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THE EFFECT OF SODIUM INTAKE ON THE URINARY HISTAMINE IN ADRENALECTOMIZED RATS

BY

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The daily excretion (μg/day) of free histamine has been measured in bilaterally adrenalectomized or sham-operated female rats. In rats developing adrenal insufficiency when kept on a "sodium free" diet, the output of free histamine in the urine fell progressively. The volume of urine was unchanged. In rats with adrenal insufficiency the oral administration of 0.9% saline caused an immediate rise in the urinary excretion of histamine. The rise was sustained for several days. Adrenalectomized rats consuming 43 mg/day of sodium chloride or less developed adrenal insufficiency and the daily output of urinary histamine fell progressively. A sodium chloride intake of 90 mg/day prevented this fall. Adrenalectomized rats kept on 0.9% saline showed no significant changes during the first 7 days after adrenalectomy. From the 8th day onwards the daily output of histamine in the urine rose progressively. After sham operation the daily output of histamine in the urine was not affected by the intake of sodium chloride. Adrenalectomized rats eat less food; a similar restriction of food intake in sham-operated rats decreased the urinary excretion of histamine. The decrease was, however, not as pronounced as in adrenalectomized rats developing adrenal insufficiency. Other sodium salts, but not glucose, had the same effect as 0.9% saline on the urinary excretion of histamine in adrenalectomized rats.

Various findings suggest that the adrenal glands influence the metabolism of histamine. Thus, in the adrenalectomized rat the content of histamine in the tissue is raised (Rose & Browne, 1941; Marshall, 1943; Bartlet & Lockett, 1959), the histaminase activity is lowered (Rose & Karady, 1939) and the rate of histamine formation is increased (Schayer, Davis & Smiley, 1955; Schayer, 1956). Furthermore the rise in the tissue histamine concentration after adrenalectomy can be reduced or prevented by the administration of sodium chloride in the drinking fluid (Rose & Browne, 1941; Hicks & West, 1958). It has also been shown that the effect of adrenalectomy on the urinary excretion of histamine in the rat (Bjurö & Westling, 1959; Angervall, Bjurö & Westling, 1961) depends upon the intake of sodium chloride. The purpose of the work described here was to study, in greater detail, the relationship between the intake of sodium chloride and the urinary excretion of histamine in adrenalectomized rats.

METHODS

White, female, nonpregnant Sprague-Dawley rats (obtained from AB Anticimax, Stockholm) weighing from 140 to 180 g were used. They were fed a semi-synthetic food (Gustafsson,

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Kahlson & Rosengren, 1957) in which the concentration of histamine base was estimated to be less than 0.01 μg/g. Sodium chloride was not included in the food, which thus contained about 0.2 mg sodium chloride/g. The rats were fed daily with 10 g of this food. The food was mixed with 10 ml. of distilled water or of saline. Thus two different types of diet were obtained: (1) 10 g of food mixed with 10 ml. of distilled water, with distilled water *ad libitum* as drinking fluid ; this diet is called " sodium free " in the text and indicated as " water " in the Figs. (2) 10 g of food mixed with 10 ml. of a sodium chloride solution, with distilled water or a sodium chloride solution *ad libitum* as drinking fluid.

The rats were weighed daily and the intake of food and drinking fluid was measured. The intake of sodium chloride could thus be calculated. The daily intake of sodium chloride varied from about 2 to several hundred mg/day. Before the experiments, the rats were maintained on semi-synthetic food containing the usual quantity of sodium chloride (3 mg/g). The rats were given the experimental diet, " sodium free " or with a known percentage of sodium chloride, starting 3 to 6 days before operation.

Throughout the experiment the rats received a daily injection of aminoguanidine sulphate (a histaminase inhibitor) in a dose of approximately 20 mg/kg of body weight.

The rats were kept in metabolism cages from 6 to 7 days before operation. The faeces were separated from the urine by a wire mesh in the collecting funnel. The urine was collected for 24 hr periods in vessels containing about 0.5 ml. of 10 N-hydrochloric acid, to bring the *p*H of the collected specimen to below 2. The urine was diluted with Tyrode solution and its free histamine content was determined by bioassay on the guinea-pig ileum (Wilson, 1954 ; Angervall *et al.*, 1961). The values given are the amounts of free histamine base excreted in 24 hr.

Sham-operation or bilateral adrenalectomy was performed in fasting rats anaesthetized with ether (Bjurö, 1963).

At the end of the experiments all adrenalectomized rats were given the " sodium free " diet. The rats developed signs of adrenal insufficiency and died, after an average of 3 to 4 days. It was concluded that the rats had been completely deprived of adrenal cortical tissue of functional importance.

RESULTS

Effects of a " sodium free " diet in adrenalectomized and sham-operated rats

Twenty-three rats on the " sodium free " diet all became less active, and by the 6th day they failed to finish their food. The average intake of solid food on the 6th postoperative day was 5 g, that is half the usual amount. This, together with the average weight loss of 6 g in 6 days, was taken to indicate adrenal insufficiency. Sham-operated rats under similar conditions increased their body weight by an average of 8 g on the 6th day after operation.

Most of the rats were kept for a maximum of 6 days on the " sodium free " food with distilled water as drinking fluid. From the 7th day after adrenalectomy sodium chloride solutions of various concentrations were given to most of the rats. A few rats were kept on the " sodium free " diet until they died with adrenal insufficiency on the 8th day after adrenalectomy. Fig. 1 shows that after the operation the urinary excretion of histamine at first rose and then fell progressively ; by the 3rd day the reduction in output was statistically significant ($P<0.01$). In six sham-operated rats kept on the " sodium free " diet the urinary histamine rose significantly ($P<0.01$) on the 1st day after operation but thereafter remained unchanged.

To see whether the decrease in urinary histamine output was related to the decrease in food intake during adrenal insufficiency, the following paired feeding experiments

were performed. The intake of food in three sham-operated rats was restricted to the same amount as that consumed by three adrenalectomized rats kept on the "sodium free" diet. The urinary histamine output in the sham-operated rats decreased by an average of 11 μg/day on the 6th day after operation. The corresponding value in the three adrenalectomized rats was 55 μg/day. Hence, the

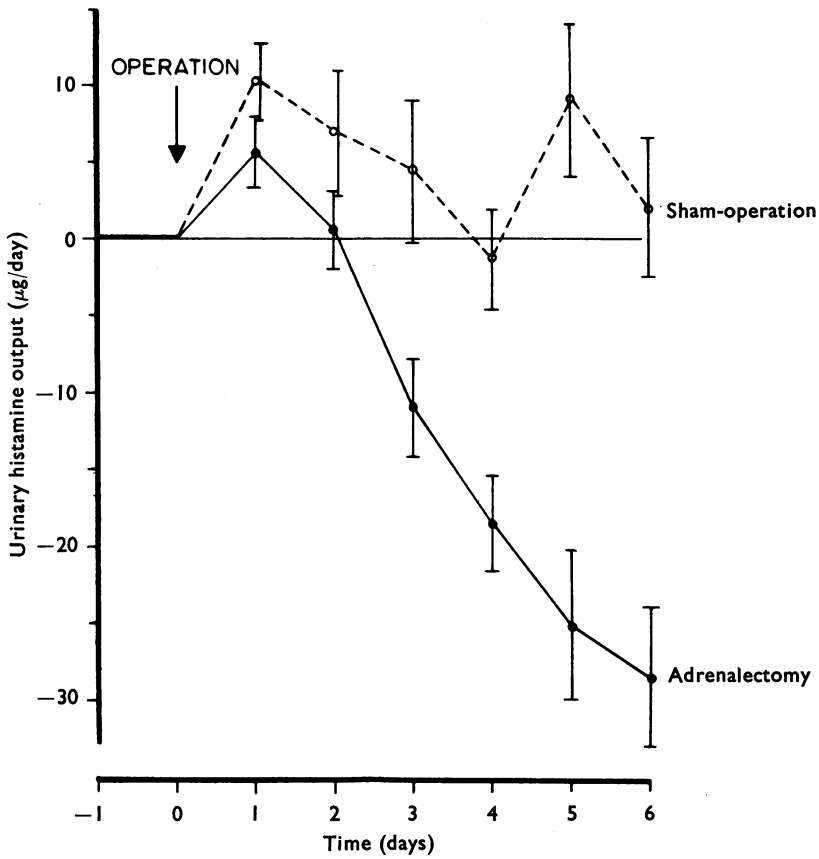

Fig. 1. The effect of a "sodium free" diet on the urinary histamine output in sham-operated and adrenalectomized rats. The amounts of free histamine (base), compared with the values on the day before operation (62·7±3·4 and 66·8±7·6 μg/day respectively), are expressed as means with standard errors. Operation time is indicated by the arrow. Note the progressive decrease in urinary histamine output in rats developing adrenal insufficiency when kept on the "sodium free" diet (water).

decrease in urinary histamine output can be partly explained by the diminished intake of food in rats with adrenal insufficiency.

The output of urine increased after adrenalectomy and then decreased to be only slightly lower on the 6th day after operation than on the day before operation (Fig. 2). The decrease in urinary histamine output was thus not caused by a diminished volume of urine.

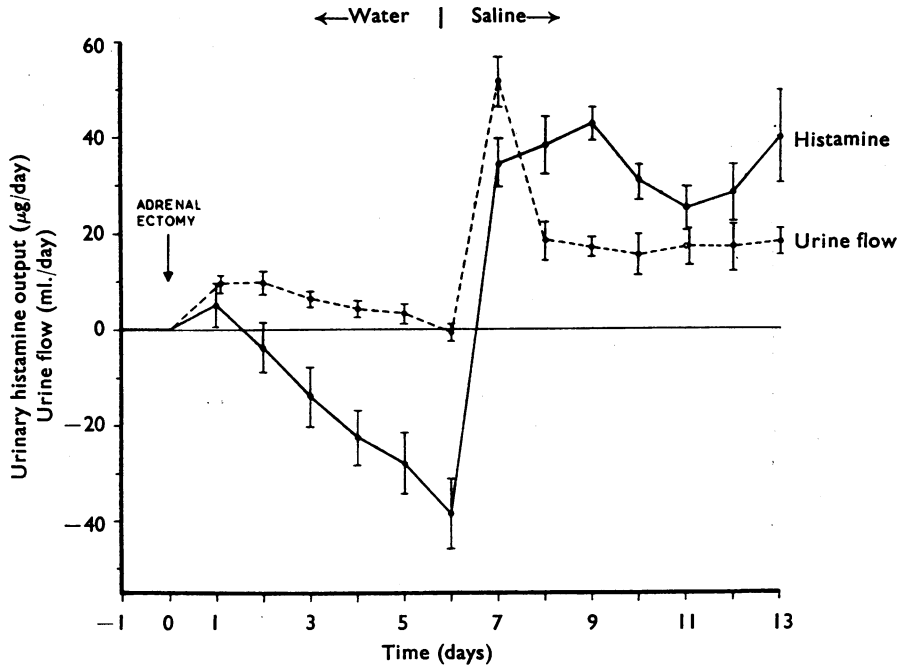

Fig. 2. The relation between the urinary histamine output and the urinary volume in adrenalectomized rats. The values are expressed as in Fig. 1. Initial values 72.1±7.3 μg/day and 8.8±1.2 ml./day respectively. 0.9% saline was given during the period marked "saline." Note that the progressive decrease in urinary histamine output in rats developing adrenal insufficiency cannot have been caused by a decrease in urinary volume.

Effects of different dietary concentrations of sodium chloride in rats kept on a "sodium free" diet for 6 days after operation

On the 7th day after adrenalectomy 0.9% saline was given to seven rats previously kept on a "sodium free" diet (see previous section). Within 24 hr the body weight of the rats increased, the signs of adrenal insufficiency disappeared and all food was consumed. The urinary excretion of histamine rose by an average of 74 μg/day from the 6th to the 7th day (Fig. 2) and remained so in all the rats. In two rats followed for five further days the urinary excretion of histamine was maintained at this higher rate. The output of urine also increased and remained about 15 to 18 ml./day higher than the value on the day before operation. Administration of 0.9% saline to two sham-operated rats on the 7th day after operation caused no noteworthy increase in the urinary excretion of histamine. The urine output in these rats increased from an average of 12.3 to 23.2 ml./day.

To see whether this sustained increase in the urinary excretion of histamine could be obtained with dietary concentrations of sodium chloride less than 0.9%, the following experiment was performed. Seven rats were adrenalectomized and kept on the "sodium free" diet for 6 days. On the 7th day after adrenalectomy two rats received 0.2% saline, two rats 0.3% saline, one rat 0.6% saline and two rats

0.9% saline *ad libitum* as drinking fluid. The signs of adrenal insufficiency disappeared and the rats excreted more histamine than before operation (Fig. 3). The intake of sodium chloride in the different groups increased from less than 2 mg/day to values between 115 and 370 mg/day. The increase in the urinary histamine output was about the same in the different groups and appears to be independent of variations in the sodium chloride intake, when the intake is between 115 and 370 mg/day. No attempt was made to study the effect of lower intakes of sodium chloride since rats given 0.1% saline developed adrenal insufficiency (see below).

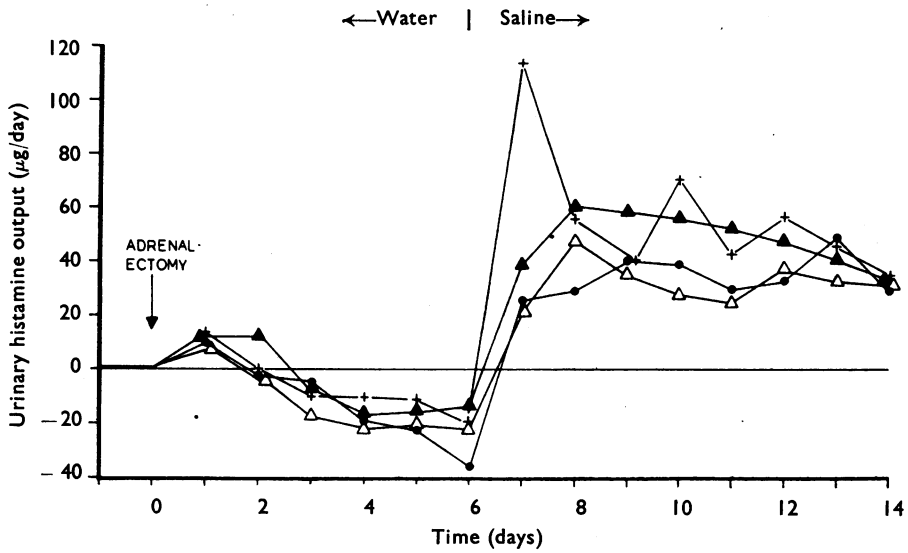

Fig. 3. The effect of different concentrations of dietary sodium chloride solution on the urinary histamine output in rats with adrenal insufficiency. The values, as compared with the day before operation (53 μg/day), are expressed as means. ▲, 0.2%; △, 0.3%; +, 0.6%; and ●, 0.9% saline. Note that all concentrations of sodium chloride caused an increase in urinary histamine output when given to rats with adrenal insufficiency.

Effects of 0.9% saline administered at various times after adrenalectomy

Fig. 4 illustrates the results. Ten rats were divided into five pairs. The rats were first kept on "sodium free" food and distilled water. The administration of 0.9% saline 2 days before adrenalectomy gave a small and transient increase in the urinary excretion of histamine. The effect was somewhat more pronounced if 0.9% saline was given on the day of operation. A change to 0.9% saline on the 3rd day after adrenalectomy gave, in one rat, a transient increase and, in another rat, a sustained increase in the urinary excretion of histamine. A change to 0.9% saline on the 5th or 7th day after adrenalectomy caused a large increase in the urinary histamine output throughout the period of observation. It seems, therefore, that adrenalectomized rats need to be maintained on a "sodium free" diet for 2 to 4 days before a high sodium intake (0.9% saline) can act to promote the excretion of histamine and to maintain it above the limit of the control values.

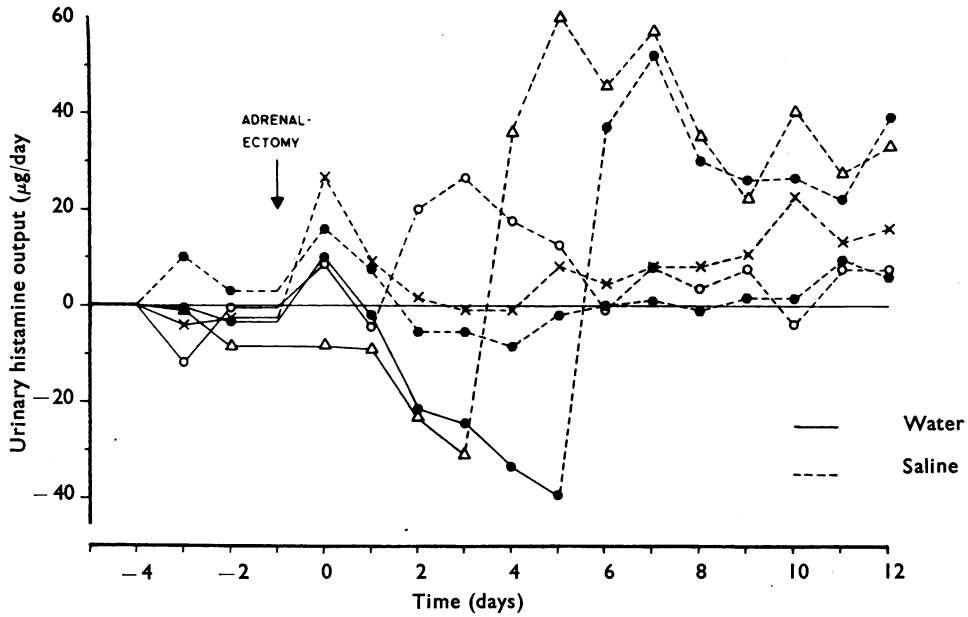

Fig. 4. The effect of 0.9% saline given at various times in relation to adrenalectomy. The values (but not the symbols) are expressed as in Fig. 3. Initial value 72.6 μg/day. The times of changing from water to 0.9% saline are shown by the changes from continuous to interrupted lines. Note that 0.9% saline caused a sustained increase in urinary histamine output when given on the 4th or 6th day after adrenalectomy.

In two other rats, the high sodium diet (0.9% saline) was started before adrenalectomy: the output of histamine was unchanged until the 5th day, when the sodium chloride intake was reduced for 2 days by giving the "sodium free" diet. During this period the output of histamine fell but, on changing back to the high sodium diet (0.9% saline), it rose again to reach a high value and remained so during the period of observation.

Effects of sodium chloride intake after adrenalectomy

To find the amount of sodium chloride which could prevent the decrease in the urinary excretion of histamine, the following experiment was done. A group of ten rats was divided into five pairs and each pair was given one of the following diets: "sodium free," 0.1%, 0.3% and 0.9% saline and 90 mg/day of sodium chloride (10 ml. of 0.9% saline mixed in the food and distilled water as drinking fluid *ad libitum*). On the 7th day after adrenalectomy 0.9% saline was given. The results are illustrated in Fig. 5. Rats which were given a "sodium free" diet or 0.9% saline behaved as described above. The two rats given 0.1% saline developed signs of adrenal insufficiency and their urinary excretion of histamine fell to 11 μg/day (mean value) on the 6th day after adrenalectomy; the mean values for the intake of sodium chloride during these 6 days after adrenalectomy were 27 and 43 mg/day of sodium chloride for each rat. The administration of 0.9% saline

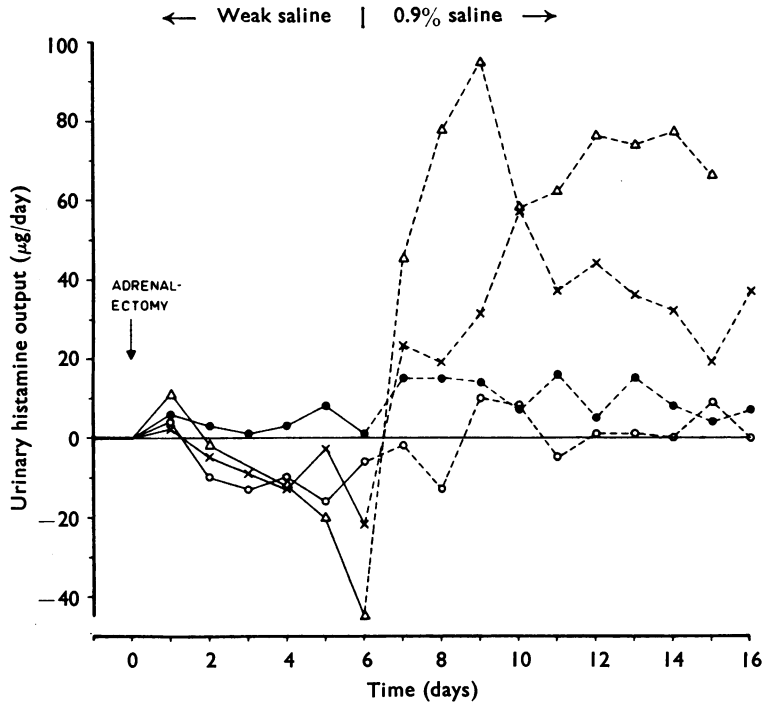

Fig. 5. The effect of 0.9% saline given to rats kept on lower levels of sodium chloride after adrenalectomy. The values (but not the symbols) are expressed as in Fig. 3. Symbols before day 6: ×, 0.1% saline; ○, 0.3% saline; ●, 90 mg sodium chloride; and △, sodium "free." Note that 90 mg/day and 0.3% saline could prevent a decrease in urinary histamine output and that 0.9% saline caused no increase in urinary histamine when given on the 6th day after adrenalectomy.

on the 7th day after operation abolished the signs of adrenal insufficiency and caused a large increase in the urinary excretion of histamine throughout the period of observation. These two rats thus behaved in a manner similar to that of rats given a "sodium free" diet for 6 days and then 0.9% saline (see above). The two rats given 90 mg/day of sodium chloride for 6 days after adrenalectomy did not develop signs of adrenal insufficiency. They showed no significant changes in the urinary excretion of histamine, and the administration of 0.9% saline on the 7th day after adrenalectomy caused only a small increase in the urinary histamine output.

The two rats given 0.3% saline developed no signs of adrenal insufficiency. They showed a small but not progressive decrease in the urinary excretion of histamine for the first 6 days after adrenalectomy. The average intake of sodium chloride during these 6 days in each rat was 180 and 200 mg/day respectively. When the rats were given 0.9% saline on the 7th day after adrenalectomy there were no significant changes in the urinary excretion of histamine.

A daily intake of 90 mg/day or more of sodium chloride prevented both the progressive decrease in the urinary histamine output during the first 6 days after adrenalectomy seen in rats on a "sodium free" diet and the large increase in

histamine excretion seen on changing over to 0.9% saline as drinking fluid. Sodium chloride intakes of 27 and 43 mg/day were not effective in presenting the changes mentioned.

Effects of continuous administration of 0.9% *saline*

The urinary excretion of histamine was estimated in nineteen adrenalectomized rats given 0.9% saline in the diet from the 7th day before operation. In seven of the rats observations were made until the 12th day after adrenalectomy. The results are illustrated in Fig. 6. It can be seen that the urinary histamine output, apart from the brief increase after operation, was unchanged until the 8th day after operation, when a progressive increase ensued. The increase was significant ($P<0.01$) from the 9th day onwards. Sham-operation in six rats did not cause a similar increase in the urinary histamine output (Fig. 6).

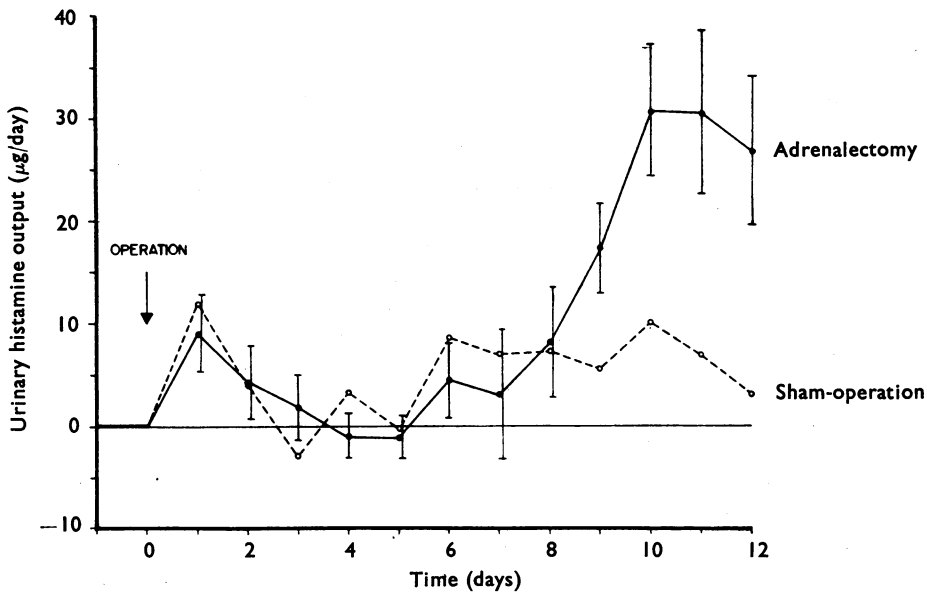

Fig. 6. The urinary histamine output in adrenalectomized rats and sham-operated rats continuously kept on 0.9% saline. The values are expressed as in Fig. 1. Note the progressive increase in urinary histamine output in the adrenalectomized rats from the 8th day onwards.

The administration of 0.9% saline thus prevents the decrease in urinary histamine output which occurs during the first 6 days after removal of the adrenal glands in rats kept on a " sodium free " diet.

Effects of intake of other sodium salts or of isotonic glucose in adrenalectomized rats

The administration of isotonic sodium bicarbonate or isotonic sodium dihydrogen phosphate solutions as drinking fluid had approximately the same effect on the urinary histamine excretion as had sodium chloride solution, and also prevented

the development of the signs of adrenal insufficiency. The adrenalectomized rat given isotonic glucose solution to drink for 3 days developed signs of adrenal insufficiency and a large decrease in the urinary excretion of histamine. Isotonic glucose solution thus has effects similar to those of distilled water in the adrenalectomized rat.

It is concluded that the sodium intake is the decisive factor in these experiments.

DISCUSSION

To explain these results we must assume that adrenalectomy in the rats leads to two principal changes in the urinary excretion of free histamine depending on whether the rat is on a low or a high sodium diet.

First, in rats maintained on a low intake of sodium chloride the daily excretion of urinary free histamine fell progressively and coincided with the development of adrenal insufficiency. How this fall occurs is not known, but it may be that circulatory changes are at least in part responsible ; however, the reduction in urinary volume was only slight. A more specific relationship between the adrenal glands and the ability to excrete histamine might lie behind the results obtained, but so far direct evidence of such a relationship is lacking. However, it is noteworthy that the tissues contain more extractable histamine in adrenal insufficiency (Rose & Browne, 1941), a finding which agrees with the fall in the urinary output of histamine.

Secondly, in rats which were given an adequate supply of sodium the output of histamine rose on about the 9th day after the operation. An increase in urinary histamine output in adrenalectomized rats given 0.9% saline can also be seen early after operation, but only in rats which have been kept on a low sodium chloride intake for 2 to 4 days. In such rats the low sodium chloride intake apparently triggers a mechanism which leads to a prolonged elevation of the urinary histamine ouput when the sodium chloride intake is raised. Rats on a high sodium chloride intake and with a high urinary histamine output had none of the signs of adrenal insufficiency and were in good condition. Previous work (Rose & Browne, 1941 ; Hicks & West, 1958) has shown that adrenalectomized rats on 0.9% saline have normal, or only moderately elevated, tissue histamine, in contrast to the elevated tissues histamine of similar rats maintained on water. The present observations on the urinary histamine output indicate that it is possible to modify histamine metabolism in rats with adrenal insufficiency. The changes observed in the urinary histamine output after adrenalectomy cannot be caused by changes in histaminase activity (Rose & Karady, 1939 ; Telford & West, 1961) because the rats received aminoguanidine in doses that would inhibit this enzyme effectively (Westling, 1958). A further analysis of the inactivation of histamine in adrenalectomized rats has been made by Bjurö (1963), who obtained evidence that the reduction in urinary histamine output, at least in severe adrenal insufficiency, was in part caused by a more efficient inactivation consequent to a prolonged exposure of histamine to the histamine-metabolizing enzymes. Such an increased inactivation could have been caused by the histamine methylating enzyme but not by histaminase, since this enzyme was blocked by aminoguanidine.

There is evidence to suggest that the increase in urinary histamine output in adrenalectomized rats which are given sodium chloride is due to an increased rate of formation of histamine in the body, notably in the stomach (Bjurö, 1963).

The results described here are slightly at variance with previously published results (Bjurö & Westling, 1959; Angervall *et al.*, 1961). In the previous series adrenalectomized rats on water had an essentially unchanged urinary histamine output while rats on 0.9% saline showed a progressive increase. These rats received *ad libitum* a diet which would have given them 40 to 60 mg/day of sodium chloride. The present results suggest that this amount of sodium chloride would have prevented the changes in urinary histamine output. It seems likely that this, together with a less perfected operative technique, explains the difference between previous and present results.

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