Serotonin metabolism in cystic fibrosis

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SUMMARY  The average blood serotonin level of 67 children with cystic fibrosis was found to be about twice that of age-matched normal children. There was no corresponding increase in the urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA). Children with cystic fibrosis were well able to metabolize serotonin taken by mouth. No significant correlations were found between the blood serotonin level and the platelet count, height, weight, skinfold thickness, and pulmonary function tests. 5 out of 44 patients had raised serum IgE levels, and their mean blood serotonin was higher than in those with normal IgE levels. No explanation for this emerged. Comparable findings (raised blood serotonin, normal platelet count, normal urinary 5-HIAA) have been reported only in severe mental retardation. Further study of this phenomenon is warranted because (a) a raised blood serotonin level is sufficiently characteristic of cystic fibrosis to explore its use in diagnosis, and (b) it may help to explain the pathogenesis of cystic fibrosis and (c) the metabolism and function of serotonin.

Kopel and Warner (1968) first reported that the blood serotonin level was increased in cystic fibrosis (CF). They found that the average blood serotonin of 28 children with the disease was about twice the normal level. There was no accompanying increase in the main metabolite of serotonin, 5-hydroxyindoleacetic acid (5-HIAA), in the urine. They suggested that there was an increased release of serotonin in CF with impaired conversion to 5-HIAA.

In man, the blood serotonin is derived from the enterochromaffin cells of the gastrointestinal tract. Most of it is carried in the blood stream by platelets; free serotonin is rapidly converted to 5-HIAA in the lungs (see Fishman and Pietra, 1974, for review) and elsewhere and excreted in the urine. Serotonin is also found in tissue mast cells and basophil leucocytes from which it may be released by cytophilic antibody of the IgE class, as occurs in patients with immediate hypersensitivity diseases (Bellanti, 1972). Recent reports indicate that CF is associated with raised serum IgE levels (Spitz et al., 1972) and an increased incidence of allergic respiratory disease (Rachelefsky et al., 1974).

We studied a number of children with CF to find out whether the increased blood serotonin level was related to impaired catabolism, the number of platelets in the blood, nutrition, severity of lung disease, or raised serum IgE levels.

Patients and methods

Sixty-seven children (35 boys, 32 girls) were divided into two groups according to when and where they were studied. Group I contained 38 children, aged 6 weeks to 16 years. 6 of these were attending the Cystic Fibrosis Clinic at Kingston General Hospital between 1970 and 1973; the remainder were at Merrywood Camp for children with CF, organized by the Ontario Society for Crippled Children, in the summer of 1974. Group II contained 43 children, aged 6 to 12 years, who attended Merrywood Camp in 1975. 14 children attended the camp in both years and so were common to the two groups. The children were all referred to Merrywood from recognized clinics for CF in Ontario, and we had no reason to doubt the diagnosis of CF in any.

For each group there were age- and sex-matched normal children as controls. Those for group I were part of a large series of normal subjects described elsewhere (Tu and Partington, 1972); those for group II were healthy children of the Faculty of Queen’s University.

Heights and weights were measured on a bar scale in light clothing without shoes. Skinfold thickness was measured in the children of group II in the left triceps and subscapular areas with a Harpenden caliper as described by Tanner and Whitehouse (1962). Pulmonary function was assessed with a portable electronic spirometer (Life Support...
Equipment Corp., Woburn, Mass.). Timed forced vital capacity (FVC, FEV₁, and FEV₁₅) and forced expiratory flow rate (FEF₂₅₋₇₅%) were measured in each subject using the best of three successive measurements.

Platelets were counted by a Coulter Counter in the same blood samples in which serotonin was measured in 41 children from group II and the amount of serotonin per 10⁶ platelets was calculated. Serotonin was measured in whole blood by the method of Ashcroft et al. (1964). In group I measurements of serotonin were also made in each sample by the method of Berman et al. (1965) because of its greater specificity for serotonin. As the results (Table 1) of the two methods were highly correlated (r = 0.94; P < 0.001) and there was no significant difference between the means (paired t = 0.92, P = 0.35), the more convenient Ashcroft technique was used for group II. In 1975 the Ashcroft method was modified slightly by making up the standard solution of serotonin in 0.5 N HCl instead of water in order to stabilize it. Variation in serial measurements was reduced but so were the absolute values for serotonin necessitating separate control subjects for group II.

Random urine samples were collected from group I patients and their controls for the measurement of 5-HIAA by the method of Korf and Valkenburgh-Sikkema (1969). In group II and their controls all urine samples were collected after walnuts and bananas had been excluded from the diet for 2 days, since these foods are rich sources of serotonin containing 17–34 mg and 1.9–3.6 mg/100 g edible portion respectively (Udenfriend et al., 1959). A rough test of the ability to metabolize serotonin was carried out as follows. A urine sample was collected the first morning. A single banana was eaten (about 3 mg serotonin) in addition to the usual breakfast, and a second urine sample was collected 4 hours later. 5-HIAA was measured in each urine sample.

Serum IgE was assessed by solid phase radioimmunoassay (RIST, Pharmacia, Montreal).

Results

Table 1 summarizes our results and those of Kopel and Warner (1968), with which they agree. The average level of serotonin in the blood of patients with CF was about twice as high as that of normal children. In group I there was no significant difference between the mean blood serotonin level of the 6 children attending the clinic and the 32 children at the summer camp (t = 0.94; P = 0.35). In the 14 children common to groups I and II all the values except one were, as expected (see under Methods), lower in 1975 than in 1974; the levels in blood from the same child were highly correlated (r = 0.84; P < 0.001).

Fig. 1 shows the frequency distribution of the blood serotonin levels of the patients in group II compared with those of normal children. About 80% of the blood serotonin levels in CF patients

![Fig. 1 Frequency distributions of the blood serotonin level in children with cystic fibrosis and in normal children of the same age.](http://adc.bmj.com/content/52/5/386)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cystic fibrosis</th>
<th>Normal children</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Method</td>
<td>n</td>
</tr>
<tr>
<td>Present series (whole blood)</td>
<td>Ashcroft</td>
<td>38</td>
</tr>
<tr>
<td>II 1975</td>
<td>Modified Ashcroft</td>
<td>43</td>
</tr>
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*P < 0.001, †P = 0.001.
were above the 90th centile of the normal values. There were no correlations between the blood serotonin level and age or sex in either the patients with CF or the normal subjects.

Blood platelet counts in the children with CF were within the normal range for the method (150–450 × 10^9/l), except in 5 patients whose counts were slightly higher (451–560 × 10^9/l). Table 2 gives the correlations between the blood serotonin level and the platelet count (not significant) and the serotonin per 10^9 platelets (highly significant), indicating that in CF the increased blood serotonin is in the platelets and not in the plasma.

Table 3 compares the urinary 5-HIAA of the children with CF in groups I and II with their respective normal controls. There were no significant differences whether the results were expressed as a concentration or whether expressed in relation to creatinine. In group II, after eating a banana, the children with CF had somewhat higher concentrations of 5-HIAA in the urine (Fig. 2) and the differences were significant (t = 3.33; P < 0.005).

The heights, weights, and skinfold thicknesses indicated that the children with CF were well nourished. Most of the measurements were scattered between the 10th and 50th centiles. In group I there were only 3 children below the 3rd centile of normal values for weight for their age and sex (Tanner et al., 1966). In group II, I was below the 3rd centile for weight, 2 were below for height, and 5 for one or the other skinfold thickness. For each measurement in turn the children were subdivided into one of four sets according to the centile limits between which the measurement fell (i.e. <10, 10–25, 26–50, >50). No differences were found between the mean blood serotonin levels of any of these sets.

Abnormal tests of pulmonary function were present in 21 out of 44 (50%) patients tested. 5 out of 44 (11%) had forced vital capacities of more than 2 SD below the predicted means for their height and sex (Dickman et al., 1971), while 20 out of 44 (45%) had significant decreases in FEV_{15%-25%} and/or FEF_{25%-75%}, indicating obstructive airway disease. No correlation was evident between the blood serotonin level and the degree of restricting or obstructive lung disease. 19 of 22 (86%) patients with normal pulmonary function tests had blood serotonin levels greater than 200 ng/ml, as did 15 of 21 (71%) patients with abnormal pulmonary function tests.

Serum IgE levels were raised above the 97th centile of the age related value (Kjellman et al., 1976) in 5 of 44 (11%) patients tested. The mean blood serotonin level of these 5 (383 ng/ml) was higher than the mean (265 ng/ml) of patients with

Table 2 Relationships between blood serotonin, platelet count, and serotonin per platelet in 41 children with cystic fibrosis

<table>
<thead>
<tr>
<th></th>
<th>Blood serotonin (ng/ml)</th>
<th>Platelet (×10^9/l)</th>
<th>Platelet serotonin (ng/10^9 platelets)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>280</td>
<td>388</td>
<td>732</td>
</tr>
<tr>
<td>SD</td>
<td>98</td>
<td>62</td>
<td>256</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>Blood serotonin</td>
<td>—</td>
<td>0.24*</td>
</tr>
<tr>
<td></td>
<td>Platelet count</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*P < 0.1; TP < 0.001.

Table 3 Urinary 5-HIAA in children with cystic fibrosis and in normal children

<table>
<thead>
<tr>
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<th>Cystic fibrosis</th>
<th>Normal children</th>
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<tr>
<td></td>
<td>n</td>
<td>Mean</td>
</tr>
<tr>
<td>Group I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>μg/ml</td>
<td>28*</td>
<td>5-24</td>
</tr>
<tr>
<td>μg/g creatinine</td>
<td>5-76</td>
<td>3-5</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>μg/ml</td>
<td>17*</td>
<td>4-04</td>
</tr>
<tr>
<td>μg/g creatinine</td>
<td>6-49</td>
<td>1-3</td>
</tr>
</tbody>
</table>

*3 children common to both groups. 5-HIAA = 5-hydroxyindoleacetic acid.
normal serum IgE levels (t = 2.7; P < 0.005). 2 of the 5 had evidence of obstructive airway disease on pulmonary function tests while the other 3 had normal lung function.

All the children with CF were receiving a pancreatic extract (Cotazym, Organon) 3 or 4 times per day, vitamin E (usually 100 IU 3 times per day) and long-term antibiotic treatment, though the type of antibiotic varied. In addition, most were receiving supplements of other vitamins and many had thrice daily inhalations of a mixture of an antibiotic, disodium cromoglycate, and orciprenaline sulphate. In the circumstances it was not possible to manipulate therapy. Numerous internal comparisons were made, none of which were significant. For example, in group II the mean blood serotonin level of the 16 children taking cephalixin monohydrate by mouth did not differ from the mean blood serotonin level of the 5 children taking lincomycin or the 5 children taking combined ampicillin and cloxacillin. In short, we were unable to discover any relationships between the type of drug therapy and the blood serotonin level.

Discussion

The present findings are similar to those of Kopel and Warner (1968) and confirm that the blood serotonin is increased in CF. The results are consistent despite technical differences in method. The close agreement, in our series, between the results of the Ashcroft and the more specific Berman method supports the belief that serotonin itself is increased and not some other 5-hydroxyindole compound (Partington et al., 1973). Furthermore, the raised blood serotonin is explained by increased serotonin in the platelets rather than an increase in platelet number. The blood serotonin level was sufficiently above normal and increased in such a large proportion of patients as to warrant exploring its use as a diagnostic test for CF in cases where other tests are equivocal.

A no increase in 5-HIAA was found in the urine of patients with CF. However, the serotonin load test using banana) showed that children with CF are well able to metabolize serotonin and excrete it as 5-HIAA. The higher concentrations of 5-HIAA in the urine of these CF patients probably does not reflect a significant difference in serotonin metabolism. Bananas vary not only in size but also in serotonin content; ripe pulp may contain twice as much serotonin per gram as unripe pulp (Waalkes et al., 1958). We studied the children with CF in the summer and the normal children in the winter when the bananas available were less ripe.

We were unable to relate the blood serotonin level to the age, sex, or nutritional state of the children with CF. Like Kopel and Warner (1968) we found no evidence that the blood serotonin level was related to drugs, but we were unable to manipulate the therapeutic regimens of the children.

Pulmonary function tests were abnormal in only 50% of the children with CF but only those with mild to moderate disease attended the camp. No correlation was found between the blood serotonin level and abnormal pulmonary function tests, suggesting that lung disease per se does not account for the increased blood serotonin.

Rachelefsky et al. (1974) suggested that allergic respiratory disease may protect the patient with CF. In their study, children with CF and allergic disease were in better clinical condition (as judged by the Shwachman score) than those with CF alone. 24% of their study group (a clinic population) had concomitant allergic disease compared to 10 to 15% of the general paediatric population; the mean serum IgE level of those with allergic disease was higher than those without. Despite the selection of our patients in favour of mild to moderate disease, only 11% had significantly raised serum IgE levels. These patients did, however, have significantly higher blood serotonin levels than those with normal serum IgE levels. We were unable to determine if there was a cause and effect relationship. If IgE antibody caused increased release of serotonin in CF, increased 5-HIAA would be expected in the urine and this does not occur.

Altered serotonin metabolism has been found in other states marked by disturbed gastrointestinal function. Total bowel removal leads to low blood serotonin levels and low urinary 5-HIAA levels presumably because the enterochromaffin system has also been removed (Haverback and Davidson, 1958). In the carcinoid syndrome there is a massive endogenous production of serotonin with very high blood serotonin levels and large amounts of 5-HIAA in the urine; diarrhoea occurs in this syndrome due to direct stimulation of the bowel wall by free serotonin in the blood (Grahame-Smith, 1972). Increased blood serotonin and increased urinary 5-HIAA (though far less than in the carcinoid syndrome) are also found in coeliac disease (Pimparkar et al., 1961; Challacombe et al., 1972) and in kwashiorkor with steatorrhoea (Teotia and Teotia, 1975). In both conditions the changes revert to normal after appropriate specific treatment of the underlying disorder but their mechanisms are quite unknown. Serotonin metabolism is normal in other forms of chronic diarrhoea (e.g. ulcerative colitis). Abnormal serotonin metabolism has not been reported in chronic lung disease other than CF.
Increased blood serotonin without increased urinary 5-HIAA levels has been observed for many years in a heterogeneous group of patients with nonspecific, severe mental and physical retardation (Pare et al., 1960; Partington et al., 1973). Considerable investigation has failed to bring out significant clinical correlations (e.g. with muscle tone, motor activity, frequency of bowel movements) and the mechanism remains obscure. In both CF and severe retardation an excess of serotonin appears to be stored in the platelets, with no overall increase in serotonin metabolism. There is no obvious reason and no obvious detrimental effect. It seems worthwhile to study serotonin uptake and release from the platelet itself in these conditions as has been done for the low platelet serotonin content found in Down’s syndrome (Lott et al., 1972; McCoy et al., 1974). At present it is not known why a similar disorder in serotonin metabolism should be found in such disparate conditions as CF and severe mental retardation, but its elucidation may further our understanding of the pathogenesis of these disorders or the metabolism of serotonin itself.

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References


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