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## Injections of Cortin on Resistance of Suprarenalectomized Rats. Biological Assay of Extracts of Suprarenal Cortex.

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The resistance of suprarenalectomized rats to toxins, poisons, bacterial and protozoan infections is markedly depressed.[1, 2, 3, 4, 5] The height of susceptibility is reached at the end of the first week following bilateral suprarenalectomy. Suprarenalectomized rats succumb to small doses of typhoid vaccine.[4] In previous experiments typhoid vaccine has been used as a gauge of suprarenal insufficiency.

In the experiments here reported the protective effect of an extract of the cortex of the suprarenal gland prepared by us according to the method of F. A. Hartman[6] was determined.

Three months old adult suprarenalectomized rats were used. Injections of a highly concentrated extract* of cortex were administered twice daily intraperitoneally. The M.L.D. for suprarenalectomized rats of a batch of typhoid vaccine was determined. The experiment was divided into 4 groups. One group of 17 rats received cortical extract twice daily from the day of operation to the end of the experiment. Of these, 4 rats were injected on the sixth day with one M.L.D., 5 were injected with 2 M.L.D., 4 with 4

[1] Scott, W. J. M., *J. Exp. Med.*, 1923, **38**, 543.

[2] Lewis, J. T., *Am. J. Physiol.*, 1923, **64**, 506.

[3] Belding, D., and Wyman, L. C., *Am. J. Physiol.*, 1926, **38**, 50.

[4] Marmorston-Gottesman, J., and Gottesman, J., *J. Exp. Med.*, 1928, **47**, 503.

[5] Marmorston-Gottesman, J., Perla, David, and Vorzimer, Jefferson, *J. Exp. Med.*, 1930, **52**, 587.

[6] Hartman, F. A., *Endocrinol.*, 1930, **14**, 229.

* 1 cc. equivalent to 40 gm. of cortex. The rats received 20-40 gm. of cortex a day.

M.L.D., and 4 with 6 M.L.D. of typhoid vaccine. A second group of 10 rats received equivalent amounts of salt solution. These were injected on the sixth day with one M.L.D. A third group of 8 rats was untreated and on the sixth day after operation these were injected with one M.L.D of typhoid vaccine. A fourth group of 4 rats received cortical extract during only the 24-hour period before and after the injection of one M.L.D. of typhoid vaccine on the sixth day.

*Results:* The rats of Group 1 that had been repeatedly injected with cortical extract survived as much as 4 M.L.D of typhoid vaccine. The rats injected with 6 M.L.D. died and one with 4 M.L.D died. The rats of Group 4 that had received cortical extract only during the last 24-hour period survived one M.L.D of typhoid vaccine. Of the 18 rats of Groups 2 and 3 that had received no cortical extract or had been injected with salt solution all but 2† were killed by one M.L.D. of typhoid vaccine. Most of the rats in these 2 groups lost from 10 to 40% of their body weight during the first week after operation. Several showed some degree of clinical insufficiency and one rat died during the week. All the rats that had been repeatedly injected with cortical extract equalled or surpassed their preoperative weight by the sixth day after the operation.

These experiments indicate that the cortical extract of Hartman raises the resistance of suprarenalectomized rats.

Hartman[7] suggested the maintenance of the weight curve in immature rats as a means of assaying the potency of his extract. Though the maintenance of the weight curve is an evidence of the activity of an extract, it cannot be utilized as a quantitative biological assay of potency. We believe that the effect of an extract of cortex on the resistance of suprarenalectomized rats to typhoid vaccine may be used as a biological assay of the potency of such extracts. A standard rat unit may thus be readily established for each batch of cortical extract. Since those rats treated during the last 24 hours prior to the injection of typhoid vaccine show increased resistance to typhoid vaccine, a rat unit of cortical extract may be established as that quantity of extract necessary to raise the resistance of suprarenalectomized rats sufficiently to survive the M.L.D. of typhoid vaccine injected on the sixth day following the operation.

Four rats were injected with the extract of 80 grams of cortex in

† Both these rats gained steadily in weight and had large accessories.

[7] Hartman, F. A., and Thorn, G. W., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 94.

divided doses only during the 24-hour period before and after the injection of one M.L.D. of typhoid vaccine administered on the sixth day after suprarenalectomy. Two rats received 40 grams of cortex in the same period and 2 rats received 30 grams of cortex in this interval. The rats that had received 30 grams were killed by one M.L.D. of typhoid vaccine, those receiving larger amounts survived. In this experiment, therefore, 1 minimal protecting rat unit (1 M.P.D.) is equivalent to 40 grams of cortex.

The effect of larger amounts of cortin administered during the last 24-hour period before and after the injection of typhoid vaccine was determined. Six suprarenalectomized adult rats were injected with 200 grams of cortex during the 24-hour period on the sixth day after suprarenalectomy. At the time the first injection was given the rats all lost 10-25% of their preoperative weight. Three of the rats received 4 M.L.D. of typhoid vaccine and 3 received 2 M.L.D. Two rats in each group survived. Three control saline treated rats were killed with 1 M.L.D. of typhoid vaccine. The effect on the resistance of suprarenalectomized rats of injections of cortin is marked in spite of the loss of weight the animal had sustained prior to the administration of cortin.

The effect of a single large dose of cortin administered immediately after suprarenalectomy on the resistance of adult rats to 1 M.L.D of typhoid vaccine administered on the sixth day after the operation was studied. Three rats received 160 gm. of cortex (4 cc. of extract) intraperitoneally in a single injection soon after suprarenalectomy. Two rats received the same quantity of saline. The weight of the treated rats was maintained during the entire week. The controls lost 12 and 15% of the preoperative weight. One of the three treated rats was killed with 1 M.L.D. as well as the controls. Though a single injection of a large amount of cortin may maintain the weight curve for a week it will not raise the resistance as high as repeated injections of small amounts of cortin.

Preliminary experiments on the effects of the injection of cortin on the resistance of suprarenalectomized rats to histamine have been made. Suprarenalectomized rats treated with cortin will resist at least three minimal lethal doses of histamine (ergotamine acid phosphate).[8] The details of these experiments will be reported in a subsequent publication.

[8] Marmorston-Gottesman, J., and Gottesman, J., *J. Exp. Med.*, 1928, **47**, 503.