THE RELATIONSHIP BETWEEN DECREASED 5-HYDROXYINDOLE METABOLISM AND MENTAL DEFECT IN PHENYLKETONURIA

BY

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In 1957 we demonstrated a defect of 5-hydroxyindole metabolism in 10 patients with phenylketonuria and suggested that, in view of the probable importance of 5-hydroxytryptamine (5HT) in normal brain function, this deficiency might be a factor in the mental defect (Pare, Sandler and Stacey, 1957). In the work described below, the previous findings are extended and the relationship of the 5-hydroxyindole deficiency to the mental defect is examined further.

Methods

In all, 49 patients with phenylketonuria and 32 mental defective controls were seen. All the controls and 26 of the phenylketonurics came from one hospital and these groups were comparable for weight, age and intelligence. The remaining phenylketonurics were drawn from four different hospitals. Tests of intelligence used included the Stanford-Binet (Terman-Merrill revision), the Merrill-Palmer and the Vineland Social Maturity scale. Serum 5HT was estimated on all children with the exception of one control, and urinary 5-hydroxyindoleacetic acid (5HIAA) and creatinine were estimated on early morning specimens of urine from all. Methods used have been described previously (Pare et al., 1957).

Results

5-Hydroxyindole Estimations. The 5-hydroxyindole deficiency in phenylketonuria was confirmed (Fig. 1). The mean value for urinary 5HIAA in 49 phenylketonurics was 2.2 mg./g. creatinine compared with 7.2 mg./g. for 32 mental defective controls. The difference between the two groups is shown by there being only one phenylketonuric with 5HIAA values greater than 4.2 mg./g. creatinine and only two controls with values below this. Similar results were

found for serum 5HT, where the mean level in 49 phenylketonurics was 71.2 ng./ml compared with 283 ng./ml in 31 controls. Six phenylketonurics had values greater than, and five controls less than, 120 ng./ml.

Relationship between 5-Hydroxyindole Values and Intelligence in Phenylketonurics. This assessment was made on patients in one hospital to exclude inter-hospital variability in estimation of intelligence and possible dietary or other effects acting on 5-hydroxyindole metabolism. As the estimation of intelligence in low-grade defectives can only be approximate, a straightforward product-moment correlation could not reasonably be applied. Instead, patients were grouped into three categories with respect to intelligence, and the mean values for age, 5HT and 5HIAA estimated for each group (Table 1). This showed there was no significant

<table>
<thead>
<tr>
<th>I.Q.</th>
<th>Patients (No.)</th>
<th>Age</th>
<th>Serum 5HT</th>
<th>Urinary 5HIAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>16</td>
<td>13.5±1.7</td>
<td>79.5±12.9</td>
<td>2.48±0.24</td>
</tr>
<tr>
<td>20–34</td>
<td>7</td>
<td>4.3±0.9</td>
<td>76.0±20.3</td>
<td>2.79±0.50</td>
</tr>
<tr>
<td>&gt;34</td>
<td>3</td>
<td>3.9±1.6</td>
<td>83.0±34.4</td>
<td>2.53±0.66</td>
</tr>
<tr>
<td>&gt;20</td>
<td>10</td>
<td>4.2±0.8</td>
<td>78.1±16.5</td>
<td>2.71±0.39</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>9.9±1.4</td>
<td>79.0±9.9</td>
<td>2.57±0.23</td>
</tr>
</tbody>
</table>

association between intelligence and either 5HT or 5HIAA.

Relationship between Age, 5HT and 5HIAA in Phenylketonurics and Other Mental Defectives. The product-moment correlation coefficient was computed for the following in both phenylketonurics and

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mental defective controls: age with 5HIAA, age with 5HT and 5HT with 5HIAA (Table 2). In both
groups of patients there was a small negative correlation between age and 5HIAA. There was no
correlation between age and 5HT but a small positive correlation between 5HT and 5HIAA.

Abnormality of 5-Hydroxyindole Metabolism in Non-phenylketonuric Mental Defectives. The variability of 5HIAA and 5HT values in normal children is not completely known. It will be seen from Fig. 1 that some of the patients had values for serum 5HT which were as high as those found in patients with the carcinoid syndrome. Many have been repeatedly examined over a period of two to three years and remain consistently high. These children, who present certain interesting features, have been further investigated and form the subject of a separate communication (Pare, Sandler and Stacey, 1959).

### Discussion
The small negative correlation between age and 5HIAA excretion was to be expected, for 5HIAA was estimated as mg./g. creatinine and the excretion of creatinine/kg. body weight increases with age (Harding and Gaebler, 1922).

The small correlation only between 5HT and 5HIAA should not occasion surprise; for although 5HIAA is the major metabolite of 5HT, 5HT follows other metabolic pathways (Chadwick and Wilkinson, 1958; McIsaac and Page, 1958; Weissbach, Redfield and Udenfriend, 1958). Likewise, some 5HIAA may be derived from other sources. The concentration of 5HT in serum is almost wholly a measure of platelet-bound 5HT (Hardisty and

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**Table 2**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Variables</th>
<th>Cases (No.)</th>
<th>'r'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylketonuria</td>
<td>Age-5HIAA</td>
<td>26</td>
<td>-0.18</td>
</tr>
<tr>
<td>Controls</td>
<td>Age-5HIAA</td>
<td>32</td>
<td>-0.23</td>
</tr>
<tr>
<td>Phenylketonuria</td>
<td>Age-5HT</td>
<td>26</td>
<td>+0.01</td>
</tr>
<tr>
<td>Controls</td>
<td>Age-5HT</td>
<td>31</td>
<td>-0.10</td>
</tr>
<tr>
<td>Phenylketonuria</td>
<td>5HT-5HIAA</td>
<td>26</td>
<td>+0.14</td>
</tr>
<tr>
<td>Controls</td>
<td>5HT-5HIAA</td>
<td>31</td>
<td>+0.40</td>
</tr>
</tbody>
</table>

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**Fig. 1.**—Serum 5-hydroxytryptamine (5HT) (ng./ml.) and urinary 5-hydroxyindoleacetic acid (5HIAA) (mg./g. creatinine) values in 49 phenylketonurics and 31 non-phenylketonuric mentally defective controls.
Stacey, 1955). Levels will depend on the number of circulating platelets and their ability to take up 5HT which in turn varies with platelet adenosine triphosphate concentration (Born, Ingram and Stacey, 1958).

Relationship between 5-Hydroxyindole Deficiency and the Mental Defect. We have been able to confirm our previous finding (Pare et al., 1957) of decreased 5-hydroxyindole production in a considerably augmented series. One of the reasons for starting this investigation was the possibility that the defect in hydroxylation of phenylalanine to tyrosine, which is known to be the primary fault in phenylketonuria, might be associated with a similarly abnormal hydroxylation of tryptophan. Little is known of the latter reaction and its further investigation is hampered, as the site in mammals of an enzyme able to carry it out has not yet been demonstrated. Recent work has indicated, however, that some aromatic acid metabolites of phenylalanine, produced in large quantities in phenylketonuria, are able to depress the synthesis of 5HT. Davison and Sandler (1958) have shown, by in vitro studies, that phenylpyruvic acid, phenylacetic acid and phenylactic acid inhibit 5-hydroxytryptophan (5HTP) decarboxylase, the enzyme responsible for the production of 5HT from its precursor 5HTP, and evidence has also accrued to indicate that at least phenylacetic acid has a similar action in vivo (Davison, Ruthven and Sandler, 1959; Sandler and Close, 1959; Sandler, Davies and Rimington, 1959). The finding of a decreased 5HTP decarboxylase activity in phenylketonuria by Pare, Sandler and Stacey (1958) must probably be interpreted then as a result of inhibition of this enzyme by aromatic acid metabolites of phenylalanine, and decarboxylase inhibition now seems a likely cause of the abnormal 5-hydroxyindole metabolism reported above. The situation, however, is still by no means clear.

5HTP decarboxylase is not the only enzyme system so affected by these phenylalanine metabolites. Fellman (1956) has shown that DOPA decarboxylase, which may be identical with 5HTP decarboxylase (Westermann, Balzer and Knell, 1958) and is essential in the synthesis of catechol amines, is similarly inhibited, and data suggesting that this might happen in vivo are also to be found. Thus, Weil-Malherbe (1955) has demonstrated a deficiency of a substance with the biochemical characteristics of adrenaline in platelets from phenylketonurics; Cawte (1954, 1957) has described an abnormal sensitivity to adrenaline in these patients, and Armstrong (1958) has found a decreased output of 3-methoxy-4-hydroxymandelic acid, the major metabolite of adrenaline and noradrenaline (Armstrong, McMillan and Shaw, 1957), in a small number of affected children he investigated. It is likely that catechol amines play an important part in the normal functions of the central nervous system as 5HT.

Another biologically active amine which is likely to prove of great importance in brain physiology is γ-aminobutyric acid (Elliott, 1958). It is of interest that the enzyme responsible for its formation, glutamic acid decarboxylase, may also be inhibited in vitro by this group of aromatic acid metabolites of phenylalanine (Hanson, 1958). Viewed in this light, it is possible that there is a multiple deficiency in phenylketonuria. If so, it is hardly surprising that we failed to demonstrate any correlation between the degree of 5-hydroxyindole deficiency and I.Q. Nor is it to be wondered at that Kirman, Pare, Sandler and Stacey (1958) were unable to show any improvement in I.Q. during a controlled trial of 5HTP in affected children. If a decreased brain concentration of biologically active amines, singly or in combination, is responsible in some degree for the mental defect in phenylketonuria, then any method of increasing their total concentration in the brain might provide a rational therapy. This can probably be achieved by treating affected children with iproniazid, which inhibits monoamine oxidase, the enzyme responsible for much of the further metabolism of biologically active monoamines (Blaschko, 1952; Davison, 1958). We are carrying out such a trial at present (Kirman, Pare, Sandler and Stacey, 1959).

Summary

The low serum 5-hydroxytryptamine and urinary 5-hydroxyindoleacetic acid previously reported in phenylketonuria has been confirmed in a larger series. There was no relationship between 5-hydroxyindole levels and intelligence.

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RELATIONSHIP OF 5HIAA DEFICIENCY TO MENTAL DEFECT

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