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Note

Histamine analysis on a single column amino acid analyzer

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Histamine toxicity, also called scombroid food poisoning, is a well-recognized syndrome which appears after eating spoiled, histamine-containing tuna and related fish[1–5]. The associated symptoms include flushing of facial areas, dizziness, cramps and nausea. Histamine is formed from free histidine by action of histidine decarboxylase. Although histamine in very small amounts is a normal body constituent[6], ingestion of histamine-containing foods can be hazardous. For this reason, accurate, fast and inexpensive methods are needed for monitoring the histamine content of foods. Various procedures for measuring the histamine content of foods have been proposed[7–18]. These are summarized by Arnold and Brown[2]. They include methods based on fluorometry, colorimetry, gas, ion-exchange and thin-layer chromatography. A major disadvantage of most of these is that histamine has to be separated before analysis. Since single-column amino acid analyzers are now widely used, we examined the possibility of using standard amino acid analysis techniques to measure histamine along with the amino acids present in hydrolysates of casein with added histamine and in tuna. The results show possible advantages of this method.

EXPERIMENTAL*

Histamine · 2HCl was obtained from Sigma (St. Louis, MO, U.S.A.). Spoiled tuna was a gift from Professor L. F. Bjeldanes, Department of Nutritional Sciences, University of California at Berkeley. This sample has been implicated in an outbreak of scombroid poisoning[5].

Amino acid analyses

A weighed sample of Animal Nutrition Research Council (ANRC) casein (5 mg + 1.6 μmol/ml histamine) or tuna fish (50 mg) was hydrolyzed in 10 ml of 6 *N* HCl in a commercial hydrolysis tube. The tube was evacuated, placed in an acetone–dry ice bath, evacuated and flushed with nitrogen before being placed in an oven at 110°C for 24 h. The cooled hydrolysate was filtered through a disc funnel and evaporated to dryness at 40°C with the aid of an aspirator. The residue was suspended in

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water and evaporated to dryness. Amino acid analysis of an aliquot of soluble hydrolysate was carried out on a Durrum amino acid analyzer, Model D-500, under the following conditions: single-column ion exchange chromatography method; resin, Durrum DC-4A (bead diameter, 8 ± 1 μm) buffer pH, 3.25, 4.25 and 7.90; photometer, 440 and 590 nm; column, 40 cm × 1.75 mm I.D.; analysis time, 130 min. Norleucine was used as an internal standard[19].

The average histamine color factor per 10 nmol for five separate determinations with histamine concentrations ranging from 1.23–2.03 μmol/ml was 275,029 ± 1628, with a coefficient of variation of 0.592%. The color factor corresponds to a leucine equivalent (ratio) of 0.476.

Histamine in serum and urine

The procedures for sample preparation were adapted from Pickering[20]. To 1 ml of mouse serum in a centrifuge tube were added 50 mg of sulfosalicylic acid. The tube was shaken manually and centrifuged for 5 min. The supernate was filtered through a 0.45-μ Millipore filter membrane. Histamine was then added to the filtrate to a final concentration of 1.5 μmol/ml. The analysis on the amino acid analyzer was carried out with a 20-μl aliquot after a second filtration.

About 0.4 ml of mouse urine was acidified to pH 2 with 12 *N* HCl. The urine was shaken manually, centrifuged and filtered. Histamine was then added to the filtrate to a final concentration of 1.5 μmol/ml. The analysis on the amino acid analyzer was carried out with a 20-μl aliquot after a second filtration.

Fluorometric assay

The official Association of Official Analytical Chemists' (AOAC) method[21] was used: 10 g of spoiled tuna were extracted with 90 ml of methanol; the methanol extract was passed through an ion-exchange column to remove the free amino acids; the histamine in the eluate was derivatized to a fluorescent compound with *o*-phthalidicarboxaldehyde (OPT); and the derivative was assayed with a Perkin-Elmer Model 203 spectrophotometer.

RESULTS AND DISCUSSION

The available methods for determining histamine in foods usually require large samples. Special precautions are often needed to avoid interference. None of the available methods can be used to measure histamine along with the free and protein-derived amino acids in a single determination. An ideal method for histamine analysis with an amino acid analyzer should meet the following requirements: (a) the analysis should be carried out with the original sample without prior isolation of histamine by extraction or chromatographic separation; (b) histamine should be eluted in a convenient position as a separate peak; (c) the intensity of the peak should be linear with concentration; and (d) histamine should be stable to protein hydrolysis conditions.

Results in Figs. 1–3 show that assay for histamine along with amino acids can be carried out on single-column amino acid analyzer by extending the time from 95 to *ca.* 125 min. Histamine appears at *ca.* 120 min as a single, well-resolved peak, as determined with histamine added to a standard mixture of amino acids (Fig. 1), cohydrolyzed with casein (Fig. 2), and in a spoiled tuna hydrolysate (Fig. 3). The

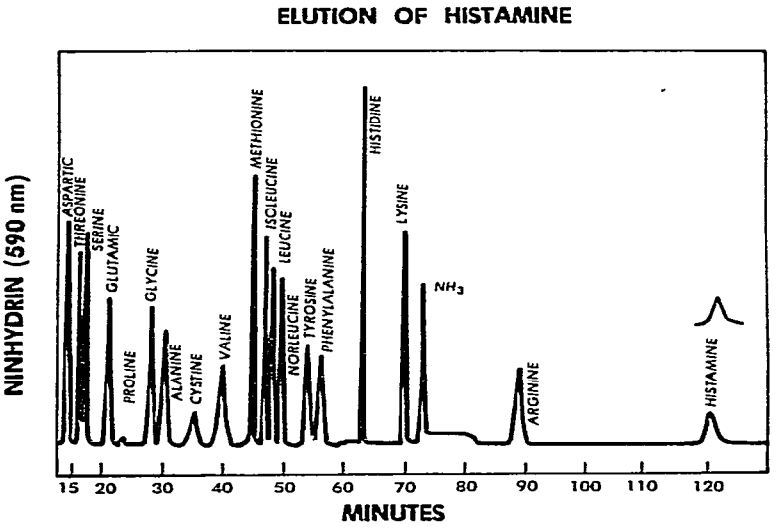

Fig. 1. Elution of histamine (0.86 μmol/ml) added to a standard mixture of amino acids. The 440 nm histamine peak is shown above the 590 nm peak.

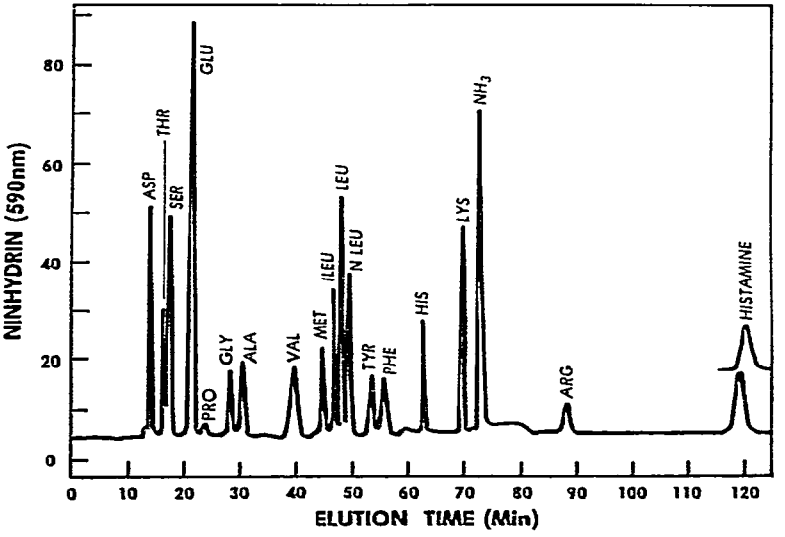

Fig. 2. Elution position of histamine cohydrolyzed with casein [5 mg casein + histamine (1.6 μmol/ml) hydrolyzed as described in the Experimental section].

calculated recovery of histamine from the cohydrolysate shown in Fig. 2 was 98.9%. This result shows that histamine is stable under acid conditions used for protein hydrolysis. The area of the histamine peak was proportional to concentration, as determined by separate analyses at five different concentrations (Fig. 4). The equation for the linear plot shown in Fig. 4 has a coefficient of determination (R^2) of 0.999 (n = 5). The lower limit of sensitivity of the analysis is estimated to be *ca.* 1 nmol of histamine on the column.

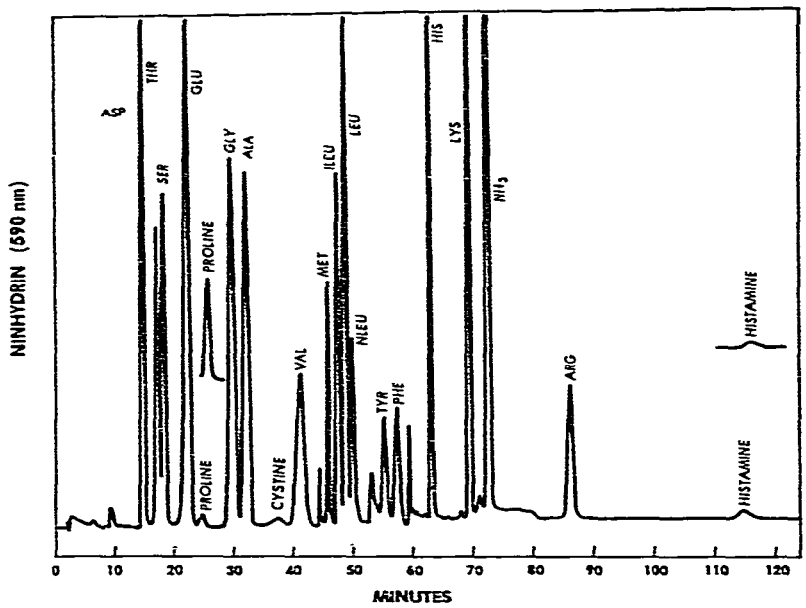

Fig. 3. Elution position of histamine in a standard amino acid hydrolysate of spoiled tuna.

Studies of the effects of varying buffer pH revealed that the elution time of histamine can be reduced to *ca.* 80 min by using a single, pH 7.9 citrate buffer as eluent instead of the three-buffer system described in the Experimental section (Fig. 5). This modification causes some of the amino acid peaks (but not histidine, which eluted at *ca.* 12 min) to coalesce on the chromatogram, permitting analysis of histamine but not most of the other amino acids.

The histamine value of spoiled tuna (154.0 mg/100 g) obtained by fluorescence analysis is somewhat lower than the value (165.7 mg/100 g) obtained by amino acid analysis of a tuna fish hydrolysate. The lower value could be due to an incomplete extraction of histamine in the AOAC method; inhomogeneity of histamine distribution in the tuna fish, especially in view of the fact that the AOAC method requires 10 g of tuna and the amino acid analysis procedure only 50 mg; or a combination of errors of both methods.

To assess the potential of the method for clinical and nutritional studies, the elution behaviour of histamine added to mouse serum and urine was also examined. Analyses of histamine added to mouse serum and urine show that components in the two physiological liquids do not affect the elution position of histamine on the short column.

The following biogenic amines (cadaverine, putrescine, tryptamine, tyramine, spermine and spermidine) do not seem to interfere with the histamine analysis since they were not found to elute between 0 and 160 min under the standard amino acid analysis conditions or with the pH 7.9 buffer.

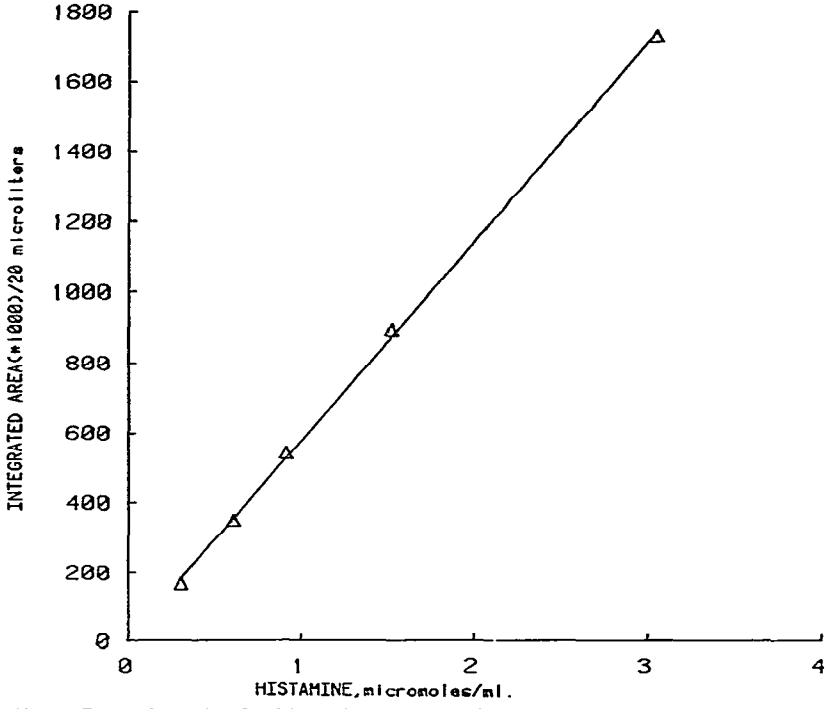

Fig. 4. Beer's law plot for histamine on an amino acid analyzer.

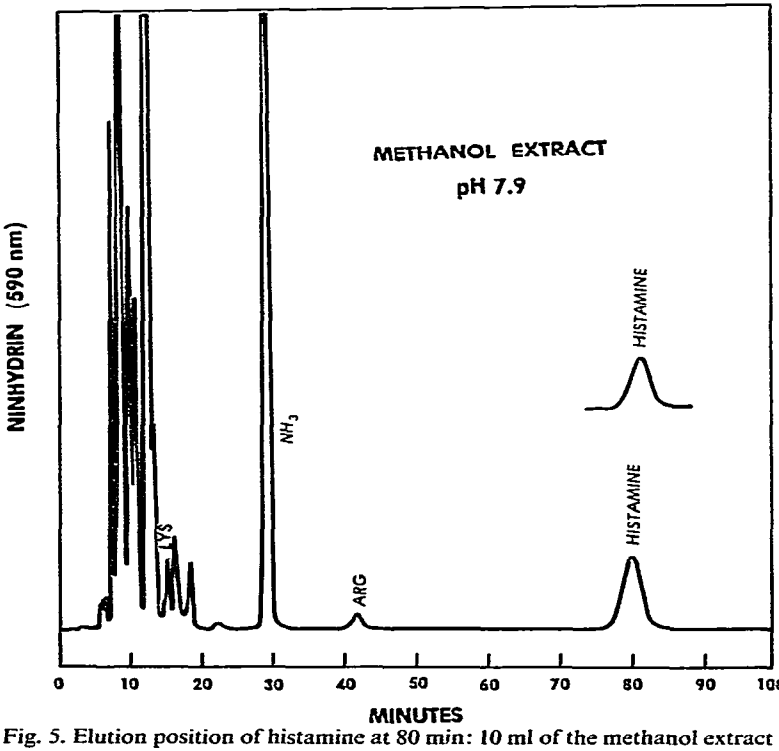

Fig. 5. Elution position of histamine at 80 min: 10 ml of the methanol extract were evaporated to dryness; the residue was dissolved in 1 ml of pH 2.2 buffer; 20 μl of this solution were applied to the column and eluted with pH 7.9 citrate buffer only.

CONCLUSIONS

The amino acid analysis method for histamine complements currently available procedures and may offer certain advantages. For instance, special extraction and purification techniques for histamine need not be applied since histamine can be measured in the presence of other amino acids. This may be important for comparing histamine to histidine (or to other amino acids) as a measure of food spoilage due to decarboxylation of histidine (or other amino acids). Application to nutritional and clinical studies designed to measure histamine along with free amino acids in body tissues and fluids should also be possible. Another advantage is that the analysis is automated, permitting after hydrolysis the sequential unattended assay of as many as 80 samples.

In summary, the described assay for histamine on a single-column amino acid analyzer complements available procedures and may have advantages in some applications. The method may be of special value for laboratories that do not have fluorometers.

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