

## BIOGENIC AMINES DERIVED FROM TRYPTOPHAN IN SYSTEMIC AND CUTANEOUS SCLERODERMA

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**Abstract.** Blood levels of serotonin (5-HT) and urinary excretion of 5-hydroxyindole-acetic acid (5-HIAA), tryptamine (T), and indole-acetic acid (IAA) were determined in 39 cases of systemic scleroderma and in 7 cases of very severe cutaneous scleroderma. The T/IAA ratio was normal and serotonin elevated in mild and rather severe acrosclerosis alike, i.e. in systemic scleroderma with pronounced vascular involvement. The T/IAA ratio was increased and serotonin normal in severe acrosclerosis, representing an intermediate form or transition to diffuse scleroderma, in severe diffuse scleroderma, and in severe cutaneous scleroderma. The T/IAA ratio and serotonin level were both elevated in a few cases of systemic scleroderma and in severe generalized morphea with concomitant vascular changes. The findings suggest impaired monoamine oxidase (MAO) activity in scleroderma with consequent accumulation in the organism of biogenic amines derived from tryptophan. An increased T/IAA ratio seems to be of prognostic significance in scleroderma, suggesting an adverse course of the disease.

Serotonin (5-HT), an amine derived from tryptophan, causes fibrosis of the skin in mice when injected peritoneally (13). In the carcinoid syndrome, in which its level in the organism is many times the normal, it is believed to lead sometimes to fibrosis and indurations (5, 21, 25). According to Winkelmann et al. the smooth muscles of skin vessels are hypersensitive to serotonin in scleroderma (23).

Our earlier studies (17) on the behaviour of urinary 5-hydroxyindole-acetic acid (5-HIAA) after oral administration of L-tryptophan in doses of 0.1 g/kg body weight suggested that metabolism of serotonin to 5-HIAA is impaired in scleroderma.

We have also studied the metabolism of the other amine derived from tryptophan, viz. tryptamine (T), by estimating the rise in urinary total indoles (TI) and indole-acetic acid (IAA) after oral administration of L-tryptophan (17). The term TI was introduced by Fischl & Rabiah (4) and covers chiefly T and IAA, the latter being usually the more abundant of the two urinary metabolites. After L-tryptophan loading, TI are normally present in

direct proportion to IAA. This is not so in scleroderma, in which their level is disproportionately high in relation to IAA (14, 17).

We thought this might have been due to an increase in tryptamine. Earlier (15), we determined the urinary levels of T and IAA (without tryptophan loading) in various types of systemic and cutaneous scleroderma and, for comparison, in other skin diseases. In some of the cases of scleroderma the T/IAA ratio, considered by LaBrosse et al. (10) to be an indirect measure of monoamine oxidase (MAO) activity, was increased.

In our earlier work (17) we suggested that in scleroderma the biogenic amines serotonin and tryptamine may perhaps accumulate. In the present studies our aim was to estimate serotonin and tryptamine metabolism directly from: (i) the levels of serotonin in blood and 5-HIAA in urine, and (ii) urinary T and IAA levels and the T/IAA ratio. Specifically, we strove to discover (a) whether the levels of these metabolites differ according to the varieties of systemic scleroderma—those with and without vascular involvement—and also change with the severity and course of the disease; and (b) whether the same will apply to systemic and cutaneous scleroderma, especially extensive morphea and linear scleroderma.

### MATERIAL AND METHODS

#### *Methods*

Serotonin blood levels were determined spectrofluorimetrically by the method of Yuwiler et al. (24) after conjugation with *o*-phthaldehyde (OPT).

Urinary 5-HIAA was assayed by the method of Udenfriend et al. (19).

Urinary tryptamine (T) was assayed spectrofluorimetrically by the method of Udenfriend (20).

Urinary indole-acetic acid (IAA) was determined by the colorimetric method of Fischl & Rabiah (4).

The T/IAA ratio was calculated as:

$$T/IAA = \frac{\mu\text{g T}}{\text{mg IAA} \times 10}$$

Table 1. Mean values of blood serotonin and some urinary tryptophan metabolites in systemic scleroderma

|                             | Blood<br>5-HT<br>( $\mu\text{g/ml}$ ) | Urinary excretion of           |                             |                             | T/IAA  |
|-----------------------------|---------------------------------------|--------------------------------|-----------------------------|-----------------------------|--------|
|                             |                                       | 5-HIAA<br>( $\text{mg/24 h}$ ) | T<br>( $\mu\text{g/24 h}$ ) | IAA<br>( $\text{mg/24 h}$ ) |        |
| <i>Systemic scleroderma</i> |                                       |                                |                             |                             |        |
| Group I                     |                                       |                                |                             |                             |        |
| $\bar{x}$                   | 0.715                                 | 1.50                           | 156                         | 11.30                       | 1.41   |
| $\pm$ S.D.                  | 0.1617                                | 1.157                          | 85.3                        | 4.75                        | 0.478  |
| <i>n</i>                    | 11                                    | 11                             | 11                          | 11                          | 11     |
| <i>p</i>                    | <0.001                                | <0.05                          | >0.05                       | <0.01                       | >0.1   |
| Group II                    |                                       |                                |                             |                             |        |
| $\bar{x}$                   | 0.294                                 | 2.01                           | 395                         | 8.90                        | 4.03   |
| $\pm$ S.D.                  | 0.0844                                | 0.982                          | 295.4                       | 3.597                       | 1.931  |
| <i>n</i>                    | 23                                    | 23                             | 23                          | 23                          | 23     |
| <i>p</i>                    | >0.1                                  | >0.1                           | <0.001                      | <0.05                       | <0.001 |
| Group III                   |                                       |                                |                             |                             |        |
| $\bar{x}$                   | 0.614                                 | 2.80                           | 351                         | 7.48                        | 4.67   |
| $\pm$ S.D.                  | 0.1016                                | 1.199                          | 200.4                       | 1.729                       | 2.550  |
| <i>n</i>                    | 5                                     | 5                              | 5                           | 5                           | 5      |
| <i>p</i>                    | <0.01                                 | >0.1                           | <0.001                      | >0.1                        | <0.01  |
| <i>Controls</i>             |                                       |                                |                             |                             |        |
| Healthy persons             |                                       |                                |                             |                             |        |
| $\bar{x}$                   | 0.357                                 | 2.55                           | 104                         | 6.37                        | 1.63   |
| $\pm$ S.D.                  | 0.1031                                | 1.15                           | 61.8                        | 2.283                       | 1.243  |
| <i>n</i>                    | 10                                    | 14                             | 14                          | 14                          | 14     |
| Other skin diseases         |                                       |                                |                             |                             |        |
| $\bar{x}$                   | 0.389                                 | 2.33                           | 132                         | 6.76                        | 2.01   |
| $\pm$ S.D.                  | 0.1598                                | 1.126                          | 82.4                        | 3.194                       | 0.627  |
| <i>n</i>                    | 8                                     | 8                              | 8                           | 8                           | 8      |
| <i>p</i>                    | >0.1                                  | >0.1                           | >0.1                        | >0.1                        | >0.1   |
| <i>Carcinoid</i>            |                                       |                                |                             |                             |        |
| $\bar{x}$                   | 1.15 <sup>a</sup>                     | 134 <sup>b</sup>               | 91                          | 8.36                        | 1.40   |
| $\pm$ S.D.                  | 0.710                                 | 106.5                          | 67.8                        | 4.788                       | 1.122  |
| <i>n</i>                    | 12                                    | 12                             | 12                          | 12                          | 12     |
| <i>p</i>                    | <0.0001                               | <0.0001                        | >0.1                        | >0.1                        | >0.1   |

<sup>a</sup> Normal range 0.07–0.22 mg/l (15).<sup>b</sup> Normal range 2–10 mg/24 h (15).

In the table mean values with standard deviation are given. In the statistical analysis, Student's *t*-test was used.

### Material

The studies covered 39 cases of systemic scleroderma and 7 of extensive cutaneous scleroderma or generalized morphea.

Since the severity criteria for scleroderma are still controversial, and the scoring proposed by Hughes *et al.* (7) does not agree with Winkelmann's (22) staging of vascular scleroderma and concept of inflammatory scleroderma, we split the cases into three grades according to the severity of the disease.

Our grading according to clinical features and course is the following.

**Grade 1.** CRST syndrome (calcinosis, Raynaud, sclerodactyly, and teleangiectasia) and acrosclerosis running a slow and relatively mild course. The interval be-

tween the onset of Raynaud's phenomenon and of the cutaneous lesions was rather lengthy in this group. The skin lesions were for the most part atrophic and confined to the hands (indurated oedema or sclerodactyly, often osteolysis) and face. Visceral involvement varied; in long-standing cases it was even extensive although not life-threatening.

**Grade 2.** Cases that began as typical acrosclerosis but ran a severe course and/or cases combining the features both of acrosclerosis and diffuse scleroderma. The skin lesions were more generalized, sclerotic and atrophic, and involved also the trunk. The Raynaud phenomenon was usually pronounced and osteolysis frequent. Visceral involvement was common and arthralgia and muscular involvement were as a rule more pronounced than in Grade 1.

**Grade 3.** Cases corresponding to diffuse scleroderma. The onset of Raynaud's phenomenon usually preceded the indurations by only a few months, or coincided with them.

The skin lesions, indurative-oedematous and more generalized, involved also the trunk and lower extremities, causing hyperpigmentations and depigmentations. Severe arthralgia and muscular involvement were a characteristic feature, whereas visceral involvement varied but, unless altogether absent, was usually more advanced.

All these groups of patients were on a hospital diet, which excluded bananas, nuts, grapefruits and tomatoes. Drugs were withheld at least 2 days before the investigations. Blood, for serotonin determinations, was drawn before breakfast into heparinized plastic test tubes. Twenty-four hour urine, if not investigated at once, was kept at  $-20^{\circ}\text{C}$ . Some patients were studied again after some months or a year.

The control group for serotonin determinations was made up of 10 healthy women aged 27–50 years and 8 patients with various skin diseases not related to scleroderma.

In determinations of the other metabolites the controls were 14 healthy persons (7 women and 7 men) and 8 patients with various skin diseases.

## RESULTS

The data on serotonin and tryptamine metabolism in patients with systemic scleroderma are set out in Table I.

There was a rise either of the blood level of serotonin or the T/IAA ratio in all patients and of both in 12.8% of them.

*Normal T/IAA ratio and elevated serotonin.* This group was composed of 11 (28%) of the 39 patients with systemic scleroderma, all of the acrosclerosis type, mild as well as severe but invariably with marked vascular involvement (grades 1–2).

*Increased T/IAA ratio and normal serotonin.* This group consisted of 23 of the 39 cases (59%) of systemic scleroderma and 3 of cutaneous scleroderma. The former were all either of severe diffuse scleroderma or of severe acrosclerosis representing an intermediate form or transition to diffuse scleroderma (grades 2–3), whereas the latter were all 3 of extensive progressive morphea or linear scleroderma.

*Both the T/IAA ratio and serotonin level raised.* Into this group fell 5 (12.8%) of the 39 patients with systemic scleroderma (grade 2) and 2 with cutaneous scleroderma (one had linear scleroderma with widespread symmetrically distributed lesions and coexistent Raynaud's phenomenon and the other generalized morphea with extensive vascular lesions and calcinosis).

Into this group were placed 2 cases of generalized grave morphea with very low 5-HIAA excretion,

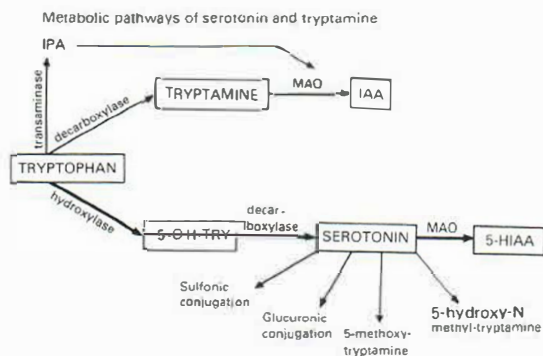


Fig. 1. Diagram of metabolic pathways of serotonin and tryptamine.

suggestive according to our findings (see Table I, group I) of an elevated blood serotonin level.

All in all the blood level of serotonin was elevated in 16 of the 39 cases of systemic scleroderma, which was 41%, and in 2 of 6 of extensive cutaneous scleroderma with concomitant vascular involvement. The T/IAA ratio was increased in 28 (i.e. 71.8%) of the patients with systemic scleroderma and in all with advanced cutaneous scleroderma.

Six patients were reinvestigated at least once after intervals of 3–12 months. The T/IAA ratio had increased in all. In one the blood level of serotonin also rose (after half a year).

## DISCUSSION

The present results seem to corroborate the earlier suggestion (17) of an abnormal metabolism of serotonin and tryptamine in scleroderma. An elevated blood level of serotonin, as also reported by Łańcucki & Nowik-Jurek (12) and/or growth of the T/IAA ratio due to impaired metabolization of T to IAA, were seen in all the patients with systemic and cutaneous scleroderma. At this point it should be noted that determination in the urine of exclusively tryptamine warrants no conclusion as to whether its conversion into IAA is undisturbed. The conversion takes place only under the influence of MAO (Fig. 1), and without determining the T/IAA ratio we can know nothing about whether or not it proceeds normally.

Serotonin too is catabolized, chiefly to 5-HIAA, first and foremost under the influence of MAO (Fig. 1), although it is also capable of being eliminated directly, e.g. as serotonin-*o*-sulphate or serotonin-*o*-glucuronate complexes (6).

Our findings seem to suggest a depressed MAO activity, as 5-HIAA excretion was low in spite of a high serotonin level in the blood. In carcinoid on the other hand, both the serotonin level and 5-HIAA excretion are greatly elevated, and in skin diseases other than scleroderma, both were normal.

However, in some patients the blood serotonin level was high yet 5-HIAA excretion was normal. Their T/IAA ratio was then as rule greatly increased, perhaps owing to a diminished activity of the MAO responsible for the conversion of tryptamine to IAA.

Depressed MAO activity can conceivably result in an accumulation in the body of the biogenic amines derived from tryptophan, whose biological effects may underlie some of the morbid phenomena in scleroderma, i.e. vascular lesions and indurations.

Being seen not only in systemic scleroderma but also in extensive morphea and in the pseudo-sclerodermatous Werner's syndrome (15) and phenylketonuria (16), increased urinary excretion of tryptamine does indeed seem to be causally related to indurations.

Serotonin on the other hand appears to increase in systemic scleroderma with pronounced vascular involvement and also in morphea with marked vascular phenomena.

As has been demonstrated on isolated arteries (23), in acrosclerosis the small arterial vessels are hypersensitive to serotonin and the smooth muscles of arterial walls react to much lower concentrations of it than in normal circumstances. Consequently, even a slight increase in the blood level of the amine may have a harmful effect on vessels in this disease. In the light of our results the increase appears to be due to a depressed activity of MAO, which is normally present in vessel walls too (1).

Serotonin is also known to promote the growth of fibroblasts in cultures. (2). Not inconceivably, therefore, it may be conducive to increased in-vivo production of collagen fibres as well (9, 11).

Storage of serotonin takes place also in the sympathetic nerve terminals of the skin (18) and in the smooth muscles of arteries (3). Its effect on the vegetative nervous system can thus be an explanation of the prolonged sensory chronaxy in the entire uninvolved skin both in systemic and in cutaneous scleroderma (8).

Studies conducted in some patients after several months showed that the T/IAA ratio, a reflection of

tryptamine accumulation in the organism, grew as disease progressed.

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*Received May 23, 1978*

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