

INCREASED URINE HISTAMINE AFTER CHALLENGE OF CONTACT SENSITIVITY IN THE MOUSE

Gösta Roupe and Göran Granerus

*Department of Dermatology and Department of Clinical Physiology,
Sahlgren's Hospital, Gothenburg, Sweden*

Abstract. The urinary excretion of histamine was observed daily during the development of contact sensitivity to picryl chloride in the mouse. After challenge, effected by painting the ears, urine histamine increased about four fold, while the histamine content in the ears did not change significantly. It is suggested that during challenge there is a general release of histamine, which may be the result either of an immediate hypersensitivity reaction or an effect of anaphylatoxin formation after complement activation.

The cellular infiltrate of contact sensitivity in mice after epicutaneous sensitization with picryl chloride (PiCl) has recently been studied (13). Already 1 hour after challenge, mononuclear cells appeared in the dermis, increasing in numbers during the first 12 hours. After 24 hours the neutrophil granulocyte was the dominant cell type of the infiltrate. This finding, together with an increase in eosinophils and a reduction in the number of mast cells, tends to suggest the effect of a skin-sensitizing antibody. The synthesis of a reaginic antibody within 7 days of epicutaneous application of PiCl has actually been described (15), supporting a possible contribution of an immediate hypersensitivity reaction in contact sensitivity in the mouse. Only occasionally were basophilic granulocytes found in the cellular infiltrate, thus refuting a cutaneous basophil hypersensitivity.

In order to evaluate the possible importance of formation of a reaginic antibody in contact sensitivity in the mouse, and to test the possibility of a concomitant type I allergic reaction, the urinary excretion of histamine was studied before and after challenge with PiCl. In humans, elevated levels of plasma histamine and of urine histamine have been reported in immediate-type allergic reactions such as provoked attacks of bronchial asthma and hay fever (2, 3). This paper presents data suggesting

that urine histamine levels are in fact increased after challenge of contact sensitivity in the mouse.

MATERIALS AND METHODS

Animals

Male, inbred, 3-month-old CBA/Lü mice were used (weight about 25 g). The animals were bred at the Karolinska Institute, Stockholm, Sweden. They were fed a standard pellet diet and water ad libitum except when urine was collected, in which case the mice were given 3 g daily of a histamine-free diet, whose composition has been described by Kahlson, Rosengren and Westling 1958. Urine collection was made in metabolism cages, which provided facilities for collecting urine and feces separately. The urine was collected every 24 hours, generally starting 2 days before sensitization and continuing 11 days after.

Immunization, challenge and quantification of contact sensitivity

were performed as described earlier (1, 12). Mice were immunized with PiCl 7% (pro analysi, British Drug Houses Ltd., Poole) in absolute alcohol by painting 0.1 ml on the clipped abdomen. Six days later (day 7) both the ears were challenged with 0.05 ml PiCl 1% dissolved in olive oil. Contact sensitivity was assessed by measuring the increase in ear thickness in units of 10^{-2} mm with an engineer's micrometer 24 hours after challenge. Mean increment of the two ears of each mouse was determined and values for groups of mice have been given as mean $\pm$ standard error of the mean. The measurement ensures contact sensitivity in epicutaneously sensitized mice.

Histamine determination

Urine histamine was measured in each 24 hour urine portion with the enzymatic double isotope method described by Beaven et al., 1972. As the inter-individual variation in urine histamine excretion was found to be small, the results have been reported as mean $\pm$ standard error of the mean for the group of mice each day.

To measure the tissue histamine content of the ears, these were cut off near the head, minced with scissors and homogenized in 0.1 M sodium phosphate buffer, pH 7.9. The homogenate was frozen overnight, thawed and

Table 1. Skin reactions in picryl chloride sensitized and control mice after challenge
In groups 1 and 2 the measurement was made 24 h after challenge; in group 3, 48 h after challenge.

Group	No. of mice	Sensitization	Challenge	Increment in ear thickness, 10^{-2} mm
1	3	PiCl 7 %	PiCl 1 %	6.9 ± 0.7
2	3	PiCl 7 %	Olive oil	0.3 ± 0.2
3	2	Absolute alcohol	Olive oil	0.0 ± 0.5

centrifugated. The histamine concentration was then determined by the enzymatic double isotope method. All samples were run in duplicate and the mean values found are given in Table II. The standard error of a single determination, calculated according to the formula $V[\Sigma(d_i)^2/2n]$ (6) was $0.048 \mu\text{g}$, which corresponds to a coefficient of variation of 3.1 %.

RESULTS

Three mice were immunized with PiCl and challenged six days later. Another 5 mice served as controls, 3 of which were sensitized but not challenged. The other 2 mice received only absolute alcohol on day 1 and olive oil on the ears on day 7. Table 1 shows the increment in ear thickness 24 hours after challenge with PiCl 1 % (group 1) and after olive oil alone (group 2). In the other control group (group 3) the determination was accidentally made 48 hours after challenge. Group 1 showed a pronounced increase in ear thickness compared with groups 2 and 3.

In Fig. 1 the urinary excretion of histamine in these three groups of mice is shown. Compared with the controls (groups 2 and 3) the histamine excretion in group 1 was increased about four fold during the 24 hours following challenge with PiCl

1 %. After the sensitizing dose of PiCl 7 % a moderate increase in urine histamine was found in groups 1 and 2, which lasted for several days.

Table II demonstrates the ear histamine content in another 5 mice. No measurements of ear thickness were performed in order not to damage the tissue. In 2 mice, tissue histamine was determined in the left and right ear on day 6 after sensitization.

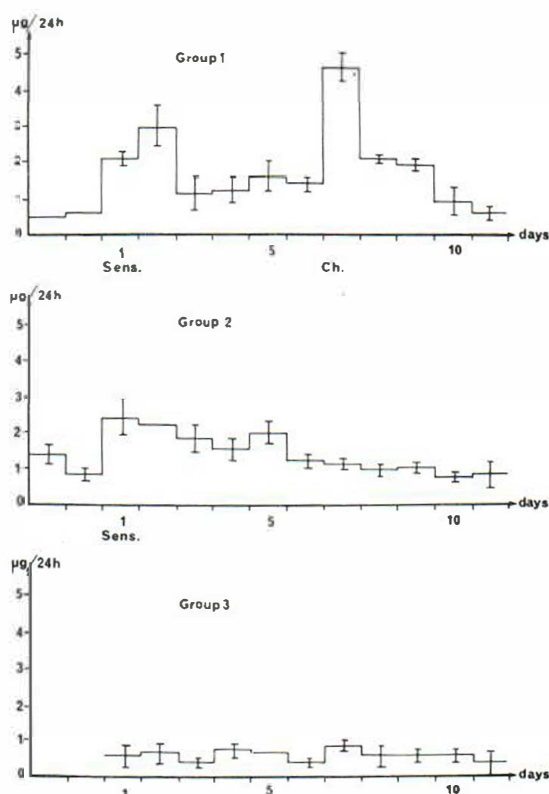


Table II. Histamine content in five ear pairs of picryl chloride sensitized mice before and 24 h after challenge

	Histamine content	
	μg	$\mu\text{g/g}$
Before challenge		
Left ear	1.32, 1.69	33, 51
Right ear	1.19, 1.60	44, 59
After challenge		
Painted left ear	1.51, 1.74, 1.92	48, 36, 46
Control right ear	1.05, 1.23, 2.30	50, 46, 60

Fig. 1. Urinary histamine excretion in picryl chloride (PiCl) sensitized mice before and after challenge (group 1), in control mice without challenge (group 2) and in non-sensitized control mice (group 3). Mean $\pm$ standard error of the mean. Sens. = sensitization with PiCl 7 % in alcohol. Ch. = challenge with PiCl 1 % in olive oil.

i.e. before challenge with PiCl. In 3 animals the left ear was challenged with PiCl 1% on the sixth day after sensitization; 24 hours later, tissue histamine was determined in the challenged left ear as well as in the untreated right ear. Because of considerable differences in ear weights, both actual histamine content (μg) and histamine content related to ear weight ($\mu\text{g/g}$) are given in Table II. Neither a decrease in histamine content after challenge (day 7), nor any difference between PiCl-painted ears and non-painted ears on day 7 could be found, compared with day 6.

DISCUSSION

In the experimental system described, the increment in ear thickness has been used as an indicator of contact sensitivity. The mean increment in ear thickness obtained in the present study in group 1 was equivalent to what has been found earlier in sensitized animals in the same system and the two control groups had ear swellings which corresponded to earlier results in non-sensitized animals (13).

In the mouse, just as in man, injected histamine is quantitatively excreted in the urine over a period of a few hours (14). Therefore a brief increase in the urinary excretion of histamine, as seen in the present study during 24 hours after challenge, probably reflects a sudden release of histamine in the tissues. In immediate-type hypersensitivity reactions, histamine release takes place rapidly, as has been demonstrated in anaphylactic shock in rats (11), in provoked allergic asthma in dogs (5), and in man (2). The prolonged and less pronounced increase in histamine excretion appearing after the sensitization procedure is believed to be an unspecific toxic histamine release in the abdominal skin, possibly followed by an increased histamine formation due to tissue damage and subsequent reparative growth (9).

Histamine released in the organism is efficiently catabolized and in the mouse it has been shown that about 10–20% of injected ^{14}C -histamine was excreted in the urine in the free form (16). In the present study, urine histamine rose by about $4\text{ }\mu\text{g}$ after challenge. That means that a total amount of at least $20\text{ }\mu\text{g}$ histamine was probably released during the day of challenge. No such histamine quantity could be derived from the PiCl-tested ear of the mouse, since the total histamine content

of the mouse ear was only around $2\text{ }\mu\text{g}$. Furthermore, there was no indication of a decreased histamine content in the ear after challenge. Large inter-individual differences in the histamine content of the mouse ears and also differences between left and right ears invalidated a closer analysis of the tissue histamine measurements. Consequently, moderate changes in histamine content after challenge, which might correspond to a reduced number of mast cells, could not be evaluated in this small material.

These results suggest a general histamine release in the body after challenge rather than a local one, and the rapid rise in urine histamine level may indicate a type 1 allergic reaction mediated by IgE antibodies, synthesized after epicutaneous application of PiCl (15). However, a mechanism which involves an activation of the complement system with anaphylatoxin formation by cleavage of complement components C 3 and C 5 must also be considered. The possible histamine-releasing effect of a reaginic antibody in this experimental system is planned to be further investigated by serum-transfer experiments.

It may be questioned whether a general histamine release is related to the local reaction of contact sensitivity in the mouse ear. In this connection, the requirement for vasoactive amines for production of delayed-type hypersensitivity skin reaction has recently been investigated in the mouse by Gershon et al., 1975. Reserpine, which depletes the mast cells of serotonin, which in turn liberates histamine (7), inhibited the ability of the mouse to produce such reactions in the skin.

ACKNOWLEDGEMENTS

The study was supported by a grant from the Edvard Welander Foundation. Painstaking assistance by Mrs Birthe Hellman and Miss Ulla Blomgren is gratefully acknowledged.

REFERENCES

1. Asherson, G. L. & Ptak, W.: Contact and delayed hypersensitivity in the mouse. I. Active sensitization and passive transfer. *Immunology* 15: 405, 1968.
2. Bath, K. N., Arroyave, C. M., Marney, S. R., Jr, Stevenson, D. D. & Tan, E. M.: Plasma histamine changes during provoked bronchospasm in asthmatic patients. *J Allergy Clin Immunol* 58: 647, 1976.
3. Beall, G. N.: Histamine in human urine. *Int Arch Allergy Appl Immunol* 26: 1, 1965.

4. Beaven, M. A., Jacobsen, S. & Horakova, S.: Modification of an enzymatic isotopic assay of histamine and its application to measurement of histamine in tissues, serum and urine. *Clin Chim Acta* 37: 91, 1972.
5. Chiesa, A., Dain, D., Meyers, G. L., Kessler, G.-F. & Gold, W. M.: Histamine release during antigen inhalation in experimental asthma in dogs. *Am Rev Respir Dis* 111: 148, 1975.
6. Dahlberg, G.: Statistical methods for medical and biological students. Allen & Unwin, London, 1948.
7. Feldberg, W. & Smith, A. N.: Release of histamine by tryptamine and 5-hydroxytryptamine. *Br J Pharmacol* 8: 406, 1953.
8. Gershon, R. K., Askenase, P. W. & Gershon, M. D.: Requirement for vasoactive amines for production of delayed-type hypersensitivity skin reactions. *J Exp Med* 142: 732, 1975.
9. Kahlson, G. J. & Rosengren, E.: Biogenesis and physiology of histamine Monographs of the Physiological Society, No. 21, Chap. 19, p. 235 and chap. 21, p. 252. Edward Arnold Publishers, London, 1971.
10. Kahlson, G., Rosengren, E. & Westling, H.: Increased formation of histamine in the pregnant rat. *J Physiol* 143: 91, 1958.
11. Mota, I.: Release of histamine from mast cells. *In* Handbook of Experimental Pharmacology (ed. O. Eichler & A. Farah), vol. 18/1, p. 569. Springer-Verlag, Berlin, 1966.
12. Roupe, G.: Modulation of age-related development of contact sensitivity in mice by adult thymectomy. *J Invest Dermatol* 71: 299, 1978.
13. Roupe, G. & Ridell, B.: The cellular infiltrate of contact hypersensitivity to picryl chloride in the mouse. *Acta Dermatovener (Stockholm)*. In press.
14. Schayer, R. W.: Catabolism of physiological quantities of histamine in vivo. *Physiol Rev* 39: 116, 1959.
15. Thomas, W. R., Asherson, G. L. & Watkins, M.: Reagin antibody produced in mice with contact sensitivity. *J Exp Med* 144: 1386, 1976.
16. Wetterqvist, H.: Histamine catabolism in male and female mice, guinea-pigs and rabbits. *Scand J Clin Lab Invest* 22: Suppl. 104: 31, 1968.

Received November 17, 1978

G. Roupe, M.D.
Department of Dermatology
Sahlgren's Hospital
S-41345 Göteborg
Sweden