THE EFFECT OF ADRENALECTOMY ON THE URINARY EXCRETION OF HISTAMINE IN THE RAT

By
L. Angervall, T. Bjurö and H. Westling

ABSTRACT

The urinary excretion of histamine was followed in female rats after adrenalectomy or sham operation.

The urinary histamine in adrenalectomized rats kept on saline showed a progressive increase from the 4th postoperative day. In rats kept on water the urinary histamine was unchanged until the 8th day when an increase took place.

At 7 to 16 days after adrenalectomy, the high urinary histamine level in rats kept on saline could be lowered by changing over to water as drinking fluid. The substitution of saline instead of water had the opposite effect on the urinary histamine excretion.

Cortisone did not prevent the increase in urinary excretion of histamine in adrenalectomized rats kept on saline.

Cortisone and cortisol caused an immediate increase in urinary histamine levels in adrenalectomized rats on saline with a subsequent slow reduction.

Sham operation induced a small temporary increase in the urinary histamine.

Several observations indicate that hormones of the adrenal cortex influence the actions and the metabolism of histamine. Thus Dale (1920) found that a cat deprived of its adrenal glands is much more sensitive to histamine than before the operation. Similar results were obtained in the rat (e.g. Wyman 1928; Marmorston-Gottesman & Gottesman 1928). These observations are in accordance with later findings in rats (Rose & Karady 1939), cats (Haeger & Kahlson 1952) and guinea-pigs (Kahlson et al. 1953) of a diminished histaminase.

* Address: Department of Clinical Physiology, Sahlgren's Hospital, Göteborg, Sweden.
activity in the tissues. This decrease is probably caused by the outflow of the enzyme into the lymph (Carlsten 1950).

The decrease in histaminase activity after adrenalectomy led Rose & Browne (1941) to study the effect of adrenalectomy on the tissue histamine content in rats. They found an increase in certain tissues. This has been confirmed by Marshall (1943), Geiringer & Hardwick (1953), Hicks & West (1958) and Bartlet & Lockett (1959), although the results have varied as to the magnitude of the increase and the tissues affected.

As entirely new aspect of the relation between histamine and the adrenal cortex has been provided by Schayer and his co-workers (Schayer et al. 1955; Schayer 1956). Using $^{14}$C-labelled histidine they found that the rate of formation of $^{14}$C-histamine in vitro by some rat tissues, notably skin and lung, was diminished after treatment with cortisone and increased after adrenalectomy. The stomach reacted in a reverse manner, showing an increase in histamine formation after cortisone and a decrease after adrenalectomy. These observations suggest that the rate of histamine synthesis is controlled by the adrenal cortex. This affords another explanation for the changes in tissue histamine content after removal of the adrenal glands.

The present paper describes experiments in which the urinary excretion of histamine was followed in rats before and after adrenalectomy. It was felt that analysis of the urinary histamine might give complementary information about histamine metabolism as a whole in the adrenalectomized animal. Some of the findings have been published in a preliminary report (Bjurö & Westling 1959).

EXPERIMENTAL

White female, non-pregnant rats were used. Most of the observations were made on 5 months' old rats. The animals were weighed daily and kept in cages, which permitted the urine to be collected with little food or fecal contamination.

The rats were given a dry cake diet ad libitum which contained less than 0.8 µg/g histamine (base). This means that the rats received less than about 10 µg histamine daily in the food. The diet was supplied by AB Konsum, Stockholm and contained about 0.4 % (w/w) sodium chloride and about 0.7 % (w/w) potassium chloride. The main series of experiments were carried out in May–December.

The rats had free access to drinking fluid, which was either distilled water or 0.9 % (w/w) sodium chloride in distilled water (called saline in the text). The daily fluid intake was measured.

As a rule the rats were injected once daily with aminoguanidine sulphate, a histaminase inhibitor, subcutaneously in a dose of 20 mg/kg body weight.

For adrenalectomy or sham operation the rats were anaesthetized with ether. A dorsal midline skin incision was made and the adrenal regions exposed through muscle incisions below the lowest ribs on each side. The adrenal glands were removed with care so as not the injure the gland capsule. Sham operations were performed in an identical way except that the adrenal glands were not removed.
All adrenalectomized animals not given hormones were kept on water as drinking fluid for some days and then developed signs of adrenal insufficiency, e.g. rapid weight loss. At autopsy careful search was made for adrenal remnants with microscopical examination of selected parts from the suprarenal region. No signs of adrenocortical tissue were found. It is understood that accessory adrenocortical tissue may still have been present in some of the rats in the present series, but from a functional point of view such tissue should have been of little importance.

The following steroids were used for substitution therapy: 21-hydroxy-pregn-4-ene-3,20-dione acetate in oil solution (cortone), 17,21-dihydroxy-pregn-4-ene-3,11,20-trione acetate in crystal suspension (cortisone) and 11β,17,21-trihydroxy-pregn-4-ene-3,20-dione acetate in crystal suspension (cortisol).* The hormones were administered intramuscularly once daily in a dose of 5 mg per rat.

The urine was collected in 24 hours' samples in vessels containing 0.5 ml 10 N hydrochloric acid which gave the collected specimen a pH below 2. After volume measurement and filtration the urine samples were stored at —20°C until assayed for histamine. The urine was then neutralized to pH 7.4 (bromothymol blue) with sodium hydroxide, suitably diluted with Tyrode's solution and assayed against a standard solution of histamine (0.1 µg base per ml) on the guinea-pig's isolated ileum in a 4.5 ml bath (Barsoum & Gaddum 1935). The gut was suspended in Tyrode's solution containing atropine and glucose and bubbled through with air.

One liter of the Tyrode's solution contained 8.0 g NaCl, 0.2 g KCl, 0.2 g CaCl₂, 0.1 g MgCl₂, 1.0 g NaHCO₃, 0.05 g NaH₂PO₄, 0.1 mg atropine sulphate and 0.15 g glucose. The addition of the glucose increased the sensitivity of the gut and also possibly made the gut respond more regularly. Duplicate assays were often performed and the evaluation of small changes in histamine excretion was made more reliable by serial assays of samples from several consecutive days. Values for histamine excretion are given as µg base excreted by the rat in 24 hours, and refer only to free histamine.

The identity of the gut-contracting substance in rat urine was checked with mepyramine as described by Reuse (1948).

RESULTS

Preliminary experiments. – Preliminary experiments were made on 7 rats, which were not injected with aminoguanidine and which were given water as drinking fluid. Five of the rats were adrenalectomized and 2 were sham operated. The latter animals showed no significant changes in the urinary histamine. In 3 of the 5 adrenalectomized rats the urinary excretion of histamine showed a moderate increase, which occurred after 8–10 days. In the other 2 rats there was no change in the urinary histamine even immediately before death in adrenal insufficiency. It was concluded that adrenalectomy caused an increase in the urinary output of histamine in some rats kept on water and not given aminoguanidine, but that the increase was sometimes small or absent in spite of severe adrenal insufficiency.

Main series of experiments. – In the following experiments all rats were in-

* The hormones were kindly supplied by Ciba-produkter AB, Stockholm, Sweden.
jected with aminoguanidine once daily and the urinary excretion of histamine was followed from 6 to 8 days before operation. The rats were initially given water to drink. The animals that were to be kept on saline after the operation were given this as drinking fluid starting 3 days before adrenalectomy or sham operation, in order to see whether the substitution of saline for water by itself caused a change in the urinary output of histamine. Each animal was kept under the same conditions during the first 6 days after as during the 3 last days before the operation. After the 6th postoperative day treatment was changed in several rats.

**Groups 1 A and 1 S. Adrenalectomy or sham operation with water as drinking fluid**

All the 8 adrenalectomized rats kept on water (group 1 A) had a loss in body weight averaging 21 g during 6 days (Table 1). The weight loss and signs of lassitude and muscular weakness indicated adrenal insufficiency. The changes in urinary histamine are shown in Fig. 1. It will be seen from the figure that there was no change in the histamine excretion during the main observation period, i.e. the first 6 days after removal of the adrenal glands. In 4 of the

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**Fig. 1.**

Changes in urinary histamine in adrenalectomized rats kept on saline (NaCl) or distilled water (H₂O) as drinking fluid. The curves give the mean individual changes (± S. E. M.) in urinary excretion of histamine (base) in μg/24 h as compared with the day before operation.

470
**Table 1.**

Body weights and initial urinary excretion of histamine in adrenalectomized or sham operated rats. The drinking fluid, either water or saline, was kept unchanged from 3 days before until 6 days after operation. The "initial" histamine excretion is given as the mean values for the day before operation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>Operation</th>
<th>Drinking fluid</th>
<th>Substitution therapy</th>
<th>Mean body weight (grams)</th>
<th>Mean urinary histamine before operation (μg/24 h)</th>
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<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
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<td>at operation</td>
</tr>
<tr>
<td>1 A</td>
<td>8</td>
<td>Adrenalectomy</td>
<td>Water</td>
<td>–</td>
<td>178 ± 4</td>
<td>157 ± 6</td>
</tr>
<tr>
<td>2 A</td>
<td>9</td>
<td></td>
<td>Saline</td>
<td>–</td>
<td>185 ± 8</td>
<td>181 ± 6</td>
</tr>
<tr>
<td>1 S</td>
<td>3</td>
<td>Sham operation</td>
<td>Water</td>
<td>–</td>
<td>197 ± 11</td>
<td>201 ± 10</td>
</tr>
<tr>
<td>2 S</td>
<td>5</td>
<td></td>
<td>Saline</td>
<td>–</td>
<td>194 ± 15</td>
<td>194 ± 14</td>
</tr>
<tr>
<td>3 C</td>
<td>6</td>
<td>Adrenalectomy</td>
<td></td>
<td>Cortisone</td>
<td>174 ± 7</td>
<td>149 ± 7</td>
</tr>
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<td>3 D</td>
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<td></td>
<td></td>
<td>Cortexol</td>
<td>175 ± 4</td>
<td>186 ± 6</td>
</tr>
</tbody>
</table>

* 4 rats cortisone, 2 rats cortisol.
rats the observations were extended until 12 days after the operation and during this period the histamine excretion increased in 2 of the rats.

Sham operation (group 1 S) induced a small and transient increase in the urinary histamine (Fig. 2).

*Groups 2 A and 2 S. Adrenalectomy or sham operation with saline as drinking fluid*

The 9 adrenalectomized animals kept on saline showed practically no change in body weight after the operation (Table 1) and were in a much better general condition than the adrenalectomized animals given water to drink. All rats, except one, showed a distinct and progressive increase in urinary histamine (Fig. 1). The increase was statistically significant from the 4th postoperative day onwards. Six adrenalectomized animals, kept on saline, were also followed after the 6th postoperative day without any change in treatment. In 5 of these rats the excretion of histamine continued to increase.

Sham operation induced a small and transient increase in the urinary histamine (Fig. 2).

The observations on groups 1 A and 2 A (compare Fig. 1) show that there is a marked difference in the urinary excretion of histamine between adrenalectomized rats kept on water and on saline. Thus, the histamine excretion of rats kept on water does not increase during the first 7 days after

![Graph](image-url)

*Fig. 2.* Changes in urinary histamine in sham operated rats kept on saline (NaCl) or distilled water (H₂O) as drinking fluid. For explanations see Fig. 1.
operation in spite of the fact that these rats show signs of severe adrenal insufficiency. Rats kept on saline, on the other hand, start to excrete increasing amounts of histamine from the 3rd or 4th day after adrenalectomy. However, at 8 to 12 days after removal of the adrenal glands there was no difference between rats kept on saline or water starting before the operation.

Effect of alternations between water and saline as drinking fluid

The difference between water and saline as drinking fluid was examined in a more direct way in 9 rats from groups 1 A and 2 A. In these rats, alternations of drinking fluid were made between the 7th and the 16th day after adrenalectomy.
Fig. 4.
Changes in urinary histamine after adrenalectomy (AE).
Upper diagram: Body weight (B.W.) in g.
Middle diagram: Urinary excretion of histamine (base) (Hi) in μg/24 h.
Lower diagram: Urinary volume (U.V.) in ml/24 h.
Drinking fluid water (H₂O) or 0.9% sodium chloride (NaCl) as indicated in the rectangles.

Adrenalectomy in a manner indicated in Fig. 4. From Fig. 4 one can see that the rat was given water during the first 7 days after adrenalectomy and lost weight. The urinary histamine did not increase until saline was supplied on the 8th day. The saline also caused an increase in body weight. Water was given again with prompt reduction in urinary histamine, which increased once more when saline was supplied after 2 days on water. Note that the ingestion of saline was accompanied by an increase in urinary volume. A change from water to saline gave an immediate large increase in the urinary histamine level in 9 out of 11 such changes. Changes from saline to water caused a reduction of the elevated urinary histamine in 7 out of 8 changes. In 4 of these 7 reductions, the urinary histamine reverted to the preoperative level. In the other 3, the urinary histamine was reduced to an intermediate level.

Alternations between saline and water as drinking fluid were also made in 4 sham operated rats from groups 1 S and 2 S (Fig. 5). In these rats small changes in urinary histamine were seen, similar in direction to those in adrenalectomized rats. Note that saline in the sham operated rat shown in Fig. 5 caused an increase in urinary volume while the urinary histamine was only slightly elevated.

In 24 rats saline was substituted instead of water 3 days before the operative procedure (groups 2 A, 2 S, 3 C and 3 D). In nearly all of these rats the change from water to saline led to a small increase in the urinary histamine. The
mean increase in all animals was $16.1 \pm 3.7 \mu g$ and was statistically significant ($P \leq 0.001$).

**Groups 3 D and 3 C. Substitution therapy in adrenalectomized animals with saline as drinking fluid**

The animals given cortisone maintained their body weight (Table 1, group 3 D) and were in a good general condition. Nevertheless, the urinary histamine increased progressively from the 4th to the 6th postoperative day up to very high levels (Fig. 3). Again, there was no relation between the outward signs of adrenal insufficiency and the output of histamine into the urine.

Four adrenalectomized rats were given substitution with cortisone and 2 were given cortisol. The results with these two steroids were very similar and the 6 rats are shown as group 3 C in Table 1 and Fig. 3. All rats were in a good general condition. However, they suffered a substantial loss in body weight. This weight loss was not caused by adrenal insufficiency but was a result of the relatively high doses of cortisone and cortisol. The urinary histamine was significantly elevated during the 2nd to the 4th day after adrenalectomy. Then a gradual reduction occurred, but in 2 rats the values were high even on the 10th to 12th day after removal of the adrenal glands.

**DISCUSSION**

The present experiments indicate that adrenalectomized rats kept on saline as drinking fluid exhibit an earlier and more marked rise in the histamine excretion than adrenalectomized animals given water to drink, in spite of the fact that rats kept on water have a more marked degree of adrenocortical insufficiency. The difference between rats on water and rats on saline tended to
disappear in animals in which there was no change in treatment for more than 8 days after adrenalectomy. However, the experiments with alternations between water and saline show that such a difference must exist even beyond the 7th day. We cannot at present offer any explanation for the discrepancy between these two sets of observations. It is possible that there is a time factor involved in the mechanism by which the drinking fluid affects the urinary excretion of histamine in the adrenalectomized rat.

These observations on the urinary histamine can conveniently be correlated with observations on the tissue histamine content in adrenalectomized rats. Rose & Browne (1941) found that adrenalectomized rats maintained on saline did not show such an elevation of the tissue histamine content as adrenalectomized rats kept on water and Hicks & West (1958) found that adrenalectomized rats on saline had the same tissue histamine content as sham operated animals, while animals on water exhibited increased tissue histamine levels. From these findings it is tempting to conclude that the administration of saline, which keeps the adrenalectomized animal in a better condition, also normalizes its histamine metabolism.

Our observations that the administration of saline leads to a sustained increase in the urinary histamine would seem to provide an entirely different explanation. We believe that the rat in adrenal insufficiency has an over-all increase in histamine formation (cf. Schayer 1956). If such a rat is kept on water the newly-formed histamine is probably largely accumulated in the tissues. If the rat is given saline a greater part of the »excess« histamine comes out in the urine. This hypothetical transfer of histamine from the tissues into the urine after giving saline may at least partly be due to a better circulatory function.

The intake of sodium chloride is apparently of great importance in experiments concerning the influence of the adrenal glands on the metabolism of histamine and 5-hydroxytryptamine (cf. Hicks & West 1958). The differences between adrenalectomized animals on water and on saline described in the present paper might have become more marked if the sodium chloride content of the food had been lower.

A small, but significant increase in the urinary excretion of histamine was also seen when normal or sham operated rats were given saline to drink. This increase may be of a similar nature as that seen after adrenalectomy, but it is also possible that the increase in urinary volume after saline caused a spurious increase in the histamine output by diminishing the small inevitable losses of urine in collection. In experiments with 14C-histamine it has been possible to estimate these losses in normal or adrenalectomized animals kept on water or saline (Bjurö, to be published). It appears that these losses can at most account for changes in the urinary histamine of the order of 10–15%.

It is unlikely that the observed increase in urinary histamine after adrenal-
ectomy is caused by a diminished activity of histamine (diamine oxidase), since it also occurs during treatment with aminoguanidine in doses which will inhibit this enzyme completely (Schayer et al. 1954; Kahlson et al. 1958; Westling 1958). As mentioned above, a reduction of histaminase activity has been observed after adrenalectomy in other species than the rat and may be of greater importance than can be inferred from the present experiments.

In the adrenalectomized female rat at least, it appears unnecessary to invoke other explanations for the increase in tissue and urinary histamine than the increased rate of formation of the amine, discovered by Schayer. It should be pointed out that Schayer (1956) found an increased histamine formation in some tissues (e.g. skin and lung) but a decreased one in the stomach. As judged from the observations on the urinary excretion, the net result seems to be an increase. Marshall (1943) also found the histamine content of the whole body to be increased by 31% in adrenalectomized rats kept on water as drinking fluid.

Cortisone, but not cortexone, can depress histamine formation in rat skin (Schayer et al. 1955) and can normalize elevated tissue histamine levels after adrenalectomy (Hicks & West 1958). The present finding that cortexone could not maintain the urinary histamine normal after adrenalectomy agrees with these observations. However, adrenalectomized animals given cortisone or cortisol also had an increased urinary histamine. The increase manifested itself soon after the operation and then tended to disappear slowly. This effect of cortisone and cortisol is difficult to explain on the basis of the observations presented and is being further studied.

The transient increase in urinary histamine after sham operation may be related to the increase in histamine content of abdominal skin observed after sham operations on the back (Geiringer & Hardwick 1953). Recently, evidence has been obtained that there is a rapid histamine formation in the skin surrounding a healing wound in rats (Kahlson et al., pers. comm.). It is possible that some of this newly-formed histamine escapes into the urine after a sham operation.

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REFERENCES