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STUDIES ON HISTAMINE

IV

ON FREE AND BOUND HISTAMINE

By

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INTRODUCTION

Histamine, though pharmacologically very active, is present in great amounts in certain tissues without causing any reaction, Tarras-Wahlberg (1936) introduced the idea of differentiating between physiologically active and physiologically inactive histamine.

In more recent literature the terms "free" and "bound" are used. On the one side it appeared that injected histamine disappears rapidly from the blood flow (Alexander, 1944) and on the other hand bound histamine can be activated by a large number of compounds such as snake poison (Rocha e Silva & Essex, 1941), curare (Alam *et al.*, 1939; Rocha e Silva & Schild, 1949) and organic bases (McIntosh & Paton, 1949) etc.

That histamine is set free during the antigen-antibody reaction forms the foundation of the histamine theory of allergy. Katz (1940) proved this *in vitro* and his results were confirmed repeatedly (Rose & Browne, 1941; Spain *et al.*, 1950).

Bartosch *et al.* (1932), Dragstedt & Gebauer-Fullnegg

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(1932) and Jimenez-Diaz *et al.* (1948) showed this mechanism in anaphylactic shock.

Several investigators have been working on the change of bound into free histamine (cf. Rocha e Silva, 1944; Rose, 1947; Rackemann, 1948). From their experiments with tubocurarine on the diaphragm of the rat Roche e Silva & Schild (1949) showed that bound histamine possibly exists under two forms. Two thirds, present in a form which is changed by tubocurarine from an in-diffusible into a diffusible modification in a rapid way. The other third, set free by several other compounds as ammonia c.q. According to these investigators the first modification is activated already in a physiological salt solution and tubocurarine would only have an accelerating influence on this process.

We may safely assume that this rapidly activated histamine is of concern in those processes where histamine is set free or is bound. In our experiments we undertook to investigate whether free and bound histamine could be distinguished from one another, by extracting part of the tissue with trichloroacetic acid and part with a Tyrode solution.

METHODS

Two amounts of approximately equal mass from the organs to be investigated were weighed. One part was ground with sand and trichloroacetic acid, the other part with sand and Tyrode solution. The suspensions obtained thus were filtered using a suction pump. In the residue histamine was estimated using the method of Code (1937).

Nieuwenhuis, Donker en Strengers (this series no. IV) demonstrated that in the extracts obtained with the Tyrode solution, compounds appear which have a disturbing influence on the estimation when carried out using Code's method. After the extraction with Tyrode solution these extracts therefore were also treated with trichloroacetic acid and afterwards analyzed using Code's method.

RESULTS AND DISCUSSION

A first series of observations were performed on three cow livers. These livers were obtained 48 hours after the cows were killed. The results are to be found in Table I.

TABLE I
*Histamine content of cow liver after extraction with trichloroacetic acid
 and Tyrode solution.*

Material	Histamine-2HCl (T.C.A.) γ/gr	Histamine-2HCl (Tyrode) γ/gr
Cow liver 1	4.1	3.1
" " 2	4.4	4.8
" " 3	3.5	3.3

From this table it becomes apparent that the two methods give the same results, within the limits of accuracy of the experiments. Obviously all histamine was free already or at least so "loosely" bound that even the grinding with Tyrode's solution sets it free. It is possible that in fresh tissue the ratio between free and bound histamine is quite different, owing to the possibility that after 48 hours, bound histamine is changed into free as a result of the autolytic processes.

To test this hypothesis in a further series of observations the histamine contents of the organs of 3 guinea-pigs were estimated.

The organs of the first animal were investigated directly after death, the organs of the second animal after keeping them for 24 hours at room-temperature, the organs of the third animal after keeping them for 48 hours at room-temperature. The results of these investigations are given in Table II.

From these analysis it appears that the total histamine content directly after death is larger than the content of free histamine—at least larger than that part of the histamine extracted by the Tyrode solution. The adrenals and muscles are an exception. The fact that in the adrenals, histamine exists only in the "free" modification is of importance when considering the recent researches of a great number of investigators, from which it became apparent that these organs have an important function in histamine metabolism (Rose & Browne, 1941; Marshall, 1943; Gray & Munson, 1951; Ingle *et al.*, 1950).

TABLE II

Histamine content of guinea-pigs organs after extraction with trichloroacetic acid and Tyrode solution.

Material	Histamine (T.C.A.) γ/gr	Histamine (Tyrode) γ/gr	Remarks
Liver	2.6	1.0	directly after death
lungs	36.—	27.5	"
intestine	23.—	7.5	"
kidneys	3.4	1.5	"
adrenals	6.9	6.1	"
brain	0.2	0.4	"
spleen	15.6	3.4	"
heart	9.2	5.2	"
muscle	2.6	3.2	"
Liver	0.3	0.2	24 hrs after death
intestine	20.—	5.8	"
kidneys	2.4	0.4	"
brain	0.9	0.7	"
spleen	2.4	2.6	"
heart	2.5	2.6	"
Liver	2.7	2.6	48 hrs after death
intestine	7.0	6.6	"
kidneys	3.4	3.7	"
spleen	7.2	8.2	"
heart	3.4	3.8	"

After the lapse of 24 hours the histamine in some organs (intestine and kidney) is still partly bound, in other organs it is only present in its free modification (spleen and heart).

After 48 hours the histamine exists only in free form in all organs. When comparing the histamine content of the same organs directly, 24 hours and 48 hours after death we were struck by the fact that the content of free histamine was approximately the same in each case. (The values found in the animal which was investigated 24 hours after death were consistently somewhat lower than those of the others.)

Obviously no bound histamine is changed into free during the autolytical process but part of it (the bound) is broken

down. This result suggests that during autolysis a histamine disintegrating enzymatical system is involved, having only bound histamine as its substrate. The autolytical breaking down of bound histamine is apparently not caused by histaminase as this breaks down free histamine in vitro. Indeed an aerobic process such as this cannot be expected during autolysis. We therefore have to presume the existence of an anaerobic histaminolytic enzymatic system as well as the histaminase aerobic system.

SUMMARY

1. By an estimation of histamine by simultaneously extracting two samples of tissue with trichloroacetic acid and with Tyrodes solution, a distinction is made between "total" and "free" histamine.
2. Directly after death there is a distinct difference between the amounts of total and free histamine in most organs, excepting the adrenals and muscles, where all histamine is free. After 24 hours this difference decreases and after 48 hours all histamine is free.
3. The phenomena observed are caused by an autolytical process whereby "bound" histamine is broken down.
4. Besides histaminase another anaerobic histaminolytic enzyme is postulated.

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