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THE RELEASE OF HISTAMINE FROM BLOOD CORPUSCLES BY ANTIGEN-ANTIBODY REACTIONS

I. *A Method for Detecting Allergy in vitro in Atopic Persons*

By

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Since 1910 a large number of studies on anaphylaxis in animals have been made and, among other things, it has been possible to show that histamine plays a central role in the release of the anaphylactic symptoms in most animal species (10, 14). One of the most important mediators in allergic phenomena of the immediate type in man is histamine, which was pointed out by Katz in 1940 (5). Some years later (6) the same author succeeded in establishing that histamine is transferred from the cells in the blood to the plasma, when blood from an allergic person is incubated with a homologous allergen. These findings have subsequently been verified by several investigators, see, for instance (10, 12, 14). Certain investigations have shown that histamine can be released from both thrombocytes (2, 3, 4) and leucocytes (1). Middleton *et al.* (11) made a closer analysis of the release of histamine from leukocytes and showed that this can be blocked by oxalate, citrate, ethylene diamine, phenol, and by heating up to 45° C, and that release is decreased at temperatures below 37° C. Similar results have been reported by several investigators (8, 9, 13).

Moreover, Lawrence *et al* (7) made comparisons between intracutaneous tests and histamine release from leukocytes *in vitro*, and assert that the latter is 20 times more sensitive.

It has thus been clearly elucidated that histamine is released *in vitro* from the thrombocytes and leukocytes of the blood when an allergen is added.

The methods used in the investigations referred to here vary somewhat, but, in principle, they are as follows: a certain amount of thrombocytes or leukocytes is suspended in serum from the patient, or leukocytes from an allergic person are washed and suspended in a saline solution. To the suspensions an antigen is added in varying concentrations and the mixture incubated at 37° C for 30–60 minutes. Incubation is followed by centrifugation, after which the histamine content of the supernatant is determined. Without analyzing these methods in detail it may be stated, however, that they are comparatively complicated and time-consuming, which reduces the possibility of utilizing them in a routine allergic work.

The aim of this and following publications is to present the results of research concerned mainly with the following questions:

1. Is histamine released from whole blood to serum after addition of an allergen?
2. Can this amount of histamine be quantitatively measured and, on comparison with the control, afford information on the occurrence of current allergy in the patient?
3. Is histamine also released in what is known as latent allergy?
4. Is the release of histamine influenced by specific anti-allergic treatment?

The questions 1 and 2 will be dealt with in this publication. The questions 3 and 4 will be briefly discussed but the main results will be published later on.

MATERIAL

- a) a group of patients with established allergic reactions to pollen of timothy grass.
- b) a group with established allergic reactions to birch pollen.
- c) a group of patients with latent allergy to pollen.

d) a group of persons allergic to pollen who were previously specifically treated.

e) controls without allergic background or symptoms.

f) a group of patients with allergic rhinitis, some of them with positive skin reactions to actual allergen, others with negative skin tests. Blood from these patients was sent under a code designation for examinations, and identification took place only after histamine determination.

METHOD

By using a coarse cannula blood is enabled to flow freely from the cubital vein into glass tubes which contained 2 ml of varying concentrations of the relevant allergic extract. Extraction was carried out with physiologic saline without the addition of a preservative. No anticoagulants were used. About 10 ml of blood were put in each tube. The blood was kept at 37° C for 20 minutes, whereupon serum was drawn off with a pipette. Histamine was subsequently determined mainly according to the method described by *Shore et al.* (15). A Beckman 77200 Ratio Fluorometer was used.

RESULTS

Tables 1 and 2 present:

1. A group of persons with manifest pollen allergy who were not treated specifically = groups a and b above.
2. a group of persons with latent allergy = group c as above.
3. a group of persons who were hyposensitized = group d as above.
4. controls.

In group 1, the mean percentage increase in the histamine content of serum, in relation to the saline controls, is 194 per cent (range 74-465) for concentration 1:1000, 208 per cent (range 80-459) for concentration 1:100 and 254 per cent (range 105-476) for concentration 1:10.

The latent allergies also show a substantial histamine release. The corresponding mean values are, 124 per cent (range 18-400) for extract 1:1000, 197 per cent (range 8-200) for extract 1:100 and 218 per cent (range 42-700) for extract 1:10. Here it should be pointed out that case 330101 had a very low histamine value which influenced the dispersion of the

TABLE
Histamine in Serum after Incubation of Blood

Patient group	Patient no.	Sex	Antigen	Skin test	History of allergy	Histamine concentration	
						Saline	Concentrated 1:1000
1	470106	F	Timothy	++	Yes	0.012	0.04
	311113	F	"	++	"	0.006	0.07
	490527	F	"	+++	"	0.017	0.09
	420223	F	"	+++	"	0.026	0.05
	550924a	M	"	+++	"	0.027	0.04
	450517	F	"	+++	"	0.040	0.07
	371109	F	"	++	"	0.044	0.09
	171223	M	"	++	"	0.017	0.02
	171223	M	Birch	—	No	0.017	0.01
	500207	F	"	+++	Yes	0.020	0.03
	400911	F	"	+++	"	0.021	0.04
2	330101	F	Timothy	+	No	0.001	0.00
	550924b	M	"	+++	"	0.031	0.04
	440812	M	"	+++	"	0.009	0.02
	440812	M	Birch	+++	"	0.009	0.02
	410330	F	"	+++	"	0.038	0.04
	331011	M	"	++	No?	0.010	0.01
3	440713	M	Timothy	+++	Yes	0.070	0.10
	300913	F	"	++	"	0.019	0.02
	231010	M	"	+	"	0.017	0.04
	390226	F	"	++	"	0.013	0.03
	390226	F	Birch	++	"	0.027	0.02
	380109	M	"	+++	"	0.021	0.03
	380109	M	Timothy	+++	"	0.021	0.03
	550110	M	"	+++	"	0.020	0
	110817	M	"	+++	"	0.016	0.02
	100721	M	"	+	"	0.022	0.01
	350109	M	"	+++	"	0.037	0.04
	331202	M	"	+++	"	0.039	0.07
	430503	M	"	++	"	0.031	0.05
	220713	M	Timothy	++	"	0.034	0.03
	300513	M	Birch	++	"	0.012	0.04
	270603	F	"	+++	"	0.048	0.07
	180415	F	"	+	"	0.019	0.01
	351113	F	"	++	"	0.031	0.05
	530104	M	"	+++	"	0.018	0.04

with Appropriate Antigen in Atopic Persons.

In serum, $\mu\text{g/ml}$		Specific treat- ment	Notes	Diff. antigen-Saline		
Concentration of antigen				1:1000	1:100	1:10
1:100	1:10					
0.046	0.046	o	Twin	0.032	0.034	0.034
0.022	0.034	o		0.026	0.016	0.028
0.095	0.098	o		0.079	0.078	0.081
0.049	0.071	o		0.027	0.023	0.045
0.086	0.084	o		0.020	0.059	0.057
0.072	0.082	o	This 2 sample taken at the same time	0.035	0.032	0.042
0.092	0.121	o		0.054	0.048	0.077
0.024	0.036	o		0.008	0.007	0.019
0.017	0.018	o		—0.003	0.000	0.001
0.049	0.063	o		0.018	0.029	0.043
0.068	0.059	o		0.027	0.047	0.038
0.003	0.008	o	Twin	0.004	0.002	0.007
0.070	0.080	o		0.012	0.039	0.049
0.020	0.025	o		0.013	0.011	0.016
0.026	0.020	o		0.016	0.017	0.011
0.083	0.096	o		0.007	0.045	0.058
0.018	0.027	o	Urticaria	0.007	0.008	0.017
0.208	0.261	Yes	Still rhinitis during sum. No rhinitis in spr.	0.034	0.138	0.191
0.055	0.091	"		0.033	0.036	0.072
0.025	0.034	"		0.007	0.008	0.017
0.031	0.048	"		0.018	0.018	0.035
0.027	0.035	"		0.003	0.000	0.008
0.030	0.055	"		0.017	0.009	0.034
0.040	0.051	"		0.037	0.019	0.030
0.076	0.085	"		—	0.056	0.065
0.034	0.046	"		0.011	0.018	0.030
0.022	0.035	"		—0.006	0.000	0.013
0.053	0.063	"		0.006	0.016	0.026
0.090	0.103	"		0.033	0.051	0.064
0.055	0.069	"		0.028	0.024	0.038
0.049	0.066	"		0.005	0.015	0.032
0.052	0.058	"		0.031	0.040	0.046
0.063	0.096	"		0.026	0.015	0.048
0.017	0.030	"		—0.002	—0.002	0.011
0.055	0.069	"		0.028	0.024	0.038
0.036	0.056	"		0.023	0.018	0.038

TABLE 2
Histamine in Serum after Incubation og Whole Blood with Extracts of Timothy of Birch Pollen in Nonallergic Persons.

Patient	Sex	Antigen	History of allergy	Histamine content in Serum $\mu\text{g/ml}$			Diff. Antigen - Saline		
				Saline	Concentration of Antigen		1:1000	1:100	1:10
460208	M	Timothy	No	0.030	0.028	0.017	0.031	0.017	0.001
481002	M	"	"	0.007	0.007	0.009	0.016	0.002	0.009
190604	M	"	"	0.007	0.016	0.007	0.013	0.000	0.006
461105	M	"	"	0.025	0.025	0.016	0.041	0.009	0.016
230306	M	"	"	0.019	-	0.020	0.036	0.001	0.017
390226	M	"	"	0.027	0.030	0.027	0.035	0.003	0.008
320524	M	"	"	0.034	0.025	0.024	0.033	0.010	0.001
470215	M	"	"	0.026	0.029	0.027	0.039	0.001	0.013
360817	F	"	"	0.020	0.016	0.022	0.028	0.002	0.008
360428	F	"	"	0.049	0.032	0.028	0.032	0.021	0.017
441120	F	"	"	0.007	0.005	0.004	0.036	0.003	0.029
360705	M	Birch	"	0.013	0.004	0.022	0.024	0.009	0.011
960113	M	"	"	0.032	0.015	0.016	0.024	0.016	0.008
340160	M	"	"	0.011	0.005	0.014	0.011	0.003	0.000
130515	M	"	"	0.011	0.015	0.015	0.022	0.004	0.011
420830	M	"	"	0.008	0.011	0.006	0.017	0.003	0.009
080710	M	"	"	0.021	0.015	0.011	0.021	0.010	0.000
161104	M	"	"	0.014	0.014	0.016	0.015	0.002	0.001
031026	M	"	"	0.010	0.009	0.012	0.020	0.002	0.010
120928	M	"	"	0.021	0.011	0.012	0.025	0.009	0.004

values in extract 1:10. In the group that was treated there were substantial divergences. Cases occur where the release of histamine is abundant despite many years' treatment. Case 390226 is interesting inasmuch as the patient received intensive treatment with an aqueous extract of birch pollen and timothy-grass pollen for two years, prior to this sampling. Her spring hayfever is worse. Analogous with this, substantially stronger release of histamine is found to take place on incubation with timothy-grass pollen extract than with birch-pollen extract. Case 380109 was also treated for several years with an aqueous extract and during recent years with "allpyral" extract. His complaint has manifestly decreased or even disappeared, but his histamine release is still high.

To judge from our results histamine release seems to have been less in the treated than in the untreated group. An analysis of the effect of treatment on histamine release will be presented in a forthcoming publication.

We had a pair of identical twins designated 550924 a and b. The former had a manifest allergy and the latter a latent allergy. Both displayed strong skin reactions and substantially high histamine release. Patient *a*, however, had a slightly larger histamine release.

The controls (Table 2) showed strikingly regular results,

TABLE 3
Histamine Contents $\mu\text{g/ml} \pm \text{S.E.}$, Differences from Placebo.

	1:1000	1:100	1:10
Control group (Table 2)	-0.0032 ± 0.0016	-0.0034 ± 0.0017	0.0064 ± 0.0022
Group 1	0.0294 ± 0.0066	0.0339 ± 0.0069	0.0423 ± 0.0071
Group 2	0.0098 ± 0.0019	0.0203 ± 0.0072	0.0263 ± 0.008
Group 3	0.0184 ± 0.0033	0.0265 ± 0.0072	0.0440 ± 0.0091

but a certain decrease in the histamine content was observed on addition of the extract in concentrations 1:1000 and 1:100.

The calculation of the mean values and dispersion between

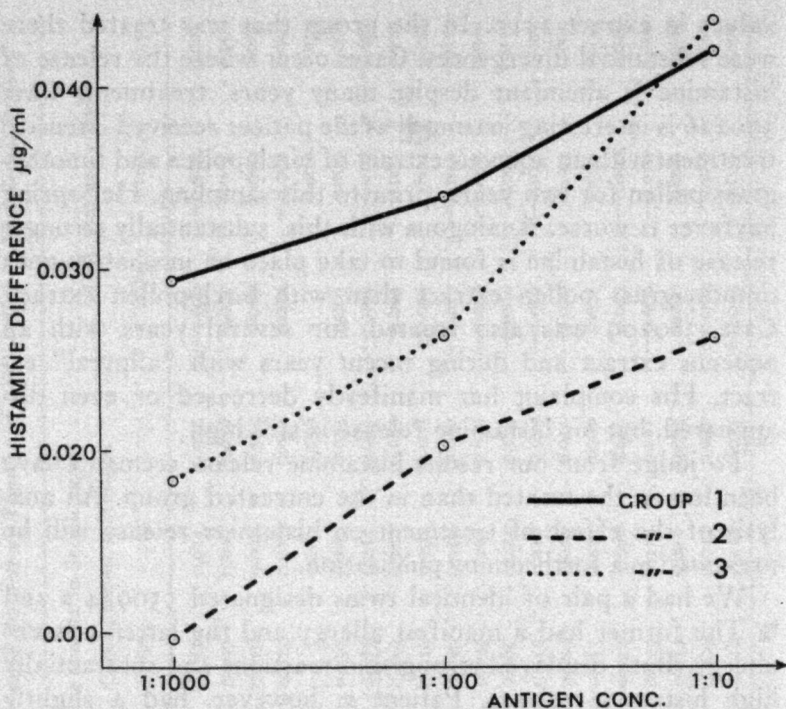


Fig. 1.

Histamine contents $\mu\text{g/ml} \pm \text{S.E.}$, differences from placebo.

the amount of histamine in the serum to which saline was added and the amount of histamine in serum to which different concentrations of the allergen were added, are shown in Table 3.

The difference is statistically significant ($P < 0.05$), if the differential values for the group of patients for the various extract concentrations are compared with the differential values for the controls, see Table 2 and Fig. 1.

Accuracy of a double determination at $P = 0.05$ is $\pm 0.008 \mu\text{g/ml}$.

The group of codified patients, who were above designated f , consisted of 32 persons. Only in one case was the amount of histamine released so large that the patient could be regarded

as hypersensitive to both birch pollen and timothy-grass pollen, despite that his history and the skin test were negative. All who were assessed as negative according to the histamine test had also a negative skin test and a negative history. In 3 cases with manifest allergies there was either insignificant or no release of histamine. These persons had previously been specifically treated and were receiving treatment at the time of the investigation. In the remaining cases the release of histamine corresponded completely with the history, the skin test and the provocation test.

TABLE 4

Differences between Treated Groups and the Control Group from Table 3
($\mu\text{g/ml} \pm \text{S.E.}$)

Group 1	0.0326 ± 0.0068 $t = 4.794 \ P < 0.001$ ($n = 30$)	0.0373 ± 0.0071 $t = 5.254 \ P < 0.001$ ($n = 31$)	0.0359 ± 0.0074 $t = 4.851 \ P < 0.001$ ($n = 31$)
Group 2	0.0130 ± 0.0025 $t = 5.200 \ P < 0.001$ ($n = 25$)	0.0237 ± 0.0074 $t = 3.203 \ P < 0.01$ ($n = 26$)	0.0199 ± 0.0091 $t = 2.187 \ P < 0.05$ ($n = 26$)
Group 3	0.0216 ± 0.0037 $t = 5.838 \ P < 0.001$ ($n = 37$)	0.0299 ± 0.0074 $t = 4.041 \ P < 0.001$ ($n = 39$)	0.0376 ± 0.0094 $t = 4.000 \ P < 0.001$ ($n = 39$)

DISCUSSION

The method which has been applied here has shown that, if pollen extract is added to whole blood from persons allergic to pollen a release of histamine is obtained which can, in certain cases, be of considerable extent. The method is relatively simple and inexpensive, but requires quite a lot of blood. Methods are now being tested which require substantially less blood. Possibly, the sensitivity of the method may be increased by adding different chemicals. Thus, in the preliminary trials we found that the addition of heparin increased histamine release, but it was then much more difficult to obtain reproducible results. A histaminase activity takes place during the incubation-period, which we have found out by adding a known

amount of histamine to the blood. This activity, however, does not disturb the reactions.

The results we obtained correspond markedly with previous investigations where cell suspensions were used in the trials.

The method can be carried out by any well-equipped chemical laboratory. By automatizing certain elements time can be saved. Even if automation is not introduced the capacity for one laboratory assistant is about 20 tests per 6 hours. The clinical interpretation of the test must, as usual, be made by a physician who is clinically active.

SUMMARY

A method is described for the determination of histamine release *in vitro*. Whole blood from persons allergic to pollen is incubated at 37° C for half an hour with pollen extract. Significant differences, in the histamine content of serum, are noted between the control serum and the incubated serum.

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