J. Physiol. (1943) 102, 180–190 612.458:547.781.5

THE INFLUENCE OF ADRENAL CORTICAL DEFICIENCY ON THE HISTAMINE CONTENT OF RAT TISSUES

BY P. B. MARSHALL (*Rammell Scholar*), *From the College of the Pharmaceutical Society, University of London*

(*Received* 9 *January* 1943)

During recent years evidence has accumulated of an antagonistic relationship between the cortical secretion of the adrenal gland and the histamine present in the tissues of the body. The beneficial results obtained in the clinical treatment with adrenal cortical extracts of shock conditions resulting from injury, burns and surgical operation appear to support this evidence, since these conditions are usually associated with a release of histamine [Barsoum & Gaddum, 1936]. Marmorston & Gottesman [1928] showed that the sensitivity of rats to histamine was increased after adrenalectomy. This observation confirmed the reports of other workers [Scott, 1927; Crivellari, 1927; Voegtlin & Dyer, 1925]. The discovery of the enzyme histaminase by Best & McHenry [1930] struck what at first seemed to be a fatal blow at the theory of cortin-histamine antagonism, and for a time the problem was not investigated further.

Subsequently it became evident that the comparatively slow histaminase reaction could not adequately account for the rapid disappearance of histamine in the body, particularly the large amounts released in certain pathological conditions. Interest was again aroused, therefore, in the possibility of an anti-histamine function of the adrenal cortex. Rose & Browne [1938] showed that the decreased resistance to histamine following adrenalectomy was associated with a loss of ability by the body to inactivate histamine. This report was followed by the discovery by Rose & Karady [1939] that adrenalectomized animals showed a decrease in the histaminase content of their lung tissue. Further, the same workers showed that both the ability to inactivate histamine and the normal histaminase content of the lungs were restored by the administration to the deficient animals of 'adreno-cortical substances' [Rose & Karady, 1939; Rose, Karady & Browne, 1940]. These results suggested that the anti-histamine activity of the adrenal cortex operates through the medium of histaminase.

If depriving animals of their adrenal cortex and its hormonal secretions reduces or abolishes their ability to inactivate histamine, then it might be

possible to show that this substance accumulates in the tissues of adrenalectomized animals. Experiments designed to test this possibility were recently carried out by Rose & Browne [1941] and by Wilson [1941]. The former workers reported that, while there was little change in the histamine content of blood, kidney or spleen, there was a moderate increase in liver and lung and a considerable increase in small intestine and stomach (see Table 3). Wilson reported an increase in blood histamine in adrenal-deficient rabbits.

The question of the relationship between adrenal cortical deficiency and tissue histamine appeared to require further investigation, and the work described here was undertaken in the hope that a more comprehensive study of the histamine content of the tissues of adrenalectomized rats in comparison with strict control experiments might throw more light upon the mechanism of cortin-histamine antagonism.

Methods

Animals. Rats were used and were obtained chiefly from a small colony bred from the Wistar-strain albino colony of the Nutrition Laboratories of the College of the Pharmaceutical Society. In a few experiments rats kindly supplied by Dr S. K. Kon from the black and white strain colony of the National Institute for Research in Dairying were used. Young rats were mostly employed, wt. 30–70 g. (3–5 weeks old).

The rats were divided into litter-mate pairs; that is, each pair from the same litter, of the same sex and as nearly as possible of equal weight. Of each pair one animal was adrenalectomized and the other 'sham' operated, undergoing the same operative treatment except that the glands were not removed.

Operative technique. The rats were anaesthetized with nembutal and ether. The nembutal was diluted 10 times with physiological saline and injected intraperitoneally, 0·15–0·2 c.c. of the dilution to young rats, 0·5 c.c. to adult rats. Anaesthesia was completed by the administration of ether, immediately before operating, by placing the animal beneath a large funnel the stem of which was connected to an anaesthetic bottle containing ether. Air was blown through the ether by a syringe bulb. Using this method, excellent control of the degree of anaesthesia could be maintained.

Access to the adrenal glands was gained by a single, mid-dorsal longitudinal incision through the skin and two lateral oblique incisions through the muscle wall immediately above each kidney. The adrenal gland, together with surrounding connective tissue, was stripped away with forceps in a caudal direction. This technique was preferable to cutting away the gland, which increases the possibility of leaving cortical tissue behind. The muscle wall and skin were ligatured with silk. As soon as they could swallow, the rats were given 25% glucose solution by mouth from a pipette, a treatment which has been found by Bruce & Wien [1940] to reduce the incidence of post-operative mortality.

Maintenance of operated rats. The operated and control rats were distributed singly into small cages each containing sawdust, a handful of wood shavings and a small pot of drinking water. All experimental rats were given the same diet from the time of weaning, consisting of a compressed cube preparation obtained from J. Thorley, Ltd., the composition and nutritional values of which have been described by Thomson [1936]. The cages were placed in a room maintained at about 70° F. For the first night after operation the animals were given physiological saline containing 0·5 % glucose to drink. This was replaced by tap water on the following morning. The animals were weighed daily.

Under these conditions young rats showed an increase in weight of 5–10 g. on the first morning after operation, due to the imbibition of large quantities of saline which is excreted more slowly than water. On the second morning (after 24 hr. on tap water) the adrenalectomized rats had usually returned to their original weight, while the controls were about the same weight as on the previous morning. From this time, the controls showed a steady normal increase in weight, while the adrenalectomized rats steadily lost weight. The latter developed symptoms of adrenal cortical deficiency usually between the 2nd and 4th day after operation. Any rats which survived this period generally increased steadily in weight and did not become deficient, probably due either to incomplete removal of the glands or to growth of accessory cortical tissue.

Symptoms of deficiency were preceded by a fall in body weight below the pre-operative level. This was followed by an increasing lassitude and a fall of temperature. Diarrhoea was not often observed in young rats. The final stage of the condition was complete prostration with occasional convulsions and irregular breathing, terminating in death. In young rats, under the conditions described, the complete course of the deficiency, from the first signs of lassitude to death, lasted only a few hours.

Adult rats showed greater fluctuations in weight among the controls, but in adrenalectomized adults there was a fairly regular decrease in weight once deficiency was established. The survival time of adult rats was longer than that of young rats, symptoms of deficiency never appearing in less than 3 days after operation.

Rats were taken for tissue extractions as they developed signs of deficiency. The limits of the conditions under which they were used ranged from loss of weight with slight lassitude to death with no rigor mortis. Animals which showed no signs of deficiency and those which had obviously been dead for some hours were rejected. Control rats were killed at the same time as the adrenalectomized litter-mates, and the two extractions carried out concurrently.

Extraction of histamine. The histamine was extracted from the tissues by the method of Best & McHenry [1930]. Hydrochloric acid extractions were carried

out in 150 c.c. Pyrex flasks heated on a sand-bath. The flasks had standard ground-glass necks. During the preliminary heating they were attached to Pyrex air condensers and afterwards transferred to a vacuum drying apparatus for removal of the acid. This apparatus accommodated four flasks, immersed in a water-bath, each flask being attached to a splash trap. Delivery tubes from the traps were brought together by T-joints and the vapour from the flasks led through a condenser-absorption chain consisting of two water-cooled condensers with ice-cooled receivers, in series, a calcium chloride absorption tower and a tube containing sodium hydroxide. The apparatus was exhausted by a power-driven vacuum pump. The absorption chain described was found to be satisfactory in preventing moisture, acid and alcohol from reaching the pump.

The rats were killed by a blow on the head except where brain was required for extraction; these were killed by an intraperitoneal injection of 5% sodium cyanide. The tissue required was rapidly removed, freed from superficial moisture and blood by gentle pressure between filter paper, and dropped into a weighed flask containing approximately the requisite volume of 10% HCl (150 c.c./20 g. tissue). Flask and tissue were then reweighed. More dense tissues such as liver, heart and kidney were finely chopped with a sharp knife before dropping into the flask. Other tissues were found to disintegrate quite readily in the whole condition, and it was considered desirable to avoid undue damage to the tissues. Blood was obtained by cardiac puncture and run immediately into HCl, without preliminary treatment with trichloroacetic acid. Sections of the alimentary tract were washed free from contents with physiological saline, except in determinations on the whole tract, when the contents were included. Whole animals were cut into small pieces immediately after killing, and extracted in 500 c.c. flasks. The extract was filtered and an aliquot part taken for evaporation.

The flasks were boiled gently for an hour. At the end of this period the tissues were completely disintegrated. The extract was dried in vacuo at an external bath temperature of 70–80° C. Some of the residual acid in the dry extract was removed by the addition of two separate portions of absolute alcohol, with subsequent distillation of the alcohol in vacuo at 50–60° C. The dry residue was extracted with three separate portions of cold distilled water and the extracts filtered through paper into clean, dry test-tubes. The tubes were plugged with cotton-wool and stored in a refrigerator at 1–2° C. until all the extracts from a group of rats had been prepared. The reaction of the stored extracts was sufficiently low (pH 1·5 or less) to prevent any bacterial growth during the period of storage, which never exceeded 5 days.

Estimation of histamine. The histamine in the extracts was measured by the isolated guinea-pig ileum method of Barsoum & Gaddum [1935]. Several modifications in the isolated-organ apparatus were devised or adopted during

the course of the work. Tyrode's solution containing 1 mg./l. atropine sulphate was fed from a reservoir to a 2 c.c. bath via a 10 c.c. graduated pipette arranged so that the pipette could be filled from the reservoir and a measured volume of solution run into the bath from the pipette. A simple drip-feed device, as demonstrated by Huggett & Mant [1941], was introduced so that, during periods when the preparation was not in use, Tyrode's solution was fed drop by drop into the bath, the excess liquid escaping over the sides into the surrounding water. This prevented the spasm of the muscle which usually developed if the preparation were allowed to stand for long periods in unchanged solution.

Guinea-pigs, wt. 300–400 g. and of either sex, were used. The piece of ileum was taken from the lower end, close to the ileo-caecal valve, as this was found to be more sensitive to histamine than muscle from higher regions of the gut.

A solution of histamine acid phosphate equivalent to 10 μg. of histamine base per c.c. was used as a standard. Small doses of this solution (0·001–0·01 c.c.) were added to the bath by means of a Wellcome 'Agla' all-glass syringe fitted with an 'Agla' micrometer attachment. Use of the syringe by hand was clumsy and, if much tilted to introduce the needle point into the bath, the piston slipped down the barrel and delivered more solution than was intended. It was therefore found more convenient to clamp the syringe in a horizontal position and connect the needle to it by a short length of fine rubber tubing, the needle being clamped with the open end held just below the surface of the solution in the bath. The point of the needle was ground flat to reduce the area of possible diffusion into the bath.

Immediately before estimation, each extract was adjusted to the reaction of Tyrode's solution with NaOH solution (5–20% according to the acidity of the extract) and the final volume of the adjusted extract was recorded. The change of *p*H of the extract during the titration was followed by transferring minimal amounts of the liquid with a glass rod on to white filter paper and treating the stain with thymol-blue solution. This method was satisfactory even with dark-coloured extracts, since the dark stain remained at the point where the rod touched the paper, while a colourless ring of moisture spread outwards from the centre. The appearance of the first trace of green was taken as the end-point of the titration (about *p*H 8·0). The adjusted extract, or a suitable dilution of it, was added to the bath in doses of 0·02–0·2 c.c. by means of a 1 c.c. syringe graduated to 0·01 c.c.

In the earlier experiments a 5 min. interval was allowed between doses of standard or extract, but subsequently it was suggested [G. B. West, private communication] that the muscle would respond quite as regularly if the interval were reduced to 2 min. In view of the large number of extracts to be tested, the saving of time by this change was very important. The fluid in the bath was changed immediately after each dose of standard or extract and again before the administration of the next dose.

Under these conditions, most of the preparations would respond to a dose of histamine as low as 0·01μg. of base, though the usual working dose was 0·05–0·1μg. The use of atropinized Tyrode's solution abolished the spontaneous activity of the muscle, so that a flat 'base-line' was recorded, which facilitated the comparison of contractions.

Control experiments. These were carried out on most of the tissues examined in order to determine whether, for each tissue, the addition of a known amount of histamine acid phosphate could be detected by the methods used. Tissues from normal rats were divided symmetrically and the halves extracted in separate flasks. To one flask was added, before extraction, the known amount of histamine in solution, the amount being roughly equal to the average proportional increase found in that tissue after adrenalectomy. The apparent histamine content per g. tissue was determined for both extracts, and the difference compared with the amount actually added to the second flask.

Water content determinations. Since adrenal cortical deficiency is accompanied by changes in the water content of some tissues, it was considered advisable to determine the relationship between the fresh and dry weights of samples of all the tissues used for histamine extraction. If the moisture content of a tissue altered significantly after adrenalectomy, the values for histamine content calculated in terms of fresh tissue alone would give an inaccurate picture of the true histamine change.

A portion of each tissue extracted was weighed on a tared watch-glass and dried to constant weight in an oven at 100° C. The histamine content of the tissues was calculated in terms of both fresh and dry material.

Results and calculations

In the experiments described, 465 rats were adrenalectomized and an equal number of control animals were 'sham' operated. Of the 465 rats operated, 259 were actually used for tissue extractions; 111 were rejected because they had been dead too long when found, or for other reasons; 74 failed to develop adrenal cortical deficiency; and 21 died during or as a result of the operation.

Since there was considerable variation between individual determinations, the mean values for histamine changes between operated and control animals were determined by calculating the mean difference between the logarithms of the histamine contents of the tissues. This method of calculation was shown by Galton [1879] to give a more accurate mean value for a series of observations where the error is large, than would be obtained by the arithmetical method. The histamine content of all tissues (including blood) was expressed in μg. histamine base per g. fresh tissue.

The results are summarized in Table 1. All the tissues examined showed an increase of histamine with the exception of stomach and the whole gut. The value of the logarithmic method of calculation is particularly well illustrated

with liver. One of the nineteen determinations showed an increase in histamine of over 1000 % in the adrenalectomized animal. This abnormal value raises the arithmetic mean for the histamine change to about 900 %. Calculated by the logarithmic method, however, the value of the mean change is about 100 %, which is much nearer the value expected from the other eighteen determinations.

TABLE 1. Histamine changes after adrenalectomy

Tissue	No. of determinations	Mean histamine content in control rats μg./g. fresh tissue	Mean histamine content in operated rats μg./g. fresh tissue	Mean % difference log. calc.
Whole body	16	14·4	18·9	+31·1
Blood	28	0·99	1·47	+51·0
Whole gut	14	11·2	10·0	− 8·61
Stomach	21	35·2	36·2	− 1·17
Small intestine	15	6·30	10·4	+64·5
Caecum	9	15·0	22·8	+52·4
Large intestine	10	4·30	6·29	+42·1
Liver	19	7·72	77·4	+104·6
Kidney	18	1·86	2·34	+24·7
Spleen	19	2·65	4·64	+70·2
Striated muscle	15	8·7	10·3	+17·8
Cardiac muscle	19	5·5	7·0	+22·8
Skin	17	53·2	66·8	+26·9
Lung	38	5·4	6·5	+23·2
Brain	—	Histamine content too small for estimation		

Changes in water content of the tissues after adrenalectomy were found to be small, and only in blood, small intestine, liver, kidney and lung was the change significant. Of these tissues lung lost water after adrenalectomy, making the histamine increase *less* when calculated in terms of dry tissue, while the other tissues gained water, which *increased* the value of the histamine change when calculated in terms of dry tissue.

TABLE 2. Control experiments

Tissue	Mean tissue histamine μg./g.	Mean tissue +added histamine μg./g.	Mean difference (added histamine found)	Histamine actually added	Mean % histamine found	Mean % histamine added
Blood*	1·34	2·46	1·12	0·57	100·0	54·4
Blood†	0·41	0·75	0·35	0·52	88·1	161·7
Stomach	43·7	78·5	34·9	21·0	87·1	53·1
Small intestine	8·78	14·4	5·6	5·1	66·5	61·2
Caecum	13·0	21·6	8·6	7·8	65·4	60·3
Large intestine	11·7	13·5	1·8	2·0	15·9	18·7
Liver	2·43	4·55	2·21	1·95	94·4	86·5
Kidney	1·04	1·62	0·58	0·60	47·9	65·2
Spleen	3·09	3·68	0·58	1·45	32·0	62·1
Striated muscle	8·73	10·20	1·45	2·06	14·9	24·1
Cardiac muscle	4·28	4·49	0·21	1·49	12·7	41·1
Skin	40·5	57·9	17·3	15·1	40·4	40·2
Lung	4·38	7·50	3·11	1·53	75·6	37·5

* Method of Best & McHenry [1930].
† Code's [1937] modification of the method of Barsoum & Gaddum [1935].

The results of the control experiments are shown in Table 2. Control experiments on blood were carried out using both Code's [1937] modification of Barsoum & Gaddum's [1935] method for the extraction of histamine from blood, and the method of Best & McHenry [1930] for tissues. The mean percentage values given in the last two columns of the table were calculated by the logarithmic method in order to make them comparable with the values recorded in Table 1. Statistical analysis of these results showed that added histamine could be recovered with reasonable accuracy from all the tissues examined with the exception of blood, spleen, cardiac muscle and lung.

Statistical analysis of results

All the results obtained were analysed statistically to ascertain how far each mean value quoted could be relied upon. In each experiment the standard deviation (σ) of the mean value of n determinations was calculated. The standard error (E) was then calculated from the formula $E=\sigma/n$, and the value of t, the significance of the mean value (M), was obtained from the formula $t=M/E$.

In the experiments on tissue-histamine change after adrenalectomy, and those on change of water content in the tissues after adrenalectomy, t was calculated for the log mean differences between tissues from control and operated rats. In the control experiments the value of t was calculated for the log mean percentage of added histamine found, and also for the log mean difference between added histamine found and histamine actually added.

All values of t were compared with values of t for $(n-1)$ determinations in a table of probabilities published by Fisher [1925]. Where the calculated value of t is equivalent to a value for P (probability) of more than 0·05, the mean is considered not to be significant; where t is equivalent to a value for P of less than 0·05, the mean value of the series of results was considered significant.

For purposes of comparison Table 3 shows the results obtained for histamine changes after adrenalectomy by Wilson, Rose and Browne, and in the present work. To make the comparison more accurate, mean values from the figures reported by the other workers have been calculated by the logarithmic method described above, and the values so obtained subjected to statistical analysis. Reference to the table of results in Rose & Browne's paper shows that, in some experiments, there are unequal numbers of observations reported on control and operated rats. Since the logarithmic method of calculation admits only of comparisons between pairs of results, the 'odd' values had to be omitted. The calculation is also based on the assumption that pairs of values on the same line are comparable as controlled pairs, which is not stated in the text. The control values reported by Wilson were obtained from the same animals before adrenalectomy, and control and experimental values may therefore be safely regarded as strictly controlled pairs.

TABLE 3. Comparative table of results for histamine changes after adrenalectomy
S = Significant change; I = Insignificant change

Tissue	Rose & Browne [1941] (rat): Maintained on saline 11 days			Rose & Browne [1941] (rat): Saline 7 days, water 4 days			Present work (rat)		
	No. of determinations	% histamine change	Significance	No. of determinations	% histamine change	Significance	No. of determinations	% histamine change	Significance
Whole body	—	—	—	—	—	—	16	+31·1	S
Blood	—	Little change	—	—	—	—	28	+51·0	S
Whole gut	—	—	—	—	—	—	14	− 8·61	I
Stomach	12	+89·0	S	6	+262·4	S	21	− 1·17	I
Small intestine	12	+108·4	S	6	+231·3	S	15	+64·5	S
Caecum	—	—	—	—	—	—	9	+52·4	S
Large intestine	—	—	—	—	—	—	10	+42·1	S
Liver	5	+53·1	—	—	—	—	19	+104·6	S
Kidney	—	Little change	—	—	—	—	18	+24·7	S
Spleen	—	Little change	—	—	—	—	19	+70·2	S
Striated muscle	—	—	—	—	—	—	15	+17·8	S
Cardiac muscle	—	—	—	—	—	—	19	+22·8	S
Skin	—	—	—	—	—	—	17	+26·9	S
Lung	8	+25·8	I	—	—	—	38	+23·2	S

Wilson [1941] in six determinations on rabbits obtained a histamine change in blood of +106·4%. This is statistically significant.

Discussion

The results obtained in the experiments described are, generally speaking, in agreement with those of Wilson [1941] and of Rose & Browne [1941], in that there is in most of the tissues examined a significant increase in histamine content after adrenalectomy.

Rose & Browne [1941] suggested that the increase of histamine in the tissues of the adrenalectomized rat was due to transference of histamine from the blood, without any increase in the histamine content of the body as a whole. Wilson [1941], however, showed that the blood histamine was increased in adrenalectomized rabbits, this result being confirmed for the rat in this communication. It has also been shown in the experiments reported here that the histamine content of the whole body is increased by about 30% after adrenalectomy, representing an increase of over 200μg. histamine base in a 50 g. rat.

Since the greatest increases of histamine were found in certain regions of the gut (large and small intestine and caecum) and in the liver, which is directly connected with the gut by the portal system, it might be suggested that the extra histamine enters the body via the alimentary tract. No significant change was found in the histamine in the whole gut with its contents. It might be, then, that there is in the adrenal-deficient animal a transference of histamine within the gut, probably from the lumen into the wall of the intestine and so by the portal blood to the liver. Histamine which passes through the liver enters the general circulation and becomes more or less evenly distributed throughout the other tissues. This transference may be brought about by the changes in the osmotic balance of the body which are characteristic of adrenal cortical deficiency. On the other hand, histamine itself is capable of altering the permeability of cell membranes. Whether the osmotic changes of Addison's disease are the cause of the change in distribution of histamine or whether increased amounts of histamine (due to suppression of a specific anti-histamine function of the hormones of the adrenal cortex) are responsible for the disturbed osmotic balance between the tissues, still remains to be established.

Summary

1. The histamine content of the whole body, blood, whole gut (with contents), stomach, small intestine, caecum, large intestine, liver, kidney, spleen, striated muscle, cardiac muscle, skin and lung was determined in adrenalectomized rats in comparison with similar determinations on litter-mate, 'sham' operated controls.

2. With the exception of whole gut and stomach, all these tissues showed a significant increase in histamine content following adrenalectomy.

3. Control experiments involving the recovery of histamine added in known quantities to the various tissues showed that the experimental methods em-

ployed were reliable except probably in the case of lung, cardiac muscle, spleen and blood.

4. All the results recorded were analysed statistically before conclusions were drawn.

5. The significance of the results is discussed, with reference to the probable source of the extra histamine present in the adrenal cortical deficient animal.

This communication contains, in abridged form, material presented in a thesis for the degree of Doctor of Philosophy (University of London, 1942). For fuller information on experimental procedures and detailed tables of results, this thesis may be consulted.

I wish to express my gratitude to Dr K. H. Coward for her helpful guidance and advice, particularly in the methods of statistical analysis, and to Prof. J. H. Gaddum, upon whose suggestion the investigation was undertaken.

REFERENCES

Barsoum, G. S. & Gaddum, J. H. [1935]. *J. Physiol.* **85**, 1.

Barsoum, G. S. & Gaddum, J. H. [1936]. *Clin. Sci.* **2**, 357.

Best, C. H. & McHenry, E. W. [1930]. *J. Physiol.* **70**, 349.

Bruce, H. M. & Wien, R. [1940]. *J. Physiol.* **98**, 375.

Code, C. F. [1937]. *J. Physiol.* **89**, 257.

Crivellari, C. A. [1927]. *C.R. Soc. Biol., Paris*, **96**, 223.

Fisher, R. A. [1925]. *Statistical Methods for Research Workers*, 1st ed. London: Oliver and Boyd.

Galton, F. [1879]. *Proc. Roy. Soc.* **29**, 365.

Huggett, A. St G. & Mant, A. K. [1941]. *Estimation of Histamine in Blood.* Demonstration, Meeting of the Physiological Society. (Private communication.)

Marmorston, J. & Gottesman, J. [1928]. *J. exp. Med.* **47**, 503.

Rose, B. & Browne, J. S. L. [1938]. *Amer. J. Physiol.* **124**, 412.

Rose, B. & Browne, J. S. L. [1941]. *Amer. J. Physiol.* **131**, 589.

Rose, B. & Karady, S. [1939]. *Amer. J. Physiol.* **126**, 614.

Rose, B., Karady, S. & Browne, J. S. L. [1940]. *Amer. J. Physiol.* **129**, 219.

Scott, W. J. M. [1927]. *Arch. Path. lab. Med.* **4**, 491.

Thomson, W. [1936]. *J. Hyg., Camb.*, **36**, 24.

Voegtlin, C. & Dyer, H. A. [1925]. *J. Pharmacol.* **24**, 101.

Wilson, A. [1941]. *J. Physiol.* **99**, 241.