

EFFECTS OF CASTRATION AND OF SEXUAL
HORMONES ON THE ADRENALS
OF MALE RATS

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THE occurrence of hypertrophied "castration adrenals" in male rats has been observed previously. The literature on the subject, which is scanty, is referred to by Andersen & Kennedy [1933], who found a wider cortex in "castration adrenals" with a less clearly demarcated zona glomerulosa and approximately the same distribution of lipoids as compared with normal controls. As to the precise effects of castration on lipid content, the authors are indefinite. More recently increase in weight of "castration adrenals" in male rats has been observed by Winter & Emery [1936].

The effect of injections of the hormones mentioned below has been studied only on the X-zone (not present in most animals including the rat and human being) of castrated mice by Deanesly & Parkes [1937] and Cramer & Horning [1937]. Callow & Deanesly [1935] could not find changes in the adrenals of mice injected with androsterone. The X-zone is only temporarily present in the female mouse, disappearing rapidly after the first pregnancy or with age. The zone does not exist in the adult male mouse, but is produced by castration [Deanesly, 1928; Cramer & Horning, 1937]. Deanesly & Parkes [1937] and Cramer & Horning [1937] found that the appearance of this zone in castrated males can be inhibited, or, if already present, caused to disappear by injections of androstenediol, testosterone, testosterone propionate and androstanediol.

In previous papers [Korenchevsky, 1930, 1932; Korenchevsky & Dennison, 1934] the effects were studied of cryptorchidism and castration on the weights of different organs including the adrenals. Castration before sexual maturity resulted in hypertrophy of the adrenals. This was confirmed in our succeeding papers, in which it was also shown that the

“castration hypertrophy” of adrenals decreases or disappears after injections of hormonal extracts from the male urine [Korenchevsky *et al.* 1933] and of the following pure sexual compounds: androsterone and androstanediol and their water-soluble esters [Korenchevsky, 1935; Korenchevsky *et al.* 1935], transdehydroandrosterone [Korenchevsky & Dennison, 1936], testosterone [Korenchevsky, 1936; Korenchevsky *et al.* 1936], testosterone propionate [Korenchevsky & Dennison, 1937; Korenchevsky *et al.* 1937*b*], Δ^4 -androstenedione and Δ^5 -androstenediol [Korenchevsky *et al.* 1937*a*]. Testosterone propionate, injected into normal male rats, did not produce definite changes in the size or weights of the adrenals [Korenchevsky *et al.* 1937*c*].

In the present paper the results are given of histological examination of the adrenals of rats described in the above-mentioned papers of Korenchevsky and co-workers. In the same papers the details of the experiments can be found. Our histological investigation was made on the adrenals of 53 normal and 150 castrated rats. The age of the rats, number of animals in each group, definition of the group, hormones injected, average weight of adrenals and width of the cortical zones and of the medulla are given in Tables I and II.

A short account of the results obtained has been given elsewhere [Hall & Korenchevsky, 1937].

TECHNIQUE

The rats were castrated when 21–27 days old, usually 21–24 days, i.e. before sexual maturity. All the sexual compounds used were artificially prepared and supplied by Messrs Ciba, Ltd. They were dissolved in sesame oil and the daily dose was injected subcutaneously twice a day in half doses. The period of the injections was 21–23 days for most of the hormones, but in about half the experiments with androsterone and androstanediol it was longer—27–59 days. At the end of the experiments the rats were anaesthetized with ether and then killed by bleeding from the abdominal aorta.

Special care was taken to dissect the adrenals and drop them into Bouin's fixative as soon as possible after death in order to prevent destructive changes in the cells. The paraffin sections were stained with hæmatoxylin and eosin, and frozen sections for lipoids with Scharlach Red.

Micrometer measurements were made at the widest diameter of the adrenals, both opposite sides of each of the cortical zones were measured, and the mean of these measurements was taken. The figures are given in Tables I and II. In Table II the weights of the adrenals of rats receiving different doses giving similar effects were averaged and therefore these data differ a little from the detailed weights given for each dose of the hormones in our previous papers, from which these data were taken. The adrenals of rats having pneumonia or other diseases were not investigated since these diseases usually change their structure. The drawings were made by Miss K. Hall from areas in the peripheral one-third of the zona fasciculata and the middle part of the zona reticularis, and were done with the aid of a camera lucida. The photomicrographs were made by Mr W. Pereira.

ADRENALS OF NORMAL UNINJECTED MALE RATS

In the rat's adrenal cortex, three zones are usually differentiated: zona glomerulosa, fasciculata and reticularis. We have invariably found a fourth zone, the "demarcation zone". This zone has been already observed by Bonnamour [1905*a*] and Jackson [1919, "transition band"] in the rat, and by Babes & Jonesco [1908] in the dog. Mulon [1912], however, in the dog found large lipid granules in this region of the adrenal. It is situated between the zona glomerulosa and fasciculata and consists of 1-4 rows of cells, considerably smaller than those of the fasciculata and usually slightly smaller than those of the glomerulosa (Fig. 1). In sections embedded in paraffin this zone is conspicuous, because in most of the cells vacuoles are absent or only few and small. The greatly vacuolated cells occasionally seen in this zone are most probably explained by penetration of cells from the fasciculata or glomerulosa. In frozen sections stained with Scharlach Red the demarcation zone is still more conspicuous, its cells almost entirely free from lipid granules, while the cells of the adjoining zones, glomerulosa and fasciculata, are crowded with granules (Fig. 7).

EFFECT OF CASTRATION

The data are given in Table I and Figs. 3 and 8. The changes appear gradually and are fully developed about 2 months after castration, in 80-day-old rats.

The increase in weight and size of the "castration adrenal" is due to hypertrophy of the zona fasciculata and reticularis, especially the latter, resulting from a considerable increase in the size of the cells composing these layers. The greater hypertrophy of the reticularis may possibly be explained by hyperæmia and also perhaps by hyperplasia.

In paraffin sections vacuolation is shown to increase slightly after castration (cp. Figs. 2 and 3) and is of a more even character as regards the numbers and size of the vacuoles, especially in the zona fasciculata. Staining with Scharlach Red shows a definite change in the demarcation zone which becomes impregnated with lipid granules and therefore appears to be absent, its cells being indistinguishable from those of the adjoining zones. A similar impregnation of this zone with lipoids was described by Jackson [1919, p. 264] in rats after inanition.

The medulla of castration adrenals was on the average only slightly larger. Since, however, the individual variations in width of medulla

were considerable, it is difficult to draw a definite conclusion without more numerous measurements. With the stains used, no changes were noticed in the cells of the medulla.

EFFECTS OF INJECTIONS OF SEXUAL HORMONES INTO
CASTRATED RATS

The data given in Table II (injected rats) have to be compared with those in Table I (control normal and castrated rats). The effect produced by all the hormones investigated was similar, varying in degree or in

TABLE I. Effect of castration on weight of adrenals and width of cortical zones and of medulla. N.=normal, C.=castrated rat

No. of group and age in days	No. of rats		Average weight of adrenals mg.		Average width, μ							
					Glomerulosa		Fascicula		Reticularis		Medulla	
					N.	C.	N.	C.	N.	C.	N.	C.
I 40-49	9	2	41	55	67	69	585	718	301	284	1303	1503
II 60-69	5	6	48	77	67	59	635	685	301	418	1336	1420
III 70-79	4	11	54	77	67	69	568	651	384	434	1319	1436
IV 80-89	10	12	58	79	67	69	568	651	384	451	1319	1486
V 90-99	7	9	65	79	67	69	586	668	334	451	1386	1486
VI 100-149	6	4	54	69	67	69	568	634	351	451	1436	1420
Average	—	—	53	73	67	67	585	668	342	415	1350	1459

TABLE II. Effect of injection of sexual hormones on weight of adrenals and width of cortical zones and of medulla

No. of group and age in days	Hormone injected	Daily doses used mg.	No. of rats	Average weight of adrenals mg.	Average width, μ			
					Glomerulosa	Fascicula	Reticularis	Medulla
I 77-149	Androsterone	0.45-0.9	7	56	75	618	317	1403
II 64-88	Androsterone	1.8-3.6	12	52	69	534	284	1286
III 91	W.S. androsterone	13.6-27	2	44	75	534	267	1386
IV 49-83	Androstanediol	0.175-0.35	9	49	75	551	317	1253
V 64-88	Androstanediol	0.7	5	49	69	584	284	1219
VI 49-91	W.S. androstanediol	1.5-5.3	6	51	69	601	267	1219
VII 68-86	Transdehydroandrosterone	1.4	19	64	59	618	351	1420
VIII 79	Androstenedione	0.5-3	11	55	69	601	367	1336
IX 69	Androstenediol	0.2-0.6	4	53	69	618	384	1319
X 98	Testosterone	0.033-0.167	8	72	69	668	351	1436
XI 98	Testosterone	0.5-1.41	8	63	75	585	367	1402
XII 98	Testosterone + androsterone	0.167-0.5 } 0.45	4	68	75	668	351	1470
XIII *89	Testosterone propionate	0.167-1.4	6	56	69	620	351	1403
XIV *89	Testosterone propionate	0.167-1.4	5	54	75	585	367	1403
XV 102	Testosterone propionate (normal rats)	0.5-1.5	12	61	59	620	434	1386

* The rats in group XIII were killed 1 day, and in group XIV 9 days after the last injection of testosterone propionate.

details only. It is noteworthy that even dehydroandrosterone, which has the weakest stimulating effect on the sexual organs, has a pronounced effect on the adrenals.

Medulla

With the stains used, no definite changes were found in the cells of the medulla and therefore the following description is of the cortical changes only. On the average, however, the width of the medulla (Tables I and II) in most of the injected groups was slightly smaller than in the control castrated rats, returning to the normal average figure.

Cortex

Width of cortical zones. All the hormones used, if injected in sufficiently large doses, caused the hypertrophied fasciculata and reticularis to return to or towards normal (Tables I and II). Complete recovery to normal width was obtained with androsterone, androstanediol and testosterone. Transdehydroandrosterone, androstenedione and testosterone propionate caused the zona reticularis to return completely to normal, but the fasciculata only partially; while androstenediol, in the doses used, in most rats produced only a partial recovery towards normal width in both zones.

It is important, however, to emphasize, that after injections of some of the hormones, the zones became abnormally narrow, e.g. the fasciculata with large doses of androsterone, and the reticularis with all doses of androsterone and androstanediol.

Size of the cells. The cells were smaller after the injections, becoming normal in size or less than normal (Fig. 6), there being in general correspondence between the changes in width of the zones and size of the cells. It is to be emphasized, however, that, with the exception of transdehydroandrosterone and the smallest dose of testosterone (0.033 mg., Fig. 5), all other hormones, in the doses used, reduced the size of the cells to normal, or smaller (Fig. 4), but in some rats there was only a partial recovery in the width of the zones. This partial recovery suggests that, besides hypertrophy of the cells, other factors may play a part in hypertrophy of the cortex, e.g. hyperæmia of the adrenals or hyperplasia of the cortical cells.

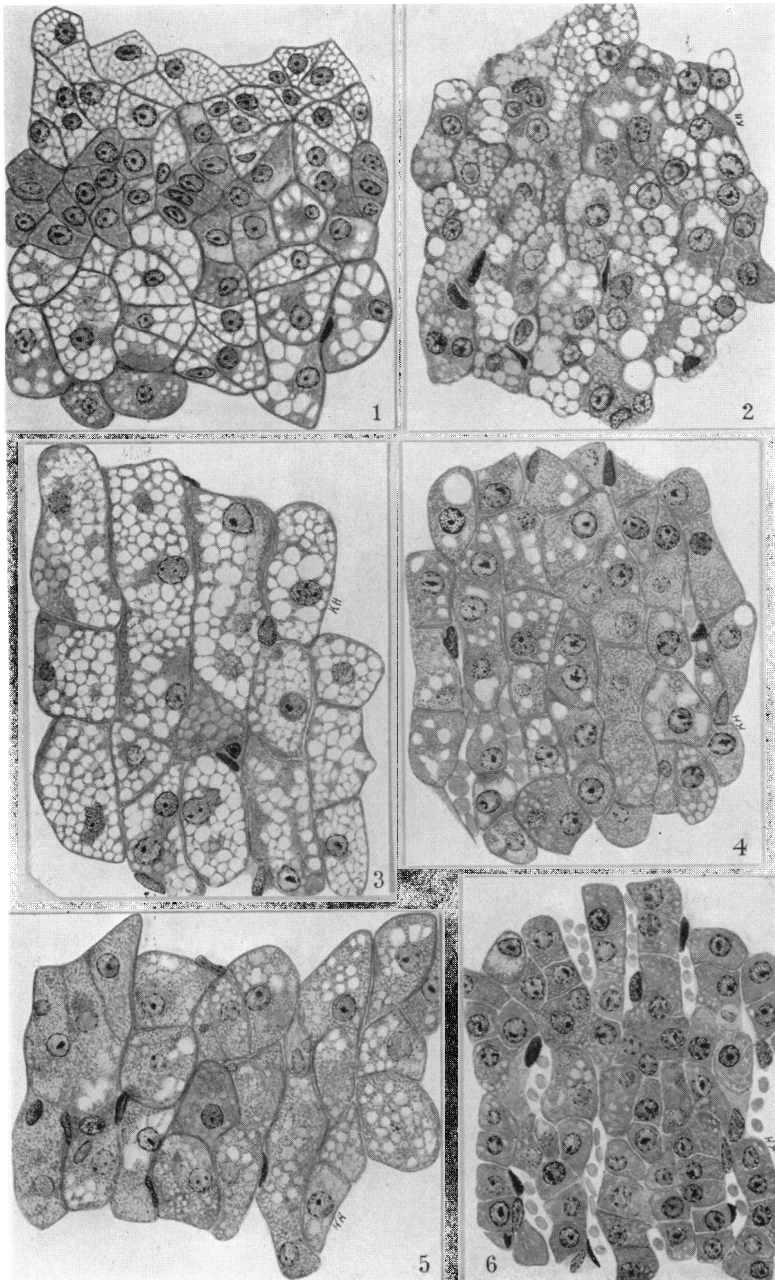
Vacuolation (as seen in paraffin preparations). After the injections, the vacuoles (with the exceptions mentioned below) decrease in number, becoming sometimes normal but more often fewer (Figs. 4 and 5), or even considerably fewer than normal (Fig. 6). Usually the size of the vacuoles also decreases. After daily injections of 0.9 mg. or more of androsterone (Fig. 6), the fasciculata cells become almost unrecognizable

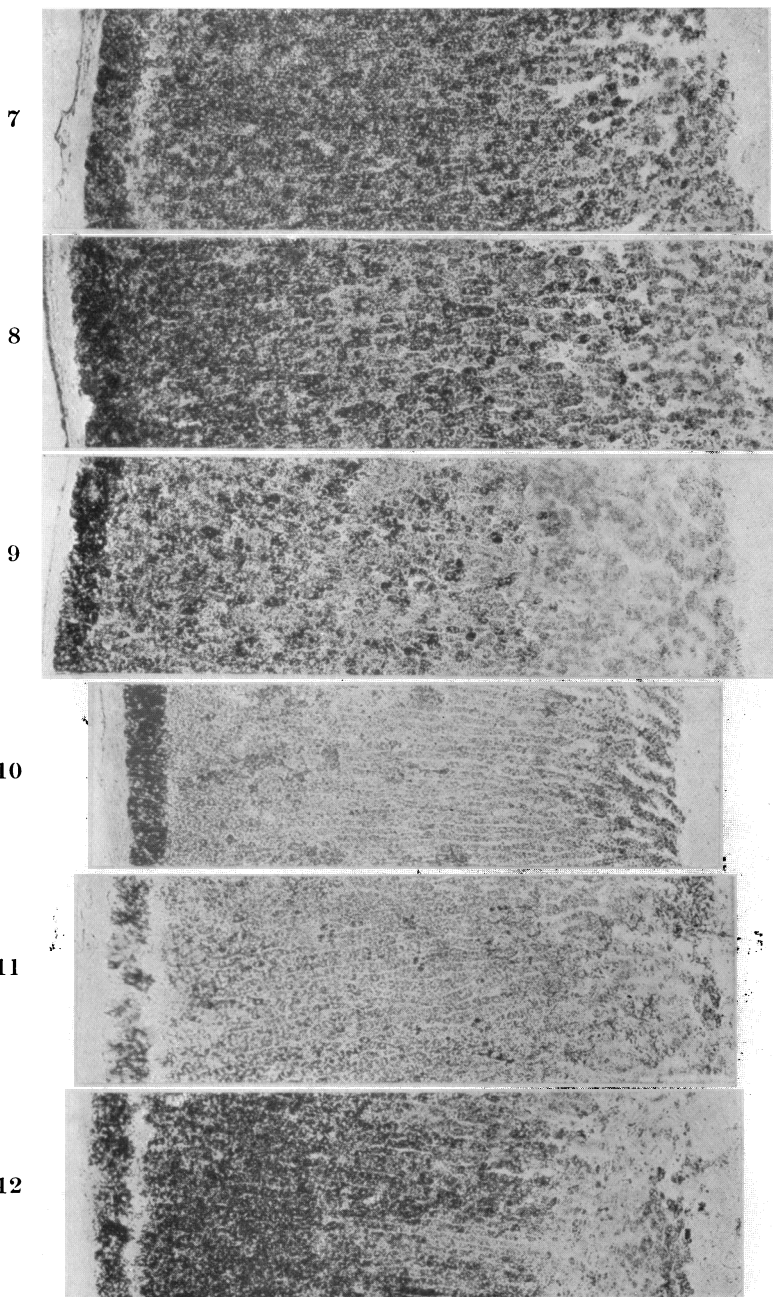
owing to their greatly reduced size and the almost complete disappearance of vacuoles. With most hormones, the higher the dose, the less marked becomes the vacuolation. Exceptions are testosterone and to a lesser degree testosterone propionate, smaller doses of which decrease vacuolation more than do larger doses.

Lipoid granules (preparations stained with Scharlach Red). All the hormones in all doses used caused a decrease below normal in the number of lipoid granules in the reticularis and especially in the fasciculata (Figs. 9–11). The greater the dose, the more pronounced was the reduction. In some cases (e.g. 0.033 and especially 0.167 mg. of testosterone) small "patches" of the fasciculata remained much impregnated with lipoid granules, which had disappeared, more or less evenly, from the larger neighbouring areas (Fig. 9). The lipoid granules in the zona glomerulosa, however, are much less decreased by the injections and remain proportionately more numerous in this zone than in the fasciculata and reticularis. In fact in some cases (e.g. 0.9 mg. androsterone, Fig. 10) the number and size of the lipoid granules in the zona glomerulosa remain apparently unchanged, being in conspicuous contrast to the lipid-impooverished fasciculata and reticularis. Jackson [1919, p. 250] describes a similar change in the adrenals of starving rats, while our injected rats had good appetites, and their gain in weight was greater than in the controls [Korenchevsky *et al.* 1935, p. 2540]. Such a difference in the resistance to the action of hormones suggests a difference in the chemical nature of the lipoids in different zones and, perhaps, a difference in their endocrinological function.

Demarcation zone. All doses of all hormones injected caused this zone in castrated rats to become conspicuous again, since the lipoid granules were absorbed and the vacuoles disappeared (Figs. 9–11). Thus the zone returns to normal.

Discrepancy between vacuolation and lipoid granules content. As a general rule, when vacuolation in the cortical cells is decreased, the number of lipoid granules as shown by lipoid staining is also decreased, and vice versa. This is, however, not always the case, since the lipoids might be present in different chemical forms, some showing positive and some negative staining reaction [Bonnamour 1905*b*; Grollman, 1936]. In our experiments an example of a discrepancy between vacuolation (pronounced) and number of lipoid granules (few) was observed after the injection of 1.4 mg. of testosterone and (to a lesser degree) of testosterone propionate. We have not made a detailed study of the nature of this discrepancy.





THE EFFECT OF A RESTING PERIOD OF 9 DAYS AFTER TESTOSTERONE
PROPIONATE INJECTIONS INTO CASTRATED RATS

When the adrenals of rats killed the day after the last injection (group XIII, Table II) were compared with those of their litter-mates killed 9 days after the last injection (group XIV), it was found that in both groups the weight and size of the adrenals returned to normal, the width of the zones (Figs. 11-12) and the size of the cells were nearly or completely normal, but there were the following striking differences. The adrenals of group XIII showed the abnormal effects usually produced by the injections, namely, decreased vacuolation and a very striking decrease, considerably below normal, in the number of lipid granules (Fig. 11). In group XIV (Fig. 12) these abnormal features had completely or, mostly with large doses (0.5 mg.), almost completely disappeared 9 days after the last injection, and the adrenal appeared to be normal. These results show that the abnormal effects of the injections of sexual hormones disappear only some time after the cessation of the injections. At the same time, however, the experiments show that testosterone propionate may have a complete recovery effect on the adrenals of castrated rats, and also that this effect may be a comparatively lasting one, being present probably more than 9 days after the last injection was given.

THE EFFECTS OF TESTOSTERONE PROPIONATE ON THE
ADRENALS OF NORMAL RATS

The data are given in group XV, Table II.

Although in injected rats the changes varied in degree, in most cases they were less pronounced with 0.5 than with 1.5 mg. The width of the zona fasciculata and reticularis, and the size of the individual cells were normal or slightly increased, a different reaction from that produced by the same hormone on the "castration adrenal". There was a definite decrease in normal vacuolation and in the numbers of lipid granules (often strikingly reduced) as compared with those in the control uninjected litter-mates. In some cases the lipid depletion took the same form as with testosterone in castrated rats, resulting in an uneven "patchy" appearance. In the zona fasciculata there was an increased number of degenerated nuclei with poorly developed and faintly stained chromatin networks.

SUMMARY

1. A histological investigation was made of the adrenals of 53 normal and 150 castrated rats, uninjected or injected with androsterone, androstenediol, transdehydroandrosterone, testosterone, testosterone propionate, Δ^5 -androstenediol, and Δ^4 -androstenedione.

2. Besides the zona glomerulosa, fasciculata and reticularis, a fourth zone, the demarcation zone, is constantly present in the cortex of the rat adrenal.

3. The following histological changes in the hypertrophied "castration adrenal" were observed: increase in the width of the zona fasciculata and reticularis, considerable increase in size of the individual cells, and a slight increase in vacuolation: an apparent disappearance of the demarcation zone due to the vacuolation of its cells and their impregnation with lipid granules.

4. Injection into castrated rats of all the sexual hormones investigated produced a comparatively lasting recovery (at least 9 days) towards, or to, normal; or, usually with larger doses, the following abnormal changes in the adrenal cortex: a decrease below normal in the width of the zona fasciculata and the reticularis and of the size of the individual cells; abnormal decrease of vacuolation and of the number of lipid granules.

5. The abnormal effects suggest some toxicity for adrenals of large doses of the hormones, but these effects had disappeared in most cases 9 days after the last injection and the adrenals had returned to normal.

6. The fact that the lipoids of the zona glomerulosa and those of the other three zones react differently suggests that they are not similar and may perhaps have a different endocrinological significance.

7. The chief effects of the doses used of testosterone propionate on the adrenals of normal rats were reduction of vacuolation and number of lipid granules in the cells of the zona fasciculata and reticularis.

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DESCRIPTION OF PLATES I AND II

PLATE I

- Fig. 1. Normal uninjected control rat. Upper rows of vacuolated cells belong to zona glomerulosa; medium rows of small unvacuolated or partly vacuolated cells—démarcation zone; lower rows of large vacuolated cells—zona fasciculata. Rat 382 g. 99 days old. Adrenals 61 mg. $\times 545$. Hæmatoxylin and eosin.
- Fig. 2. Normal uninjected control rat. Cells of zona fasciculata, with uneven vacuolation, and occasional degeneration of nuclei. Rat 365 g. 99 days old. Adrenals 52 mg. $\times 545$. Hæmatoxylin and eosin.
- Fig. 3. Castrated uninjected control rat. Cells of zona fasciculata much enlarged, with greater but more even vacuolation than in Fig. 2. Rat 325 g. 99 days old. Adrenals 82 mg. $\times 545$. Hæmatoxylin and eosin.
- Fig. 4. Testosterone propionate (0.167 mg. daily $\times 23$ days) injected castrated rat. Zona fasciculata. Medium effect. Cells are smaller or equal to normal without any or with reduced vacuolation. Rat 308 g. 84 days old. Adrenals 61 mg. $\times 545$. Hæmatoxylin and eosin.
- Fig. 5. Testosterone (0.033 mg. daily $\times 23$ days) injected castrated rat. Zona fasciculata. Comparatively weak effect of small dose. Cells smaller than in Fig. 3. Vacuolation reduced below that in castrated (Fig. 3) and normal (Fig. 2) rats. Rat 338 g. 93 days old. Adrenals 73 mg. $\times 545$. Hæmatoxylin and eosin.
- Fig. 6. Androsterone (0.9 mg. daily $\times 47$ days) injected castrated rat. Zona fasciculata. Strong effect. Cell-cords narrower, making blood vessels more conspicuous. Cells reduced in size much below normal. Vacuoles few or absent. Rat 258 g. 77 days old. Adrenals 39 mg. $\times 545$. Hæmatoxylin and eosin.

PLATE II

- Fig. 7. Normal control uninjected rat. From left to right: (1) unstained adrenal capsule; (2) zona glomerulosa—rich in lipid granules (black); (3) demarcation zone, very narrow—lipoid granules few or absent; (4) zona fasciculata—widest zone, with columnar arrangement of cells rich in lipoids; (5) zona reticularis—narrow network of cells, with fewer lipid granules than in zona fasciculata and glomerulosa; (6) unstained medulla. Rat 323 g. 99 days old. Adrenals 59 mg. $\times 72$. Scharlach Red.
- Fig. 8. Castrated control uninjected rat. Demarcation zone appears absent because of impregnation with lipoids. Because of the hypertrophy only the upper part of zona reticularis is seen. Rat 325 g. 99 days old. Adrenals 82 mg. $\times 72$. Scharlach Red.
- Fig. 9. Testosterone (0.033 mg. daily $\times 23$ days) injected castrated rat. Weak effect of small dose. Glomerulosa with normal amount of lipoids, fasciculata impoverished in "patches", less lipid in reticularis. Reappearance of demarcation zone. Rat 338 g. 93 days old. Adrenals 73 mg. $\times 72$. Scharlach Red.
- Fig. 10. Androsterone (0.9 mg. daily $\times 47$ days) injected castrated rat. Strong effect. Lipid content in glomerulosa normal, but much decreased in fasciculata and less in reticularis. Rat 258 g. 77 days old. Adrenals 39 mg. $\times 72$. Scharlach Red.
- Fig. 11. Testosterone propionate (0.167 mg. daily $\times 23$ days) injected castrated rat, killed 1 day after last injection. Strong effect. Number of lipid granules much decreased in all zones. Effect much stronger than that with the same dose of testosterone. Rat 327 g. 73 days old. Adrenals 58 mg. $\times 72$. Scharlach Red.
- Fig. 12. Testosterone propionate (0.167 mg. $\times 23$ days) injected castrated rat, killed 9 days after last injection. Number of lipid granules restored to normal in fasciculata, slightly below normal in reticularis and glomerulosa. Rat 267 g. 73 days old. Adrenals 54 mg. $\times 72$. Scharlach Red.