THE MAINTENANCE OF NITROGEN EQUILIBRIUM IN DOGS
BY INTRAVENOUS ALIMENTSATION WITH AN ACID HY-
DROLYSATE OF CASEIN FORTIFIED
WITH TRYPTOPHANE

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Since McCoy, Meyer, and Rose's (1) discovery of threonine and its in-
dispensability to the animal system, numerous studies in animals and in
man have been reported on the administration of amino acids derived from
various sources. Using a complete mixture of purified amino acids in a
synthetic diet, Rose et al. (2, 3) have shown that it is possible to keep dogs
and human beings in nitrogen equilibrium. Also Madden et al. (4) have
obtained positive nitrogen balance with hypoproteinemic dogs injected with
mixtures of the ten essential amino acids.

Amino acids are normally present in the blood stream. Therefore it is
to be expected that they can be safely administered parenterally. In the
normal animal amino acids are absorbed from the gastrointestinal tract.
They are circulated in the blood stream and utilized in part for the syn-
thesis of body proteins. A complete mixture of amino acids administered
intravenously should thus be metabolized in the same manner. How-
ever, in malnutrition and starvation, when pathologic changes have oc-
curred in the intestinal wall, the administration of amino acids or proteins
may not be as effective orally as parenterally (5).

Recently investigators (6-15) have studied the parenteral adminis-
tration of hydrolysates and mixtures of pure amino acids. Although bene-
ificial results were clinically observed, many of the experiments were limited
to short periods of time. This raises the question of retention of the nitro-
gen rather than utilization. Also most of the published work has not taken
into account the excretion of nitrogen in the feces. Some investigators
used the hydrolysate of proteins as a supplement to the diet. In these
instances the role that such preparations play in the maintenance of nitro-
gen balance is open to question. Furthermore, in almost all of the above
studies large amounts of nitrogen were administered.

The present investigation was undertaken to determine whether or not it is possible to maintain nitrogen balance in dogs over a sufficiently long
period of time on known, low nitrogen intakes by (1) the oral administra-
tion of a complete mixture of amino acids produced by the acid hydrolysis
of casein, fortified with dl-tryptophane,¹ and (2) the parenteral administration of such a preparation. The method for making a sterile, pyrogen-free acid hydrolysate of casein has been described elsewhere (16) and the quantitative estimation of its indispensable amino acids has been reported by Block (17).

EXPERIMENTAL

The procedure employed was the same as that used by Rose et al.² in their investigation of the utilization of mixtures of purified amino acids. Three adult female dogs were used throughout the duration of the experiment and were kept in individual metabolism cages. Each animal was catheterized once daily and the 24 hour urine samples were diluted to a standard volume and preserved with thymol in the refrigerator. The feces were divided into 7 day periods by the use of carmine capsules and were kept in acid alcohol. Total nitrogen of the urine and feces was determined by the Kjeldahl procedure as modified by Scales and Harrison (18). A methylene blue, methyl red indicator was used in the saturated boric acid (19). Creatinine and creatine analyses were carried out by the colorimetric methods of Folin (20) with Peters’ (21) modification of time factors. Consistent daily creatinine excretion was used to indicate that a complete 24 hour urine sample had been obtained.

The diet presented in Table I is an adaptation of Cowgill’s (24). The protein fraction of the diet was omitted and the amino acids were fed either by stomach tube or by vein. Nitrogen of the food other than that furnished by the amino acids amounted to 60 mg. per 100 gm. of diet, of which approximately 40 mg. were contained in the vitamins. The 20 mg. of nitrogen of unknown origin represent less than 2 per cent of the total nitrogen intake even though the total nitrogen intake is very low. Thus the nitrogen in the diet is substantially derived from the acid hydrolysate of casein fortified with tryptophane.

At the beginning of each experiment the animal was fed a 6 per cent casein diet supplemented with methionine³ for at least 2 weeks. The casein and methionine were then replaced by the acid hydrolysate fortified with tryptophane for 1 or more weeks. Subsequently this preparation was injected either intravenously or intraperitoneally for 7 day periods respectively. The remaining portions of the diet were fed orally. Following the period of parenteral administration, the hydrolysate was again fed by stomach tube. Throughout the experiment the dog was given approximately 80 calories per kilo of body weight.

¹ Paremamine, Frederick Stearns and Company Division.
³ Courtesy of U. S. Industrial Chemicals, Inc.
Results

Since all the animals reacted very similarly, the complete data for only one dog are given in Table II. The nitrogen balance data are summarized in Figs. 1 to 3.

It can be seen from the data given in Table II that the creatinine excretion is constant. This has been noted in all normal adult dogs studied. The creatine excretion, however, is quite variable. The small amount of nitrogen in the feces represents a rather large percentage of the total nitrogen excreted (15 to 20 per cent). The fecal nitrogen is probably close to an irreducible minimum. When no appreciable nitrogen is fed,

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Composition of Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>calories</td>
</tr>
<tr>
<td>Sucrose</td>
<td>160</td>
</tr>
<tr>
<td>Dextrin</td>
<td>86</td>
</tr>
<tr>
<td>Methylcellulose</td>
<td>2.50</td>
</tr>
<tr>
<td>Salt mixture*</td>
<td>4.00</td>
</tr>
<tr>
<td>Lard</td>
<td>177.75</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>27.00</td>
</tr>
<tr>
<td>Wheat germ oil</td>
<td>2.25</td>
</tr>
<tr>
<td>Liver extract†</td>
<td>2.00</td>
</tr>
<tr>
<td>Casein hydrolysate fortified with tryptophane (fed separately)</td>
<td>28.00</td>
</tr>
<tr>
<td></td>
<td>481.00</td>
</tr>
</tbody>
</table>

Approximately 15.5 gm., per kilo of body weight of the dog, of the protein-free diet were fed.

* Jones and Foster (22).
† Conger and Elvehjem (23). Fullers' earth treatment omitted.

the feces obtained still contain approximately the same amount of nitrogen. The largest animal, receiving more nitrogen, excreted a greater amount in the feces, but a slightly lower proportion of the total nitrogen eliminated (14 per cent). In no case did the nitrogen in the feces fall below 12 per cent of the total nitrogen output.

The data presented in Table II show that Dog 2 retained nearly 20 per cent of the nitrogen fed, whereas the others kept 10 to 15 per cent. During the week of intraperitoneal injections of Dog 2, only 1.7 per cent of the nitrogen administered was held (Fig. 1). However, during all the other parenteral feedings, at least 15 per cent of the nitrogen was not excreted,
which is approximately the same as when the amino acids are fed orally. As was anticipated, the dogs slowly gained weight. While there are days

![Fig. 1. Dog 2, 4.9 kilos. Nitrogen balance with an acid hydrolysate of casein fortified with tryptophane as the sole source of amino acids. Nitrogen intake 1125 mg. per day = 230 mg. of nitrogen per kilo of body weight. Days 1 to 14, oral administration; days 15 to 21, intraperitoneal administration; days 22 to 28, oral administration.](image)

![Fig. 2. Dog 3, 16.3 kilos. Nitrogen balance with an acid hydrolysate of casein fortified with tryptophane as the sole source of amino acids. Nitrogen intake 3850 mg. per day = 230 mg. of nitrogen per kilo of body weight. Days 1 to 21, oral administration; days 22 to 28, intravenous administration; days 29 to 35, oral administration.](image)

![Fig. 3. Dog 1, 5.6 kilos. Nitrogen balance with an acid hydrolysate of casein fortified with tryptophane as the sole source of amino acids. Nitrogen intake 1200 mg. per day = 214 mg. of nitrogen per kilo of body weight. Days 1 to 14, oral administration; days 15 to 21, intravenous administration; days 22 to 28, oral administration.](image)

of negative nitrogen balance, these are the usual variations one obtains with normal adult animals, and the over-all picture is one of good positive
balance. Figs. 1 to 3 show that good positive nitrogen balance has been maintained in every dog used, with the acid hydrolysate of casein, to which

TABLE II
Nitrogen Balance with Casein Hydrolysate Fortified with Tryptophane As Sole Source of Amino Acids

<table>
<thead>
<tr>
<th>Amino acids fed (total N intake, 1000 mg.)</th>
<th>Date</th>
<th>Body weight</th>
<th>Urine N</th>
<th>Creatinine N</th>
<th>Fecal N*</th>
<th>Total N output</th>
<th>N balance</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>kg.</td>
<td>mg.</td>
<td>mg.</td>
<td>mg.</td>
<td>mg.</td>
<td>mg.</td>
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<tr>
<td>Orally</td>
<td>Mar. 14</td>
<td>5.3</td>
<td>676</td>
<td>32</td>
<td>16</td>
<td>142</td>
<td>818</td>
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<tr>
<td></td>
<td>&quot; 15</td>
<td>5.3</td>
<td>705</td>
<td>32</td>
<td>14</td>
<td>142</td>
<td>847</td>
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<tr>
<td></td>
<td>&quot; 16</td>
<td>5.4</td>
<td>648</td>
<td>32</td>
<td>6</td>
<td>142</td>
<td>790</td>
</tr>
<tr>
<td></td>
<td>&quot; 17</td>
<td>5.4</td>
<td>655</td>
<td>31</td>
<td>7</td>
<td>142</td>
<td>797</td>
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<tr>
<td></td>
<td>&quot; 18</td>
<td>5.4</td>
<td>675</td>
<td>32</td>
<td>3</td>
<td>142</td>
<td>817</td>
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<td></td>
<td>&quot; 19</td>
<td>5.4</td>
<td>633</td>
<td>31</td>
<td>3</td>
<td>142</td>
<td>775</td>
</tr>
<tr>
<td></td>
<td>&quot; 20</td>
<td>5.4</td>
<td>728</td>
<td>32</td>
<td>3</td>
<td>142</td>
<td>870</td>
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<tr>
<td>Total</td>
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<td>4720</td>
<td>222</td>
<td>52</td>
<td>994</td>
<td>5714</td>
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<tr>
<td>Average</td>
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<td></td>
<td>5.4</td>
<td>674</td>
<td>32</td>
<td>7</td>
<td>142</td>
</tr>
<tr>
<td>Intravenously</td>
<td>Mar. 21</td>
<td>5.4</td>
<td>766</td>
<td>32</td>
<td>10</td>
<td>125</td>
<td>891</td>
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<tr>
<td></td>
<td>&quot; 22</td>
<td>5.4</td>
<td>870</td>
<td>31</td>
<td>14</td>
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<td>995</td>
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<td>&quot; 23</td>
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<td>583</td>
<td>34</td>
<td>3</td>
<td>125</td>
<td>708</td>
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<td>&quot; 24</td>
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<td>5.3</td>
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<td>32</td>
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<td>125</td>
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<td>512</td>
<td>33</td>
<td>2</td>
<td>158</td>
<td>670</td>
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<td></td>
<td>&quot; 29</td>
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<td>571</td>
<td>33</td>
<td>2</td>
<td>158</td>
<td>729</td>
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<td></td>
<td>&quot; 30</td>
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<td>631</td>
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<td>3</td>
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<td>&quot; 31</td>
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<td>35</td>
<td>2</td>
<td>158</td>
<td>981</td>
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<td>Apr. 1</td>
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<td>773</td>
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<td>34</td>
<td>2</td>
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<td>806</td>
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<td>232</td>
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<td>1106</td>
<td>5572</td>
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<tr>
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<td>5.4</td>
<td>638</td>
<td>33</td>
<td>3</td>
<td>158</td>
</tr>
</tbody>
</table>

* The total fecal nitrogen was determined once for each period; thus the daily figures are averages, which accounts for their uniformity.

1 per cent of tryptophane has been added, as the sole source of amino acids. The nitrogen intakes are near minimal and were chosen because of
the practical importance of maintaining balance with the least amount of fluid administration. It is possible to obtain much larger nitrogen retention for short periods at high nitrogen intakes, but in view of the clinical conditions which must normally be met low levels of nitrogen intake were used.

SUMMARY

Three different dogs have been maintained for 21 to 35 days in positive nitrogen balance by the administration of purified diets wherein practically the sole source of nitrogen was derived from an acid hydrolysate of casein fortified with tryptophane. The preparation was given both orally and parenterally to supply a nitrogen intake of approximately 200 mg. per kilo of body weight.

BIBLIOGRAPHY

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