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# 5–HYDROXYTRYPTAMINE IN THE BRONCHIAL SPUTUM OF ASTHMATIC PATIENTS

*By*

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The rôle played by serotonin (5–hydroxytryptamine) in asthma has not yet been sufficiently clarified and concerning this problem there are different opinions.

Serotonin is released in the course of certain pathological reactions, mainly after anaphylactic reactions in certain animals.

Discussions have been made by *Weissbach et al.* on the rôle of serotonin in the pulmonary aspects of hypersensitivity.

*Humphrey* & *Jaques* after *in vitro* studies found that in the rabbit serotonin takes an active part in anaphylactic phenomena. Identical verifications were made by *Waalkes* & *Coburn in vivo*. *Lecomte* & *Fisher* showed that 5-hydroxytryptamine (5–HT) is thereafter catabolized and an excessive amount of 5-hydroxyindol-acetic acid (5-HIAA) is excreted in the urine.

According to *Herxheimer*, guinea-pigs submitted to aerosols of serotonin produced bronchospasm and similar findings might occur in human asthma. Indeed, human beings submitted to aerosols of serotonin showed decrease of vital capacity and respiratory flux, according to *Michelson et al.*

Serotonin is a broncho-constrictor and it is generally admitted that this is the cause of the asthmatiform symptoms in patients with malignant carcinoid.

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[1] With the technical assistance of *A. Germano Silva.*

*Christenson* & *Ekwall* found significant increase of urinary 5-HIAA in 2 patients with hay-fever.

However, *Brocklehurst* found that isolated human bronchi do not contract by the action of 5-HT but relax under high doses of the same drug; thus there is no evidence that 5-HT has any participation in the anaphylactic reaction of the human lung.

*Lecomte* thinks that serotonin has no action whatsoever in human allergy.

Considering all these facts, we think it is opportune to find out if serotonin, as represented by 5-hydroxyindol-acetic acid, is found in the sputum of asthmatic patients and if there is any quantitative difference of this substance during the attacks.

## MATERIAL AND METHODS

We used bronchial sputum of 10 asthmatic patients who had positive allergic tests for several kinds of inhalants and food. These patients constituted a relatively homogenous group with a type of mixed asthma, that is allergy and infection. One of them (No. 10 of the table No. I) showed bronchiectasis.

The patients had not eaten any kind of food that would increase the excretion of 5-HIAA, such as banana.

The volumes of sputum collected varied from 5 to 14 ml. Determination of 5-HIAA was made both during and out of attacks.

For controls we used 4 patients with other lung diseases: chronic cor pulmonale, Hamman-Rich's syndrome, smoker's bronchitis and chronic bronchitis.

The sputum was preserved in freezer at —5° C for 5 to 10 days and the method used was that of *Udenfriend et al.* with the following modifications: total volume of sputum, 5 to 14 ml with 6 ml of 2-4-dinitro-phenyl-hydrazine; the mixture was left standing for 30 minutes. Then, 25 ml of chloroform was twice added to the mixture; this was agitated for 5 minutes and then centrifuged; chloroform was poured away and 4 g of sodium chloride were added; extraction was made twice with

15 ml of ethylic ether. The mixture now was agitated for 5 minutes, centrifuged and the ether (about 30 ml) was passed to another tube where 1.5 ml of Sørensen phosphate of pH 7.0 was added and agitated for about 4 minutes; then the ether was poured away after centrifugation. To the buffer containing the substance under investigation 0.5 ml of a solution of 1-nitrous-2-naphtol reagent and also 0.5 ml of nitrous acid was added; this mixture was warmed in double boiler at 37° C for 5 minutes. The excess of dye was removed by 5 ml of ethyl acetate with agitation for 3 minutes and repeated twice. Afterwards, the colorimetric reading was made.

TABLE I

*Mg/100 ml of 5-hydroxytryptamine in the sputum of 10 asthmatic patients and in 4 controls.*

| Asthmatic patients | During an attacks | Free of an attack |
|---|---|---|
| 1. A. F. M. ..... | Undetectable | Undetectable |
| 2. A. R. M. ..... | 0.050 | 0.080 |
| 3. J. P. S. ..... | 0.228 | 1.34 |
| 4. H. S. ..... | 0.150 | 0.075 |
| 5. D. P. P. ..... | 0.330 | Undetectable |
| 6. M. L. C. ..... | 0.133 | Undetectable |
| 7. M. R. M. ..... | 0.130 | Undetectable |
| 8. M. X. R. S. ..... | 0.130 | Undetectable |
| 9. M. J. J. ..... | 0.140 | Undetectable |
| 10. A. L. ..... | 0.99 | 0.614 |
| | Control group | |
| 1. A. B. F. ..... | Chronic "cor pulmonale" | Undetectable |
| 2. J. H. ..... | Smoker's bronchitis | 0.688 |
| 3. S. L. F. ..... | Chronic bronchitis | Undetectable |
| 4. C. A. M. ..... | Hamman-Rich's disease | 0.863 |

## RESULTS

Table I condenses the results obtained. It was found that the bronchial sputum of several asthmatic as well as non-asthmatic patients contains 5-hydroxyindol-acetic acid. During asthma

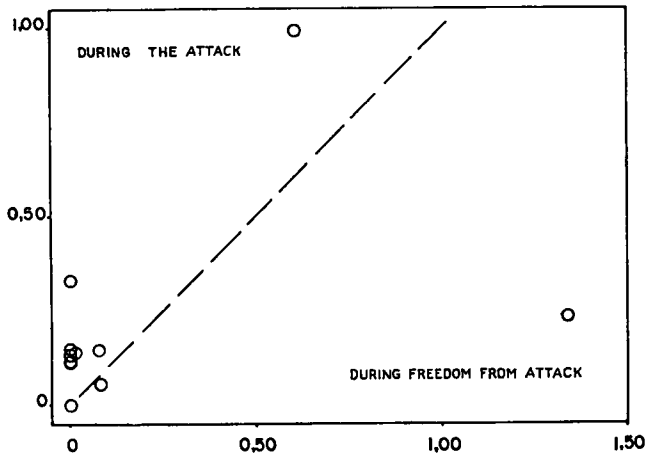


*Fig. 2.*
mg/100 ml. of 5-hydroxytryptamine in the sputum during an attack and during freedom.

attacks 5-HIAA was found in the sputum of all the asthmatic patients, except one.

In the asthmatic patients not in a attack the quantities of 5-HIAA, ranged from a maximum of 1.34 mg/100 ml of sputum to undetectable quantities.

Among the 10 asthmatic patients, only 1 (No. 1) showed undetectable quantities during the attack; 2 patients (No. 2 and 3) showed greater quantities of 5-HIAA when free but patient No. 3 continued to be wheezy at the second estimation (out of an attack)). During the asthmatic attacks the quantities of 5-HIAA ranged from 0.99 mg/100 ml to 0.050 mg/100 ml of sputum, except case No. 1 (undetectable).

Among the 7 patients who presented greater quantities of 5-HIAA in sputum during attacks (cases No. 4 to 10) only 2 presented detectable quantities when free (cases No. 4 and No. 10) In the 5 remaining patients (cases No. 5 to 9), the values during the attack ranged from 0.330 mg/100 ml to 0.130 mg/100 ml of sputum; when free, they showed undetectable quantities.

In the control group, we obtained undetectable quantities as

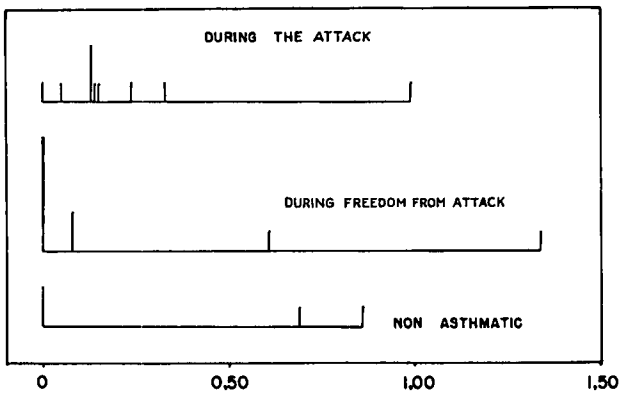


*Fig. 3.*
Amount of 5-hydroxytryptamine in the sputum of asthmatic and non-asthmatic patients.

well as relatively high values like 0.688 and 0.863 mg/100 ml of sputum.

## DISCUSSION

According to *Waalkes* & *Coburn,* the lung contains serotonin and histamine; the release of these amines in man should be a vital factor during allergic reactions.

Since serotonin takes part in the allergic mechanisms and is found in lung tissue, it is obvious that we should find this substance in the bronchial mucus during acute asthma. Actually, in 5 asthmatic patients without detectable quantities of 5-HIAA in the bronchial mucus, this substance appeared in such quantities as 0.330 mg in the course of the attack. However, the results were not uniform in the 10 patients studied, since in two cases, there was decrease of 5-HIAA during the attack.

Estimation of 5-HIAA during periods of freedom and during attacks shows (Fig.s 2 and 3) in general, an increase during the attack. Although extreme cases were present, we found that there was a general tendency toward higher values during attacks.

Apparently, the clinical type of asthma was the same in all

patients studied. Therefore, no conclusion can be drawn on the relationship between the presence of serotonin in the bronchial mucus and the clinical type of asthma.

In non-asthmatic conditions serotonin was also found in the bronchial mucus. In fact, relatively high quantities of 5-HIAA were found in the bronchial mucus of a case of Hamman-Rich's syndrome and a case of smoker's bronchitis. In contrast with these findings, serotonin was undetectable in the other two cases of lung disease one of which was "cor pulmonale".

These observations may suggest and stimulate future investigations on the determination of 5-hydroxy-indol-acetic acid in the bronchial mucus in lung diseases.

## SUMMARY AND CONCLUSIONS

1. 5-HIAA was determined by Udenfriend's technique in the bronchial sputum of 10 asthmatic patients, both during and out of asthma attacks.

2. During asthma, the bronchial mucus showed 5-HIAA in quantities that ranged from 0.050 to 0.99 mg/100 ml. Only in one case, 5-HIAA was undetectable during the attack.

3. Out of the attack, one half of the patients did not have detectable 5-HIAA in the bronchial mucus.

4. In two patients there was a decrease of 5-HIAA in the bronchial mucus during the asthmatic attack.

5. In non-asthmatic lung disease taken as controls 5-HIAA was found in quantities of 0.688 mg (in a case of smoker's bronchitis) and 0.863 mg (in a case of Hamman-Rich's syndrome). In two other cases of lung disease, no detectable 5-HIAA was found in the bronchial mucus.

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