THE AMINO-ACID CONTENT OF THE BLOOD IN NORMAL AND PATHOLOGIC CONDITIONS.

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The present day concepts of the amino-acid metabolism in normal persons have been formulated largely on the basis of the studies of Folin and Denis, Van Slyke and Meyer, and Folin and Berglund. These authors have been particularly interested in the relation of the amino-acids in the blood to the processes of protein metabolism in normal persons.

The behavior of the amino-acids in the blood in disease has not been investigated so thoroughly. On the basis of his study of normal persons, Folin says: "The deamination process appears to be such a fundamental process that one cannot expect to find many pathological conditions in which the amino nitrogen of the blood filtrates will vary very much from the normal." Various analyses of the blood in disease have been reported, but not in a series sufficiently large to establish this generalization. Furthermore, the results reported have not been sufficiently uniform to be of great value in the study of the deamination process.

The quantitative study of the amino-acids in the blood has been difficult because of the inadequacy of the earlier methods of analysis; indeed it was not until 1914 that Abel, Rowntree, and Turner first isolated crystalline amino-acids from the blood stream by their method of vividiffusion. The present methods of analysis are more satisfactory, but the exact amount of amino nitrogen found in a sample of blood depends largely on the details of the analytic method used. Amino-acids are more concentrated in the blood corpuscles than in the serum.
The method of protein precipitation is important. Hiller and Van Slyke have recently compared the more commonly used reagents, most of which permit similar amounts of amino nitrogen to pass into the blood filtrate. After alcohol precipitation, however, the filtrate contains only about two-thirds as much amino nitrogen as the filtrates after trichloroacetic acid, or tungstic acid precipitation, although the proportion of the amino nitrogen in the alcohol filtrate is fairly constant. This objection to the method of alcohol precipitation in no way affects the validity of the conclusions drawn from comparative physiologic experiments, but the results of Van Slyke and Meyer, and of others using the method cannot be compared directly with analyses of filtrates prepared according to the methods of Bock or of Folin and Wu. The postalimentary amino-acidemia must also be considered in interpreting analytic values found in blood samples not taken under conditions of fasting, although Hammett found practically the same amino nitrogen content in blood samples taken from 3 to 4 hours after a meal, as in samples after fasting.

In this study, analyses were made of whole blood from a series of disorders as extensive as possible, with special reference to known disorders of metabolism. All blood samples were taken in the morning, before breakfast. The colorimetric method of Folin was used for determining the amino nitrogen. Duplicate analyses of standard solutions of alanine by the nitrous acid method of Van Slyke gave constant analytic values, although a somewhat more uniform series of readings was obtained by the colorimetric method. Duplicate analyses of a small series of blood filtrates indicated that the results by the Folin method were, on the average, slightly higher (0.5 mg.) than those by the Van Slyke method. Because of the greater convenience, the colorimetric method was used routinely in the present study.

DISCUSSION OF RESULTS.

A summary of the analytic data is given in Table I. The average, minimal, and maximal values for the amino nitrogen found in each condition are given. The constancy of the analytic values is striking, and in none of the conditions studied was there a significant deviation from the normal. Certain of these conditions warrant individual discussion.
Normal.—In a series of twenty observations on twenty normal persons, the amino nitrogen in the whole blood was found to vary between 5.2 and 7.2 mg. in 100 cc., the average amount being 6.37 mg. This compares favorably with the analyses of

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Observations</th>
<th>Amino-acid nitrogen in 100 cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Min.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mg.</td>
</tr>
<tr>
<td>Normals</td>
<td>20</td>
<td>5.2</td>
</tr>
<tr>
<td>Renal insufficiency; blood urea greater than 50 mg. in 100 cc.</td>
<td>56</td>
<td>4.9</td>
</tr>
<tr>
<td>Chronic glomerular nephritis</td>
<td>21</td>
<td>5.1</td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>17</td>
<td>4.9</td>
</tr>
<tr>
<td>Hypertension and arteriosclerosis; blood urea normal</td>
<td>20</td>
<td>4.8</td>
</tr>
<tr>
<td>Myocardial degeneration with cardiac decompensation</td>
<td>20</td>
<td>4.7</td>
</tr>
<tr>
<td>Exophthalmic goiter; basal metabolic rate above +15</td>
<td>44</td>
<td>4.6</td>
</tr>
<tr>
<td>Adenoma of the thyroid with hyperthyroidism; basal metabolic rate above +15</td>
<td>27</td>
<td>5.1</td>
</tr>
<tr>
<td>Myxedema and hypothyroidism; basal metabolic rate below −10</td>
<td>25</td>
<td>4.9</td>
</tr>
<tr>
<td>Diabetes; blood sugar over 0.2 per cent</td>
<td>86</td>
<td>4.6</td>
</tr>
<tr>
<td>&quot; &quot; &quot; below 0.2 &quot; &quot;</td>
<td>30</td>
<td>5.1</td>
</tr>
<tr>
<td>Obesity</td>
<td>17</td>
<td>5.6</td>
</tr>
<tr>
<td>Chronic arthritis</td>
<td>11</td>
<td>5.9</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>19</td>
<td>5.3</td>
</tr>
<tr>
<td>Hepatic insufficiency as shown by the phenol-tetrachlorophthalein test</td>
<td>19</td>
<td>4.7</td>
</tr>
<tr>
<td>Anemia</td>
<td>6</td>
<td>5.1</td>
</tr>
<tr>
<td>Tetany</td>
<td>5</td>
<td>5.0</td>
</tr>
<tr>
<td>Duodenal ulcer; sippy regime</td>
<td>5</td>
<td>5.7</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>3</td>
<td>5.5</td>
</tr>
<tr>
<td>Typhoid fever</td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>Addison's disease</td>
<td>3</td>
<td>5.8</td>
</tr>
<tr>
<td>Acromegaly</td>
<td>1</td>
<td>5.9</td>
</tr>
<tr>
<td>Gout</td>
<td>2</td>
<td>6.3</td>
</tr>
<tr>
<td>Total</td>
<td>458</td>
<td>4.6</td>
</tr>
</tbody>
</table>
Folin and Berglund. In a series of twelve normal, fasting subjects, they found that the blood contained from 5.7 to 7.8 mg. of amino nitrogen, an average of 6.4 mg. These two series of estimations were made by the same method under similar conditions. The analyses of Bock gave slightly higher amounts, 7.72 mg., while those of Hammett were slightly lower, 4.9 mg. Blau, also using the gasometric method, obtained values for the normal comparable with those of Hammett. The other reported analyses of normal human blood come within the foregoing limits.

Fig. 1. Frequency curve showing the distribution of different concentrations of amino nitrogen in human blood over the range of occurrence.

Frequency curves were constructed, showing the distribution of amino nitrogen in the blood of normal persons, and that of the entire group studied. As there was no significant divergence between the two series, only the curve for the larger group is shown (Fig. 1). This frequency curve is fairly symmetric. 90 per cent of the observations are between the values of 4.8 and 7.8 mg. The most probable value for the amino nitrogen content of the blood is between 6.0 and 6.4 mg., while the average is 6.3 mg.
Renal Insufficiency.—Bock, in particular, emphasized the possibility of an increase in the amino nitrogen in the blood accompanying the retention of other forms of nitrogen in cases of chronic nephritis and uremia. Okada and Hayashi have also reported such an increase in dogs, following ligation of both ureters. Folin and Berglund, on the other hand, did not find evidence of amino-acid retention in a small series of patients with high blood urea. In our study, the observations were classified according to the clinical diagnosis. Also, all cases in which the blood urea was more than 50 mg. were grouped. The relation between the amino nitrogen and the blood urea is shown in Fig. 2. There is no significant departure from normal in any of the foregoing groups, and no apparent correlation between the amino-acids and the degree of renal insufficiency or urea retention.

Fig. 2. The amino nitrogen in the blood compared with the blood urea in patients with renal insufficiency.

The similarity between the results obtained by the colorimetric and gasometric methods of analyzing the blood of normal persons has been mentioned. Urea reacts slowly with nitrous acid, and, if present in moderate amounts, will lead to high readings
by the Van Slyke method unless appropriate corrections are made. Comparison between the two methods when tested against a known alanine solution and the solution with 300 mg. of urea added in 100 cc. is shown in Table II. The colorimetric method gives similar analytic values in the different solutions, and the accuracy is not affected by the excess of urea. No attempt was made to remove the urea by treatment with urease, but the usual corrections were made in the gasometric analysis. Nevertheless,

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\begin{array}{|l|l|l|l|l|}
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\text{TABLE II.} & \text{Comparison between the Folin and Van Slyke Methods for Determining Amino Nitrogen.} \\
\hline
& \text{Folin.} & \text{Van Slyke.} & \text{Folin.} & \text{Van Slyke.} \\
\hline
\text{Alanine solution.} & \text{mg. per 100 cc.} & \text{mg. per 100 cc.} & \text{mg. per 100 cc.} & \text{mg. per 100 cc.} \\
\hline
8.25 & 8.75 & 8.49 & 12.20 & 8.59 \\
8.58 & 8.65 & 8.69 & 11.10 & 8.70 \\
8.26 & 8.20 & 8.27 & 9.40 & 8.72 \\
8.45 & 8.34 & 8.23 & 11.80 & 8.54 \\
8.44 & 8.47 & 8.92 & 11.50 & 8.68 \\
8.50 & 8.20 & & & 8.76 \\
8.42 & 8.61 & & & 8.68 \\
8.54 & & & & 8.58 \\
\hline
\text{Average} & 8.45 & 8.45 & 8.50 & 11.40 \\
\text{“ deviation...} & \pm 0.15 & \pm 0.20 & \pm 0.20 & \pm 0.50 & \pm 0.10 \\
\hline
\end{array}
\]

the gasometric method shows a relatively large error as a result of the added urea, and the possibility of such error must be considered in interpreting previous analyses by that method.

We have repeated the experiments of Okada and Hayashi, but were unable to confirm their finding of an increase in the amino-acids in the blood following nephrectomy. The changes in the blood urea and in the amino nitrogen in a dog, following bilateral nephrectomy, are shown in Fig. 3. The increase in the blood urea apparently is directly proportional to the length of time after operation. The amino-acids, on the other hand, show no change during life. Blood taken post mortem contained nearly double the previous amount of amino nitrogen. Whether this
increase is strictly post mortem or develops during the last 12 hours of life we are not prepared to say. In any event it is essentially a terminal condition. Haden and Orr found no change in the amino nitrogen content of the blood following intestinal obstruction, although there was a marked increase in the blood urea, with accompanying evidence of protein destruction.

**Thyroid Disorders.**—The metabolic changes in diseases of the thyroid gland are well recognized. The metabolic rate is an accurate index of the degree of the disturbance, especially in cases of exophthalmic goiter. However, no correlation was found between the amino nitrogen in the blood and the metabolic rate (Fig. 4). The amino-acid content of the blood does not differ from the normal in cases of hyperthyroidism or hypothyroidism.

**Diabetes.**—Desqueyroux has suggested that the amino-acids in the blood are increased in cases of diabetes, especially in obese persons. Neither in diabetes of any type nor in obesity did we find significant changes from the normal. The amino-acids are not correlated with the level of the blood sugar (Fig. 5). This was true both of patients under treatment and of those with diabetic coma and acidosis.

Folin and Berglund noted a slight reduction in the nitrogenous constituents of the blood, including the amino-acids, after the ingestion of 200 gm. of glucose. They said: "As was to be expected from the absence of nitrogen intake and from the protein-
Fig. 4. The amino nitrogen in the blood compared with the basal metabolic rate.

Fig. 5. The amino nitrogen in the blood compared with the blood sugar level.
sparing action of the sugar we meet here with small, but definite reductions in the different nitrogenous constituents in the blood." Through the courtesy of Drs. Wilder and Kitchen, we were able to follow the amino-acids in the blood in their study of the effects of insulin on the utilization of different sugars. There was a slight, though definite, fall in the amino nitrogen after the ingestion of 100 gm. of sugar. This change was entirely similar to that reported by Folin and Berglund, the period of minimal amino nitrogen in general coinciding with the maximal blood sugar levels. There was no change in the hemoglobin during the experiment, showing that the fall in the amino-acids could not be referred to changes in the blood volume or in the relative proportions of plasma and corpuscles present. Levulose produced less change than did glucose. Insulin had no effect on the amino-acids. The degree of fall in the amino nitrogen bore no apparent relationship to the respiratory quotient or to the increase in metabolism accompanying the oxidation of the sugar. We do not believe that the relation between the sugar and the amino-acids is as simple as that postulated by Folin and Berglund, but are unable to account satisfactorily for these changes.

Hepatic Insufficiency.—Opinions regarding the effect of hepatic disease on the amino-acids in the blood have varied in proportion to the degree of emphasis placed on the rôle of the liver in amino-acid metabolism. Amino-acid excretion in cases of acute yellow atrophy of the liver may be so increased that leucine and tyrosine crystals appear in the urine. The amino nitrogen in the blood was greatly increased in the cases studied by Feigl and Luce and by Stadie and Van Slyke. In cases of acute yellow atrophy, the hepatic insufficiency is complicated by the rapid autolysis of that organ and the consequent flooding of the system with amino-acids of autolytic origin. An accumulation of amino-acids need not necessarily be ascribed to a failure of deamination and urea formation.

Changes in the blood with acute yellow atrophy alone cannot be used as a standard for determining hepatic insufficiency in other disorders of the liver. Rowntree, Marshall, and Chesney have made the most extensive study of the amino-acids in the blood in hepatic disease. They suggest that the amino nitrogen may be increased in a considerable proportion of such cases. They
used blood serum in their study and also the alcoholic filtrate for analysis; hence the values they obtained for the amino nitrogen cannot be compared directly with the present data. The increases they found were not striking, especially in view of the low value for amino nitrogen taken as the upper limit of normal. Marshall and Rowntree also found a slight increase in the amino-acids in the blood of dogs after phosphorus poisoning. They point out that this increase is largely terminal, and examination of their protocols shows that the highest values were obtained in postmortem samples of blood.

In this study an attempt was made to confirm the degree of hepatic insufficiency by means of the Rosenthal modification of the phenoltetrachlorophthalein test of Rowntree, Hurwitz, and Bloomfield. This test is not an absolute gauge of hepatic function, but in conjunction with the accompanying clinical findings it affords a convenient index of severe functional disturbance. In all the cases reported, largely cirrhosis or chronic hepatitis, there was marked retention of dye in the blood plasma 2 hours after injection. No evidence of disturbance in the amino-acid content of the blood was found. Unfortunately, no cases of acute yellow atrophy were available.

Leucemia.—Martin and Denis found evidence of an increase in the rest nitrogen in the blood in cases of leucemia. They suggested that this increase might be due to amino-acids, and this suggestion has since been abundantly confirmed by Okada and Hayashi, and by Sandiford, Boothby, and Giffin. The latter found that in leucemia the values for the amino nitrogen in the whole blood ranged between 5.0 and 16.0 mg., with an average value of 10.0 mg., and were interested in a possible relationship between this finding and the increased metabolism usually occurring in this disease.

The increased excretion of uric acid in cases of leucemia has long been recognized as evidence of a rapid destruction of leucocytes. Collip, in particular, has emphasized the large amount of amino-acids present in the cell nucleus. It seems plausible, therefore, to ascribe the high amino-acid content of the blood in leucemia to the increase in the leucocytes and an associated flooding of the organism with amino-acids of autolytic origin.

1 A detailed study of the metabolism in leucemia is in progress.
Miscellaneous.—In the other conditions, such as carcinoma, gout, and febrile diseases (Table I), noteworthy changes in the amino-acid content of the blood are not shown, and an examination of the literature did not indicate that changes were to be expected.

CONCLUSIONS.

The amount of amino nitrogen in the blood varies between 4.8 and 7.8 mg. in 100 cc., the average amount being 6.3 mg. This was found to be true in normal persons, and in a series of more than 400 observations covering twenty pathologic conditions. The level of amino nitrogen may be increased by flooding the organism with amino-acids arising during digestion or from the rapid autolysis of body tissue, as has been noted in cases of leucemia and acute yellow atrophy of the liver. In general, the quantity in the blood is maintained within the foregoing limits with remarkable constancy. Such disease conditions as uremia, diabetes, exophthalmic goiter, or hepatic insufficiency are not exceptions to this rule.

The observed constancy of this regulation in the presence of such severe metabolic disturbances is direct evidence of the widespread and fundamental nature of the deamination processes in the body.

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