TABLE I.
Acute Oral Toxicity for White Mice of Derris Root and Various Extracts of It.

	Sample	Rotenone, %	Total Chloroform Extractives, %	Red color % of total	L.D.$_{50}$ white mice, mg/kg
1.	Powdered derris root	4.6	14.0	100.00	350
2.	Marc, after extraction of No. 1 with water			70.5	470
3.	Marc, after extracting No. 1 with petroleum ether 20 hr	2.44	6.8	50.7	570
4.	Water extract of No. 1			31.0	600
5.	Petroleum ether extract of No. 1 on talc having mean diameter of 1 μ	2.20	7.2	56.0	600
6.	Petroleum ether extract of No. 1 on talc having mean diameter of 2 μ	2.20	7.2	55.0	700
7.	Total chloroform extract of No. 1 on 2 μ talc	4.6	14.0		700
8.	Chloroform extract of No. 3 on 2 μ talc	2.4	6.8	44.0	1,100
9.	Marc after extracting No. 3 with water			40.5	3,000
10.	Marc from complete extraction of No. 1 with chloroform	0	0	0.0	>3,600*
11.	Marc from No. 3 extracted 20 hr with cyclohexane	1.5	4.5	20.0	>1,200†
12.	Talc control, 2 μ				>4,200*

* No fatalities. † 10% fatalities.

fractions, or from whole derris.

Summary. From studies dealing with the acute toxicity of derris and several extracts thereof for mice, it appears that this toxicity is due to the insecticidally active materials represented by the rotenone content, total chloroform extractives content, and red color index. No single one of these three determinations, however, seemed uniformly to indicate the relative toxicity of the various fractions studied. Materials not extractable with chloroform were found to be practically non-toxic. Thus far no active alkaloid or glycoside has been isolated from derris.

14338

Changes in the Adrenals Following Prolonged Treatment with Methyl-Testosterone.

HANS SELYE, E. M. ROWLEY, AND C. E. HALL.*

From the Department of Anatomy, McGill University, Montreal, Canada.

The fact that adrenal cortical tumors are often accompanied by clinical signs of "virilism" called attention, at an early date, to possible relationships between the adrenal cortex and the production of testicular or "testoid" hormones. It has been found that the zona reticularis or "x-zone" of the adrenal cortex in the mouse undergoes rapid involution under the influence of testoid hormones.[1-3] In other species, however, this is not the case. For instance in the rat testosterone and its propionate fail to elicit specific changes in the reticularis although they decrease the weight of the organ due to a slight involution of the cortex as a whole.[4,5] It has been espe-

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1 Howard, E., *Am. J. Physiol.*, 1940, **129**, P 297.

2 Burrows, H., *J. Path. and Bact.*, 1936, **43**, 121.

3 Starkey, W. F., and Schmidt, E. C. H., Jr., *Endocrinology*, 1938, **23**, 339.

4 McEuen, C. S., Selye, H., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 390.

5 Selye, H., *Canad. M. Assn. J.*, 1940, **42**, 113.

cially emphasized that in the rat testosterone treatment during the first few weeks of life causes a particularly pronounced involution of the glomerulosa without eliciting any significant change in the reticularis.[6] In acute experiments on rats Nathanson and Brues[7] found, on the other hand, that testosterone propionate stimulates mitotic activity especially in the glomerulosa as judged by the colchicine technic.

The object of this communication is to report upon a series of experiments which indicate that the rat adrenal responds just as specifically to chronic overdosage with testoids as that of the mouse, although the type of the resulting morphological changes is entirely different.

Twelve male albino rats weighing 60-75 g (average 68 g) were subdivided into 2 groups of six. The animals of the first group received 2 daily injections of 0.1 cc of peanut oil containing 5 mg of methyl-testosterone in the form of finely ground crystals in suspension. The remaining 6 rats were treated with cholesterol in the same manner and dosage, since cholesterol is devoid of any hormonal activity they served as controls. The animals were killed 76 days after initiation of treatment and their adrenals were weighed after fixation in Suza mixture. The average weight of the adrenals in the methyl-testosterone treated group was 32 mg as compared with 38 mg in the controls, a difference which was not statistically significant ($P = 0.1$).

Histological examination of these adrenals, on the other hand, showed very characteristic changes. The glomerulosa appeared narrower than that of the controls and consisted of small cells which were very poor in cytoplasm. The connective tissue capsule of the gland was greatly thickened. The other layers of the cortex did not participate in the atrophy but likewise showed typical alterations. Throughout the cortex a large number of epithelial cells was filled with voluminous vacuoles. In frozen sections stained with Sudan III these proved to contain lipid material. In many cases a single large lipid granule distended the cell body and pushed the nucleus to one side so that "signet ring" types resulted resembling the fat cells of ordinary adipose tissue (Fig. 1 and 2). Fat-laden cells are usually also

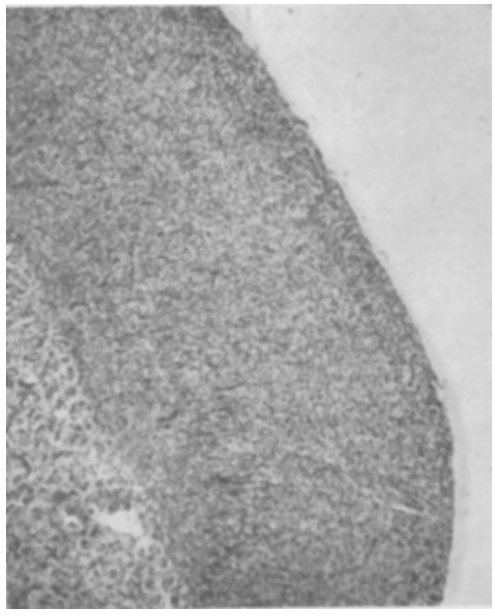

FIG. 1.
Normal adrenal cortex of control rat.

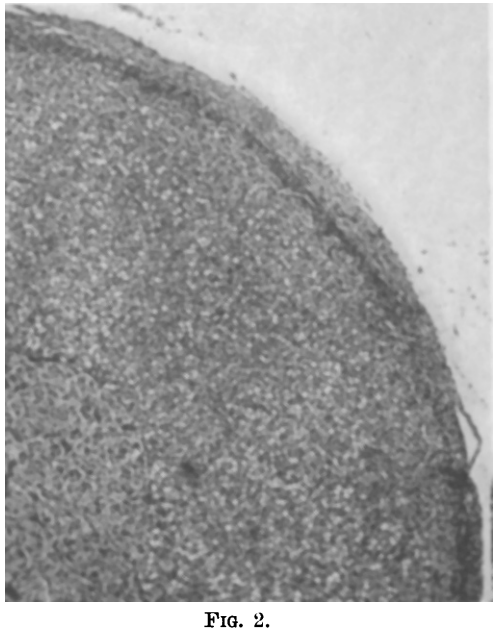

FIG. 2.
Adrenal cortex of rat treated with methyl-testosterone. Note thickening of connective tissue capsule, involution of glomerulosa (dark zone) and vacuoles in remaining cortical cells.

[6] Selye, H., *Anat. Rec.*, 1940, **76**, 145.

[7] Nathanson, I. T., and Brues, A. M., *Endocrinology*, 1941, **29**, 397.

found in the adrenal medulla of the rat and in the hormone-treated group these medullary cells were likewise characterized by unusually coarse lipid granules. This change was not observed in animals treated with steroids devoid of testoid properties. It will be recalled that folliculoids cause hypertrophy of the cortical cells accompanied by an actual loss of lipid granules while corticoids induce a generalized atrophy of the cells in all 3 layers of the cortex; thus the response of the adrenal to treatment with testoids appears to be rather specific.

The influence of the adrenal cortex on potassium metabolism is well known and in connection with the adrenal changes described above it is of interest to note that Butler *et al.*[8] observed a pronounced fall in the serum potassium of patients treated with methyl-testosterone. We therefore determined the serum potassium concentration (method of Kramer and Tisdall[9] modified by Jacobs and Hoffman[10]) of our rats on several occasions during the course of the experiment. However, in this respect there was no statistically significant difference between the two groups.

It is also of interest to note that in spite of this long continued treatment with high doses of methyl-testosterone, these animals showed no other manifest signs of damage except for a slight inhibition of somatic growth, their average final body weight being 196 g while that of the controls was 236 g.

Summary. Experiments on male albino rats indicate that chronic treatment with large doses of methyl-testosterone elicit changes in the adrenal cortex which are characterized by an involution of the glomerulosa, marked thickening of the connective tissue and deposition of coarse fat granules in the cells of the fasciculata and reticularis. In many cases these fat granules fuse into a single voluminous lipid globule which distends the cytoplasm and pushes the nucleus to one side. Consequently the epithelial cells of the cortex assume the appearance of ordinary fat cells. Although in the rat testoid compounds do not cause a specific involution of the reticularis as they do in the mouse, the above mentioned changes are considered to be a typical response of the cortical cells to overdosage with testoid substances.

Contrary to previous observations in man the serum potassium showed no significant change following treatment with methyl-testosterone in the rat.

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8 Butler, A. M., Talbot, N. B., and MacLachlan, E. A., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 378.

9 Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, **46**, 339.

10 Jacobs, H. R. D., and Hoffman, W. S., *J. Biol. Chem.*, 1931, **93**, 685.

14339 P

Use of Bacterial Asphyxia for Homologous Antigen Production in Experimental Immunization Against Human-Type Tuberculosis.*

TRUMAN SQUIRE POTTER.

From the Laboratory of Preventive Medicine, Department of Anatomy, University of Chicago.

In tuberculosis no wholly satisfactory immunity of man and domestic animals has been obtained. This may be due in part to the unsatisfactory constitution of the vaccines employed. It is therefore restated that human, bovine, and avian type bacilli may be killed

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