CHEMICAL AND METABOLIC STUDIES ON PHENYLALANINE

II. THE PHENYLALANINE CONTENT OF THE BLOOD AND SPINAL FLUID IN PHENYLPYRUVIC OLIGOPHRENIA*

By GEORGE A. JERVIS, RICHARD J. BLOCK, DIANA BOLLING, and EDNA KANZE

(From the Departments of Neuropathology and Biochemistry, New York State Psychiatric Institute and Hospital, New York, and from Letchworth Village, New York State Department of Mental Hygiene, Thiells, New York)

(Received for publication, February 14, 1940)

Phenylpyruvic oligophrenia is an inborn error of metabolism characterized clinically by mental deficiency and chemically by the excretion of phenylpyruvic acid in the urine. It was shown previously that the feeding of a diet rich in protein or the ingestion of phenylalanine, phenylpyruvic acid, and of phenyllactic acid to patients afflicted with this syndrome resulted in an increased excretion of phenylpyruvic acid in the urine (1). The present paper contains observations on the phenylalanine and phenylpyruvic acid contents of the blood of normal and oligophrenic individuals, on the phenylalanine and phenylpyruvic acid content of the spinal fluid of these patients, and on the effects upon these values of feeding protein, phenylalanine, phenylpyruvic acid, phenyllactic acid, and tyrosine.

EXPERIMENTAL

Methods

For the quantitative determination of phenylalanine the following procedure was used. The blood or spinal fluid proteins were precipitated with 5 volumes of 10 per cent trichloroacetic acid; the precipitate was removed by centrifugation and washed

* Aided by a grant from Child Neurology Research (Friedsam Foundation).
twice with 1 volume of 5 per cent trichloroacetic acid. The combined filtrates and washings were diluted to volume and suitable aliquots were evaporated to dryness in a porcelain dish on the steam bath. The estimation of phenylalanine was carried out on the residue by means of the nitration method previously described (2, 3), by use of the Evelyn colorimeter with Filter 560 mJ. 

It was shown (Table I) that the amount of color developed is proportional to the amount of phenylalanine used and that tyrosine, tyrosine and glycine, an excess of an amino acid mixture consisting of tyrosine, glycine, glutamic acid, arginine, and histidine, or a protein hydrolysate does not interfere with the determination. The use of the color filter makes unnecessary the removal of tyrosine and histidine as described by Kapeller-Adler (2).

Moreover, various amounts of phenylalanine were added to samples of blood from normal individuals and the samples were treated as described above. The recovery of phenylalanine is shown in Table II, in which the results are expressed in net values after deduction of the blank.

Phenylpyruvic acid was determined colorimetrically by the green color which develops when ferric chloride is added to an

TABLE I

Proportionality of Amount of Color Developed by Phenylalanine

All reactions were carried out four or more times; average values are given.

<table>
<thead>
<tr>
<th>Amount of phenylalanine</th>
<th>Optical density; ( L = 2 - \log G ) (( G = ) galvanometer readings)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg.</td>
<td>Alone†</td>
</tr>
<tr>
<td>0.5</td>
<td>0.194</td>
</tr>
<tr>
<td>1.0</td>
<td>0.409</td>
</tr>
<tr>
<td>1.5</td>
<td>0.585</td>
</tr>
<tr>
<td>2.0</td>
<td>0.824</td>
</tr>
</tbody>
</table>

* From the table in "Notes on operation of the Evelyn photoelectric colorimeter."
† These figures are in terms of optical density \( L \) and when divided by the respective concentration \( C \) of phenylalanine give a constant value \( (L/C = K) \).
aqueous solution of the acid (4). The test was performed as follows: 2 cc. of a 1 per cent aqueous solution of FeCl₃ were added to 20 cc. of the trichloroacetic acid blood or spinal fluid filtrates. As the green color is unstable, the reading was made in the Evelyn colorimeter (Filter 620 mp) at the point of maximum deflection of the galvanometer. The reaction was found to detect as little as 0.005 mg. per cc. of phenylpyruvic acid or about 0.04 mg. per cc. of blood under the experimental conditions here followed.

Feeding Experiment—Seven male and nine female patients were studied during the course of this investigation; their ages varied between 4 and 40 years, their intelligence quotients being between 5 and 50. Four normal subjects were included as controls. The

| Phenylalanine | Recovery | Experiment 1 | | | Experiment 2 |
|---------------|----------|--------------|--------------|--------------|
| mg.           | mg.      | mg.          | mg.          |--------------|
| 0.5           | 0.45     |              | 0.45         |              |
| 1.0           | 1.0      |              | 1.0          |              |
| 2.0           | 2.0      |              | 2.0          |              |
| 5.0           | 5.0      |              | 4.5          |              |
| 10.0          | 9.7      |              | 9.9          |              |

The phenylalanine content of the blood and spinal fluid was determined after ingestion by fasting (16 hours) subjects of (a) a protein-rich meal consisting of 250 gm. of meat, 50 gm. of cheese, 200 cc. of milk, and various vegetables, (b) dl-phenylalanine, (c) phenylpyruvic acid, (d) dl-phenyllactic acid, and (e) l-tyrosine. In each experiment phenylalanine and phenylpyruvic acid were determined at the fasting level and 2, 4, and 6 hours following the ingestion of the compound. For each estimation four replicate determinations were carried out on two different samples of blood drawn at the same time.

The following results were obtained. Under the conditions of analysis, the blood obtained from normal fasting individuals contained so little phenylalanine that it could not be determined with
even a fair degree of accuracy. In contrast, blood from sixteen fasting patients afflicted with phenylpyruvic oligophrenia contained from 15 to 41 mg. of phenylalanine per 100 cc. The spinal fluid of five fasting patients contained from 4 to 12 mg. of phenylalanine per 100 cc. Phenylpyruvic acid was not found in the blood or spinal fluid in any instance.

The ingestion of a high protein meal by eleven patients resulted in an appreciable increase of blood phenylalanine in nine experiments, averaging 6 mg. per 100 cc. of blood. In one instance, the level rose from 27 to 45 mg. per 100 cc. No phenylpyruvic acid was detected in the blood in any of these experiments.

The feeding of 5 gm. of \( dl \)-phenylalanine to five oligophrenic individuals resulted in an appreciable rise in blood phenylalanine, with a maximum of 30 per cent of the fasting level (Table III).

5 gm. portions of phenylpyruvic acid were fed to four oligophrenics. A distinct rise in blood phenylalanine was found in

<table>
<thead>
<tr>
<th>Substance fed</th>
<th>Blood phenylalanine, mg. per 100 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr.</td>
</tr>
<tr>
<td>( dl )-Phenylalanine, 5 gm.</td>
<td>M. M.</td>
</tr>
<tr>
<td></td>
<td>M. B.</td>
</tr>
<tr>
<td></td>
<td>C. S.</td>
</tr>
<tr>
<td></td>
<td>W. C.</td>
</tr>
<tr>
<td></td>
<td>W. K.</td>
</tr>
<tr>
<td>Phenylpyruvic acid, 5 gm.</td>
<td>M. K.</td>
</tr>
<tr>
<td></td>
<td>M. B.</td>
</tr>
<tr>
<td></td>
<td>W. R.</td>
</tr>
<tr>
<td></td>
<td>W. K.</td>
</tr>
<tr>
<td>( dl )-Phenyllactic acid, 5 and 10 gm.</td>
<td>M. K.</td>
</tr>
<tr>
<td></td>
<td>W. R.</td>
</tr>
<tr>
<td></td>
<td>M. B.</td>
</tr>
<tr>
<td>( l )-Tyrosine, 10 gm.</td>
<td>Average, 3 patients</td>
</tr>
<tr>
<td>Controls</td>
<td>Average, 3 controls</td>
</tr>
</tbody>
</table>

5 gm. portions of phenylpyruvic acid were fed to four oligophrenics. A distinct rise in blood phenylalanine was found in
each instance (Table III) but no detectable quantity of phenylpyruvic acid appeared in the blood after either phenylpyruvic acid or phenylalanine was fed.

The data presented in Table III show that the ingestion of tyrosine had no apparent influence on the phenylalanine content of the blood. The ingestion of phenylalanine or of phenylpyruvic acid by normal individuals resulted in a slight increase in the blood phenylalanine (Table III).

The phenylalanine content of the spinal fluid was determined in two patients 6 hours after the ingestion of 5 gm. of the amino acid. 12 and 9 mg. per 100 cc. of fluid were found as compared with 5 and 3 mg. respectively in the same patients under fasting conditions. It should be pointed out, however, that the latter values were not obtained on the same day. No phenylpyruvic acid was found in the spinal fluid before or after the feeding of phenylalanine.

While this work was in progress, it was found that phenyllactic acid gives almost as much color in the nitration procedure as does phenylalanine. It was necessary, therefore, to rule out the possibility that phenyllactic acid and not phenylalanine was measured in the foregoing studies. This was accomplished by the following experiments. 15 mg. of phenylalanine were added to 30 cc. of normal serum, the proteins were precipitated with trichloroacetic acid, and the filtrate was extracted with ether in a continuous extractor for 8 hours. The residue from the ether extract gave no color with the nitration method, while all of the color remained in the aqueous layer. 6 mg. of phenyllactic acid, as the calcium salt, were added to 15 cc. of normal serum. The proteins were precipitated with trichloroacetic acid and the filtrate was extracted with ether for 4 hours. All the phenyllactic acid was found in the ether layer. Other experiments of a similar nature carried out on normal blood after the addition of phenylalanine, phenyllactic acid, or both, showed that, on extraction with ether, phenylalanine remains in the water layer while phenyllactic acid is completely extracted by ether.

With this ether extraction procedure, it was found that no phenyllactic acid is present in the blood of fasting patients affected with phenylpyruvic oligophrenia. Moreover, the ingestion of 5, 5, and 10 gm. of dl-phenyllactic acid by three patients resulted
in an increase of blood phenylalanine, as shown in Table III, while neither phenyllactic acid nor phenylpyruvic acid was found in these samples of blood. Finally, the trichloroacetic acid filtrates from one each of the phenylalanine and phenylpyruvic acid feeding experiments were extracted with ether for 3 hours and the colorimetric procedure was applied to both aqueous and ethereal solutions. The purple color developed only in the aqueous solution, thus confirming that in each instance phenyllactic acid was not responsible for the increased values of the colorimetric reading.

Comment

The data presented confirm in general the results of Fölling, Closs, and Gammes (5) who were the first to observe an increase in phenylalanine in the blood of patients with phenylpyruvic oligophrenia. The method of estimation used by these investigators, however, was not quantitative, since it was based upon the ability of Bacillus proteus to convert phenylalanine into phenylpyruvic acid, which was then determined qualitatively by the ferric chloride reaction. The experiments here reported seem to exclude the presence of appreciable amounts of phenylpyruvic acid in the blood and spinal fluid and of phenyllactic acid in the blood of these patients. It appears, therefore, that the essential biochemical characteristic of the disease consists in an inability of the subjects to dispose of phenylalanine at a normal rate rather than in a failure to break down phenylpyruvic acid, as had been previously assumed (1, 6). The presence of phenylpyruvic acid in the urine may be considered, then, as an incidental phenomenon resulting from the deamination of a portion of the blood phenylalanine by the kidney tissue. The observation that ingestion of d-phenylalanine results in a significantly higher urinary output of phenylpyruvic acid than does an equivalent quantity of the l acid (1) may be explained by assuming that a portion of the natural amino acid was utilized for some physiological process which may not have involved deamination to phenylpyruvic acid (cf. (7)).

The formation of phenylpyruvic acid in the kidney represents an alternative path in the catabolism of phenylalanine. This route would be available to the organism when the normal pathway is blocked. Some evidence which suggests that the normal
route of phenylalanine catabolism may be through tyrosine and not via phenylpyruvic acid is as follows: (a) studies in alkaptonuria have shown that both phenylalanine and tyrosine cause an increased elimination of homogentisic acid (8); (b) when the liver is perfused with phenylalanine, tyrosine is found in the perfusion fluid (9); (c) in tyrosinosis, the ingestion of phenylalanine causes an increased urinary excretion of tyrosine and of \( p \)-hydroxyphenylpyruvic acid (10); (d) experiments with tissue slices indicate that phenylpyruvic acid fails to give acetoacetic acid under conditions in which phenylalanine and tyrosine yield this compound (11). Other evidence of the close metabolic relationship between tyrosine and phenylalanine is given by the studies on experimental alkaptonuria (12, 13) and by the finding of \( l-p \)-hydroxyphenyllactic acid in the urine of vitamin C-deficient premature infants following the ingestion of either phenylalanine or tyrosine (14). In this connection, the apparent failure of the feeding of 10 gm. of \( l \)-tyrosine to change the level of blood phenylalanine of the oligophrenic patients is of interest. The opinion that different metabolic pathways which may vary according to the requirements of the organism are available is in agreement with recent observations on the metabolism of sulfur compounds (cf. (15)).

The results of feeding phenylpyruvic acid and phenyllactic acid are worthy of especial interest. These compounds, while increasing the phenylpyruvic acid output in the urine (1), fail to induce a rise of the ketonic acid in the blood, but cause instead an appreciable increase of blood phenylalanine. This finding appears to indicate that the patients are able to aminate the keto and hydroxy acids and that the phenylalanine so formed is eventually deaminated in the kidney to phenylpyruvic acid. Within the past few years evidence which indicates that keto acids probably are transformed into the corresponding amino acids in the animal organism has been accumulating. Thus, certain keto acids are able to support the growth of animals maintained on a diet deficient in the corresponding essential amino acid (16). Likewise, the methyl derivatives of many essential amino acids can be used for purposes of growth in lieu of the corresponding amino acid. The mechanism of their conversion appears to consist in the oxidation of the \( N \)-methylamino acid to the corresponding
ketonic acid, followed by amination to the amino acid (17-20). Our data also indicate the occurrence of such amination and deamination processes in man. The possibility that the alteration of the anabolic pathway of phenylalanine indicated by this investigation may result in a diminished availability of the amino acid in the building up of proteins remains the subject for a later paper of this series.

SUMMARY

1. Quantitative determinations of phenylalanine and of phenylpyruvic acid in the blood of sixteen patients with phenylpyruvic oligophrenia showed a content of phenylalanine varying from 15 to 41 mg. per 100 cc. and the absence of phenylpyruvic acid. Neither compound could be estimated in appreciable amounts in the blood of normal individuals by the procedures described.

2. The blood phenylalanine showed a significant increase following ingestion of proteins, of phenylalanine, of phenylpyruvic acid, and of phenyllactic acid.

3. The ingestion of these substances did not result in the appearance of determinable quantities of phenylpyruvic acid or of phenyllactic acid in the blood.

4. Patients with phenylpyruvic oligophrenia have phenylalanine but no phenylpyruvic acid in the spinal fluid.

5. Ingestion of phenylalanine causes an increase in the amount of phenylalanine in the spinal fluids of these patients.

We wish to thank Professor Hans T. Clarke and Dr. Warren M. Sperry for their interest and helpful advice in the preparation of this manuscript.

BIBLIOGRAPHY

CHEMICAL AND METABOLIC
STUDIES ON PHENYLALANINE: II.
THE PHENYLALANINE CONTENT OF
THE BLOOD AND SPINAL FLUID IN
PHENYLPYRUVIC OLIGOPHRENIA
George A. Jervis, Richard J. Block, Diana
Bolling and Edna Kanze


Access the most updated version of this article at http://www.jbc.org/content/134/1/105.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/134/1/105.citation.full.html#ref-list-1