophin which increases cortical hormone production. It is noteworthy that although the primary effect of testoids is to cause fatty metaplasia, under certain circumstances, (especially when given in conjunction



#### PLATE XII. GLOMERULOSA CHANGES

*Figure 59.* Atrophy of all cortical layers in a rat receiving excessive amounts of DCA (compare with control shown in Fig. 55). Although glomerulosa cells are small, there is no disproportionate atrophy of this layer or any intense basophilia such as is noted on high Na diets (Fig. 58). The cortex is very similar to that of a hypophysectomized rat (magnification x 100).

*Figure 60.* Wide glomerulosa with hypertrophy of individual cells in a rat kept on a Na-free diet, but receiving the same amount of DCA as the animal whose adrenal is illustrated in Fig. 59. Note that the mineralo-corticoid compound failed to counteract the effect of sodium deficiency upon the glomerulosa (magnification x 100).

*Figure 61.* Adrenal cortex of a rat immediately after it received 38 mg. of Armour's (32-D) ACTH preparation in divided doses during 48 hours. Note that the cells in all layers of the cortex are proportionately enlarged. The glomerulosa cells are not insensitive to the corticotrophic preparation. Sudan stained sections of this same adrenal revealed no loss of lipid granules, comparable to that which would have been produced by non-specific stress (during an alarm reaction) causing a corresponding adrenal enlargement (magnification x 100).

*Figure 62.* Adrenal cortex of a rat with severe pantothenic acid deficiency. Section stained with Sudan III. Sudanophilic granules are selectively retained in the glomerulosa, while the other layers of the cortex are free of lipids. This suggests that in pantothenic acid deficiency, the glomerulosa also reacts differently from the other cortical layers (magnification x 100).

with thyroxine and APE), they can cause the accumulation of considerable quantities of adrenal colloid (Figures 23, 26, 27 and 28).

## Fibrinoid Degeneration

In several of our experiments a peculiar degenerative change occurred in many adrenal cortical cells; we termed it "fibrinoid degeneration." This is characterized by the accumulation of an intricate fibrillar network within the cytoplasm of large signet-ring-type cells. At the intersections of the fibrils, nodules are formed so that the reticulum resembles that of a freshly precipitated fibrin mesh. This intracellular network gives the characteristic histochemical reactions of fibrin; e.g., it stains dark with Mallory's hematoxylin (Figure 25). This change can occur simultaneously with colloid formation or with granular degeneration of cortical cells. Sometimes the impression is gained that fibrinoid degeneration may be a precursor of colloid body formation. It occurred with particular frequency in animals treated simultaneously with APE and methyl-testosterone, especially if the effect of the pituitary preparation was potentiated by simultaneous thyroxine administration. We have seen similar changes in the adrenals of man, dying from various diseases.

## Cytolysis

Complete disintegration of certain cytoplasmic areas, or of entire cells, is quite common in the adrenal cortex, especially if the gland is under the influence of acute intense corticotrophic stimuli. In our experimental material, it occurred so regularly that we believe it to be a normal response to extreme stimulation; an endocrine

counterpart of the apocrine or holocrine secretion among exocrine glands. It often occurs under the influence of intense systemic stress (e.g., cold) or APE administration, especially if the effect of the latter is augmented by simultaneous thyroxine treatment. When only part of the cytoplasm undergoes cytolysis, vacuoles appear within the cytoplasmic mass and these are filled with granular protein-like eosinophilic cell-debris (Figure 17). When the whole cell is affected, the nucleus undergoes pyknosis and chromatolysis, with eventual disappearance of all nuclear debris so that a space, in a fasciculata column, which would normally be occupied by a single cell, is taken up by granular eosinophilic cell detritus. Sometimes the cell membrane remains visible even after complete disintegration of cytoplasm and nucleus (Figure 19).

This appears to be an entirely non-specific reaction to any intense corticotrophic stimulus. It may well be the morphologic expression of an extreme response to an acute need for cortical compounds, which results in the discharge not only of the hormones (and accompanying lipid, ascorbic acid, plasmalogens and other specific substances), but also of parts of the cytoplasm or of whole disintegrated cells. The significance of this response is not known, perhaps in conditions of urgent need, a holocrine discharge of cell-materials is necessary because time does not suffice for the selective elimination of the required hormonal cytoplasmic constituents. Another possible interpretation is that extreme stimulation causes damage by overwork. This would be comparable to the degeneration due to exhaustion seen in the islets of Langerhans after treatment with diabetogenic anterior-lobe extracts or extensive partial pancreatectomy.

The phenomenon of cytolysis is common in adrenals which also show holocrine secretion of entire, compara-

tively well preserved, cells and the formation of lumina within the cortical parenchyme (see below).

### “Chromidiosis”

Many glandular cells are known to contain basophilic granules or threads in their cytoplasm. These exhibit essentially similar tinctorial reactions as the nuclear chromatin itself and have often been regarded as material extruded from the nucleus. They have variously been described under the names of “cytoplasmic chromatin,” “chromidia,” or “ergastoplasm.” In serous glands, especially in the pancreas, they form filaments coiled up in the form of a nucleus-like, spherical body beneath the true nucleus. These structures are the “Nebenkerne” or “accessory nuclei” of the literature (Matthew, 1899; Seguchi, 1920; Garnier, 1900). In some preparations they tend to stain like the nucleolus.

We are not aware of any description of such granules in the cytoplasm of adrenal cortical cells, although in our material “chromidia” appear quite commonly even under normal conditions in untreated animals. They are particularly abundant in the cortical cells of rats whose adrenals were stimulated by chronic exposure to stress (stage of resistance), long continued treatment with corticotrophic anterior pituitary extracts or with folliculoid hormones. They were rare in the atrophic adrenals of animals treated with DCA and we have never seen them after hypophysectomy.

For lack of a better term, we shall refer to them as “chromidia” since this designation emphasizes their resemblance to nuclear chromatin. We do not wish to imply, however, that they consist of the same material as is found in the serous exocrine glands. Their development is

so irregular that they may even be postmortal artefacts. Even in this event they would be of interest in connection with the morphology of the adrenals, since they rarely occur in other organs similarly prepared for histologic study.

Sometimes these chromidia exhibit essentially the same tinctorial properties as the nucleolus; on hematoxylin-eosin sections, therefore, they may differ somewhat from the nuclear chromatin itself. Experiments with ribonuclease and desoxyribonuclease are now under way in our Institute in order to establish whether these chromidia consist of ribonucleic acid, a possibility which appears plausible as judged by many of their characteristics.

It is somewhat difficult to establish how these chromidia are formed. They occur not only in cortical, but also in adrenal medullary cells, although, generally, the basophilic granules in the cytoplasm of cortical cells are fine and dust-like, those in the medulla, coarse and granular. In glands in which intense chromidiosis is manifest, we often find groups of apparently agglutinated erythrocytes in the sinusoids — especially in the cortico-medullary border line and in the medulla itself — which appear to be incrusted with intensely basophilic material (Figure 32). In such instances, the endothelium of the medullary sinuses tends to be intensely basophilic and hence, in hematoxylin-eosin preparations, it appears in the form of very sharply drawn blue lines (Figure 32). This extraordinary affinity of the basophilic material for the surfaces of erythrocytes and endothelia was always striking.

In the cortex, incipient chromidiosis is often manifested by the deposition of basophilic material at one point on the inside of the nuclear membrane. Thus a dark homogeneous "chromatin cushion" is formed; from here fine fibrils or granules of chromidial material appear to

emanate into the cytoplasm where they accumulate in larger granules (Figures 29 and 31). Not unfrequently, the newly formed chromidia have a tendency to migrate towards the cell membrane (Figure 30). Usually, however, there is no obvious connection between the nucleus and these cytoplasmic granules which then assume a more or less branching, star-shaped or "snowflake-like" appearance, often in immediate contact with cell membranes. Very often when such a chromidial star is seen near one cell membrane, a similar structure is detectable in the cytoplasm of the adjacent cortical cell (Figure 31). This distribution pattern further emphasizes the apparently great affinity of the chromidial material for cell surfaces.

It is evident that mere observation of histologic sections can not furnish definite evidence concerning the dynamics of the histogenesis of these basophilic granules. Our observations would be equally compatible with the assumption that the chromidia are produced in the cytoplasm itself or invade the latter from the blood stream. Nevertheless, the simultaneous appearance of the "chromatin cushion" inside the nucleus and of the chromidial granules in its immediate vicinity, suggests some relationship between the two structures. This may be in the nature of an actual extrusion of chromidia from the nucleus, or an induction of their formation within the cytoplasm under the influence of adjacent nuclear chromatin. In view of the great importance of chromatin-like materials in the function of the cell (enzyme formation, protein synthesis, secretion, etc.) we feel that these structures should be carefully examined in all investigations dealing with the experimental morphology of the adrenal, especially since they do not appear to have received attention up to the present time.

The possible relationship of these chromidia with the

so-called "corps siderophiles" or siderophilic bodies of Guieysse (1901) should perhaps also be considered. The latter are basophilic granules in cortical cells which may be stained with iron-hematoxylin or preferably with molybdenum-hematoxylin (Takechi, 1926). They are particularly numerous in the zona reticularis of the guinea pig adrenal, but are not prominent in the suprarenal tissue of the normal rat. Since they are more developed in male than in female or male castrate guinea pigs (Takechi, 1926) these granules have been claimed to represent a secondary sex-characteristic of the male (Kolmer, 1912; Kolmer, 1918).

### Myeloid Metaplasia

Perhaps one of the most interesting and specific adrenal cortical reactions which we observed was the formation of myeloid tissue within the adrenal cortex. This process has been the subject of a previous communication (Selye and Stone, 1950) and hence it will suffice merely to outline its most prominent features.

In rats receiving injections of necrotic tissues (e.g., the necrotic parts of tumors, autolyzed kidneys) as well as in animals in which extensive necrosis of their own tissues is produced (e.g., by the injection of impure extracts or locally irritating protein precipitants), both lymphoid and myeloid elements appear in the adrenal cortex, and to a lesser extent, also in the medulla (Selye, 1947).

Such tissue necrosis fails to cause lymphoid or myeloid reactions in hypophysectomized animals, but is particularly effective in rats simultaneously treated with necrotic tissue and corticotrophic preparations; pure corticotrophin itself does not produce this change. It has therefore been as-

sumed that the non-specific corticotrophic effect of hypophyseal corticotrophin is so modified by the presence of necrotic cell material in the body that the adrenal is induced to undergo this type of change (Selye and Stone, 1950).

So-called round-cell infiltration, lymphocytic infiltration, formation of lymphoid islets, myeloid infiltration, formation of hemopoietic islets and so forth, has often been described in human suprarenal tissue (for literature see: Selye and Stone, 1950). In man, the frequent occurrence of such "round-cell islets" in combination with extensive tissue breakdown and necrosis had already been noted by pathologists (Paunz, 1923) many years ago. Diffuse infiltration with round cells is also quite common in the so-called primary contracted adrenal of Addisonian patients. Recently Gülvzow (1948) described an interesting case of a woman with the adreno-genital syndrome in whom an adrenal tumor was found to consist partly of cortical cells and partly of bone marrow.

The etiology and pathogenesis of this change remains to be elucidated. In our experimental material we have found that the formation of lymphocytic and myeloid islets often occurs simultaneously in the same adrenal; there are also many intermediate, transitional stages between these two types of response. Most intense reactions of this type have been obtained in animals simultaneously receiving LAP (which is both rich in corticotrophin and contains much contaminating tissue-protein material) and thyroxine. The change was quite regularly observed in the adrenals of animals so treated among the experiments reported in the first part of this communication.

If LAP and thyroxine are given in combination with methyl-testosterone, the fatty metaplasia produced by the latter compound occurs simultaneously with the develop-

ment of hemopoietic elements. Thus large parts of the adrenal cortical tissue assume the typical appearance of ordinary bone marrow. Only the outer fasciculata and glomerulosa regions remain essentially unaffected by this change (Figure 34).

Usually, the first change to be observed is the development of diffuse cytoplasmic basophilia in certain circumscribed foci of the adrenal cortex (Figures 35, 36 and 37). Later, some of the adrenal cells appear to assume the characteristics of lymphocytes; others resemble polymorphonuclear leucocytes inasmuch as their nucleus becomes lobed or perforated. Annular, "doughnut shaped" nuclei are common among the neutrophils of the rat under normal conditions and the gradual transformation of the rather vesicular nucleus of the adrenal cortical cell into the annular or multilobed leucocyte nucleus is quite striking in such sections (Figures 36, 37, 38 and 39). Megakaryocyte-like giant-cells are likewise common. The many transitional types between ordinary cortical cells and all types of hemopoietic elements (hemocytoblasts, myeloblasts, erythroblasts, lymphocytes, polymorphonuclear leucocytes, etc.) suggest a process of direct metaplasia of cortical cells into lymphoid and hemopoietic elements. This does not exclude the possibility that the reticulo-endothelial system of the adrenal cortex may also contribute to this type of blood formation.

It has been emphasized by pathologic anatomists that in septicemias the adrenals are especially rich in micro-organisms, yet their tissue is unusually resistant to the direct, damaging actions of bacteria (Paunz, 1923). The local proliferations of lymphatic, myeloid and reticulo-endothelial elements described above, may play a rôle in protecting this important gland against such local damage.

### CHART III

#### SCHEMATIC DRAWINGS ILLUSTRATING MECHANISM THROUGH WHICH VARIOUS AGENTS COULD CAUSE SPECIFIC CHANGES IN THE ADRENAL CORTEX

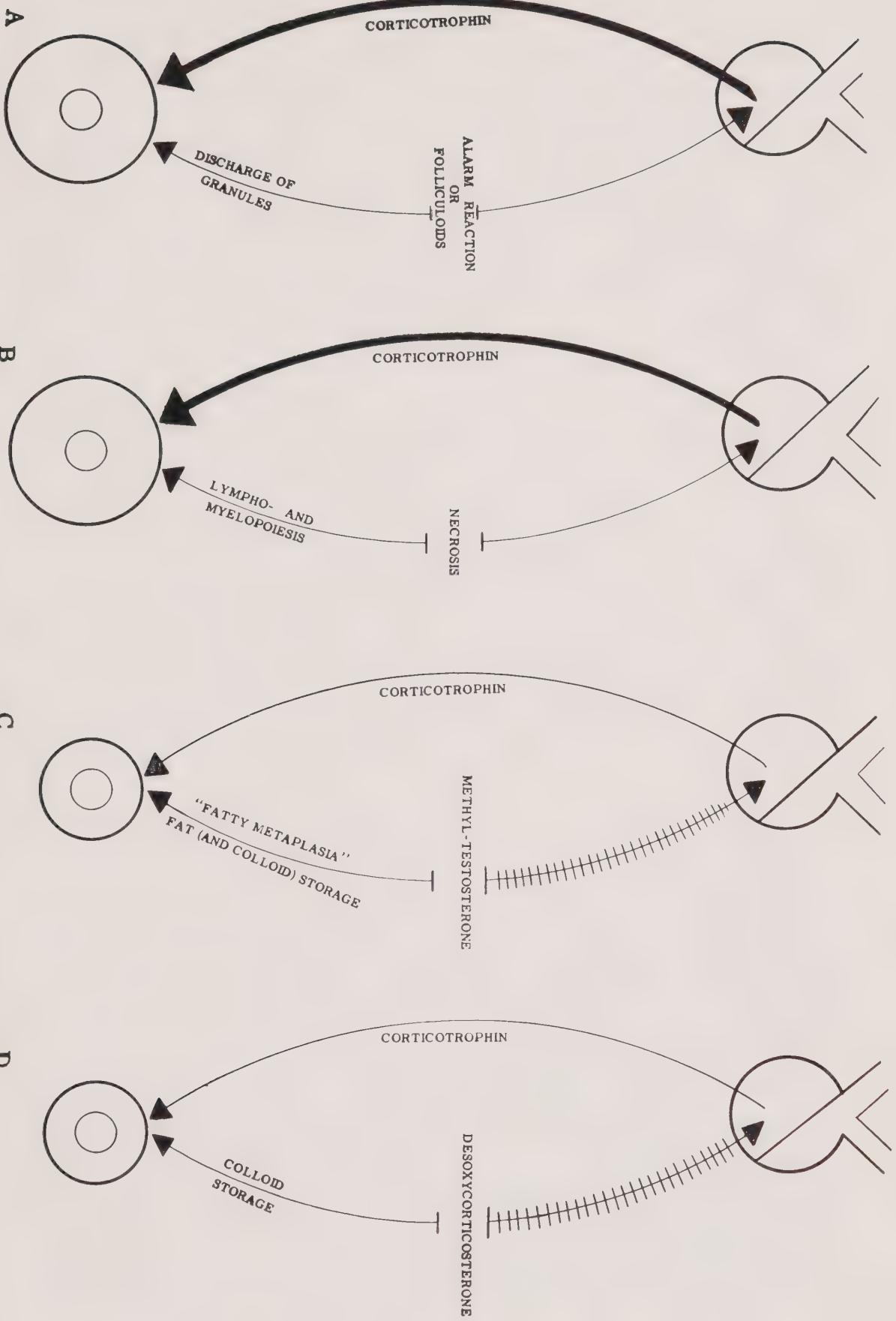
Not all the interrelations depicted in these graphs can be considered as definitely proven, but the mechanisms illustrated here appear to be compatible with all observations made to date. The drawings are merely intended to act as graphic expressions of a working hypothesis. This is, of necessity, based only on present knowledge, but by precisely formulating the problems involved, it may help to formulate the points requiring further experimental analysis.

*Graph A:* During the alarm reaction or under the influence of folliculoids, the pituitary is stimulated to produce an excessive amount of corticotrophin. The latter tends to increase both storage and discharge of secretion granules. However, under these conditions, the cortex, though enlarged, discharges its lipids. Hence, we must assume that these agents do not act merely through corticotrophin production, but also exert a direct granule-discharging effect.

*Graph B:* Widespread tissue necrosis acts as an alarming stimulus and as such, increases corticotrophin production. Yet, it also causes lympho- and myelopoiesis in the adrenal. This is not obtained with pure corticotrophin. It is assumed that this latter effect is direct, though dependent upon the adequate maintenance of the cortex by corticotrophin. It cannot be obtained after hypophysectomy and is particularly pronounced in animals simultaneously receiving both corticotrophic anterior-lobe extracts and necrotic tissue preparations.

*Graph C:* Methyl-testosterone (and other testoids) decrease corticotrophin production since they produce an involution of the adrenal cortex. This effect is presumably due to an inhibition of corticotrophin secretion, since testoids do not interfere with adrenal enlargement caused by exogenous corticotrophin. Nevertheless, the fatty metaplasia caused by testoids presumably results from a direct peripheral synergism between these steroids and corticotrophin; it is not seen after hypophysectomy and is particularly pronounced in animals simultaneously receiving both corticotrophic anterior-lobe extracts and methyl-testosterone.

*Graph D:* Corticoids (e.g., DCA) cause adrenal cortical atrophy, with colloid storage, presumably through a similar mechanism. They induce adrenal atrophy by inhibiting corticotrophin production, since they do not interfere with the corticotrophic effect of exogenous ACTH. Their ability to induce colloid storage in the adrenal cortex, is probably due to a direct synergism between these steroids and corticotrophin; it cannot be elicited in hypophysectomized animals and is especially pronounced if DCA and corticotrophic anterior lobe extracts are simultaneously given.



Blood formation in the adrenal is especially pronounced, but other tissues are also the site of hemopoiesis in these same animals. This is especially true of the spleen and the so-called brown fat of the "hibernating gland" tissue (Selye and Stone, 1950).

The following diagram may help to visualize the probable interrelations between the hypophysis and certain stimuli capable of eliciting highly specific structural changes in the adrenals.

## Formation of "Lumina" Within the Cortical Parenchyme

In many of our animals, chronically treated with crude anterior-pituitary extracts, the cells of the adrenal cortex degenerated in certain areas and became liquefied. This process of cytolysis has already been considered above. When it affected many cells, it led to the formation of "lumina" resembling tubules or acini. We should like to emphasize that similar findings have also been reported in man.

Beckmann (1914) was probably one of the first authors who gave these "lumina" special attention. He emphasized that the cavities may contain granules of precipitated, structureless material or even degenerating cells. These formations have been studied by several investigators (Ritter, 1944; Paunz, 1923; Dietrich and Siegmund, 1926; Rich, 1944; Golden, 1945; Gossman, 1927; Thomas, 1911; Dietrich, 1918). Lucadou (1938) pointed out that they are particularly common in pregnancy, the diseases of old age (arteriosclerosis, atherosclerosis, etc.) and the very acute infections. This led him to suspect that they may be connected with excessive demands upon adrenal cortical function. Zamcheck

(1947) who saw such structures in patients after exposure to a great variety of fatal stresses (infections, etc.) considers them as a rather typical manifestation of the alarm reaction in man.

In the rat we produced similar "lumina" by overstimulating the adrenal cortex with very intense systemic stress (e.g., cold, trauma, infection), or with excessive doses of corticotrophin or folliculoid compounds (e.g., estrone, estradiol, stilbestrol); occasionally, they were seen in the rat during late pregnancy and lactation (Selye, 1947). Folliculoids are known to cause especially exhaustive degranulation of the adrenals, with manifest morphologic and functional signs of extreme overstimulation. Since folliculoids are produced in excessive amounts during gestation, all known facts seem to be compatible with the assumption that the formation of such "lumina" is the result of wide-spread cell-disintegration due to exhaustive stimulation. In this sense, the process of "lumen" formation would merely be due to the spreading of the cytolytic reaction described above.

We have not been able to convince ourselves that such tubule and acinus-like lumina can maintain themselves and become permanent structures. The impression is gained that this process is due to a transient acute exacerbation of intense overstimulation. The lumina are always lined by cells in the process of being cast off into the cavities and the latter appear to be filled with disintegrating cortical cells and blood-cells. These disintegrating cells appear to enter the lumina due to erosion of the lining endothelium at the places where cytolysis is particularly active. In this manner, communications are established between the vascular system and the newly formed lumina; through these, the debris of disintegrating cortical cells and blood-cells is flushed down towards

the medullary sinuses (Figures 40, 41, 42, 43 and 46). Correspondingly, the process of lumen formation is usually accompanied by holocrine secretion of cells; these rapidly become digested within the system of "tubules" or in the sinusoids with which the latter communicate.

Some entire cells, free nuclei and chromatin-like material ("chromidia"?) remain distinguishable within the "tubules" and sinusoids. Thus this change is closely related to the holocrine secretion of comparatively intact cells, which we shall discuss below. It is remarkable, however, how quickly the cast-off cells disintegrate. This process recalls the postmortal autolysis of tissues which is unusually rapid in the adrenal. That is why the ancient anatomists — who rarely had the opportunity to dissect fresh cadavers — believed the organ to be a capsule, filled with fluid and hence named it "*capsula suprarenalis*." This special proteolytic power of the adrenal cells may be involved in the cytolytic phenomena underlying the process of cell disintegration and lumen formation associated with exhaustive over-stimulation.

## Holocrine Secretion

In addition to the intravascular discharge of cytolized cortical elements, holocrine secretion of more or less intact cells is also frequently seen. This is particularly obvious in adrenals which have undergone myeloid metaplasia, since here, the newly formed blood-cells are discharged into the blood in essentially the same manner as in the normal hemopoietic organs. These cells are then readily discernible within the large sinuses of the medulla. However, in the case of intense overstimulation by severe stress, or by heavy overdosage with corticotrophic anterior-pituitary extracts, even essentially unaltered adrenal

cortical cells may detach themselves and be cast off into the sinusoids. This process is often accompanied by the formation of the above-mentioned "lumina" in the fasciculata region. Occasionally, the sinusoids in the cortico-medullary boundary line and even the larger sinuses within the medulla itself, are filled with epithelioid cells, many of which contain numerous lipid granules and exhibit the characteristics of the cortical cells.

It would be premature to make any definite suggestions concerning the possible physiologic rôle of this "holocrine secretion." In agreement with what we have said about other cytolytic phenomena it is suggestive to assume, however, that cytolysis, the formation of "lumina" in the cortex, the establishment of communications between these lumina and the vascular system, as well as the discharge of intact or cytolized cortical cells into the blood, are all responses to severe and acute overstimulation. Perhaps during acute emergencies, when the need for corticoids is so great that time does not permit the selective elimination of secretion granules, the whole cell is cast off into the blood stream or the cells become the victims of lethal overstimulation.

## Hyperemia

Hyperemia of the adrenal cortex is a common accompaniment of all types of overstimulation (Figure 41). However, certain stimuli, for instance, folliculoid hormones, appear to cause a disproportionately intense dilatation of the sinusoids in the fasciculata so that one gains the impression of a rather specific reaction-form. Sometimes large portions of the fasciculata may thus be transformed into an almost cavernous tissue (Selye, 1947).

## Hemorrhagic Infarction

Occasionally, extreme corticotrophic stimulation by anterior-pituitary extracts, or exposure to stress, results in an almost complete hemorrhagic infarction of the cortex, similar to that seen in man following fulminating infections (e.g., Waterhouse-Friderichsen syndrome, extensive burns). In such instances, it appears that widespread cytolysis, sometimes accompanied by "lumen formation," is associated with intense hyperemia. The resulting increased vascularity of the cortex, conjointly with the accompanying weakening of its tissue resistance (due to cytolysis and endothelial damage) leads to multiple and extensive communications between the vascular system and the tissue spaces occupied by disintegrating cortical cell masses. Thus, widespread hemorrhagic infarction of the adrenal cortex ensues. Only the comparatively dense glomerulosa region seems to be particularly resistant to this type of change. As such infarction causes severe nutritional disturbances in the adrenal it either kills the animal—if the lesion is widespread and bilateral—or results in necrosis with scar formation (Figure 44).

## Focal Necrosis

Focal necrosis of the adrenal was especially common in those of our animals which were chronically treated with alarming stimuli or with heavy doses of corticotrophic anterior-pituitary extracts. In the latter event their incidence was particularly high in rats in which the corticotrophic effect of these preparations was increased by high protein diets, or simultaneous thyroxine treatment. Often periarteritic changes were distinctly visible in the adrenal vessels of such animals and we had every reason

to ascribe the necrosis to local nutritional disturbances, due to complete occlusion of arteries by hyaline and granulomatous material. In other instances the necrosis was secondary to the diffuse infarction of the cortex described above (Figure 50).

### “Toxic Involution”

Certain drugs exert a highly specific effect upon the adrenal cortex, which has variously been referred to as “cytotoxic atrophy,” “zonal degeneration” or simply cortical atrophy. Germanin (Humphreys and Donaldson, 1941; Weis and Gaunt, 1946) is claimed to possess this effect in various laboratory animals and according to a brief preliminary report (Nelson and Woodard, 1948), “DDD,” a derivative of the well-known insecticide “DDT,” causes severe adrenal cortical atrophy in the dog, although the rat and the monkey appear to be immune to this action of the compound.

Thanks to the kindness of Dr. P. S. Larson and his colleagues of the Medical College of Virginia, we had the opportunity of examining the adrenals of DDT-treated dogs. We found that the cortical involution and the decrease in the total weight of the adrenals is mainly due to a rather selective involution of the reticularis region, in which the fatty metaplasia occurs and numerous islets of lymphatic and hemopoietic cells appear. Figures 52 and 54 illustrate the adrenals of such a DDD-treated dog. The animal weighed 10 kg. at the beginning of the experiment and only 9.1 kg. at its termination, although it maintained an excellent appetite throughout the treatment period. This dog received 50 mg./kg. of DDD daily (six days a week during five weeks), incorporated in the diet which consisted of finely ground “Purina Dog Chow.”

The extent of the adrenal-cortical involution is readily discernible when the gland is compared with that of a similar control animal (Figures 51 and 53). Even under low magnification, the reticularis stands out as a dark, irregular zone (Figure 52). At a higher magnification the presence of numerous fat cells, lymphoid and hemopoietic elements is prominent in the reticularis region (Figure 54). In other sections, intense phagocytosis of a brown pigment ( hemosiderin?) was noticeable in the reticulo-endothelial cells of the reticularis region. The glomerulosa and outer fasciculata cells were greatly enlarged, presumably due to "compensatory hypertrophy," in an effort to substitute for the damaged cells of the inner layers. The medulla remained essentially intact.

Nothing is known about the pathogenesis and functional significance of this peculiar change, but the selective degeneration of the inner layers represents a noteworthy exception to the general rule that drugs and other toxic agents stimulate the development of the adrenal cortex.

## ✖ 5 ✖

# Summary

IN THIS communication we report on a series of experiments (on rats and dogs), designed to study some of the most important factors which regulate the structure of the adrenal cortex. The experimentally produced changes have been compared with similar lesions occurring in man. The data have been analyzed mainly as regards their functional significance. It was found that qualitative changes in adrenal structure can help to recognize factors which act upon the gland and at the same time, can aid in the evaluation of adrenal function.

The changes in the gross weight of the adrenals, which lend themselves to strictly objective statistical evaluation and histologic changes, which require a necessarily more subjective description, have been discussed in separate sections.

## I. Changes in Adrenal Weight

It was found that — within a certain range — the increase in adrenal weight occasioned by a given dose of *lyophilized anterior pituitary tissue* (LAP) is directly proportional to the *protein* content of the diet.

METHYL-TESTOSTERONE causes a pronounced decrease in adrenal weight and is highly effective in inhibiting the adrenal enlargement normally caused by thyroxine or exposure to non-specific stress. On the other hand, methyl-testosterone does not prevent the adrenal enlargement

caused by LAP. Hence it is concluded that this testoid compound inhibits the endogenous production of hypophyseal corticotrophin, but does not interfere with the action of exogenously administered corticotrophic preparations.

The decrease in adrenal weight occasioned by methyl-testosterone is not significantly influenced by high protein diets.

THYROXINE, in itself, causes only moderate enlargement of the adrenal cortex, but it greatly potentiates the corticotrophic effect of LAP. In this respect the action of the thyroid principle resembles that of high-protein diets. It is suggested that the sensitizing action may in both instances be occasioned by protein catabolytes (e.g., amino-acids); these could result from increased protein-turnover due to the metabolic action of thyroxine in one case and from a greater dependence upon calories derived from ingested protein in the other.

Neither *unilateral nephrectomy* nor a high *sodium* intake exert any important effect upon the adrenal weight changes induced by thyroxine, LAP or methyl-testosterone. This is mentioned because both unilateral nephrectomy and sodium-rich diets had previously been shown to increase the toxicity of corticoid hormones; furthermore, dietary sodium can change the histologic structure of the cortex (see below).

The *gonads* do not significantly influence the sensitivity of the adrenals to thyroxine, LAP, methyl-testosterone and combinations of these hormone-preparations, since females, males and castrate males respond in essentially the same manner.

Even high doses of *desoxycorticosterone acetate* (DCA) fail to inhibit the adrenal enlargement caused by anterior pituitary extract.

An amount of DCA which causes "compensatory atrophy" of the adrenal cortex on a Na-free diet, fails to do so in animals receiving large quantities of NaCl. Presumably on high-Na diets the non-specific (adrenal-enlarging) stress effect of this corticoid compound can be so intense as to overcompensate for its tendency to induce adrenal-cortical involution. These two actions of DCA are therefore entirely independent and indeed mutually antagonistic.

## II. Changes in the Histologic Structure of the Adrenal Cortex

In addition to the usual non-specific, progressive and regressive, changes caused by simple stimulation and inhibition respectively, *the adrenal cortex can respond to certain stimuli with various highly specific reaction-forms.* This fact is incompatible with the view that all stimuli, which affect the adrenal cortex, act merely by increasing or decreasing the production of a single corticotrophic hormone (ACTH).

An analysis is given of the experimental conditions which permitted the production in the adrenal cortex of the following qualitatively distinct morphologic changes: atrophy (various types), hypertrophy (various types), hyperplasia, capsular adenomas, storage or discharge of various granules (sudanophilic lipids, cholesterol, colloid, plasmal, ascorbic acid), fibrinoid degeneration, cytolysis, "chromidiosis," fatty metaplasia, lymphoid and myeloid metaplasia, formation of "lumina" within the cortical parenchyme, holocrine secretion of cell debris or entire cells into the blood, hyperemia, hemorrhagic infarction, focal necrosis and "toxic involution." The very existence of these manifold reaction-forms clearly demonstrates

that the structure of the adrenal cortex cannot depend exclusively upon the action of a single corticotrophic hormone.

Certain experiments strongly suggest that *the quality of the adrenal response to hypophyseal corticotrophin can be modified by additional stimuli*. The fatty metaplasia of adrenal cortical cells caused by testoid hormones, or the lymphatic and myeloid metaplasia occasioned by the parental administration of necrotic tissue preparations, cannot be produced after hypophysectomy. The development of all these changes is enhanced by simultaneous treatment with corticotrophic hypophyseal extracts. By themselves, neither testoids nor DCA cause any accumulation of colloid granules in the cortical cells in intact or in hypophysectomized animals. Yet, both these steroids are highly effective in this respect, when given in conjunction with corticotrophic pituitary preparations.

For the evaluation of the adrenal lesions seen during the general-adaptation-syndrome the following observations are of special importance. In acute (48 hrs.) experiments, *even very high doses of purified corticotrophin failed to reproduce the characteristic discharge of sudanophilic granules, which regularly occurs during the alarm-reaction*. The cortex remained rich in lipids, although its enlargement was greater than that caused by non-specific stress. It could even be shown that the loss of cortical lipids occasioned by non-specific stress or by folliculoid hormones is partially inhibited by corticotrophic anterior pituitary preparations. These observations are incompatible with the assumption that during the alarm reaction non-specific stress depletes the cortex of its sudanophilic lipid granules merely as a result of increased corticotrophin secretion. On the other hand, previous experiments had proven that non-specific stress does not

cause loss of cortical lipid granules in the hypophysectomized animal. Hence it is probable that during the alarm reaction some hitherto unidentified factor is responsible for the discharge of cortical lipids, although this factor is ineffective in the absence of hypophyseal corticotrophin.

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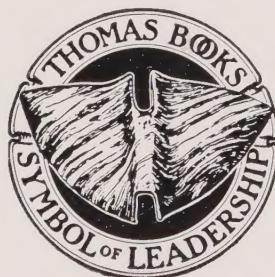
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ON THE  
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OF THE  
ADRENAL CORTEX

*By*

HANS SELYE, M.D., Ph.D., D.Sc., F.R.S. (C)  
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