# THE EFFECT OF CORTISONE AND GLUTAMINIC ACID ON THE EXCITATION OF THE NERVOUS SYSTEM IN RATS

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The therapeutic action of cortisone is known to be often accompanied by a number of side effects, one of which is an increase in the excitation of the nervous system. In patients receiving cortisone for a long time, mild euphoria and insomnia and — more rarely — psychoses may develop.

These clinical findings of a change in the excitation of the nervous system under the influence of cortisone were confirmed by animal experiments [5] in which it was shown that this hormone lowers the threshold of electric shock in rats and also disturbs the central nervous activity of dogs [1].

In a previous paper [2] we showed that the administration of cortisone to rats is accompanied by accumulation of ammonia in the brain, and for this reason the hypothesis was put forward that the increased excitation of the central nervous system under the influence of the hormone was associated with the increased concentration of $NH_3$ in the brain tissue. It was shown in the same paper that administration of glutaminic acid at the same time as the cortisone lowers the concentration of this toxic product.

The aim of the present investigation was to find out whether a change in the excitation of the nervous system takes place under the influence of cortisone if, at the same time, glutaminic acid is administered, thereby preventing the accumulation of ammonia.

## EXPERIMENTAL METHOD

Experiments were carried out on male white rats weighing 180-200 g. Cortisone was injected intramuscularly in a dose of 2.5-5.0 mg daily, and neutralized L-glutaminic acid subcutaneously in a dose of 100 mg daily.

In determining the degree of excitation by the duration of sleep, the rats were given intraperitoneal injections of sodium amytal solution in a dose of 8 mg/100g body weight. Measurement of the time began from the moment when the animal stopped trying to turn over from the supine to the prone position. Sleep was considered to have ended when the rat turned to the prone position.

In determining the degree of excitation by the magnitude of the threshold of electric shock, we used an apparatus assembles in our Institute along the lines suggested by Woodbury [4]. This apparatus enables the current strength and duration of action to be accurately controlled.

The magnitude of the threshold of electric shock was determined by the method of the author cited above [4]. Stainless steel electrodes were applied to the animal's ears and a current passed for 0.6 seconds. In the measurement of the threshold of electric shock an accuracy of 0.25 ma was attained.

TABLE 1

The Effect of Cortisone and Glutaminic Acid on the Duration of Sleep in Rats (in minutes)

| Animal No. | Control | Cortisone | Cortisone + glutaminic acid |
|---|---|---|---|
| 1 | 95 | 32 | 65 |
| 2 | 60 | 30 | 48 |
| 3 | 85 | 43 | 65 |
| 4 | 46 | 20 | 33 |
| 5 | 85 | 35 | 63 |
| 6 | | 40 | 62 |
| 7 | | 38 | |
| Mean . . . . . | 74 | 34 | 56 |

Usually in the course of the two weeks before the start of the experiment, 3-4 determinations of the threshold of electric shock were made in the rats. This value was taken as the control level, and animals which did not give more or less constant values in the control period were excluded from the experiments. Animals were subjected to the action of the electric current not less than 2-3 days after the preceding experiment.

## EXPERIMENTAL RESULTS

It was shown in preliminary experiments that after administration of cortisone, the sleep of the rats became shorter and more restless (Table 1). The simultaneous administration of glutaminic acid increased the duration of sleep, although not to normal. Whereas the sleep period in the control rats lasted an average of 74 minutes, and cortisone decreased it to 34 minutes, after administration of glutaminic acid the animals' sleep lasted 56 minutes.

Having obtained results which showed that glutaminic acid to some degree prevented the increase in the excitation of the nervous system due to cortisone, we decided to verify and to augment these findings by determining the degree of excitation by a more accurate method , by the magnitude of the threshold of electric shock.

Three series of experiments were carried out. In each series, at the end of the control period the animals were divided into three groups. The rats in the first group received 0.2 ml of 0.9% NaCl solution daily, the second group 2.5-5.0 mg of cortisone daily and the third 100 mg of glutaminic acid as well as the hormone daily. It must be pointed out at once that the changes in the threshold of electric shock in the control rats of all three series were insignificant, amounting to only 2-3% at the end of the experiment, and they will not, therefore, be considered further.

The first series of experiments was conduced on 12 animals, 4 in each group. It can be seen from Fig. 1 that cortisone increased the excitation of the nervous system, as shown by a lowering of the threshold of electric shock, which reached 28% on the 15th day of the experiment. The administration of glutaminic acid together with with cortisone to the other group of rats almost completely neutralized the action of the hormone on the level of excitation during the first week, but in these rats a fall in the threshold of electric shock subsequently took place, so that it amounted to 24% at the end of the experiment.

The second series of experiments was performed on 34 animals. As shown in Fig. 2, administration of cortisone in a dose of 5 mg daily lowered the threshold of electric shock on only the third day, by an average of 30%. On the 9th day of administration, the decrease was 20%, and after 12 days it reached 30% once again. The sharp change in the threshold of electric shock in the first 3 days may be accounted for by a rise in the air temperature to 30° , whereas during the whole course of the experiment the temperature varied between 15 and 20° .

In order to ascertain the effectiveness of glutaminic acid during such intensive changes in excitation, the rats were divided into two subgroups. One subgroup continued to receive cortisone, and the other received glutaminic acid together with the cortisone. As may be seen from the figures in Table 2, in the first subgroup the fall in the threshold of electric shock continued and reached 36%, whereas in the second subgroup no further increase in excitation took place.

The other group of rats received glutaminic acid in addition to cortisone from the beginning of the experiment, and this somewhat prevented an increase in excitation. For instance, the fall in the threshold of electric shock after 3 days was only 15% , after 9 days 11% and on the 12th day it reached 15% .

In order to find out the duration of this amino acid, in some of the rats its administration was discontinued, although cortisone continued to be given. After only 2 days the value of threshold of electric shock in these rats

TABLE 2

The Effect of Cortisone (2.5 mg a Day) and Glutaminic Acid on the Threshold of Electric Shock in Rats (threshold values are expressed in milliamperes)*

| Animal No. | Preparations administered | Before admonistration | | | | During administration | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 25/VI | I/VII | 4/VII | 9/VII | 16/VII | 19/VII | 23/V II | 26/V I | |
| 1 | 0,9% NaCl | 8,25 | 8,75 | 9,0 | 9,0 | 9,25 | 9,25 | 9,0 | 8,75 | |
| 2 | | 7,75 | 8,0 | 7,75 | 8,25 | 8,25 | 8,0 | 8,25 | 8,0 | |
| 3 | | 10,5 | 9,75 | 10,25 | 10,5 | 10,75 | 10,5 | 10,5 | 10,5 | |
| 4 | | 9,0 | 9,25 | 9,0 | 9,0 | 9,0 | 9,25 | 9,0 | 8,75 | |
| 5 | Cortisone | 8,0 | 9,25 | 9,5 | 9,75 | 8,25 | 7,5 | 7,5 | 8,25 | Cortisone + |
| 6 | | 7,25 | 7,0 | 8,5 | 8,75 | 7,25 | 7,0 | 8,0 | 8,0 | glutaminic |
| 7 | | 7,5 | 7,25 | 7,75 | — | 6,5 | 6,0 | 6,25 | 6,0 | acid after |
| 8 | | 8,75 | 9,0 | 9,0 | 9,0 | 7,75 | 7,0 | 6,0 | 6,0 | July 19 |
| 9 | | 8,25 | 9,0 | 9,25 | 9,25 | 8,0 | 7,25 | 7,0 | 7,25 | |
| 10 | | 7,0 | 8,5 | 9,0 | 9,0 | 7,75 | 7,0 | 6,5 | 6,25 | |
| 11 | Cortisone + | 10,0 | 10,5 | 9,5 | 10,5 | 12,0 | 11,25 | 10,0 | 9,75 | Cortisone |
| 12 | glutaminic | 10,0 | 10,25 | 10,5 | 10,5 | 12,0 | 11,0 | 9,25 | 9,0 | alone after |
| 13 | acid | 7,75 | 9,0 | 8,75 | 8,75 | 8,75 | 9,0 | 7,0 | 7,75 | July 19 |
| 14 | | 8,75 | 10,0 | 9,75 | 10,0 | 11,0 | 10,0 | 10,0 | 9,25 | |
| 15 | | 8,5 | 8,25 | 8,75 | 9,0 | 10,0 | 9,5 | 9,0 | 8,0 | |
| 16 | | | | 7,25 | 7,0 | 7,0 | 7,5 | 7,5 | 6,25 | |

* Administration of the preparations began on July 12, 1958 .

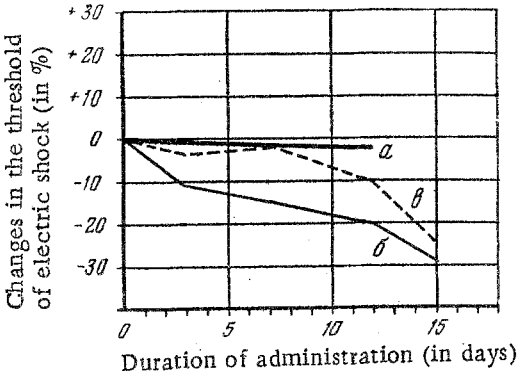


Fig. 1. Changes in the threshold of electric shock under the influence of cortisone ( 5 mg a day) and glutaminic acid . a) Control b) administration of cortisone; c) administration of cortisone + glutaminic acid .

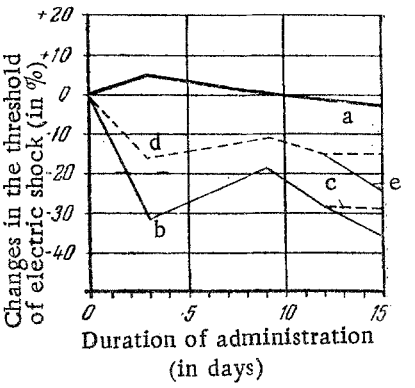


Fig. 2. Changes in the threshold of electric shock under the influence of cortisone (5 mg a day) and glutaminic acid. a) Control; b) administration of cortisone; c) administration of glutaminic acid in addition to cortisone ; d) administration of cortisone + glutaminic acid; e) continued administration of cortisone and discontinuation of glutaminic acid.

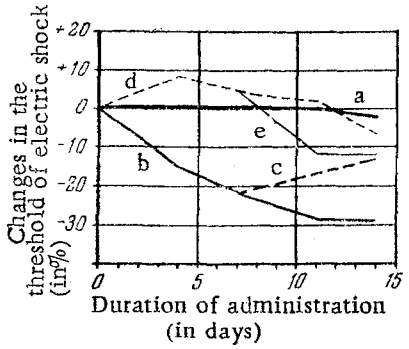


Fig. 3. Changes in the threshold of electric shock under the influence of cortisone (2.5 mg a day) and glutaminic acid. a) Control; b) administration of cortisone; c) administration of glutaminic acid in addition to cortisone; d) administration of cortisone + glutaminic acid; e) continued administration of cortisone and discontinuation of glutaminic acid.

was 10 % below the previous value, whereas in the animals which continued to receive glutaminic acid the excitation was virtually unchanged.

To investigate the relationship between the effectiveness of action of the glutaminic acid and the dose of cortisone, in the third series of experiments the dose of cortisone was reduced to 2.5 mg a day. As may be seen from Table 2 and Fig. 3, on halving the dose of the hormone, the changes in the threshold of electric shock under the influence of cortisone were the same as in the previous experiments. At the same time, the action on the glutaminic acid was more effective with a dose of 2.5 mg a day, for on giving the amino acid to rats hitherto receiving only cortisone an appreciable fall in the level of excitation took place, which was not observed if the rats received a dose of 5 mg of the hormone daily.

When glutaminic acid was given from the beginning of the experiment, the action of cortisone on the excitation of the nervous system was completely neutralized; under these circumstances the value of the threshold of electric shock was even slightly above that that of the controls, and the increase in excitation began only on the 11th day of the experiment.

The results obtained leave no doubt that glutaminic acid prevented the increase in excitation of the nervous system during administration of cortisone. Several suggestions may, however, be made about the mechanism of its action.

It was shown in a previous paper [2] that the administration of cortisone to rats is accompanied by the accumulation of ammonia in the brain, and that glutaminic acid removes this toxic metabolic product. In view of these findings it may be supposed that this factor is responsible for the lowering of the excitation of the brain. However, in spite of the complete normalization of the $NH_3$ content which was obtained in the above-mentioned work, in our investigation the effectiveness of this amino acid fell toward the end of the experiment, and in one experiment the curve showing changes in the threshold of electric shock was slightly below the control curve after the first few days.

Since the repeated action of an electric current may lead to a number of irreversible changes in nerve tissue it may be postulated that it is this effect which lowered the effectiveness of the glutaminic acid. This hypothesis was overthrown, however, by the experiment in which the criterion of excitation was the length of sleep caused by injection of sodium amytal, since in this case the amino acid only partially restored the length of the period of sleep in the rats. There is no doubt that it is not only ammonia metabolism that is disturbed by cortisone. It is, therefore, a more likely hypothesis that glutaminic acid neutralizes the $NH_3$ but does not remove the products of certain other changes in metabolism, which gradually accumulate and lead to the increased excitation. These disturbances may, possibly, be connected with changes in the electrolyte balance of sodium and potassium, which is known to be affected by cortisone [3].

## SUMMARY

The effect of cortisone and glutaminic acid on the excitability of the nervous system was studied in white rats. The weight of the animals varied from 180 to 200 g. The degree of excitability of the animals was determined by the duration of sodium amytal-induced sleep and by the value of the electric shock threshold (EST). The latter was determined with the aid of an instrument suggested by Woodbury. The results obtained demonstrated that cortisone administration (2.5-5.0 mg per day) was accompanied by a considerable rise of the excitation of the nervous system. With the simultaneous action of the glutaminic acid, the excitability changed insignificantly or not at all. Interruption of the glutaminic acid injections without discontinuance of the hormone administration reduced the EST value sharply.

## LITERATURE CITED


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