# PROTECTION OF ADRENALECTOMIZED ANIMALS AGAINST BACTERIAL INTOXICATION BY AN EXTRACT OF THE ADRENAL CORTEX*

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In 1924, Scott (1) demonstrated the protective power of the adrenal cortex against intoxication with pyogenic bacteria, both staphylococcus and streptococcus. Because of the probable importance of such non-specific resistance in human infections, repeated attempts to obtain a cortical extract which would increase the lowered resistance of adrenalectomized animals, were made at that time but without success. Marine and his coworkers have amply demonstrated this increased susceptibility of adrenal-deficient animals to bacterial intoxication (2–4), Jaffe in their laboratory being successful in replacing this function of the normal adrenal cortex by that of autoplastic transplants. Recent progress with extraction methods has produced cortical extracts of proven efficiency in maintaining life in the presence of a total adrenal deficiency (5, 6).

Recently Scott and Bradford (7) found a slight increase in the resistance to bacterial intoxication conferred by the administration of one such extract, that of Swingle and Pfiffner (6). The chief difficulty encountered was that no satisfactory criterion of adequate substitution was available. Hartman and Thorn (8) have introduced a test for the adequacy of substitution by the cortical hormone after adrenalectomy in rats. The present study was undertaken therefore to determine whether an extract of the adrenal cortex could adequately protect adrenal-deficient animals from bacterial intoxication.[1]

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[1] A preliminary report of this work was presented before the Society for Experimental Biology and Medicine (9).



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## *Experiment I*

The animals used were all young male albino rats taken from uniform stock which was free from infection and which had been reared in the laboratory under the same conditions as during the experiment proper. In the first test the animals were all fairly uniform in size, weighing between 90 and 135 mg. with three exceptions. They were fed on a standard diet and kept in a constant temperature room at 27–28°C. The growth curves were determined for a period of 2 weeks preceding adrenalectomy. The entire group was then operated upon on the same afternoon, both adrenals being removed from each animal by a single operator. Following this the animals were divided into two groups of sixteen members each, having matched weights. All rats were injected with the same small volumes of fluid at the same times after adrenalectomy. Group A received cortin obtained by the ether-alcohol method (5). Group B, serving as controls, received a corresponding volume of isotonic sodium chloride solution. All received the same number of injections subcutaneously. The extract used in the 1st week was prepared by a modified method which, as we have learned, greatly reduced its potency. Two rats in each group died during this period. Beginning with the 2nd week however, extract of known potency was used, under the influence of which the weight curves of the extract-injected animals rapidly improved, and nearly recovered the preoperative slope. 1 cc. of the extract was the product of 50 gm. of fresh adrenal cortex. For the first 11 days after operation, the rats were given 0.5 cc. twice daily. In order to increase the protection at the time that it would be most needed, injections of the same volume were made more frequently during the 3 days before the test with bacterial intoxication, at first three times daily and in the final 24 hours, five times.

2 weeks after adrenalectomy, the fourteen surviving animals of each group were in good condition. The animals of Group A (cortin-injected) had gained on the average 21.6 per cent of their initial weight following operation while in Group B the gain was 17.2 per cent. The average gain was not greatly different in the two series, owing in part to large gains in a few animals in the NaCl series. Six of this series had an average gain of 8 per cent or less while only two of the cortin series had gains so small and none showed as large weight increases as the maximum of the NaCl series (Chart 1).

Each rat was now given 1.5 cc. of standard typhoid vaccine (1 billion killed organisms per cubic centimeter intraperitoneally). This dose was administered in two injections, the first of 1 cc. and the second 0.5 cc. 1½ hours later. Each animal in the two groups was given 0.5 cc. cortin or saline respectively about every 2 hours until it died. There was considerable difference in the behavior of the animals in the two groups which became obvious soon after the injection of typhoid vaccine. 4 hours after the first typhoid injection all of the cortin-treated rats would react to a needle prick by moving away in the cage. Three of the fourteen saline-injected rats did likewise but the rest appeared indifferent to this stimulus. 6 hours after the injection of the test dose of bacterial vaccine all

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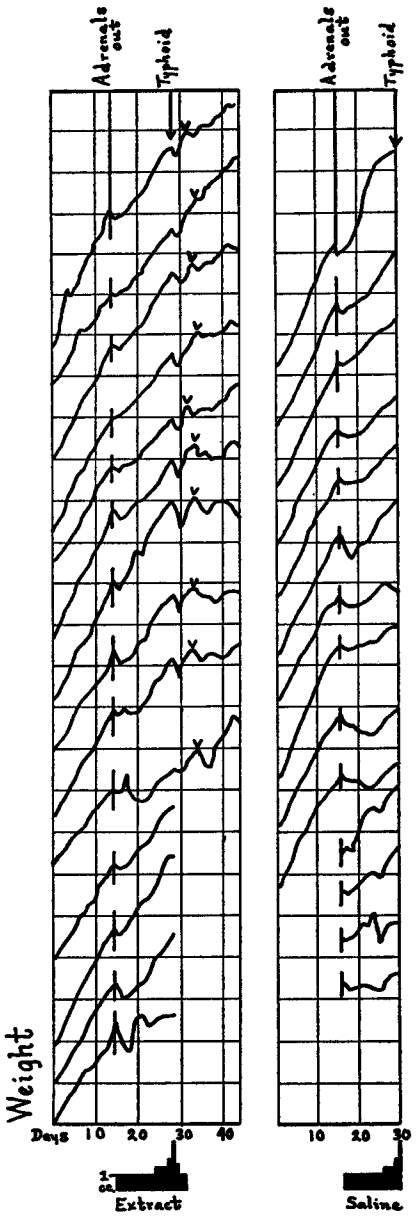


CHART 1. Weight curves of rats before and after adrenalectomy. Those on the left injected with cortin (see text) while those on the right were injected with the same volumes of isotonic NaCl solution. All rats receiving NaCl died following the injection of typhoid vaccine.



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animals in Group B (saline-injected) were dead and all in Group A (extract-injected) were alive. Four of Group A died later, but ten of the fourteen in this group entirely recovered from the effects of intoxication with typhoid bacilli and survived the test. 6 hours after the first injection of typhoid vaccine and shortly after the death of the last control animals two of the extract-injected animals appeared very ill. They were each given an extra 0.5 cc. of cortin. The condition of one improved at the end of 1 hour while the other one died some time during the next 11 hours. A third cortin-injected rat became prostrate and went into convulsions 7 hours after the first cortin injection. 1 cc. of cortical extract was given intravenously but respirations did not return. Two others died during the night (11+ hours after the typhoid administration). One of these apparently had a respiratory infection. He had failed to regain his growth curve under the administration of cortin and his lungs at autopsy showed mottled areas not found in the other three animals of Group A that died. Beginning 10 hours after the injection of the typhoid vaccine, the surviving ten rats of Group A (cortin-injected, all saline-injected animals having died) received no injections overnight, that is to say for a period of 7 hours. It was during this time that three out of the four cortin-injected rats died. It is possible that if the animals had been closely watched during this interval and injected at critical times some would have survived. This is borne out by the outcome in the rat mentioned above that recovered from a critical condition after an extra injection. In spite of this overnight intermission, ten of the fourteen rats in Group A (cortin-injected) survived. These were given cortin twice daily until their growth curves indicated complete recovery, when the injections were discontinued. After discontinuing the extract, thereafter the growth rate markedly diminished. 16 days after the typhoid injection they were killed and a careful search made for evidence of cortical tissue. This was found in only one animal, which showed a mass of tissue about 1.5 mm. in diameter, proven histologically to be made up of cortical cells. No cortical tissue had been found in the animals which had died as the result of typhoid injections.

*Discussion.*—This experiment seemed to us conclusive evidence that the extract of the adrenal cortex which was used afforded a definite degree of protection against bacterial intoxication in the diminished resistance of adrenal insufficiency. This we consider is important to prove conclusively, as we believe that the hormone of the adrenal cortex plays a very important rôle in human pyogenic infections. In the first experiment, killed typhoid organisms were used because a somewhat better standardized intoxicant can be obtained with them than with other bacteria. In order to make certain that the same protection was afforded against intoxication with the ordinary pyogenic organisms, another experiment was tried.

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*Experiment II*

Forty rats were available that had been used in estimating the potency of various lots of cortical extract though they were now no longer receiving it. They were divided into two corresponding groups of twenty animals each, and kept under the standard conditions of the first experiment except that their weight curves were not followed. Every animal in both groups was injected daily with killed *Staphylococcus aureus* in ascending dosage, beginning with 2 billion organisms on each of the first 2 days, increasing to 5 billion on the 5th, 6th and 7th days, jump-

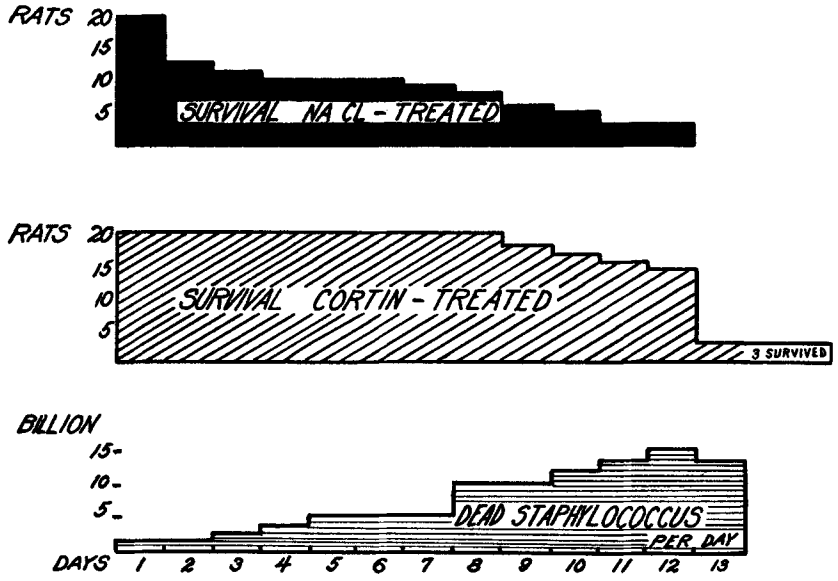


CHART 2. Influence of daily injection of killed *Staphylococcus aureus* on survival of adrenalectomized rats.

ing to 10 billion on the 8th and 9th days and increasing by 2 billion daily to 16 billion organisms on the 12th and 13th days. One group received cortin injections subcutaneously, 0.5 cc. twice a day, while the animals in the other group were given a corresponding volume of isotonic sodium chloride solution at the same intervals. Chart 2 shows graphically the results of this experiment. Six animals in the control group (receiving salt solution) died on the 2nd day and by the 9th day twelve animals, or 60 per cent of this group, had died while none of the animals receiving cortin had as yet succumbed. The first animals of the cortin group died on the 9th day having received by that time 46 billion killed staphylococci in this interval. By the 11th day of the experiment, sixteen of the twenty control animals (80 per cent) had succumbed while only four of the twenty cortin-treated

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rats (20 per cent) had done so. With the progressively increased dosage of dead bacteria the protection afforded to the animals in the cortin group by the limited amount of extract given was overcome in most cases. On the 13th day, the remaining four animals in the control group and eleven of the rats in the cortin group died. Two of the four control animals surviving to the 13th day but dying on that day were found on autopsy to have small accessory adrenals. The other two had no masses large enough to see grossly. That they had microscopic accessories is possible because they both gained in weight, one showing a considerable gain. None of the eleven animals in the cortin group dying on the 13th day showed any accessories. Three cortin-injected animals survived the entire experiment and were sacrificed. One of them showed an accessory gland, the other two did not.

*Discussion.*—The effect of the bacterial intoxication was so different in the two groups that it is hardly necessary to treat the results mathematically. The three animals that showed gross adrenal cortical tissue were excluded from statistical analysis. The average fatal dose was 34 billion staphylococci for the control animals compared to 95 billion for those treated with extract. Furthermore, two cortin-injected rats survived and eleven of them succumbed only after they had received more than 100 billion killed staphylococci, while none of the saline-injected rats survived and all but two succumbed to 58 billion organisms or less. Even the two saline-injected rats in which cortical tissue was found died after receiving 103 billion staphylococci in 13 days. This fact is a functional verification of a previous histological observation made by one of us, *viz.*, that repeated injections of killed staphylococci caused an exhaustion of cortical accessories (1).

It is our impression that we could have afforded still more protection to the animals in Group A by increasing the dosage of cortin. However, we purposely restricted the extract to the amount used because we desired to show how much protection can be obtained from a limited dose. This second experiment shows the protection in adrenal insufficiency that can be obtained for chronic pyogenic intoxication. It is our belief that all severe pyogenic infections with constitutional effects lasting over a period of 2 to 3 weeks may be associated with inadequate production of cortin. Further work on this hypothesis is in progress.

At the same time that we were studying the influence of an adrenal cortical extract on the diminished resistance of adrenal deficient animals this question was being investigated independently by Perla

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and Marmorston-Gottesman in Marine's laboratory, using the same type of extract (10). Their results are in complete agreement with ours.

## CONCLUSIONS

1. The resistance of adrenal-deficient rats to bacterial intoxication has been significantly increased by an extract of the adrenal cortex.

2. This is shown both for acute intoxication with killed *Bacillus typhosus* and for chronic intoxication with killed *Staphylococcus aureus*.

3. During the height of the bacterial intoxication relatively large amounts of the cortical hormone are apparently required to maintain the animals.

4. It is considered probable that a human pyogenic infection imposes a severe load upon the adrenal cortex.

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