

From the Departments of Biochemistry and Allergy,
Hacettepe University, Medical School, Ankara, Turkey.

IN VITRO HISTAMINE RELEASE FROM LEUKOCYTES IN PENICILLIN ALLERGIES

By

Y. SARAÇLAR, G. CILIV & K. ÖZKARAGÖZ

Definite diagnosis of penicillin allergy still remains a problem, and a safe and reliable method has not yet been established. Since *in vivo* tests can be dangerous, allergists find it necessary to use *in vitro* laboratory tests for the diagnosis of drug allergies. Among these the detection of specific IgE by the RAST method, and the quantitative determination of histamine released from sensitized leukocytes by antigen stimulation are the most promising for Type I allergy to penicillin (14, 16). The latter method has been used successfully in the diagnosis of atopic hypersensitivity and the follow-up of hypersensitisation (5, 6, 7, 8, 10, 11, 14). Experimental work is still required to investigate its value in drug allergies (4). This work was done to show the extent of its use in the final diagnosis of Type I allergy against penicillin.

MATERIAL AND METHODS

For this study nine cases were selected from patients who had presented at the Department of Allergy with either anaphylactic shock or other Type I allergies to penicillin. The group comprised seven adults and two children, their ages ranging from 8 to 60 years. Only those cases were selected whose last reaction had taken place at least 6 weeks before the test.

The control group comprised seven adults and two children who had received penicillin and had no history of penicillin allergy; their ages ranged from 9 to 55 years.

Care was taken to ensure that neither groups received any drug in the two days prior to the test.

In Vitro Histamine Release from Leucocytes

All chemicals used were analytically pure. Organic solvents were distilled before use (1). Histamine dihydrochloride was obtained from Merck, dextran from Baxter, *N*-butanol and *N*-heptane from British Drug House, *O*-phthaldialdehyde (OPT) from Fluka, and penicillin G from the Ibrahim Etem firms.

Plastic syringes, tubes and pipettes were used for leukocyte separation. Glassware was washed with dilute nitric acid for fluorimetric determinations. Aminco Bowman fluorimeter was used for fluorimetric readings (1).

The separation of the leukocytes from the whole blood of the control and patient groups was made according to the method of Rodey et al. (3, 13).

Histamine released from leukocytes was determined mainly by the method of May et al. with some modifications made by us (10). These consisted of the different way of isolating leukocytes as mentioned above, and preparation of a "blank" tube for each incubation mixture. The contents of the incubation mixtures and their "blanks" are shown in Table 1.

Calculation: Due to the variation in the histamine content of a constant number of cells derived from different donors, the results obtained in this study are expressed in terms of per cent histamine released. Eight tubes were used for each case. The value for the total extractable histamine in the cells is obtained from the readings of Tube 2. Tube 1 is the "blank" of Tube 2 and its reading (if any) gives the fluorescence that may derive from HBSS. Therefore the value of Tube 2 minus the value of Tube 1 gives the net total histamine in the incubation mixture. The spontaneous (non-specific) release of histamine by the cells is determined by the difference of the readings between Tube 4 (control) and Tube 3 (blank of control). The difference gives the net non-specific release of histamine and is termed net control FU (fluorescent units). The reading of Tube 6 gives the value of the release of histamine by penicillin and Tube 5 is its blank. The difference between the readings of Tube 6 and Tube 5 gives net antigen FU.

When these values are applied to the formula below, one gets the specific release of histamine:

$$\% \text{ net release} = \frac{\text{net antigen FU} - \text{net control FU}}{\text{net total histamine FU}} \times 100.$$

Tube 8 gives the value of a known concentration of histamine (standard) and Tube 7 is its blank. These tubes are used to check the precision of the histamine determination method.

Phosphoglucosomerase activity was determined spectrophotometrically (2).

RESULTS

The type of penicillin reaction seen in all patients was the Type I hypersensitivity reaction. Three of the patients had

TABLE I
Incubation Mixtures for Histamine Release from Leukocytes.

	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7	Tube 8§
	Blank for total hist.	Total hist.	Blank for control	Control	Blank for antigen	Antigen	Blank for internal standard	Internal standard
AB group, human serum (ml)*	—	—	0.1	0.1	0.1	0.1	—	—
HBSS (ml)§	1.2	0.2	1.1	0.1	1.0	—	—	0.2
Antigen (ml)†	—	—	—	—	0.1	0.1	—	—
Leukocyte suspension (ml)‡	—	1.0	—	1.0	—	1.0	—	—
Histamine standard (60 µg/l) (ml)	—	—	—	—	—	—	—	1.0
0.01 N HCl (ml)	—	—	—	—	—	—	1.2	—

The amount of reagents (in ml) in each tube is indicated under each tube number. Dash lines indicate no reagent in tube.

* From donors with no history of penicillin allergy or atopy (Hacettepe University blood bank).

§ Hank's balanced salt solution.

† Penicillin G prepared in HBSS used as antigen.

‡ Mean leukocyte count in 1 ml of leukocyte suspension was ca. 3×10^6 .

§ cf. ref. 9.

TABLE 2
Histamine Release in Cases with Penicillin Allergy.

Case No.	Name	Age in years	Sex	Histamine release (%)					
				Penicillin concentration (U/ml)					
				0.062×10^6	0.125×10^6	0.250×10^6	0.5×10^6	1×10^6	2×10^6
1	R.Ç.	60	F	—	7.08	7.08	11.25	23.75	—
2	Ş.G.	38	F	0	—	4.90	—	—	39.53
3	Ş.T.	24	M	—	—	—	—	27.74	51.52
4	U.B.	29	M	—	—	—	—	10.46	—
5	N.T.	30	F	—	—	—	—	13.66	—
6	S.E.	12	M	—	—	3.99	—	23.94	—
7	A.Ç.	9	F	—	—	—	—	—	20.21
8	E.T.	43	M	—	—	—	—	—	18.29
9	B.S.	29	F	—	—	—	—	8.8	—

The Table shows the percentage of histamine released from the leukocytes of patients with penicillin allergy, when incubated separately with six different concentrations of penicillin. The horizontal values indicate the percentage of histamine released when the leukocytes were incubated with the penicillin concentration (U/ml) shown at the top of the corresponding column. For the determination of these values see Materials and Methods.

TABLE 3
Histamine Release in the Control Cases.

Case No.	Name	Age in years	Sex	Histamine release (%)						
				Penicillin concentration (U/ml)						
				0.062×10^6	0.125×10^6	0.250×10^6	0.5×10^6	1×10^6	2×10^6	
1	A.T.	24	F	—	—	—	—	0	0	0
2	G.A.	33	F	—	—	0	0	0	—	—
3	A.I.	55	M	—	—	0	0	0	—	—
4	Ö.T.	9	F	—	0	0	0	0	—	—
5	M.T.	33	M	—	—	—	—	0	0	0
6	Y.S.	38	F	—	—	0	0	0	—	—
7	U.T.	23	M	—	—	—	—	0	0	0
8	H.O.	12	M	0	0	0	0	—	—	—
9	M.T.	24	M	0	0	0	0	—	—	—

See footnote Table 2.

had anaphylactic shock (case Nos. 1, 3, 8) and the others had had urticaria and/or angioedema. The time interval between the reaction and the test was at least 2 months and at most 2 years, except in one patient (case No. 8) who had had the last reaction 5 years before the test.

The amounts of histamine released from the leukocytes of patients are shown in Table 2. The maximum histamine release was found with a 2×10^6 U/ml penicillin concentration. The histamine release was zero in all cases in the control group (Table 3) because the net antigen FU never exceeded the net control FU in this group and therefore, according to the formula for calculation, the result is expressed as "0".

Phosphoglucosomerase activity remained the same throughout the incubation period.

DISCUSSION

Shelley & Comaish in 1965 were the first to use fluorimetric determination of histamine for the diagnosis of immediate-type penicillin allergy (15).

These authors used rabbit thrombocytes and leukocytes which had been passively sensitized by sera from humans with penicillin allergy. They suggested this test to be reliable for the diagnosis of penicillin allergies because 80 per cent of the 40 cases with penicillin allergy and 15 per cent of 81 normal cases gave positive results.

On the other hand, Lichtenstein et al. suggested that the system using human leukocytes isolated and washed from serum was the best system for the histamine release test. Using this leukocyte preparation, they applied this test successfully for pollen allergies (5, 6, 7, 8). May et al., using principally this method, proved that with less blood more antigen can be tested (9).

We used the method of May et al. with some modifications in our work. In this test the isolation of leukocytes is very important. We used the method of Rodey et al. for leukocyte

preparations (13) and could each time isolate leukocytes in great yield from less blood by this method.

A blank tube for each case was prepared to eliminate the non-specific fluorimetric readings produced by every chemical put in the incubation mixture (Table 1).

The result of phosphoglucosomerase activity showed that there was no rupture of leukocytes during the incubation period. Therefore, by eliminating fluorescence from chemicals other than histamine and discounting the possibility that the histamine in the incubation mixture is formed by leukocyte rupture rather than by antigenic stimulation, our fluorimetric readings strongly suggest that penicillin stimulates release of histamine from leukocytes.

Perelmutter & Eisen used the test of histamine release from leukocytes in 14 cases with penicillin allergy, in 16 cases with ragweed allergy and in six controls (12). They found 30-90 per cent and 16-43 per cent histamine release in cases with ragweed allergy and penicillin allergy, respectively. We found a 3.9-51.7 per cent histamine release in cases with penicillin allergy and zero in the control group. On the other hand, Perelmutter & Eisen found up to 7.6 per cent histamine release in their control group (12).

The test of *in vitro* histamine release from sensitive leukocytes can be used for the diagnosis of Type I hypersensitivity reactions. Our results seem to prove this test to be useful for detecting Type I hypersensitivity reactions to penicillin. The results of this test may help to reevaluate the findings in the case history and physical examination of doubtful cases. This test can also indicate which cases should be given penicillin.

SUMMARY

The definite diagnosis of Type I allergy against penicillin is very important. The *in vivo* tests used for this purpose may have some disadvantages for the patient. Among the *in vitro* tests used for the evidence and differential diagnosis of Type I allergy against penicillin, the determination of histamine re-

lease from leukocytes and the detection of IgE by the RAST test are the most promising.

We have used the test of histamine release from leukocyte in this study of 18 cases, nine of whom had Type I hypersensitivity reactions to penicillin and comprised the study group, the rest were control cases. The per cent of histamine released in the control group was "zero" while in the study group it ranged from 3.9 to 51.7 per cent.

The results indicate that the method used in this study is a sensitive and reliable method for the diagnosis of Type I penicillin allergies.

ACKNOWLEDGEMENT

We wish to thank A. Türkeş for his excellent technical assistance.

REFERENCES

1. *Aminco Bowman spectrophotofluorometer. Instruction 768-H*, pp. 27-28; 60-61. American Instrument Co., 8030 Georgia Avenue, Silver Spring, Maryland 20910, U.S.A.
2. Colowich, S. P. & Kaplan, N. O. (1955): *Methods in Enzymology* 1, 299-301. Academic Press, New York.
3. Cutt, J. H. (1970): Cell separation. *Methods in Hematology* 169-174. Academic Press, New York.
4. Halpern, B. N. (1972): Antibodies produced by drugs and methods for their detection. In Samter, M. & Parker, C. W. (eds.): *Hypersensitivity to drugs* (I.E.P.T., Section 75,1) 113-147, Pergamon Press, Oxford.
5. Lichtenstein, L. & Osler, A. G. (1964): Histamine release from human leukocytes by ragweed pollen antigen. *J. exp. Med.* 120, 507-530.
6. Lichtenstein, L., King, T. P. & Osler, A. G. (1966): *In vitro* assay of allergenic properties of ragweed pollen antigens. *J. Allergy* 38, 174-182.
7. Lichtenstein, L., Norman, P. S., Osler, A. G. & Winkenwerder, W. L. (1966): *In vitro* studies of human ragweed allergy. Changes in cellular and humoral activity associated with specific desensitization. *J. clin. Invest.* 45, 1126-1136.
8. Lichtenstein, L., Norman, P. S. & Winkenwerder, W. L. (1968): Clinical and *in vitro* studies on the role of immunotherapy in ragweed hay fever. *Amer. J. Med.* 44, 514-524.

9. May, D. G., Lyman, M., Alberto, R. & Cheng, J. (1970): Procedures for immunochemical study of histamine release from leukocytes with small volumes of blood. *J. Allergy* 46, 12-20.
10. Norman, P. S., Winkenwerder, W. L. & Lichtenstein, L. (1972): Trials of alum precipitated pollen extracts in the treatment of hay fever. *J. Allergy* 50, 31-44.
11. Osler, A. G., Lichtenstein, L. & Levy, D. A. (1968): *In vitro* studies of human reaginic allergy. *Advanc. Immunol.* 8, 183-231.
12. Perelmutter, L. & Eisen, A. H. (1970): Studies on histamine release from leukocytes of penicillin sensitive individuals. *Int. Arch. Allergy* 38, 104-112.
13. Rodey, G. E., Ford, D. K., Gray, B. H. & Good, R. A. (1970): Defective bactericidal activity of peripheral blood leukocytes in lipochrome histiocytosis. *Amer. J. Med.* 49, 322-327.
14. Sadan, N., Rhyne, M. B., Mellits, E. D., Goldstein, E. O., Levy, D. A. & Lichtenstein, L. (1969): Immunotherapy of pollinosis in children. *New Engl. J. Med.* 280, 623-627.
15. Shelley, W. B. & Comaish, J. S. (1965): New test for penicillin allergy. Fluorimetric assay of histamine release. *J. Amer. med. Ass.* 192, 36-40.
16. Wide, L. & Zuhlin, L. (1971): Detection of penicillin allergy of the immediate type by radioimmunoassay of reagins (IgE) to penicilloyl conjugates. *Clin. Allergy* 1, 171-177.

Authors' address:

Dr. Y. Saraçlar
Hacettepe University
Faculty of Medicine
Department of Pediatrics, Allergy Section
Ankara
Turkey

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.