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## SEROTONIN IN NASAL ALLERGY

By

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The allergic diseases of the nose are among the commonest and most intractable diseases seen in an ear, nose and throat outpatient clinic. In spite of their frequency and of the ready accessibility of the nose to clinical investigators little is known about their underlying pathophysiology, and in the absence of such knowledge there can be no rational therapy.

Recent work implicating serotonin in some anaphylactic reactions in certain animals (8) and in bronchial asthma in man, (11,14) as well as the recent availability of potent serotonin antagonists, led us to measure the serotonin concentration in the nasal mucosa of patients suffering from allergic diseases of the nose and in a control group free from any nasal or allergic disease.

### MATERIAL AND METHODS

The control group consisted of 56 subjects free from any sign of disease of the nose or paranasal sinuses and from any history of allergic disease. The allergic group consisted of 54 patients, 42 suffering from perennial nasal allergy and 12 suffering from seasonal nasal allergy.

A full personal and family history was taken from each of the patients, who were then carefully examined. For each patient 5 symptoms: sneezing, rhinorrhoea, nasal obstruction, diminution of smell sensation, and conjunctival irritation, and two signs: swelling of the nasal mucosa and nasal discharge, were looked for and recorded as either absent (—), mild (+), or severe (++) . The clinical state of each patient was summarised in a clinical index. This was obtained by adding all the + signs, each + being counted as two points for the symptoms and as five points for the signs. This

system of scoring thus gives equal weight to subjective and objective findings.

Each subject had a urine and stool analysis to exclude parasites, and a full blood count, including platelets (5). In addition a smear was taken from the nasal mucosa and stained for eosinophils (13), the smear being graded as —, +, ++ or +++ according to the number of eosinophils observed.

Finally a biopsy was taken (under local 2 per cent pantocaine anesthesia) from the nasal mucosa covering the middle third of the lower edge of the inferior turbinate. This was extracted by the method of *Amin et al.* (1), and the serotonin content estimated by its effect on the fundus of the rat stomach, as described by *Vane* (24) and modified by *Lin and Yeoh* (19). A specially designed lever resting on knife-edge supports was used (6), and amounts of serotonin as low as 50 pg ( $50 \times 10^{-12}$  g) could be estimated. Eight of the patients had polypi, and in them a biopsy was also taken from a polyp with a nasal snare and treated in the same way.

Appropriate statistical methods were used, including the Chi squared test, the "exact" test for  $2 \times 2$  contingency tables (7), the t test and its modification in cases with unequal variances (9), analysis of variance and linear regression. Results for which  $p > 0.05$  are considered not significant, those for which  $0.05 > p > 0.01$  are considered significant, while those for which  $0.01 > p$  are considered highly significant.

The results are given in the text as mean  $\pm$  standard deviation.

## RESULTS

The control group consisted of 28 males and 28 females, the perennial allergy group of 24 males and 18 females, and the seasonal allergy group of two males and ten females. This excess of females over males in the seasonal allergy group is highly significant.

The signs and symptoms in the allergic patients are summarised in Table 1. From that Table it is clear that seasonal nasal allergy is a more severe condition than perennial nasal allergy, and this is confirmed by the clinical index (Table 2), which is very significantly higher in the seasonal than in the perennial group.

The mean duration of the complaint was significantly shorter in the seasonal group ( $3.9 \pm 2.1$  years) than in the perennial group ( $6.8 \pm 4.1$  years).

In the control group the eosinophil and platelet counts

TABLE 1

*Number of allergic patients with different degrees of severity of the clinical features.*

|                                  | Perennial (42) |    |    | Seasonal (12) |   |    |
|----------------------------------|----------------|----|----|---------------|---|----|
|                                  | —              | +  | ++ | —             | + | ++ |
| Sneezing                         | 6              | 23 | 13 | 1             | 0 | 11 |
| Rhinorrhea                       | 0              | 22 | 20 | 0             | 1 | 11 |
| Nasal obstruction                | 0              | 9  | 33 | 0             | 3 | 9  |
| Diminution in<br>smell sensation | 1              | 21 | 20 | 0             | 6 | 6  |
| Conjunctival<br>irritation       | 24             | 12 | 6  | 2             | 6 | 4  |
| Swelling of<br>nasal mucosa      | 0              | 18 | 24 | 0             | 4 | 8  |
| Nasal discharge                  | 1              | 34 | 7  | 0             | 1 | 11 |

were all within the generally accepted normal range (Table 2).

The platelet count was very significantly higher in the perennial group than in the controls, while there was no difference between the seasonal group and either the perennial group or the controls. As is obvious from Table 2, the eosinophil count was very significantly raised in both allergic groups as compared with the controls. There was, however, no significant difference between the two allergic groups. None of the controls showed any eosinophils in their nasal smears, while all the allergic patients showed some, those with seasonal allergy having significantly more eosinophils than those with perennial allergy.

In the controls the concentration of serotonin in the nasal mucosa ranged from 5.0 to 57.3 ng/g, with a mean of  $27.0 \pm 14.6$  ng/g. There do not appear to be any published values with which to compare these results.

The nasal serotonin concentration showed no significant correlation with either the eosinophil or platelet counts. It did, however, show a highly significant negative correlation

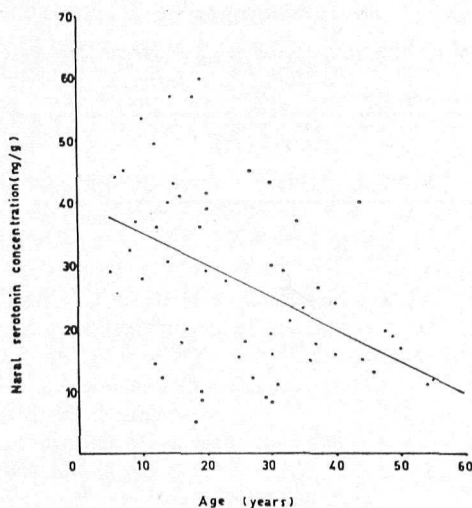


*Fig. 1.*

Relation between nasal serotonin concentration and age in control group.

( $r = -0.470$ ) with age, the regression equation being:  $S = 40.22 - 0.517 A$ , where  $S$  is the nasal serotonin concentration in ng/g, and  $A$  is the age in years (Fig. 1).

In the patients with perennial allergy, the nasal serotonin concentration ranged from 21.5 to 193.2 ng/g, with a mean of  $89.2 \pm 33.5$  ng/g, while in those with seasonal allergy it ranged from 42.9 to 175.4 ng/g, with a mean of  $111.8 \pm 37.7$  ng/g. As is obvious, the serotonin concentration in the nasal mucosa of both allergic groups is very significantly higher than that in the controls. The difference between the two allergic groups just fails to reach the 5 per cent significance level. There was no significant correlation between the nasal serotonin concentration and either age, clinical index, eosinophil or platelet counts in either allergic group.

Eight of the patients with perennial allergy had nasal polypi, and in these patients the serotonin concentration was measured in both the polypi and the mucosa covering the turbinate. The results are shown in Fig. 2, which shows the re-

TABLE 2

*Data in the control and both allergic groups*

|                        | Controls (56)   | Perennials (42) | Seasonals (12)   |
|------------------------|-----------------|-----------------|------------------|
|                        | Mean $\pm$ S.D. | Mean $\pm$ S.D. | Mean $\pm$ S.D.  |
| Age (years)            | 25.6 $\pm$ 13.2 | 24.9 $\pm$ 8.0  | 20.4 $\pm$ 7.8   |
| Duration (years)       | — —             | 6.8 $\pm$ 4.1   | 3.9 $\pm$ 2.1    |
| Clinical index         | — —             | 26.5 $\pm$ 6.5  | 34.2 $\pm$ 6.7   |
| Eosinophils/c mm       | 101 $\pm$ 81    | 498 $\pm$ 264   | 663 $\pm$ 203    |
| Platelets ('000/c mm)  | 246 $\pm$ 52    | 294 $\pm$ 91    | 272 $\pm$ 52     |
| Nasal serotonin (ng/g) | 27.0 $\pm$ 14.6 | 89.2 $\pm$ 33.5 | 111.8 $\pm$ 37.7 |

markably close correlation between the serotonin concentration in the polypi and in the mucosa covering the turbinate ( $r = 0.913$ ). The regression equation is  $P = 4.3 + 0.156 T$ ; where  $P$  is the concentration of serotonin in the polyp and  $T$  that in the mucosa covering the turbinate, both in ng/g.

## DISCUSSION

The clinical index used in this study requires a word of justification. It was devised in an attempt to summarise the entire clinical picture of each patient in a single figure, which would be more amenable to statistical treatment than an assortment of + signs (see Appendix).

The patients suffering from seasonal nasal allergy had a shorter history, a higher clinical index, more eosinophils in their nasal secretions and a higher (but not significantly so) serotonin concentration than those suffering from the perennial form. Thus we may say that they had a more severe form of the disease, but one which burned out faster.

The marked eosinophilia in both allergic groups, manifested in both the blood and the nasal secretions, is in accord with the well known eosinophilia of allergic disease. Its role is not clear, but it is possible that eosinophils antagonise histamine or serotonin (2).

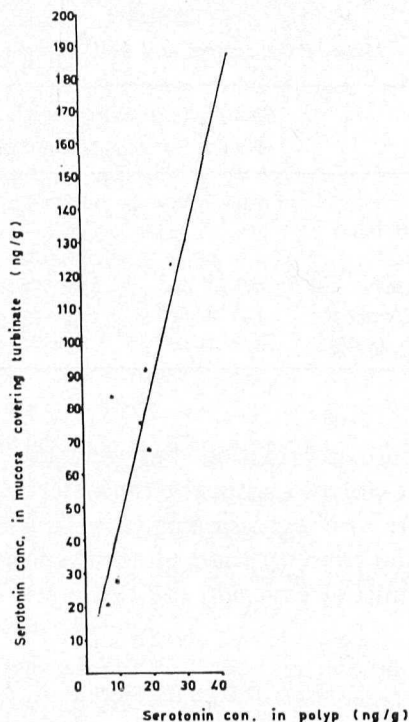


Fig. 2.

Relation between serotonin concentration in polypi and in mucosa covering the turbinate.

The meaning of the negative correlation between serotonin concentration and age in the controls is not clear, but it may be related to the fact that the incidence of allergic nasal diseases decreases with increasing age. In the present series of 54 allergic patients only three were more than 40 years old. The absence of this correlation in the allergic patients is probably due to its masking by the enormous variability due to the disease. A similar negative correlation between age and blood serotonin was observed by *Hinterberger et al.* (15).

The close correlation between the serotonin concentrations in the mucosa covering the turbinates and the polypi is very

reasonable, as both the general mucosa and the polypi partake in the same allergic process. Since the polypi contain much more water and less cellular material than the general mucosa, the fact that the concentration of serotonin in them is much lower (about one sixth) than in the general mucosa may suggest that the serotonin is more related to the cellular structures than to the interstitial fluid. It is interesting to note that a different result was obtained by *Baxter & Rose* (3) in the case of histamine. They found the concentration of histamine to be the same in the polypi and general mucosa.

The role of serotonin in clinical allergic disease, as opposed to experimental anaphylaxis, is far from clear. *Herxheimer* (14), *Hajos* (11), and *Panzani* (23) showed that some asthmatic patients responded to a serotonin aerosol by an asthmatic attack, and that this response was inhibited by known serotonin antagonists in some cases. *Marcelle et al.* (20) found the bronchoconstrictor effect of serotonin in asthmatics to be much less than that of histamine. On the other hand, *Melon & Lecomte* (21) failed to produce any local nasal reaction after spraying the nasal cavity with 5 to 10 mg serotonin. Such results have, however, little direct bearing on the role of endogenous serotonin in the production of spontaneous asthmatic attacks.

The introduction of potent serotonin antagonists was followed by their use in the treatment of allergic diseases. *Panzani* (23) used Methysergide in the treatment of asthmatic patients, but concluded that a placebo effect could not be ruled out. Other reports (4, 10, 25) were more enthusiastic, but in the absence of adequate controls it is difficult to reach any firm conclusions about the reported benefit. In any case, the fact that one of the drugs used (cyproheptadine) is also a histamine antagonist makes the interpretation of these results very difficult.

The present work has shown that in one allergic disease, at least, there is a marked increase in the serotonin concentration in the affected tissue. Whether this is specific to nasal allergy or occurs in some or all other allergic diseases, remains

to be studied. It is well known (8) that platelets carry serotonin, and it has been postulated (16) that both histamine and serotonin are released from platelets during the antigen-antibody reaction. No correlation was found between the level of serotonin in the nasal mucosa and the platelet count in the control or either of the allergic groups. In fact, the patients with perennial nasal allergy had a very significantly elevated platelet count, whereas thrombocytopaenia would have been expected.

A last question is: what is the relation, if any, between the increased serotonin concentration and the local and general allergic process? The lack of correlation between the serotonin concentration and either the clinical index (as an indicator of the local process) or the eosinophil count (as an indicator of the general allergic process) shows that this relation is unlikely to be a direct one.

#### SUMMARY

The serotonin concentration was measured in the nasal mucosa of 54 patients with nasal allergy (perennial in 42 and seasonal in 12) as well as in 56 controls. In the controls, a negative correlation was found between age and the nasal serotonin concentration, and was believed to be related to the decrease in the incidence of allergic nasal disease with increasing age.

In both groups of allergic patients, the concentration of serotonin in the nasal mucosa was very significantly higher than in the controls. It was, however, uncorrelated with either the clinical state or the eosinophil or platelet counts. This lack of correlation is believed to indicate that the relation between the raised serotonin concentration and the local and general allergic process is not a direct one.

#### APPENDIX

It is clear that a clinical index, such as that used in the present work, does help to summarize the entire clinical picture of each patient in a single figure. Many different indices may be constructed, and it is not obvious to what

extent they will agree amongst themselves, though they all aim to measure the same underlying "clinical state". It was therefore decided to calculate 4 different indices and to examine the degree to which they were correlated with each other.

The first and simplest index (C.I.<sub>1</sub>) was obtained by adding all the plus signs. *A priori* it appeared to give too much weight to the patient's subjective impression, as we recorded 5 symptoms and only 2 signs. The index used in the paper (C.I.<sub>2</sub>) was designed to overcome this apparent bias by giving an equal total weight to the signs and symptoms.

In an attempt to obtain absolutely objective indices, the first principal component of the variance-covariance matrix of the clinical features was calculated (22). This was used as a clinical index either as such (C.I.<sub>3</sub>) or after giving the signs the same total weight as the symptoms (C.I.<sub>4</sub>). The correlation coefficients between the various indices are given in Table 3.

TABLE 3  
*Correlations between the clinical indices.*

|                   | C.I. <sub>1</sub> | C.I. <sub>2</sub> | C.I. <sub>3</sub> | C.I. <sub>4</sub> |
|-------------------|-------------------|-------------------|-------------------|-------------------|
| C.I. <sub>1</sub> | 1.000             | 0.979             | 0.969             | 0.972             |
| C.I. <sub>2</sub> |                   | 1.000             | 0.929             | 0.973             |
| C.I. <sub>3</sub> |                   |                   | 1.000             | 0.976             |
| C.I. <sub>4</sub> |                   |                   |                   | 1.000             |

From this we may conclude that all "reasonable" clinical indices are highly correlated with one another. This is to be expected, since they all measure the same overall clinical process. Kendall (18) quotes his previous results (17) to show that most indices of agricultural productivity are highly correlated, and that an index obtained by ranking was strikingly correlated with the first principal component (Spearman's rank correlation coefficient was 0.99). More recently Hamilton has stated (12) that adding ratings (e.g. the number of plus signs) will give a result highly correlated with the first principal component.

We are thus justified in considering that the index used does give a valid estimate of the overall "clinical state" of each patient.

#### ACKNOWLEDGEMENT

*The serotonin standard used in the bioassay was generously supplied by Sandoz S.A., through the courtesy of Dr. Khozam.*

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