Radioimmunoassay for plasma histamine: a study of false positive and false negative values

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Summary
In order to achieve a correct diagnosis of drug anaphylaxis using a radioimmunoassay devoid of interferences, we have studied factors leading to false positive or false negative values of plasma histamine. Different steps in sample collection were studied systematically in 30 normal volunteers. False positive values were found in haemolysed samples, with histamine concentrations being correlated with haemoglobin concentrations, and where plasma was aspirated from the white-cell layer. There was no significant increase when a tourniquet or vacuum tubes were used, or when blood tubes were left at 4 °C overnight. In 12 patients who experienced an anaphylactic reaction, histamine disappeared from blood 10 times more slowly than expected. False negative values were found in two pregnant women and one heparinized patient. Histamine was remarkably stable in vitro in blood or plasma samples, whereas it disappeared rapidly when plasma from a pregnant woman or a heparinized patient was added to the sample. We conclude that false positive and false negative values are rare when using this radioimmunoassay.

Key words
Histamine. Measurement techniques, radioimmunoassay.

Materials and methods
CONTROL SUBJECTS AND SAMPLING PROCEDURE
The study was approved by the local Committee for the Protection of Volunteers for Biomedical Research and informed consent was obtained from all subjects. Blood was obtained consecutively in seven tubes, within 1 or 2 h after lunch, from each of 30 healthy volunteers (12 male) aged 27.3 (range 18-45) yr. On the left forearm, a tight tourniquet was placed 5 min before introducing a 22-gauge Vacutainer needle into an antecubital vein, and connecting it to 2-ml glass vacuum tubes (Vacutainer, Becton Dickinson). These tubes contained, in order: tube 1, ethylenediaminetetraacetic acid (EDTA); tube 2, EDTA; tube 3, heparin; last tube, called No. 7, EDTA. At the same time a fluoroethylene-propylene catheter (Abbocath T 20G) was placed, without a tourniquet, DOMINIQUE LAROCHE*, MD, PHD (Service of Nuclear Medicine); FRÉDÉRIC DUBOIS, MD, JEAN-LOUIS GÉRARD, MD, CLAIRE LEFRANÇOIS, MD, BERNARD ANDRÉ, MD, LAURENT DUBUS, MD, HENRI BRICARD, MD (Department of Anaesthesiology); MARIE-CLAUDIE VERGNAUD, MD (Department of Pneumonology); Centre Hospitalier Régional Universitaire, Caen, France. Accepted for publication: November 5, 1994.

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into a vein of the right forearm and blood was obtained by gravity through the catheter into three EDTA-containing open tubes, numbered consecutively 4 to 6 (inclusive). Tube Nos 4 and 6 were constructed from polypropylene and tube 5 from sterile glass (Vacutainer, Becton Dickinson). The whole sampling procedure took less than 5 min for each subject. Tube Nos 1–6 were placed immediately in ice-cold water and centrifuged at 4 °C and 800 g (1500 rpm) for 10 min. Thereafter, plasma was aspirated gently without approaching the white cell layer and frozen at −20 °C until assayed. Subsequently, from tube No. 2, another plasma sample was aspirated near or inside the white cell layer (tube No. 8) and frozen at −20 °C. During the course of the study, spontaneous haemolysis occurred in three samples from two subjects and catheterization failed in two others. Tube Nos 1–6, but not tube No. 7 obtained from these four subjects were rejected. Whole blood in tube No. 7 was left either at room temperature (20 °C) for 1 (n = 8) or 18 h (n = 10), or at 4 °C for 18 (n = 9) or 40 h (n = 7) before centrifugation. Furthermore, blood samples obtained from seven other volunteers were divided into three aliquots which were centrifuged at either 4 °C or 15 °C or without refrigerating the centrifuge.

SAMPLES WITH INCREASED HISTAMINE CONCENTRATIONS

Two types of samples with increased concentrations of histamine were used: plasma samples obtained in EDTA-containing tubes from patients who had anaphylactoid reactions, which were stored frozen at −20 °C, and blood samples from normal volunteers challenged in vitro with anti-IgE immunoglobulin (approximately 8 ml of blood was obtained in heparinized tubes (Vacutainer, Becton Dickinson) and incubated at 37 °C. Subsequently, 50 μl of monoclonal anti-human-IgE immunoglobulin (Immunotech) was added and incubation was prolonged for 30 min. Histamine concentrations in the plasma samples were 10–1100 nmol litre⁻¹.

Stability of histamine in whole blood
After in vitro degranulation, blood samples were separated into two aliquots. One was centrifuged immediately and the other was left for 2 h at room temperature (n = 9) or overnight (18 h) at 4 °C (n = 7). In another experiment, five blood samples were divided into three aliquots which were centrifuged at 4 °C, 15 °C or without refrigeration.

Stability of histamine in plasma
Three aliquots of plasma, obtained after in vitro degranulation of blood from five volunteers, were placed in polypropylene, polyethylene or sterile glass tubes. The tubes were left for 30 min at room temperature, then frozen at −20 °C until assayed. Plasma from three patients who experienced anaphylactoid reactions and plasma obtained after in vitro degranulation were left in polypropylene tubes at room temperature for up to 48 h or at 4 °C for up to 72 h, before measurement of histamine. Four plasma samples obtained during anaphylactoid reactions were subjected to five freeze–thaw cycles, with measurement of histamine after each cycle. Seven samples obtained during anaphylactoid reactions were diluted 1/10 or 1/50 in kit buffer before assay. The remaining volume of diluted plasma was frozen at −20 °C and re-assayed 1 week later.

IN VIVO HISTAMINE METABOLISM

Patients with increased histamine concentrations
Two consecutive blood samples were obtained in EDTA-containing tubes from 12 patients (six male), aged 21–82 yr, during anaphylactoid reactions for measurement of plasma histamine and tryptase. All had immediately reacted after i.v. administration of a drug (neuromuscular blocker, n = 8; beta-lactam, n = 1; gelatine, n = 1; protamine, n = 1; aprotinin, n = 1). Ten patients recovered well but two who had experienced severe and prolonged anoxia died some weeks later.

IN VITRO HISTAMINE METABOLISM

In order to evaluate the rate of histamine metabolism in the sample tube during specific clinical conditions, a plasma sample containing histamine 300 nmol litre⁻¹, obtained through in vitro degranulation, was mixed (vol/vol) with plasma from a pregnant woman (34 gestational weeks), from a heparinized patient (after 40 min of extracorporeal circulation) or from a normal control. The mix was incubated at 37 °C for 80 min or at 4 °C for 22 h.

ASSAYS

Histamine was measured after alkylation by radioimmunoassay [8] (Immunotech, Luminy, France). In this study, the lower limit of detection of the assay was 0.5 nmol litre⁻¹ (1 nmol litre⁻¹ = 0.11 ng ml⁻¹). The usual limit for pathological values is 9 nmol litre⁻¹ [3]. The within-assay coefficient of variation is 10% for a plasma sample containing histamine 10 nmol litre⁻¹. For each control subject, the eight tubes were processed within the same assay series. In haemolysed samples, haemoglobin was measured by differential spectroscopy (normal range < 5 μmol litre⁻¹) [14]. Tryptase was measured by an immunoradiometric assay [15] and urinary methylhistamine by a competitive radioimmunoassay [16] (Kabi Pharmacia Diagnostics, Uppsala, Sweden). The normal range is < 2 μg litre⁻¹ for tryptase and < 380 μmol per mole of creatinine for urinary methylhistamine [17]. All measurements were made in duplicate. When necessary, samples were diluted in the buffer provided with the kit.

STATISTICAL ANALYSIS

Data are expressed as mean (SD). Means were compared by analysis of variance. Values were compared by Wilcoxon or Kruskal–Wallis tests when
the number of compared samples was small. Linear correlation was performed using the method of least squares. \( P < 0.05 \) was considered statistically significant.

### Results

**SEARCHING FOR FALSE POSITIVE RESULTS IN NORMAL CONTROLS**

**Blood sampling**

Sampling conditions are summarized in table 1. Individual histamine concentrations measured in the 30 volunteers are shown in figure 1, according to the different sampling conditions.

Mean histamine concentrations in tubes centrifuged immediately were, respectively (\( n = 30 \)): tube 1, 2.04 (1.02) (range 0.7-5.2) nmol litre\(^{-1} \); tube 2, 1.80 (0.80) (0.8-3.7) nmol litre\(^{-1} \); tube 3, 2.04 (0.72) (1.1-3.7) nmol litre\(^{-1} \); tube 4, 2.06 (1.11) (0.7-6.2) nmol litre\(^{-1} \).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Summary of the different withdrawal conditions used for obtaining blood samples in 30 volunteers for measurement of plasma concentrations of histamine. X = condition fulfilled, G = glass, P = polypropylene, H = heparin; E = EDTA.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling condition</td>
<td>Tube number</td>
</tr>
<tr>
<td>First ml discarded</td>
<td>1</td>
</tr>
<tr>
<td>Tourniquet</td>
<td>X</td>
</tr>
<tr>
<td>Catheter</td>
<td>X</td>
</tr>
<tr>
<td>Vacuum</td>
<td>X</td>
</tr>
<tr>
<td>Tube material</td>
<td>G</td>
</tr>
<tr>
<td>Anticoagulant</td>
<td>E</td>
</tr>
<tr>
<td>4 °C</td>
<td>X</td>
</tr>
</tbody>
</table>

Table 2 shows the results of the analysis of variance performed on the histamine concentrations in tubes centrifuged immediately and those left at room temperature for various times. The mean histamine concentrations in tubes left at room temperature for 4.5 h were 2.01 (0.92) nmol litre\(^{-1} \); tube 5, 1.90 (0.94) (0.4-5) nmol litre\(^{-1} \); and tube 6, 2.19 (0.91) (0.5-3.9) nmol litre\(^{-1} \). The overall mean was 2.01 (0.92) nmol litre\(^{-1} \). Analysis of variance showed no significant difference between means.

One of the patients (represented by filled triangles in fig. 1) had increased values in tubes 4 and 5 (6.2 and 5 nmol litre\(^{-1} \), respectively). The values in the other tubes were 1.4-3.3 nmol litre\(^{-1} \).

**Stability of histamine in whole blood**

Blood tube No. 7 was left at room temperature for 1 or 18 h, or at 4 °C for 18 or 40 h. Histamine concentrations were compared with concentrations in tube No. 2 centrifuged immediately from the same patient. There was no significant difference between histamine concentrations in tube Nos 7 and 2 except for a slight increase after 18 h at room temperature (2.67 (1.07) vs 1.81 (1.02) nmol litre\(^{-1} \); Wilcoxon test, \( P < 0.01 \)).

**Centrifuge temperature**

Blood aliquots from seven subjects were centrifuged at different temperatures: mean histamine concentrations were 3.66 (1.64) nmol litre\(^{-1} \) (range 2.0-7.4 nmol litre\(^{-1} \)) at 4 °C; 1.81 (0.48) (1.1-2.5) nmol litre\(^{-1} \) at 15 °C; and 2.41 (0.57) (1.6-3.4) nmol litre\(^{-1} \) without refrigeration (fig. 2). The difference was significant (\( P < 0.01 \), Kruskal-Wallis test). In one case, a large increase in plasma histamine concentration was observed at 4 °C. When this patient was excluded, the difference was still significant (\( P < 0.02 \)); values found after centrifugation at 15 °C were consistently smaller than in the other conditions.

**Plasma aspiration**

Histamine concentrations exceeded 9 nmol litre\(^{-1} \) in six of 30 samples obtained near the buffy coat (tube No. 8). The concentration range was 0.6-150 nmol litre\(^{-1} \). The mean concentration was 12.3 (28.3) nmol litre\(^{-1} \), whereas in plasma aspirated from the same tube far from the buffy coat (tube No. 7) it was 1.5 (1.5) nmol litre\(^{-1} \).
Histamine: false positive and false negative values

Stability of histamine in plasma. The effect of storage of plasma in polypropylene, polyethylene or sterile glass tubes was studied. No significant difference was found for histamine concentrations in the different tubes (Kruskal–Wallis test).

No significant difference was found between histamine concentrations measured immediately after centrifugation or after incubation of plasma at room temperature for up to 48 h, or at 4 °C for up to 72 h (Kruskal–Wallis test).

Five successive freeze–thaw cycles were performed for four plasma samples. There was no significant difference between the values after 1, 2, 3, 4 or 5 cycles (Kruskal–Wallis test). Moreover, a plasma sample has been stored frozen at −20 °C for 3 years, with histamine concentrations of 1116 nmol litre⁻¹ after the reaction, 1060 nmol litre⁻¹ 4 months later and 1047 nmol litre⁻¹ 3 years later.

Diluted plasma samples were assayed immediately after the dilution process or frozen at −20 °C before assay. The measured histamine concentrations were smaller in frozen diluted samples (Wilcoxon test, P < 0.05); the difference was greater when the initial concentration was smaller (fig. 4).

In vivo histamine metabolism

Blood was obtained twice from 12 patients who experienced anaphylactic reactions. The selection criteria were an increase in histamine and tryptase concentrations during the reaction. All had increased plasma histamine concentrations in the first sample (853 (1343) (38–5100) nmol litre⁻¹), obtained 5–60 min (30 (21) min) after the onset of the reaction, and plasma tryptase concentrations were 7–500 μg litre⁻¹. The mean histamine concentration in the second sample was 65 (95) (5–300) nmol litre⁻¹, 30–200 min (72 (43) min) after the first. As shown in figure 5, all but one patient had persistently increased histamine concentrations 60 min after the onset of the reaction.

Clinical cases with false negative results

Case 1. A pregnant woman, aged 35 yr, was administered suxamethonium and thiopentone for Caesarean section. Immediately, erythema, hypotension and mild bronchospasm occurred. Blood was obtained 30 min after the onset of the reaction. Plasma histamine concentration was < 0.5 nmol litre⁻¹, whereas plasma tryptase was 35 μg litre⁻¹ (normal

2), it was 1.8 (0.8) nmol litre⁻¹ (Wilcoxon test, P < 0.05). The measured value was related to the level of plasma reached by the pipette. The value remained unchanged when approaching the cell layer until the tip of the pipette entered the white cell layer, then the value increased dramatically.

Spontaneous haemolysys

Two control subjects were rejected from the study because haemolysis occurred, respectively, in 1 or 2 samples: histamine concentrations were 5.5, 12.4 and 11.4 nmol litre⁻¹, whereas the mean values in the other tubes from the same subjects were, respectively, 1.74 (0.48) and 3.18 (0.70) nmol litre⁻¹. In order to evaluate the effect of haemolysis, we measured histamine and haemoglobin concentrations in 10 spontaneously haemolysed plasma samples obtained for other purposes. Histamine concentrations ranged from 2.1 to 32 nmol litre⁻¹ and haemoglobin from 3.2 to 49 μmol litre⁻¹. Two samples had increased haemoglobin (13.5 and 33.6 μmol litre⁻¹, respectively) but normal histamine concentrations (2.8 and 5.5 nmol litre⁻¹). A linear correlation (r² = 0.992) was found for the eight other pairs of values (fig. 3).

Figure 3 Linear correlation between histamine and haemoglobin concentrations in eight spontaneously haemolysed plasma samples (r² = 0.992).

Figure 4 Effect of freezing diluted plasma samples at −20 °C on histamine concentrations (values corrected for dilution). 1 = Immediate assay, 2 = assay after freezing.

SEARCHING FOR FALSE NEGATIVE RESULTS

In vitro stability studies

Stability of histamine in whole blood. We compared histamine concentrations in blood aliquots centrifuged immediately after degranulation with concentrations in aliquots left for 2 h at room temperature or 18 h at 4 °C before centrifugation. No significant difference was found between the values in tubes centrifuged immediately and in the other tubes (Wilcoxon test). Similarly, the effect of temperature during centrifugation was studied at 4 °C, 15 °C or without refrigeration. The Kruskal–Wallis test showed no significant difference between histamine concentrations in the three aliquots.

Stability of histamine in plasma. The effect of storage of plasma in polypropylene, polyethylene or sterile
value < 2 μg litre⁻¹). A second blood sample, obtained 1 h later, contained histamine < 0.5 nmol litre⁻¹ and tryptase 47 μg litre⁻¹. Urine samples contained methylhistamine 1180 μmol per mole of creatinine (normal range < 380 μmol mol⁻¹). Eight weeks later, skin tests and specific IgE measurements showed evidence of anaphylaxis to suxamethonium.

Case 2. A pregnant woman, aged 32 yr, had a Caesarean section for a twin pregnancy. She received suxamethonium and thiopentone and developed acute bronchospasm, followed by diffuse urticaria and severe hypotension 10 min later. A blood sample was withdrawn immediately, but left at 4 °C overnight. Plasma histamine concentration was 0.6 nmol litre⁻¹ and tryptase 63 μg litre⁻¹. In a second sample, 1 h later, histamine concentration was 0.6 nmol litre⁻¹ and tryptase 86 μg litre⁻¹. Urinary methylhistamine concentration was 2160 μmol per mole of creatinine. Neuromuscular blocker-specific IgE was present in high concentrations and skin tests were positive for suxamethonium.

Case 3. A 56-yr-old man was administered fentanyl, flunitrazepam, thiopentone, pancuronium, Haemacell and heparin before extracorporeal circulation (ECC) for coronary bypass operation. When ECC was started, acute hypotension was observed, which was treated by acceleration of ECC, ephedrine and fluid perfusion. A blood sample was obtained within 10 min of the reaction. Histamine concentration was 1.5 nmol litre⁻¹ and tryptase 4.3 μg litre⁻¹. One hour later, histamine concentration was < 0.5 nmol litre⁻¹ and tryptase 9.4 μg litre⁻¹. Urinary methylhistamine concentration was 1665 μmol per mole of creatinine. The molecule responsible was not identified.

In vitro histamine metabolism

We studied the rate of disappearance of histamine from a plasma sample containing histamine, 300 nmol litre⁻¹, mixed with plasma from a pregnant woman (mix No. 1), from a heparinized patient (mix No. 2) or from a normal subject (mix No. 3). When incubation was performed at 37 °C, histamine disappeared from mix Nos 1 and 2 with an apparent half-life of 20 min (fig. 5). At 4 °C, the half-life was 130 min for mix No. 1 and 110 min for mix No. 2. After 22 h at 4 °C, histamine concentrations were 4.2 nmol litre⁻¹ in mix No. 1 and 1.8 nmol litre⁻¹ in mix No. 2. Histamine concentrations did not change in mix No. 3 during the whole experiment.

Discussion

The aim of this study was to determine which conditions could lead to false positive or false negative values for plasma concentrations of histamine when using the radioimmunoassay, in order to achieve an accurate diagnosis of anaphylaxis. The monoclonal antibody used here is highly specific for histamine; the cross-reactivity ratio for N-methylhistamine is 1/14500 and for histidine 1/250000 [8]. Numerous compounds known to be potentially capable of interfering with measurements of histamine failed to interfere in this assay [9]. This assay compares favourably with other techniques, according to a quality control study [18]. It was shown to have equal sensitivity and accuracy as the fluorimetric assay, and to be convenient to use for plasma samples at the Munich Consensus Development Conference on Histamine Determination [19]. According to the manufacturer, the sensitivity is 0.2 nmol litre⁻¹. In this study it was 0.5 nmol litre⁻¹ (78 determinations over a 2-yr period). The variation coefficient was 10% for a concentration of 10 nmol litre⁻¹ (21 determinations) and in the normal concentration range [9]. False positive results were investigated in normal volunteers after lunch, as food may increase plasma histamine concentrations. The mean histamine concentration was 2.01 (0.92) nmol litre⁻¹ in 13 fasting controls [10] (P < 0.01). Other laboratories reported values of 3.33 (1.62) nmol litre⁻¹ [8] in 14 non-allergic subjects or 1.74 (0.72) nmol litre⁻¹ [9] in 40 normals using the same reagents.

From our results we suggest that a simplified sampling procedure may be used comprising direct venous puncture and sampling into glass vacuum tubes containing either EDTA or heparin. The
Histamine: false positive and false negative values

threshold usually reported for pathological levels, obtained with the fluorometric method, is 1 ng ml⁻¹; that is 9 nmol litre⁻¹ [3]. This value appears to be appropriate for the radioimmunoassay also. The stability of basophils in blood has been studied at room temperature and during centrifugation at various temperatures. Although there was a tendency for histamine to increase when blood was left overnight at room temperature or for 2 days at 4 °C, the samples could be left for 1 h at room temperature or for 1 night at 4 °C without significant changes in histamine concentrations, indicating that histamine does not leak from basophils readily enough to give false positive values. The values obtained after centrifugation at different temperatures were very close, except when the centrifugation temperature was 4 °C. This could be explained by poor temperature regulation, with variations of ±2 °C or 3 °C, and potential freezing leading to cell lysis. Thus we suggest the use of an intermediate temperature of 15 °C. Although use of a non-refrigerated centrifuge is possible, it induces a slight increase in histamine concentrations. As centrifugation was performed at 4 °C for tubes 1–6, it is possible that the higher values obtained apparently at random in some samples could result from an excessively low temperature.

During this study, false positive values were observed in two different situations: (i) when spontaneous haemolysis occurred (three of 224 samples, i.e. 1.2 %), histamine concentrations ranged from normal to moderately increased. Histamine increased concomitantly with an increase in haemoglobin in most cases. Spontaneous haemolysis during sampling affects a few cells: haemoglobin concentrations are in the micromolar range in haemolysed plasma and millimolar in whole blood, and histamine concentrations in haemolysed plasma are 20–50 times smaller than in whole blood. However, the red colour is readily noticed. Measurements of haemoglobin may be useful in interpreting an increased histamine concentration in a reddish sample obtained after an anaphylactoid event; (ii) when plasma was aspirated from the upper cell layer, a dramatic increase in histamine concentration occurred. In such cases, no apparent change was noticed in the tested plasma, contrary to the haemolysed samples.

We were also interested in detecting possible false negative results caused by the disappearance of histamine either in vivo or in vitro. The in vivo half-life of plasma histamine has been calculated as 1–2 min in volunteers receiving histamine infusions [4] and was shown to be in the minute range during challenges [20] or protocols [21]. All of these values were obtained in subjects with moderately increased peak histamine concentrations. We selected for this study 12 patients with increased concentrations of plasma histamine during anaphylactic reactions to i.v. administered drugs. All had increased concentrations of tryptase, which is a specific marker of mast cell activation virtually absent from normal serum [22]. The initial histamine concentrations were increased markedly in the majority of patients (38–5100 nmol litre⁻¹). As no intermediate values were obtained, the exact half-life could not be calculated; however its value was probably closer to 20 than to 2 min. The longer half-life could be explained by saturation of enzymatic metabolism, by continued release or by reduced clearance because of the disease process. The combination of a high initial histamine concentration and a longer half-life than expected allowed histamine to remain at pathological levels 1 h after the onset of reaction in 11 of 12 patients.

The stability of histamine was studied further in vitro. As large quantities of different plasma obtained during anaphylactoid reactions were not easily available, in some experiments we used normal blood challenged in vitro with anti-IgE. On such occasions the anticoagulant was heparin instead of EDTA. However, heparin has no effect in vitro on histamine metabolism and we have verified in one patient that histamine concentrations were identical in EDTA and heparin-containing tubes obtained at the same time after an anaphylactoid event. Whole blood could be left for 2 h at room temperature or overnight at 4 °C without significant change in histamine concentration. Blood could be centrifuged at 4 °C, 15 °C or without refrigeration, without changing histamine values. It had been suggested that histamine could be adsorbed on tube walls, especially where sterile glass is used, and that some contaminants of the tube material could interfere with the assay [23], but we could not confirm this. It is likely that contaminants interfered with light emission during the fluorimetric assays, whereas radioimmunoassays are not affected by such conditions, as they are highly specific for the measured molecule. Dyer and colleagues reported that histamine added to normal plasma was stable for 30 min at room temperature or at 4 °C, but did not experiment further [24]. In this study, endogenous histamine was stable in separated plasma for 48 h at room temperature or 72 h at 4 °C.

It is usual to suggest that one should not re-freeze plasma samples that have previously been frozen. However, five freeze–thaw cycles did not change histamine concentrations in this study. When diluted plasma samples were frozen, a dramatic decrease in histamine concentrations was observed. The radioimmunoassay used in this report proceeds through an alkylation step leading to stable alkylated histamine [8]. When diluted histamine samples were alkylated before freezing, there was no decrease in histamine concentration (results not shown). Thus, when in vitro histamine-release tests are performed from diluted blood samples, supernatants should be either assayed immediately or alkylated before freezing if the assay cannot be performed immediately.

False negative values have been identified in two different clinical conditions: pregnancy and extracorporeal circulation. Tryptase concentrations are increased in patients undergoing anaphylactoid reactions [10, 25]. Thus the finding of increased concentrations of tryptase in each of our three patients indicated mast cell degranulation, and obviously in vitro histamine release, which was confirmed by increased methylhistamine concen-
trations in urine. There are two reasons for the low value of histamine: a shorter half-life in vivo and in vitro degradation. Several reports have shown that diamine oxidase activity increases during pregnancy, with maximum activation during the third gestational trimester [26, 27]. It increases to similar levels 30 min after injection of large doses of heparin [28]. In patients administered low doses of heparin, the effect appears to be dose-dependent [29]. We tried to appreciate the in vivo catabolism of histamine under similar clinical conditions: mixing plasma containing large quantities of endogenous histamine with plasma from a pregnant woman or a heparinized patient led to the disappearance of histamine from the sample within 80 min at 37 °C, or 1 night at 4 °C, whereas histamine was remarkably stable in plasma from subjects with normal metabolism. Our results confirm those of Morel and Delage who showed that exogenous histamine added to plasma from a pregnant woman disappeared from the sample within 10 min whereas alkylated histamine did not [8]. These findings indicate that precautions should be taken in order to avoid false negative values for histamine in pregnant women and heparinized patients: blood should be refrigerated and plasma frozen as soon as possible, preferably after alklylation of histamine. In contrast, patients with normal metabolism of histamine do not require such precautions, as histamine is remarkably stable in their plasma samples.

This study has shown that a great number of precautions used with the fluorimetric assay are not necessary with the radioimmunoassay, and that false positive results are rare. Moderately increased concentrations (< 50 nmol litre⁻¹) may be found in haemolysed samples. High values are found where plasma is aspirated from the white cell layer. Thus laboratory staff must be well informed of the dramatic consequences in subsequent diagnosis. During anaphylactic events, histamine disappeared from plasma at a slower rate than expected, and high values were found 1 h, and in some patients 2 h, after the onset of the reaction. This allows emergency treatment to be administered before sampling is performed. Clinical conditions where histamine metabolism is activated are rare. They include pregnancy and high-dose heparinization. In such cases false negative values are likely to occur. Plasma tryptase should be measured in order to ensure an accurate diagnosis, although the clinical sensitivity of this marker is lower than that of histamine [17]. Histamine measurements should be undertaken every time a clinical reaction suggesting anaphylaxis occurs after drug administration. When resuscitation is unsuccessful or after-effects are anticipated, such measurements may give forensic evidence [11]. Where the reaction mechanism is unclear, they may be helpful. Furthermore, during drug or food challenges they can be of great interest for linking together mechanisms and symptoms. As routine, reliable techniques are available, and as false negative, and also false positive, results are rare and well documented, there is no justification, apart from cost, for not performing histamine measurements every time drug anaphylaxis is suspected.

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References

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