loss of weight was greater in the males than in the females. There was no correlation between ascorbic acid content and adrenal weight. It will be seen that although the adrenals were larger in the females than in the males, their ascorbic acid content was less (Table I). The drop in ascorbic acid was less precipitous in the female 1-hour group than in the corresponding male series and the return to the normal level was more rapid in females. Another difference between the two sexes was a secondary rise in ascorbic acid in the female. Repetition of the experiment yielded identical results. The adrenals of the male were more hypertrophic than those of the females. While there is no complete explanation of the changes which follow X-ray irradiation, the observation of Sayers and Sayers(5) that progesterone blocks ACTH release, combined with the experiments of Snyder and Wyman(6), who have shown that progesterone has twice the potency of DOCA in maintaining adrenalectomized hamsters, are of interest in that they suggest a possible explanation of the less precipitous fall in adrenal ascorbic acid in the female rat. Finally, the possibility that the gonads may play a role in this mechanism is suggested by the experiment of Wexler(7) which showed less depletion of ascorbic acid in animals with intact ovaries.

*Summary.* Total body X-ray irradiation is associated with a marked depletion in adrenal ascorbic acid. The decrease in ascorbic acid is more marked in the male rat than in the female.

1. Sayers, G. and Sayers, M., *Recent Progress in Hormone Research*, Academic Press, New York, 1948, Vol. II, p81.
2. Selye, H., *Stress*, Acta, Inc., Montreal, 1950.
3. Long, C. N. H., *Fed. Proc.*, 1947, v6, 461.
4. Bessey, Q. A., *J. Biol. Chem.*, 1938, v126, 771.
5. Sayers, G. and Sayers, M. A., *Endocrinology*, 1947, v40, 265.
6. Snyder, J. G. and Wyman, L. C., *Endocrinology*, 1951, v49, 205.
7. Wexler, B. C., unpublished data, 1951.

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## Tolerance of Normal and Adrenalectomized Rats for Cortisone Acetate.* (19315)

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Howard(1) has commented upon "the almost uniform finding that an organism is highly oversensitive to a hormone that it lacks, and the corollary that the normal is relatively insensitive to doses of a hormone which would seriously injure the deficient animal." Thus, the depancreatized rat has a less-than-normal tolerance to insulin(2), and removal or destruction of the thyroid decreases the tolerance for large doses of thyroxin(3). Selye(4) has reviewed the evidence that adrenalectomized animals and patients with Addison's disease are more sensitive than normal to over-dosage with 11-desoxycorticosterone acetate.

In the present experiments large doses of cortisone acetate were administered to adrenalectomized and to sham-operated force-fed rats. The adrenalectomized rats lost more weight, excreted more glucose and showed a greater incidence of gross pathologic changes than did the non-adrenalectomized rats.

*Methods.* Male rats of the Sprague-Dawley strain having an initial weight of approximately 300 g were used in these experiments. The animals were placed in metabolism cages and were force-fed a medium carbohydrate diet(5) by stomach tube each morning (8:30 to 9:15 A.M.) and afternoon (4:15 to 5:00 P.M.). The technics and diet were modifications of those described by Reinecke, Ball, and Samuels(6). The experiments were carried

* We wish to express our appreciation to Dr. Augustus Gibson, Medical Division of Merck and Co., who supplied the cortisone acetate.

TABLE I. Comparison of Normal and Adrenalectomized Rats Given Cortisone Acetate for 21 Days.

| Operation | No. | Daily dose, mg | Number showing gross pathology: Died | General infection | Lungs | Heart | Liver | Kidneys | Stomach ulcers | Avg and range*: Wt loss, g/rat | Total urine glucose/rat |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Adrenx | 12 | 5 | 1 | 0 | 0 | 0 | 0 | 11 | 4 | 37(19-60) | 6.5( .0-20.2) |
| Sham | 12 | 5 | 1 | 1 | 1 | 1 | 1 | 9 | 2 | 26(15-41) | 7.1( .0-20.3) |
| Adrenx | 23 | 10 | 2 | 5 | 9 | 3 | 7 | 18 | 18 | 59(37-98) | 51.6(12.1-97.4) |
| Sham | 23 | 10 | 1 | 3 | 6 | 2 | 3 | 13 | 12 | 47( 7-71) | 30 ( .0-62 ) |

* See text for statistical calculations.

out in an air-conditioned room at a temperature of 74 to 78°F. Twenty-four hour samples of urine were collected at the same hour (8:00 to 8:30 A.M.) and were preserved with toluene. Urine glucose was determined by the method of Benedict(7). The rats were adrenalectomized under aseptic conditions by the method of Ingle and Griffith(8). In the sham-operated animals the adrenals were exposed but were not manipulated. The adrenalectomized rats were treated with 3 cc of beef adrenal cortex extract per rat per day during the control period only. Following a control period of 14 days, all of the rats were given twice daily subcutaneous injections of cortisone acetate (Merck) in aqueous suspension. All of the rats were subjected to necropsy either when they were found to be dying or on the 21st day following the beginning of injections.

*Experiments and results.* In Exp. 1, 12 pairs of adrenalectomized and sham-operated rats were given 5 mg of cortisone acetate per rat daily for 21 days. In Exp. 2 and 3, 12 and 11 pairs respectively of adrenalectomized and sham-operated rats were given 10 mg of cortisone acetate per rat daily for 21 days.

*Body weight* (Table I). In each of the 3 experimental groups the average loss of weight was greater in the adrenalectomized rats than in the sham-operated rats. When the data for Exp. 2 and 3 were pooled together, the average loss of weight for the adrenalectomized rats was 59.5 $\pm$ 2.7 g and the average loss of weight for sham-operated series was 47.0 $\pm$ 3.3 g. The difference of 12.5 $\pm$ 4.26 g is statistically significant.

*Glycosuria.* In Exp. 1 (Table I), 7 adrenalectomized rats and 8 sham-operated rats excreted glucose on one or more days during the injection of 5 mg daily of cortisone acetate. There was no significant difference in the average amount of glucose excreted by the 2 groups. In Exp. 2 (Table I) all 12 of the adrenalectomized rats and 11 of 12 sham-operated rats excreted glucose on one or more days during the injection of 10 mg of cortisone acetate daily. The average amount of glucose excreted by the adrenalectomized rats was greater than that shown by the non-adrenalectomized rats. In Exp. 3 (Table I), all 11 pairs of adrenalectomized and sham-operated rats developed glycosuria during the experiment. The average amount of glucose excreted by the adrenalectomized rats was greater than that of the non-adrenalectomized rats. The total amounts of glucose excreted by each of the rats in Exp. 2 and 3 were compared. The average for 23 adrenalectomized rats was 51.6 $\pm$ 4.2 g and the average for 23 sham-operated rats was 30.0 $\pm$ 4.1 g, a statistically significant difference of 21.6 $\pm$ 5.87 g. The distribution of gross tissue changes among the experimental groups is shown in Table I. The data for Exp. 2 and 3 are pooled together in this summary.

*Survival.* Deaths were related to either a rapid exacerbation of glycosuria or to a generalized infection. *Infection.* Some of the rats treated with cortisone acetate developed diffuse infections which involved several of the intrathoracic and intra-abdominal organs

as well as the pleura, the pericardium and the peritoneum. *Lung lesions.* The pathologic changes in the lungs always represented infection which varied from tiny discrete nodules to massive abscesses which caused consolidation of most of the lungs. *Heart.* Visible damage to the heart consisted of white or gray spots which penetrated the surface of the heart. *Liver.* The signs of liver damage consisted either of localized abscesses or of gray patches on the surface of the liver. In addition, the livers of rats given 10 mg of cortisone acetate daily were frequently very yellow, suggesting an abnormally high content of fat. *Kidneys.* Signs of renal pathology included a mottled appearance of the kidney with gray patches penetrating the surface. Tiny nodules representing hypertrophied tubules together with tiny pits were seen on the surface of most kidneys. In a few rats the surface of the kidney was very rough due to the presence of many pits and nodules. *Stomach.* Ulcers appeared in the pyloric portion of the stomach in many of the rats. The lesions varied from tiny ulcers and shallow erosions to a few deep bleeding ulcers which filled the lower gut with blood and caused black tarry feces.

*Discussion.* If the rate of secretion of steroids by the adrenal cortices were unaltered by the administration of cortisone acetate, the summation of endogenous and exogenous steroids in the non-adrenalectomized animal should produce signs of hypercorticalism more readily than does an equal amount of exogenous hormone in the adrenalectomized animal. Actually, as was first reported by Ingle and Kendall(9), the administration of exogenous cortical hormones causes a compensatory atrophy of the adrenal cortices. The present data strongly suggest that signs of cortisone acetate overdosage are more easily induced in adrenalectomized rats than in similar animals having their adrenal glands intact. What role does the atrophic adrenal gland play in determining this apparent difference in resistance to the exogenous steroid? Does it have the capacity to inactivate some exogenous cortisone or does it continue to secrete substances which either balance or inhibit the effects of cortisone?

Although moderate doses of cortisone may support a subnormal rate of growth in young adrenalectomized rats(10), the characteristic effect of large doses is to inhibit growth. It must be assumed that the normal adrenal cortex secretes one or more compounds other than cortisone or 17-hydroxycorticosterone which supports growth(11). There is some evidence that 11-desoxycorticosterone, which may or may not be secreted by the adrenal cortex in physiologically important amounts, has effects which modify those of cortisone in rats(12,4).

Signs of gross pathology following overdosage with cortisone acetate occurred somewhat more frequently in the adrenalectomized rats than in the non-adrenalectomized rats. Since there is some difficulty in appraising these changes quantitatively and objectively no claim is made that we have proven a true difference in the incidence and extent of pathology between the two groups. None of the lesions which were observed has ever been found to occur in untreated control rats.

The signs of hypercorticalism reported in these experiments are similar to those observed in rats given either an excess of ACTH (13) or of cortisone acetate(5). There are an increasing number of reports of ulcers of the gastrointestinal tract in patients given large doses of either ACTH or cortisone acetate. Ingle, Sheppard, Evans, and Kuizenga(14) first reported the experimental production of gastric ulcers by 17-hydroxycorticosterone and Ingle, Li, and Evans(15) first reported the experimental production of a gastric ulcer by ACTH.

*Summary.* Male rats having an initial weight of approximately 300 g were force-fed a medium carbohydrate diet by stomach tube twice daily. One rat of each pair was adrenalectomized and the other was subjected to a sham-adrenalectomy. A comparison was made of the resistance of the two groups of rats to large doses of cortisone acetate. In Exp. 1, 12 pairs of rats were each treated with 5 mg of the steroid per rat per day for 21 days. The loss of weight in the adrenalectomized animals was greater than in the non-adrenalectomized animals, but there was no group difference in the extent of glycosuria. In Exp. 2 and 3, a total of 23 pairs of rats were given 10 mg of cortisone acetate per rat

per day for 21 days. The loss of weight and the average amount of glucose excreted were significantly greater in the adrenalectomized animals. The incidence of gross pathology, especially kidney changes and stomach ulcers, was greater in the adrenalectomized animals of all 3 experimental groups. It was concluded that adrenalectomized rats are more sensitive to an excess of cortisone acetate than are non-adrenalectomized rats.

1. Howard, J. E., *American Practitioner,* 1948, v2, 674.

2. Ingle, D. J., and Nezamis, J. E., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 315.

3. Kunde, M. M., *Am. J. Physiol.,* 1927, v82, 195.

4. Selye, H., *Stress,* Montreal, Canada, Acta, Inc., 1950.

5. Ingle, D. J., Prestrud, M. C., and Nezamis, J. E., *Am. J. Physiol.,* 1951, v166, 171.

6. Reinecke, R. M., Ball, H. A., and Samuels, L. T., PROC. SOC. EXP. BIOL. AND MED., 1939, v41, 44.

7. Benedict, S. R., *J.A.M.A.,* 1911, v57, 1193.

8. Ingle, D. J., and Griffith, J. Q., Jr., Chapter 16. *The Rat in Laboratory Investigation.* J. B. Lippincott Co., Philadelphia, 1942.

9. Ingle, D. J., and Kendall, E. C., *Science,* 1937, v86, 245.

10. Kuizenga, M. H., and Cartland, G. F., *Endocrinology,* 1939, v24, 526.

11. Ingle, D. J., *Progress in Clinical Endocrinology,* 1950, pp. 1946, Grune and Stratton, New York.

12. Woodbury, D. M., and Sayers, G., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 398.

13. Ingle, D. J., Prestrud, M. C., and Li, C. H., *Am. J. Physiol.,* 1951, v166, 165.

14. Ingle, D. J., Sheppard, R., Evans, J. S., and Kuizenga, M. H., *Endocrinology,* 1945, v37, 341.

15. Ingle, D. J., Li, C. H., and Evans, H. M., *Endocrinology,* 1946, v39, 32.

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## Ovarian Retention Cysts in Hypothyroid Rats Treated with Diethylstilbestrol.* (19316)

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It was reported earlier (Janes)(1) that cystic ovaries were encountered in rats which had been thyroidectomized for 8 months and that the incidence of their occurrence was increased when diethylstilbestrol was given for 20 days at the end of the period. Recently, Bourg and Simon(2) found cystic follicles in normal rats which had been given relatively large doses of estradiol for 10 or 20 days.

The present work is a continuation of the earlier study and includes observations on more thyroidectomized rats as well as rats treated with thiourea.

*Materials and methods.* Two hundred and twenty-nine adult female rats of the Long-Evans strain were used. Ninety-eight of these were thyroidectomized for 8 to 12 months, and 46 were rendered hypothyroid by the administration of 0.5% thiourea in the drinking water for 8 months. The remaining 85 were normal as far as thyroid function was concerned. For the last 20 days of the experiment, some of the rats in each group were given diethylstilbestrol either as daily injections of 100 $\gamma$ or were implanted subcutaneously with 10 mg pellets (95% diethylstilbestrol, 5% cholesterol). At the end of the experiment, the ovaries were fixed for microscopic study. In certain instances the pituitaries were saved and assayed for gonadotrophins in immature female Sprague-Dawley rats.

*Results.* Rats with a chronic hypothyroidism, resulting from the addition of thiourea to their drinking water or from thyroidectomy, had a greater number of ovarian cysts than normal rats. The incidence of cyst formation in normal rats was increased when diethyl-

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