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STUDIES ON HISTAMINE
III

INVESTIGATIONS ON THE ESTIMATION
OF HISTAMINE IN TISSUES¹

By

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INTRODUCTION

For the determination of histamine in blood most investigators use the method described by Code (1937). The Code's method is also used for the estimation of histamine in tissues. It is, however, questionable if this method, unmodified can be applied to tissues. Unlike tissues, blood has a relatively constant composition. For this reason we have investigated the Code-method in extenso, firstly in aqueous solutions of histamine, and secondary on tissue.

A. INVESTIGATIONS ON AQUEOUS
HISTAMINE SOLUTIONS

I. *Influence of the ratio and concentration of the
reagents.*

Code deproteinizes 10 ml of blood with 15 ml of 10 % trichloroacetic acid and boils the proteinfree filtrate with 10 ml of 38 % HCl. For the histamine-estimation in tissue Dekanski

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(1949) uses 1 to 2 gm of starting material and adds 15 ml trichloroacetic acid. Of the proteinfree filtrate he boils 10 ml with 10 ml of 38 % HCl.

It is not always possible to maintain the same volume ratios when applying Code's method to tissue, so we have investigated the influence that changes in the volume ratios of the starting material, to both the acids, has on the estimation. Various volumes of 10 % trichloroacetic acid were added to a number of solutions consisting of 10 γ histamine-dihydrochloride dissolved in 10 and 1 ml of water. After one hour 20 ml of 38 % HCl were added and the solutions boiled for 1½ hours. The histamine was then estimated in the usual way. The results of these determinations will be found in table I.

TABLE I

Histamine dissolved γ	Water (ml)	10 % T. C.A. (ml)	38 % HCL (ml)	Histamine estimated γ
10	1 ml	20 ml	20 ml	10.2
"	"	"	"	10.4
"	"	"	"	10.1
"	"	"	"	10.2
"	10 ml	30 ml	"	10.5
"	"	"	"	10.5
"	"	"	"	10.2
"	"	"	"	10.5
"	"	20 ml	"	9.7
"	"	"	"	10.1
"	"	"	"	9.8
"	"	"	"	9.9
"	"	10 ml	"	9.2
"	"	"	"	10.3
"	"	"	"	9.5
"	"	"	"	9.6

¹ Throughout these investigations our results have been expressed in terms of histamine-2HCl.

The results show firstly that histamine is quantitatively recovered when the ratio of trichloroacetic acid to hydrochloric acid varies from 1/2 to 3/2. Secondly that a relatively smaller volume of the starting material than that prescribed by Code, can be used.

II. *Influence of grinding methods on aqueous histamine solutions.*

The tissues have to be ground. In order to investigate possible losses of histamine when using certain methods, the following experiments were carried out:—In a first series 1 ml of water containing 10 γ of histamine was treated 10 minutes in a tissue grinder together with 10 ml of trichloroacetic acid. In another series the histamine-solution was treated similarly, but instead of trichloroacetic acid, a Tyrode solution was used, and trichloroacetic acid was added afterwards. In both series a large part of the histamine was not recovered when estimated by Code's method (see table II). In the next series grinding was performed with ordinary sand, washed with water. Some of the histamine was again lost. The histamine-solutions were then ground with chemically pure sand, which had been washed several times with concentrated hydrochloric acid and afterwards rinsed with water. After washing, part of the sand had been heated. Histamine was recovered totally when ground with trichloroacetic acid; the same result was obtained, when ground with Tyrode solution, using unheated sand; when using the heated sand with the Tyrode solution, part of the histamine was lost. Apparently the sand when not heated after washing, still contains traces of acid, as the pH of the Tyrode solution during the grinding process decreases to pH 5.0.

The influence of alterations in pH on the stability of histamine was therefore investigated further (see III).

The above mentioned investigations show that traces of heavy metals, originating from the tissue grinder or from the impure sand, promote the destruction of histamine.

TABLE II
Influence of grinding methods on aqueous histamine solutions¹
(γ histamine estimated).

Tissue-grinder		Ordinary sand		Pure sand heated		Pure sand unheated	
T.C.A.	Tyrode	T.C.A.	Tyrode	T.C.A.	Tyrode	T.C.A.	Tyrode
9.5	10.4	8.0	6.0	10.3	8.4	9.7	10.9
7.0	7.4	3.8	5.8	9.8	9.5	10.1	9.8
5.3	7.0	3.7	2.0	9.9	7.6		9.7
4.2	4.0	2.8	1.2	10.2	7.9		9.6
7.0	3.8	2.5	0.8	9.6	8.0		

III. Influence of the pH on the stability of histamine.

It is known that histamine solutions of low concentration at increased pH are very unstable.

Schmitterl w (1948) found that at pH 8, 30 % of the histamine disappears in 30 min.

TABLE III
Influence of the pH on the stability of histamine (all tests started with 10 γ histamine dissolved in 20 ml of Tyrode-solution).

pH Tyrode solution		Histamine estimated (γ)
Before shaking	After shaking	
5.0	6.4	9.9-9.8-9.9-10.0
5.8	6.5	10.0-9.9
6.2	6.8	10.0
6.4	7.0	9.9-10.0
6.6	7.2	10.0
6.8	7.4	9.8-10.0-10.0
7.0	7.6	9.8-9.7
7.2	7.8	8.4-9.5-7.6-7.9-8.0 ¹

In the above mentioned tests in which histamine solutions were ground with sand and Tyrode solution at a pH of 5.0 and 7.8 resp., our results confirm those of Schmitterl w. In the next series of tests, 10 γ of histamine were dissolved in 20 ml Tyrode solutions, which by adding different amounts

¹ All tests started with 10 γ histamine-2HCl.

of 0.1 N HCl had different pH values. Thereafter the solutions were shaken for 10 minutes, and ultimately histamine was again estimated according to Code's method (see table III). (The pH values indicated in the table are those of the solutions before and after shaking, as during shaking the pH increases owing to loss of CO_2 .)

These tests show that histamine is stable at $\text{pH} < 7.5$ and is destroyed at $\text{pH} > 7.5$.

IV. Influence of "Code's method" on aqueous histidine solutions.

According to Åkerblom (1941) and to Schmiterlöv (1949) histidine might have a disturbing effect on the estimation of histamine by being decarboxylated.

Emmelin (1941) on the other hand was unable to demonstrate the formation of histamine from histidine in Code's method.

By repeating their experiments with aqueous solutions of dl-histidine and l-histidine we did obtain a compound which contracts the guinea-pig's intestine. Histamine was identified by demonstrating that the contraction could be suppressed by the histamine antagonist "neoantergan".

Results are summarized in Table IV.

TABLE IV
Forming of histamine by subjecting histidine to "Code's method".

Starting with		Histamine estimated (γ)
l-histidine	dl-histidine	
mg	mg	
20		0.0-5.3-0.4
10		0.6-0.6-0.5
1		0.4-0.5-0.4
0.1		0.0-0.4-0.4
	20	0.0-7-3.6-0
	10	0.4-0.4-0.4
	1	0.8-0.4-0.4
	1.0	0.0-0.2

These results are in agreement with those of Schmitterl w, who also found that quite different amounts of histamine were formed from equal amounts of histidine. We also gained the impression that histamine is formed more easily from l-histidine than from dl-histidine. One must also take into account the fact that the original solutions contained an excess of histidine.

According to Edlbacher, Jucker and Baur (1937) histidine itself is an antagonist of histamine, therefore the amount of histamine formed might have been even greater.

In their experiments, however, Edlbacher et al. suppressed the contraction of the intestine brought about by 1 γ of histamine, by applying 1 ml of a 0.5 N solution of histidine ($\pm$ 75 mg histidine). In our experiments we started off with 0.1–20 mg histidine, but only, at the most, 1/10 of it was added to the intestine-vessel at a time, i.e. 0.01–2 mg. However, the quantities of histamine in our experiments were also smaller than those of Edlbacher et al. In order to study the effect of these smaller amounts of histidine, we compared the contractions resulting from pure histamine solutions with those

TABLE V
Influence of histidine on the estimation of histamine.

Histamine in γ /ml	l-histidine in mg/ml	Histamine found in γ /ml
0.30	2	0.22
0.40	2	0.34
0.16	2	0.13
0.12	2	0.10
0.12	2	0.08
0.18	2	0.04
0.12	0.5	0.10
0.12	0.5	0.12
dl-histidine in mg/ml		
0.16	2	0.11
0.16	0.5	0.13
0.12	0.5	0.11
0.12	0.13	0.08
0.12	0.1	0.12

resulting from the same solutions after the addition of histidine. The results are summarized in Table V.

From the table it can be seen, that in our experiments, both l- and dl-histidine are antagonistic towards the contracting effect of histamine on the guinea-pigs intestine.

In Code's method, however, histidine and histamine are not added separately to the intestine, but jointly. In the following experiment we therefore took 20 mg l-histidine or 20 mg dl-histidine, and applied it, together with 0.8 γ histamine to the intestine. In this case an intestine-contraction occurred which was equally as strong as the contraction caused by a histamine solution with no histidine added. It thus appears that in this case the histidine had no influencing effect. The histamine had probably already acted on the intestine before the histidine was able to decrease its irritability.

As a result of these experiments we may suppose that the presence of histidine does not affect the determination. We therefore conclude that traces of histamine are possibly formed from histidine during the process of extracting the histamine from the tissues, but the quantities of histidine used in our above mentioned experiment (see Table IV) are probably much larger than the quantity of histidine already present in tissue. Moreover the influence of the histidine which has not been decarboxylated, is, under the conditions of the experiment, to be regarded as negligible.

B. INVESTIGATIONS ON TISSUE

In accordance with the above mentioned experiments the estimation on tissues was carried out along the following lines: 0.2–2.0 gms of tissue are ground with $\pm$ 2 gms pure sand (which should not contain traces of heavy metals and to which 10 ml 10 % trichloroacetic acid has been added). The mass obtained in this way is filtered by suction and washed twice with 2 ml of 10 % trichloroacetic acid. The combined filtrates are boiled for one hour with 10 ml 38 % HCl. The consequent estimation is executed as usual provided that the last

dry residue before testing is dissolved in hot water and not in cold. If we have only a small amount of tissue at our disposal we use Gooszen-Strengers' micro method. (This series no. III.)

In a number of experiments using cows and guinea-pig's livers good duplicate values were obtained. In most cases after adding histamine to the tissues, the amounts recovered were within the limits of accuracy. In some cases, however, we found less and in some cases more histamine than was added. The cause of this was found by accident. According to "Code's method" the resulting residue is dissolved in water prior to the test. It appeared that in the first series of observations the residue was dissolved in water at room-temperature, in the second series it was dissolved in boiling water. The dry residue

TABLE VI
*Histamine content of organs before and after addition of histamine
(residue dissolved in hot water).*

Materiel	Histamine γ /gm	Histamine added γ /gm	Histamine found γ /mg
Liver of guinea-pig	1.0-1.0-1.0	1.0	1.6
Liver of guinea-pig	2.4-2.35	1.0	4.4-4.3
Cowliver	16.0-16.0-16.0	10.0	55.0
		20.0	54.0
		30.0	85.0
		60.0	75.0
Cowliver	32.0-36.0	83.0	127.0
		330.0	317.0

TABLE VII
*Histamine content of organs before and after addition of histamine
(residue dissolved at room temperature).*

Materiel	Histamine γ /gm	Histamine added γ /gm	Histamine found γ /gm
Cowliver	9.1-9.4-9.4-10.0	2.0	12.2-12.0-12.0
	31.0-30.0	20.0	50.0-49.0-46.0
Liver of guinea-pig	2.8-2.7		

apparently often contains interfering substances which do not dissolve at room-temperature. Histamine on the other hand, dissolves quantitatively at room-temperature. In the following experiments, only when the residues were dissolved at room-temperature, were the recovery values compatible with the amount of histamine added. The results of all the experiments are summarized in tables VI and VII.

I. *Comparison of Code's method with that of Jimenez-Diaz.*

In a paper by Jimenez-Diaz et al. (1948) a method of estimating histamine in tissue was proposed whereby the tissues are ground with sand and Tyrode solution for 5 min. After being filtered the solutions are heated in a boiling water-bath for 10 minutes and tested immediately on guinea-pig intestine, the pH having first been adjusted. The advantage of this procedure, is that the histamine is extracted in a more physiological manner.

In order to compare this method with Code's, we applied both methods to the same tissue. The results fluctuated considerably. In some cases estimated by Jimenez-Diaz's method less histamine was found than by Code's method, in other cases more.

TABLE VIII
Histamine content of cow liver estimated using the methods of Code and Jimenez-Diaz.

No.	Histamine in γ /gm (Code)	Histamine in γ /gm (Jimenez-Diaz)
1	32.0-36.0	24.3-24.3-27.0
2	13.0-10.3	11.0-10.7
3	10.0-9.0	13.5-13.5-21.0

The extractions obtained using the method of Jimenez-Diaz, were slightly turbid. It subsequently appeared that this turbidity was due to the presence of proteins, for a precipitate was formed on the addition of trichloroacetic acid.

That these interfering proteins had a contracting influence

on the intestine was proved by testing some of the extracts obtained using the method of Jimenez-Diaz immediately, and some after they had also been treated by Code's method.

These last extracts gave the same results as the samples on which only Code's treatment had been applied. That the large values found after Jimenez-Diaz's method was used, resulted from interfering substances, which themselves had a contracting influence on the intestine, could moreover be proved by suppressing the contractions with neo-antallergan. In this case only part of the contractions were suppressed.

SUMMARY

1. The reliability of applying Code's method to the estimation of tissue-histamine was investigated.
2. In aqueous histamine solutions it appeared that the ratio of the amount of starting material, to that of trichloroacetic acid and hypochloric acid could be varied within wide limits.
3. During the tissue grinding process traces of heavy metals from the tissue grinder or from chemically impure sand, have a disturbing influence on the estimation.
4. In all primary manipulations of the tissue, the pH must be < 7.5 as histamine is destroyed at a pH > 7.5 .
5. The dry residue must be dissolved at room-temperature as at a higher temperature interfering substances are also dissolved.
6. In Code's method, small, but varying amounts of histamine are formed from histidine. The amount of histidine in tissue, however, is so small that it does not interfere with the estimation of histamine.
7. Even in small concentrations histidine has an antagonistic effect on the contractions of the intestine brought about by histamine, but only when the histidine and histamine are applied consecutively.

When applied simultaneously this interfering effect of histidine is not observed.

8. Using the method of Jimenez-Diaz, in which the tissue is extracted by means of a Tyrode solution, which is afterwards heated for 10 minutes at 100° C, substances are found in the extract which themselves have a contracting influence on the intestine of a guinea-pig.

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