Development of a Sensitive Radioassay of Histamine for In Vitro Allergy Testing

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A radioenzymatic assay for the measurement of histamine is described, based on the incubation of histamine in the presence of histamine-N-methyl-transferase from rat kidney and [^{3}H-methyl]-*S*-adenosyl-L-methionine (sp act 15 Ci/mmol) in phosphate buffer, 0.05 mole/l, pH 7.9, at 37°C for 60 min. The *N*-[^{3}H]-methyl]histamine generated was selectively extracted into toluene/isoamyl alcohol (3:2) and the quantity of the tritium in the sample was determined by liquid-scintillation counting. As little as 1 nmol/l of histamine can be detected. The assay is specific, with no cross-reactivity noted for several compounds closely related to histamine. The assay was used to measure the released histamine of a group of allergic subjects following the incubation of their blood with various allergens. A good correlation was found between histamine release from whole blood and the response of skin mast cells to intradermal antigen administration.

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Histamine release from human leukocytes represents an important model for in vitro studies of allergic reactions (*1–5*). This is reflected by marked similarities between blood basophils and tissue mast cells, including:

1. They are equally endowed with surface IgE antibody.
2. They contain relatively similar complement mediators. Both cell types are largely filled with histamine-containing granules.
3. There is a quantitative relationship between the response of basophils to antigen, manifested by histamine release, and the response of skin mast cells to intradermal antigen administration (*6*).
4. There is a similar quantitative correlation between either skin-test results or basophil histamine release and the response of lung mast cells (*7*).
5. Inhibition of histamine release occurs in both systems in the presence of drugs that act on adenylate cyclase (such as beta agonists and prostaglandins) and drugs that inhibit phosphodiesterase, such as methylxanthines (*8–11*).

In view of the important role of histamine in allergy-mediated reactions, we decided to develop a specific and sensitive radioenzymatic assay for the quantitative estimation of histamine. The method is based upon the use of the enzyme histamine-*N*-methyltransferase (HNMT), isolated from rat kidney, to transfer a radioactive methyl group from the cofactor [^{3}H-methyl]-*S*-adenosyl-L-methionine (SAM) to an endogenous histamine acceptor molecule to form the radioactive metabolite [^{3}H-methyl]-N-histamine. This was followed by selective extraction of the liberated metabolite and its subsequent quantification by liquid-scintillation counting. The assay was used to measure the histamine released from the blood of a group of atopic subjects following the incubation of the blood with different allergens including grasses, trees, ragweed mixture, mite dust, house dust, and dog and cat dander. A comparative study was carried out between the response of the skin test to intradermal antigen administration and the in vitro whole-blood histamine release in these subjects.

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MATERIALS AND METHODS

Reagents. Compounds and solvents were obtained commercially: histamine·2 HCl, 1-methyl-histamine·HCl, bovine serum albumin, Trizma base*, sodium chloride, potassium chloride, calcium chloride, magnesium chloride, toluene, isoamyl alcohol†, Liquifluor, and

[^{3}H-methyl]-*S*-adenosyl-L-methionine (sp act 15 Ci/mmol)‡.

Allergens. The allergens were obtained from one commercial source§ as an aqueous buffered solution. Mixed grasses contained equal amounts of timothy, orchard, june, Bermuda, Johnson and Bahia; mixed ragweed pollen contained equal amounts of tall and short ragweed; mixed tree pollen contained equal amounts of oak, maple, hickory, and pecan. Mite dust, house dust, and cat and dog dander were also used. The house dust and the cat and dog dander were obtained at a concentration of 20,000 protein nitrogen units (PNU) per ml; the grass and tree pollens at a concentration of 10,000 PNU/ml; mite dust and ragweed mixture were at 5,000 and 40,000 PNU/ml.

Histamine standard solution. The reference standard histamine solution was prepared by dissolving 1.8 mg of histamine·2 HCl in 100 ml of 0.01 *N* HCl to give 10 μg of histamine base per ml. The solution was then divided into 1-ml fractions and stored at −80°C.

Collection of specimens. Venous blood (10 ml) was drawn into evacuated tubes containing sodium heparin (143 USP units/tube). The blood was studied immediately or kept at 4°C up to 48 hr.

Clinical evaluation. Thirty patients seen in an allergy clinic were evaluated by history and intradermal skin tests. The skin tests were performed with the same antigens used for in vitro histamine release. The concentrations used for skin testing were 1,000 PNU/ml for house dust, dog dander, and tree pollen, and 100 PNU/ml for mite dust, cat dander, ragweed mixture, and tree pollen. For skin tests, 0.02 ml of allergen was injected at each site on the upper arm with a disposable syringe, with reactions read at 15 min. Skin-test grading was based on measurements of the greatest diameter of the wheal following the usual criteria: negative, (site no different from a diluent control), 3+ (8–12 mm of wheal), and 4+ (>12 mm wheal).

Antigen-mediated histamine release from whole blood. Histamine release reactions were carried out in 12- by 75-mm plastic tubes. To each of the tubes was added 0.5 ml of Tris-albumin buffer¶, an allergen (in PNU/ml: house dust, 200; mite dust, 5; cat dander, 60; dog dander 1,000; ragweed mixture, 100; tree pollen, 150; and, grasses, 150), and 0.5 ml of heparinized blood. Control tubes were prepared by mixing buffer with blood alone. The tubes were incubated in a shaking water bath for 15 min at 37°C. This was followed by centrifugation at 500 *g* for 10 min. The plasma was separated and stored at −30°C until analyzed.

Total blood histamine was determined by diluting 0.5 ml whole blood with 1.5 ml of 0.05 mole/l phosphate buffer (pH 7.9) and heating in a boiling-water bath for 20 min. After centrifugation at 15,000 *g* for 30 min, the supernatant was removed and stored at −30°C until analyzed.

Enzyme preparation. An enzymatic preparation containing histamine-*N*-methyltransferase was formulated according to a procedure modified from Brown et al. (*13*). Rat kidneys or guinea-pig brains (40 g), were purchased frozen (−70°C).** The tissues were homogenized with ice-cold sucrose solution (360 ml, 0.25 mole/l, pH 7.4) and centrifuged at 62,000 *g* for 30 min at 4°C. To the supernatant was added ammonium sulfate (0.26 g/ml, enzyme grade). The mixture was stirred for 10 min, allowed to stand for 1 hr at 4°C, then centrifuged at 10,000 *g* for 20 min at 4°C. The precipitate was discarded and ammonium sulfate (0.156 g/ml) was added to the supernatant fraction with stirring. This solution was allowed to stand for 1 hr, then centrifuged at 10,000 *g* for 10 min at 4°C. The supernatant fraction was discarded and the precipitate was dissolved in 40 ml of ice-cold sodium phosphate buffer (40 ml, 0.1 mole/l, pH 7.4). After dialysis for 72 hr against sodium phosphate buffer (0.01 mole/l, pH 7.4), 40 ml of the dialyzed enzyme solution was divided into 1-ml fractions and stored at −80°C in plastic tubes. The enzyme preparation was stable for at least 6 mo.

Enzymatic assay. The reaction mixture was composed of sodium phosphate buffer (0.350 ml, 0.05 mole/l, pH 7.9). To this would be added one of the following: (a) for the development of a standard curve for histamine, 0.010 ml of 2.0–60.0 n*M* solution of histamine, pH 7.9; (b) for total blood histamine, 0.05 ml of 1:5 dilution of supernatant fraction obtained from boiled blood as described earlier; (c) for antigenic release of histamine from blood, 0.050 ml of plasma; (d) for internal standards, 0.01 ml histamine solution (11.3 n*M*) was added to samples in c; and (e) blank tubes prepared by substituting 0.05 ml of buffer for substrate or sample material. In each case, the mixture was vortex-mixed and 0.026 ml of a mixture containing 0.025 ml of enzyme preparation and 166 n*M* (1 μCi) [^{3}H-methyl]-*S*-adenosyl-L-methionine, sp act 15 Ci/mole, was added to each tube. We incubated the reaction mixture for 60 min at 37°C in a shaking water bath. The reaction was then stopped by the addition of 0.2 ml of borate buffer (0.5 mole/l sodium borate, containing per liter 0.4 mmol of 1-methylhistamine, pH 10.5). The *N*-[^{3}H-methyl]histamine generated in the reaction tubes was extracted by shaking the samples with 4 ml of toluene/isoamyl alcohol (3:2) for 20 min, then centrifuging at 500 *g* for 10 min. Rapid freezing in an isopropyl alcohol/dry-ice bath facilitated the transfer of all the organic layer into a scintillation vial. The toluene/isoamyl alcohol extract containing the methylated histamine was evaporated to dryness under a stream of air (20 min at 60°C), then 10 ml of liquid-scintillation fluid (toluene/Liquifluor, 10:0.5 by vol) containing 1 ml of ethyl alcohol was added. The radioactivity of samples was counted to ±2% error. Each sample was assayed in duplicate.

Percentage of histamine release. The percentage of

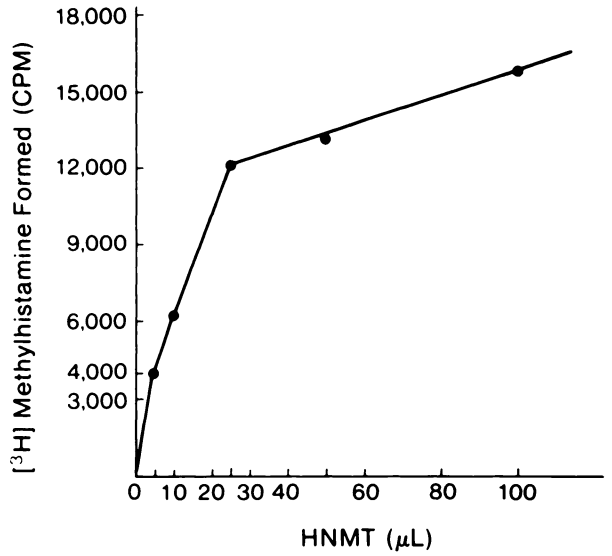

FIG. 1. Correlation between concentrations of histamine-*N*-methyltransferase isolated from rat kidneys and [^{3}H-methyl]histamine formed (cpm) following incubation of enzyme in aliquots of 2–100 μl in presence of 360 nmol/l of histamine standard and [^{3}H-methyl]-*S*-adenosyl-methionine (166 n*M*, 1 μCi, sp act 15 Ci/mmol). Each point represents average of six determinations.

histamine release from 1 ml of blood by each allergen was determined from the following formula: 100 × A/B = % histamine release, where A = concentration of histamine in plasma following the incubation of 1 ml of whole blood with allergen, minus concentration of histamine in plasma following incubation of 1 ml of blood alone; B = concentration of total histamine obtained from 1 ml of boiled blood minus blank sample.

Specificity study. A variety of imidazoles and catecholic and noncatecholic phenethylamines were examined as possible substrates or inhibitors of histamine-*N*-methyltransferase isolated from rat kidneys. These compounds included L-histidine, imidazole, arocanic acid, methylhistidine, imidazoleacetic acid, norepinephrine, and octopamine. They were tested in concentrations ranging between 55 n*M* to 55,000 n*M*, in a manner analogous to that described above.

RESULTS

pH Optimum. We examined the radioenzymatic assay of histamine in various buffers over a pH range of 6.5 to 8.5. The enzyme activity was optimum in 0.05 mole/l sodium phosphate buffer at pH 7.9. This is in agreement with previously reported values (*16–19*).

Preparation of the histamine-*N*-methyltransferase. The histamine-*N*-methyltransferase, isolated from rat kidney as described above, was found to be most suitable. After treatment with ammonium sulfate, dialysis (4°C) against repeated changes of 0.01 mole/l sodium phosphate buffer (pH 7.4) over a period of 72 hr was found essential to maintain an enzyme with optimal methylating capacity. The purity of the enzyme in terms of its specific activity was tested by measuring enzyme activity in the presence of 360 nmole/l of histamine in aliquots (2–100 μl) of the enzyme preparation. The enzyme preparation was fairly pure, since a kinetic relationship was noted between 0 to 25 μl concentrations of the enzyme and the *N*-[^{3}H-methyl]histamine generated. A 25-μl aliquot of the enzyme preparation was found to produce maximal enzyme activity (Fig. 1).

Effect of storage of the enzyme. The dialyzed enzyme, when divided into 1-ml portions and stored at −80°C, was found to be stable over a period of 6 mo, as validated by the reproducibility of the results (for histamine standards 91–270 nmol/l) with the same batch of enzyme in ten consecutive assays over a period of 6 mo. The respective CVs were 4, 5, and 4.5%. Furthermore, three different batches of histamine-*N*-methyltransferase prepared at different times gave identical results with respect to the standards and the samples of histamine in plasma.

Effect of storage on the histamine concentration. Plasma samples containing basal histamine levels, and others also containing allergically released histamine, were stored at 4°C to −30°C. The histamine concentrations were determined daily for 10 days with no noticeable decrease in concentration when stored at −30°C.

Standard stock solution. The concentration of the standard stock solution of histamine was checked during storage at pH between 1 to 10 at different temperatures (−80°C, −10°C, and +4°C). The histamine concen-

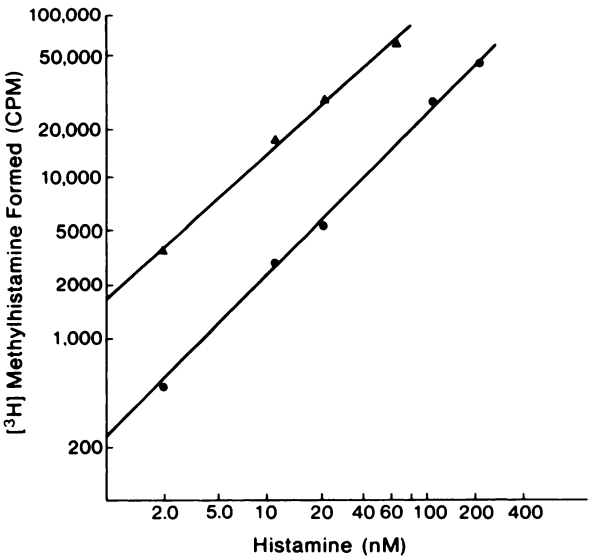

FIG. 2. Typical standard curve (double log) showing relationship between amount of histamine added (2–200 mmol) using partially purified histamine-*N*-methyltransferase isolated from rat kidneys (▲—▲) or guinea-pig brains (●—●) in presence of [^{3}H-methyl]-*S*-adenosylmethionine (166 n*M*, 1 μCi, sp act 15 Ci/mmol). [^{3}H-Methyl]histamine formed represents net cpm obtained after subtracting the blank (av. 4770 cpm) from each value. Each point represents average of ten determinations.

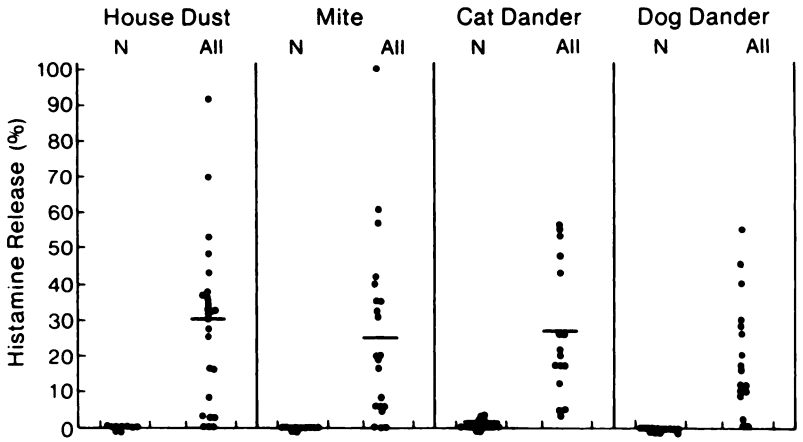

FIG. 3. Allergen-mediated histamine release (in % of total blood histamine) from whole blood of allergic (All) patients sensitive to house dust, mite dust, cat and dog dander. Clinical evaluation of patient was based on history and skin test. Positive patients reacted ≥3+ to allergen by intradermal test. Concentrations of allergens used for histamine blood release were 200, 5, 60, and 1000 PNU/ml for house dust, mite dust, cat and dog dander. N denotes nonallergic subjects with no histamine release.

tration was least affected when the sample was acidified and kept frozen at −80°C.

Sensitivity. The standard curve for histamine was linear between 2.0 and 60.0 n*M* (Fig. 2). After subtracting the cpm of the enzyme control from the sample, concentrations of histamine down to 0.5 n*M* could be reliably measured by the above procedure with methylating enzyme isolated from rat kidney. In contrast, the radioenzymatic assay of histamine using histamine-*N*-methyltransferase isolated from guinea-pig brain is less sensitive than that of rat-kidney enzyme, since the limit of sensitivity of this assay with the former is only 5 nmol/l (Fig. 2).

Analytical variables. *Specificity.* None of the related compounds (histidine, imidazole, urocanic acid, methylhistidine, imidazole-acetic acid, norepinephrine, and octopamine) activated histamine-*N*-methyltransferase and thus did not liberate [^{3}H-methyl]histamine from the incubation of histamine with [^{3}H]SAM. The counts extracted were not significantly different from the blank.

Analytical recovery. Known amounts of histamine were added to plasma in concentrations ranging from 10 n*M* to 300 n*M*. The samples (n = 25) were prepared by an independent group and sent to our laboratory for analysis. Mean analytical recovery, expressed as the ratio of histamine added to that measured, was 85 ± 8.1 (s.d.) percent. Furthermore, the use of the standard curve for the determination of histamine content of plasma samples gave results ranging from 70% to 100% of that obtained by the internal-standard method.

Precision. Within- and between-assay variation was assessed for histamine by assay of 50 plasma histamine samples in the same as well as in a different assay (n = 50), according to the method of Rodbard (*12*). Intra- and interassay CVs were 10.7% and 12%, respectively.

Histamine release. Heparinized blood may be stored at 4°C for 48 hr without modification of its histamine content and without alteration in its capacity to release histamine on contact with the allergen. Blood release is rapid, since 90% of the histamine "reliazable" by antigen was found after 15 min of incubation time.

Working time. The time involved in setting up an assay comprised of 100 tubes is 6 hr, including time spent in pipetting standards, enzymatic incubation, extraction, counting, and calculation of data. The enhanced sensitivity of the assay allows testing of 18 different allergens in 10 ml of blood.

Studies in humans. A typical assay for the determination of histamine released by basophils after in vitro provocations with suspected allergens involved the following: (a) measurement of endogenous plasma histamine; (b) measurement of total blood histamine; (c) measurement of basal release of histamine; and (d) measurement of histamine released by incubations with different allergens. Our studies showed that total blood histamine (mean ± s.d.) was variable, ranging from 450 n*M* to 1351 n*M* (920 ± 250 n*M*, 50 samples). The mean basal release of histamine, when blood was incubated with buffer alone at 37°C for 10 min, was only (2.84 ± 0.5)% (s.d.) of total blood histamine. The basal histamine release into plasma should not be confused with the endogenous plasma histamine level, which was 4.5 ± 0.4 n*M*.

The histamine release and skin-test data for house dust, mite dust, cat dander, dog dander, grass and tree pollens, and ragweed mixture are demonstrated in Figs. 3 and 4, and listed in Table 1.

The results indicate that there is good correlation between in vitro histamine release from whole blood and the response of skin tests on these patients. This was demonstrated by an average of 28–38% histamine blood release following antigenic challenge in vitro when the skin test was 4+ or greater, compared with an average histamine blood release of 11.1–21% in patients with skin-test results of 3+. The allergen-mediated histamine release from whole blood is highly dependent on antigen concentration (Fig. 5). Furthermore, the test is also specific, since nonallergic blood from donors with negative skin tests released no histamine following incuba-

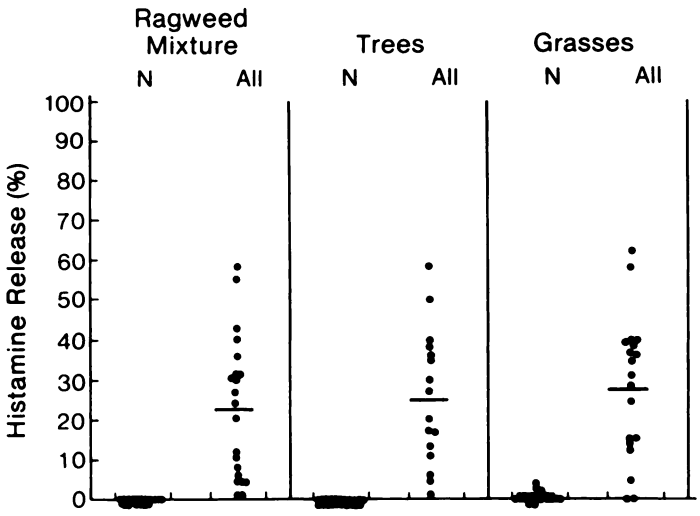

FIG. 4. Allergen-mediated histamine release (in % of total blood histamine) on whole blood of allergic (All) patients sensitive to ragweed mixture, trees, and grasses. Patient's clinical evaluation was based on history and skin test. Positive patients reacted ≥3+ to allergen by intradermal test. Concentrations of allergens used for histamine blood release were 100, 150 PNU/ml for ragweed, trees and grasses. N denotes nonallergic subjects with no histamine release.

tion with house dust, mite dust, dog dander, ragweed mixture and tree pollen; and only 1.5% of total blood histamine following incubation with grasses and cat dander (Figs. 3 and 4, and Table 1).

DISCUSSION

The need for a fast, reliable procedure for the measurement of plasma histamine is increasingly apparent as more correlations become established between the

TABLE 1. CORRELATION OF HISTAMINE BLOOD RELEASE WITH SKIN-TEST RESULTS (Average)

Allergen	Skin-test response	Total No. patients	Histamine release (%)	% Agreement with skin test
House dust	≥3+	27	30.0	85
	≥4+	12	38.3	100
	3+	15	21.6	80
	Negative	9	0	100
Mite dust	≥3+	21	25.0	86
	≥4+	14	31.0	93
	3+	71	11.1	71
	Negative	11	0	100
Cat dander	≥3+	16	27.0	100
	≥4+	7	34.4	100
	3+	9	21.0	100
	Negative	18	1.2	95
Dog dander	≥3+	17	20.0	82
	≥4+	6	30.0	100
	3+	11	14.3	73
	Negative	13	0	100
Ragweed mixture	≥3+	21	23.0	90
	≥4+	12	28.0	83
	3+	9	16.1	100
	Negative	17	0	100
Trees	≥3+	16	25.0	94
	≥4+	8	34.6	100
	3+	8	16.0	87
	Negative	22	0	100
Grasses	≥3+	21	28.0	90
	≥4+	12	28.0	83
	3+	9	16.1	100
	Negative	17	1.2	98

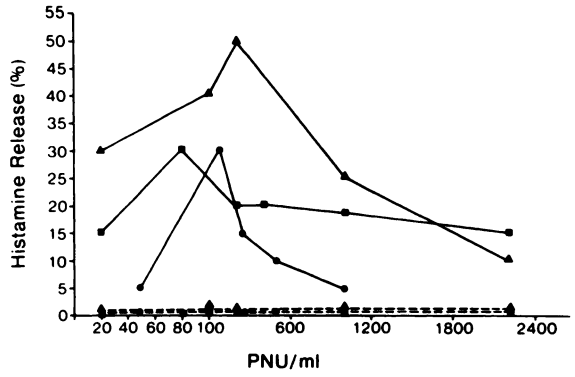

FIG. 5. Relation between antigen concentration (PNU/ml) and allergen-mediated histamine release from whole blood (in % of total blood histamine) of donors clinically diagnosed as sensitive (●—●, ■—■, ▲—▲) or not sensitive (●- -●, ■ - - ■, ▲- -▲) to trees, ragweed, and housedust. Each point represents average value obtained from ten patients in each category.

concentrations of histamine released from whole blood in vitro and allergic reactions in atopic diseases (*1*,*2*).

The purpose of this communication is to offer a sensitive and specific method for the determination of histamine in plasma by radioenzymatic technique. Histamine-*N*-methyltransferase (*13*), an important enzyme in the metabolism of histamine in the central and peripheral nervous systems, was isolated from rat kidney, and partially purified through repeated ammonium sulfate precipitations. The method used for the determination of histamine-*N*-methyltransferase activity was based on the principle that the enzyme catalyzes the transfer of methyl groups to histamine in the presence of [^{3}H-methyl]-*S*-adenosylmethionine as the methyl donor. The end product of the enzymatic reaction, [^{3}H-methyl]histamine was measured by liquid-scintillation counting.

A notable feature of this method is its specificity for histamine, since closely related metabolites and analogs—including L-histidine, imidazole, arocanic acid, methylhistidine, and imidazoleacetic acid—did not liberate [^{3}H-methyl]histamine upon incubation with histamine, and the extracted radioactive counts were not significantly different from those of the blank.

Our assay for the measurement of histamine is precise, sensitive, reproducible, inexpensive, and amenable to routine application. Key elements associated with the assay have made this possible. It included the use of the following items: (a) methylating enzyme isolated from rat kidney compared with guinea-pig brain; (b) tritiated high-specific-activity methylating cofactor compared with [^{14}C]-*S*-adenosylmethionine; (c) toluene/isoamyl alcohol compared with chloroform for the selective extraction of formed methylated metabolite; and (d) rapid transfer of the organic phase by freezing of the aqueous phase compared with removal of aliquots by pipetting. The enhanced sensitivity of the assay was partly the effect of using methylating enzyme isolated from rat kidney, since it caused a substantial increase in the amount of [^{3}H-methyl]-histamine formed in the presence of an equivalent amount of histamine with only one-fourth of the enzyme concentration required by that isolated from guinea-pig brain. This made it possible to detect as little as a 0.5-n*M* concentration of histamine in a sample and thereby allow the measurement of endogenous as well as released blood histamine in reduced volume of diluted plasma samples. Moreover, it was found that histamine content in a sample can be estimated by using an internal standard, which requires only that the sample alone and sample plus internal standard be incubated the same length of time, as well as by a standard curve. The agreement between the two methods is an indication of the absence of substances that may inhibit the enzyme reaction. Furthermore, the ease and simplicity of the assay procedure allows the processing of many samples per day.

The conventional assay of histamine in biological fluids has depended on time-consuming procedures including fluorometric analysis (*14*) and gas chromatography with mass spectrometry (*15*). Although these procedures are promising, problems inherent in preparing biological samples for gas chromatography, and interfering side reactions in the derivatization of histamine by fluorometric analysis, make these methods less attractive for routine clinical analysis for histamine.

The radioenzymatic assay of histamine described by Snyder et al. (*16*) is based on the enzymatic methylation of histamine using a histamine methyltransferase isolated from guinea-pig brain, [$^{14}CH_3$]-*S*-adenosylmethionine as the methyl donor, and labeled histamine as an internal standard. We found that, in principle, this method is adequate, but is not suitable for practical applications. The method is cumbersome and lacks sensitivity, reproducibility, and accuracy. Several problems associated with the Synder et al. assay include (a) ratios of C-14 to H-3 could not be linearly related to histamine concentrations at all times; and (b) it is not suitable for measurement of histamine in diluted plasma samples. Other modifications of the assay have been described (*17*-*19*). Even though the use of [^{3}H]SAM in the majority of these methods has increased the sensitivity of the histamine method, the essential requirement of a thin-layer chromatography step and use of *N*-methylhistamine as an internal standard in each sample has made these methods cumbersome, and they lack a considerable degree of uniformity.

Antigen-mediated histamine release from washed leukocytes has been used extensively for in vitro studies of allergy. Although this technique has proven to be a reliable index of the clinical sensitivity of a patient, it has been given only limited clinical application. This was due mainly to the large amount of blood required, the slow kinetics of histamine release, and the difficulty of the histamine determination.

In this investigation we used the radioenzymatic assay of histamine for measurement of histamine released from small samples of whole blood after in vitro provocations with the suspected allergens. We found that this method can be routinely applied and easily performed. The sensitivity of the system allows in vitro quantitative studies with 18 allergens to be performed with only 10 ml of blood. Furthermore, the storing of blood for 48 hr at 4°C had no effect on the allergen-induced histamine release from whole blood. This is a clear-cut advantage for both patient and allergist.

A good correlation was noted between histamine release from whole blood and the response of skin mast cells to intradermal antigen administration. A significant allergen-mediated histamine release, ranging from 28–38% of the total histamine content, was observed in 83–100% of patients with skin test sensitivity of 4+ or greater to grasses, trees, ragweed mixture, house dust, mite dust, and dog and cat dander. There was a good agreement between the intensity of the skin reaction and magnitude of histamine release, since patients with skin-test sensitivity of 3+ had a histamine blood release upon antigen challenge in vitro of only 11.1% to 21%. By contrast, all patients who were clinically negative did not release histamine from blood. Allergen-mediated histamine release from whole blood is highly dependent on antigen concentration. The dose-effect relationship is a bell-shaped curve showing a strong inhibitory effect at high antigen concentration. It is important, then, to establish the dose-response curve for each antigen to find out the optimal antigen concentration. In our experimental conditions, it is 150 PNU/ml for grasses and trees, and 100, 200, 5, 6, and 1,000 PNU/ml for ragweed mixture, house dust, mite dust, and cat and dog dander.

The histamine-release results compare favorably with published reports of the use of the RAST technique in allergy diagnosis (*20–23*). Several reports have shown that the RAST has an overall accuracy of about 70% when correlated with a patient's clinical history. The present results showed that the histamine release was positive in over 90% of the patients who were allergic by clinical evaluation. Furthermore, histamine release from whole blood probably is a biologically more sensitive index of a patient's sensitivity than RAST test because it is the cell-attached IgE that reacts with allergen, resulting in the release of allergic mediator, histamine. Furthermore, the basophil histamine-release test seems to be superior to RAST because no allergen-coupling procedure is needed, and it can utilize the same allergens used for skin testing with a comparable concentration range.

In summary, the approach presented here for determination of plasma histamine is rapid and reliable. The method is specific and easily applicable to the measurement of antigen-mediated histamine released from whole blood. The finding of a good correlation between histamine release and skin-test data make the in vitro histamine release from whole blood a potentially useful technique for the diagnosis and study of allergy. It is a safe in vitro adjunct to the traditional in vivo diagnostic method for differentiating between offending allergens. It can provide accurate, reproducible confirmatory results in pediatric and adult cases where (a) positive skin tests do not correlate with case history; (b) provocation tests may be considered too hazardous or inconvenient; and (c) confirmation of negative or inconclusive skin tests is needed.

FOOTNOTES

* Sigma Chemical Co., St. Louis, MO 63178.

† Fisher Scientific Co., Fairlawn, NJ 07410.

‡ New England Nuclear Corp., Boston, MA 02118.

§ Center Laboratories, Port Washington, NY.

¶ 25 mmol/l Tris[hydroxymethyl]-aminomethane·HCl, 120 mmol/l NaCl, 5.20 mmol KCl, 0.60 mmol/l $CaCl_2$, 1.00 mmol/l $MgCl_2$, and 4.00 mole/l bovine serum albumin, adjusted to pH 7.4 with dilute HCl solution.

** Pel-Freez Biological, Inc.

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