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DETERMINATION OF
5-HYDROXY-INDOLACETIC-ACID (5-HIAA)
IN URINE AND NASAL
MUCUS IN PROVOKED ALLERGIC RHINITIS

By

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The disturbances in both vascular tone and permeability and also in glandular secretion of the nasal mucosa in patients with acute allergic rhinitis have generally been related to a number of substances acting on the vessels and glands; these include 5-HT (5-Hydroxy-tryptamine, serotonin, enteramine) and its metabolite 5-HIAA (5-Hydroxy-indolacetic acid), produced by the enterochromaffin system (*Erspamer*, 1961).

A 5-HT solution, administered in drops, has been shown to cause an acute nasal vasomotor crisis in normal individuals and also in patients with nasal allergy (*Celestino & Tripodi* 1965). However, 5-HT in a spray did not provoke any symptoms in patients with nasal allergy (*Melon & Lecomte* 1967).

Increased serum levels of 5-HT have been found in individuals with asthma and allergic rhinitis (*Mac Haffie et al.* 1960, *Hauna* 1961). It has been assumed that 5-HT is released from circulating platelets (*Rocha & Silva* 1950). Some authors have occasionally observed a decrease in the number of platelets after subcutaneous injection of allergens

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(*Hoigné & Storck 1953, Johansson et al. 1960*) and also after topical nasal administration in patients with allergy (*Celestino & Cerqua 1966*).

Investigations of the urinary excretion of 5-HIAA have given contradictory results. Increased amounts of 5-HIAA in 24 hour urine specimens were found in 12 out of 18 patients with bronchial asthma (*Pipitone et al. 1963*). The severity of symptoms, in patients with respiratory allergy showed a correlation with the 24 hour 5-HIAA excretion in a series investigated by *Vaccarezza & Bochi (1963)*. However, these results do not agree with data previously reported by *Band et al. (1961)* who, in a small series, found normal values of the 24 hour urinary 5-HIAA excretion after spontaneous or provoked crises.

Christensson & Ekwall (1960) measured the urinary excretion of 5-HIAA during two consecutive 2-hour periods in 15 patients with hay fever before and after provocation. A significant increase occurred only in two subjects; repeated testing in these two patients showed no increase.

5-HIAA has also been determined in the sputum of asthmatic patients; the substance has seldom been found during asymptomatic periods. Higher concentrations were present in bronchial secretions only during attacks (*Levy et al. 1961*).

The somewhat contradictory results of the 5-HIAA excretion in patients with allergic rhinitis initiated the present investigation. The purpose of the investigation has been to determine:

- 1) the urinary excretion of 5-HIAA in short time intervals after provocation tests in patients with allergic rhinitis
- 2) the number of circulating platelets before and after provocation
- 3) the amount of 5-HIAA in nasal secretions before and after provocation.

MATERIAL

14 patients, 5 men and 9 women, aged between 15-35 years were investigated. During the spring or summer months all had previously complained of allergic nasal discomfort which was evoked by pollens. In 7 patients the releasing allergen was grass pollen, in 7 birch pollen. The diagnosis was confirmed in all patients after a full case history, skin-tests and provocation experiments.

During the period when the present investigation was performed (October-December 1966) all patients were free from discomfort. The provocation experiments were performed with nasal instillation of extracts of grass pollen or birch pollen. All patients developed typical allergic nasal symptoms immediately after administration.

METHOD

Experimental Procedures

1) Before provocation: blood sampling for platelet count and serum electrophoresis; urine collection for 5-HIAA and creatinine estimation; nasal mucus collection for 5-HIAA determination.

2) Provocation of a vasomotor crisis by nasal instillation of a few drops of the previously identified specific antigen.

3) Administration of 300-500 ml water to increase diuresis.

4) Collection of blood and urine samples for platelet count and 5-HIAA estimation every 20 minutes until 100 minutes after provocation. Blood for serum electrophoresis was taken 40 minutes and nasal mucus collected 20-25 minutes following provocation.

The patients were starved for at least three hours prior to testing. They were also carefully advised not to take such food as bananas, nuts or spinach in the week preceding the test, as these are known to increase 5-HIAA excretion.

Control experiments were performed in 4 patients on other occasions. The schedule was identical to that mentioned above except that provocation was not performed.

Analytical Procedures

Urinary creatinine was determined according to *Folin* (1904) as modified for the Autoanalyser® (Method N 11a, Technicon Instruments Corp., Chauncey, New York, U.S.A.), 5-HIAA in urine, by the *Hanson & Serin* method (1955). Blood platelets were counted microscopically after capillary sampling.

Paper-electrophoresis of serum proteins was performed according to *Schneider & Wallenius* (1951).

RESULTS

Platelets: The number of platelets before provocation was within the lower limit of the normal range and did not change significantly after provocation.

5-HIAA/creatinine: Since the creatinine excretion is con-

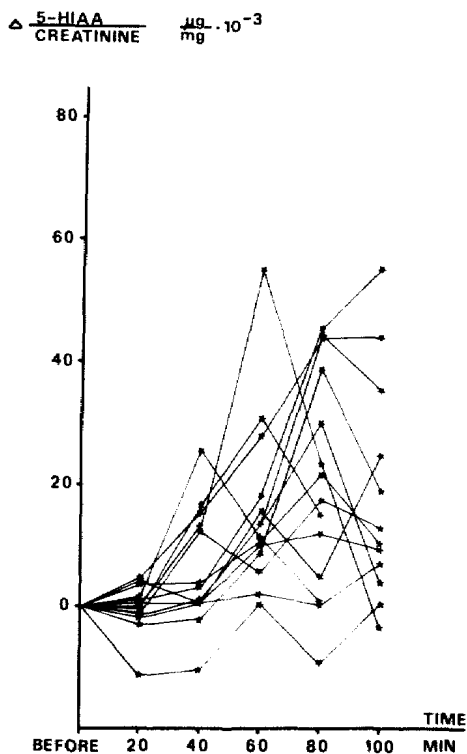


Fig. 1 a.

The changes of 5-HIAA/creatinine ratio from the original value in 14 allergic patients following provocation and waterloading. The quotient between the concomitant amounts of 5-HIAA (in μg) and creatinine (in mg) in urine volumes before and during different periods after provocation and water loading were calculated. The quotient (R-value) for the period before was subtracted from R-values after provocation and waterloading.

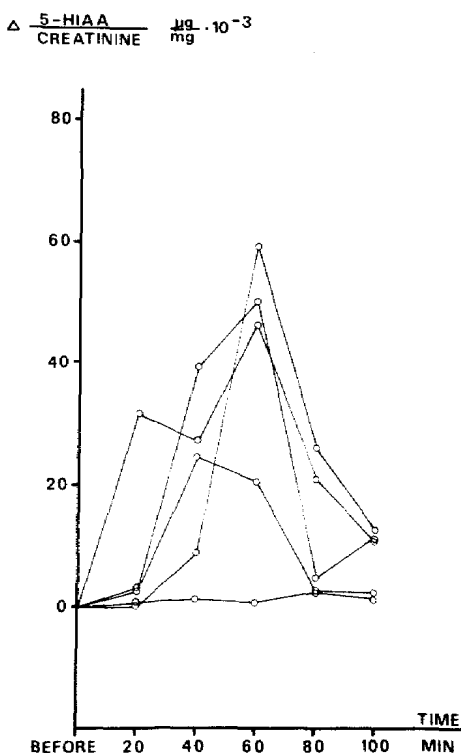


Fig. 1 b.

The corresponding results in 4 allergic patients following waterloading, but without provocation.

sidered to be relatively constant at rest, the urinary 5-HIAA/creatinine ratio (R-value) was calculated. The R-value before provocation (in allergic patients) and before administration of water (in the controls) was subtracted from the corresponding R-values after provocation and water administration respectively. Figs. 1a and 1b illustrate the relative change of the R-value with time, in the two groups.

It is evident, that the difference between the R-values increases initially in most individuals, subsequently decreasing

5HIAA/CREATININE

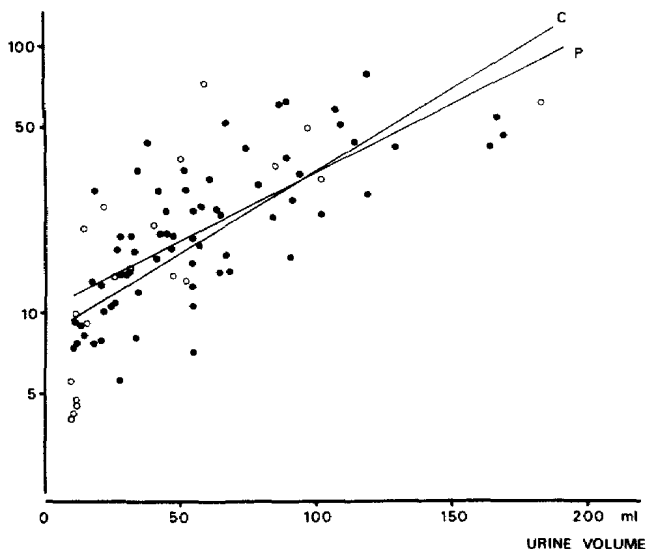
 $\frac{\mu\text{g}}{\text{mg}} \cdot 10^{-3}$ 

Fig. 2.

Correlation between the urinary 5-HIAA/creatinine ratio and urinary volume. ● = values obtained in 14 allergic patients following provocation and water-loading.

○ = the same values in 4 allergic patients following waterloading, but without provocation.

The urine was collected at intervals of 20 minutes in each subject.

The correlation fitted the expressions $y = 0.0064x + 0.9112$, $r = 0.73$, $n = 20$, $p < 0.001$ and $y = 0.0050x + 1.0175$, $r = 0.71$, $n = 68$, $p < 0.001$ for control subjects (c) and allergic patients (p), respectively.

in some subjects; though the time course differs considerably.

The mean of the highest value of difference between the R-values obtained in each individual differs significantly from zero both in the subjects with allergic rhinitis and the controls ($p < 0.01$ and $p < 0.05$, respectively).

The R-values have been correlated with the volume of urine of appropriate samples (Fig. 2). A highly significant

TABLE 1

5-HIAA (mg/100 ml) in Nasal Mucus in 5 Patients Before and 20-25 Minutes after Nasal Provocation with Pollen

Before	After
0,94	1,03
1,80	1,80
1,32	2,08
0,47	2,08
3,76	0,85

direct correlation could be established between these two variables both in the allergic patients and the controls ($r = 0.71$, $n = 68$, $p < 0.001$ and $r = 0.73$, $n = 20$, $p < 0.001$, respectively). No significant difference existed in this correlation between the control and the allergic groups of patients.

Electrophoretic protein fractions: Electrophoresis of serum proteins showed normal and constant values of the different protein fractions (albumin, alpha-1-, alpha-2-, beta-1-, beta-2- and gammaglobulin) before and after provocation.

5-HIAA in nasal mucus: Due to difficulties in obtaining a sufficient quantity of nasal mucus especially before provocation, only 5 patients could be used for measurements of 5-HIAA in nasal mucus both before and after provocation (Table 1). In two of these increased values were found after provocation, one had a decreased value and the remaining two had about the same value before and after provocation.

DISCUSSION

This investigation showed that the ratio of urinary 5-HIAA/creatinine increased after provocation not only in patients with allergic rhinitis but also in control subjects. This change is correlated with changes in the magnitude of the diuresis. Why the ratio of 5-HIAA/creatinine is correlated to the magnitude of the diuresis remains to be explained. From the theoretical point of view the result can be explained by (1) an increased excretion of 5-HIAA in the urine with increased

diuresis or (2) a decreased excretion of creatinine. As it is known that water administration increases creatinine elimination (*Monasterio* 1963, *Rehnberg* 1926) the results indicate that the relative increase in excretion with increasing diuresis is greater for 5-HIAA than for creatinine.

It seems probable that an increased 5-HIAA elimination reported by other authors after provocation, may be due to the increased diuresis following waterloading to obtain sufficient urine for analysis.

Christensson & Ekwall (1960) found an increase in the mean values for the 5-HIAA/creatinine ratio after provocation in patients with allergic rhinitis. This increase however, was caused only by an increase in 2 of the 20 patients examined. The schedule in that investigation also included waterloading. Repeated testing in these two patients showed no such increase. However, the latter tests were performed during hyposensitization and could not therefore be directly compared with the tests before treatment. The 5-HIAA/creatinine ratios were slightly higher in the present investigation than in that of *Christensson & Ekwall*. The difference is probably due to differences in the methods of determination of 5-HIAA.

The electrophoretic analysis of serum proteins was performed to study a possible increase of beta-globulins as described by *Vaccarezza* (1960) in asthmatic subjects with and without nasal symptoms. The present investigation showed no change after provocation.

This investigation is the first in which 5-HIAA in nasal mucus has been determined. The quantity of nasal secretion was however usually too small before provocation to permit analysis of 5-HIAA. Only 5 patients could therefore be examined both before and after provocation. The results were inconclusive.

SUMMARY

The blood platelet level and the excretion of 5 hydroxy-indolacetic acid (5-HIAA) were determined before and several

times after provocation in 14 patients with nasal allergy. The platelet level was constant. The urinary 5-HIAA/creatinine ratio was found to increase, but it also increased in control experiments without provocation. Both series included water-loading to obtain sufficient urine for analysis. A correlation between the urinary 5-HIAA/creatinine ratio and urine volume was found, indicating that excretion of 5-HIAA increases to a greater extent with increasing diuresis, than does creatinine excretion.

Determination of 5-HIAA in nasal mucus was performed but the number of patients with sufficient nasal mucus before and after provocation was too small to permit any conclusion regarding possible changes following an attack of allergic rhinitis.

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