STUDIES IN AMINO ACID METABOLISM

VI. THE METABOLISM OF DL-VALINE AND DL-ISOVALINE IN THE NORMAL RAT*

BY JOSEPH S. BUTTS AND RUSSELL O. SINNHUBER

(From the Department of Chemistry, Oregon State College, Corvallis)

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The indispensability of valine in the diet of the rat has been demonstrated (1). However, experiments designed to study the disposal of this amino acid when fed in large amounts have failed to yield satisfactory information as to its fate. Thus, Embden, Salomon, and Schmidt (2) by using liver perfusion methods could find no acetone bodies in the perfusing fluid on the addition of valine. Dakin (3) in three phlorhizin experiments was unable to demonstrate the production of an appreciable amount of glucose after feeding valine. He concluded that this amino acid did not give rise to sugar. He did make one rather significant statement: "The excretion of acetoacetic acid was distinctly lowered on giving valine." Chase and Lewis (4) in a study concerned primarily with rates of absorption failed to find any increase in liver glycogen 6 hours after feeding valine or isovaline (α-amino-α-methylbutyric acid).

The work here reported is a study of the glycogenic property of the valines and the ketolytic property of dl-valine.

EXPERIMENTAL

The experimental procedures used in this study are the same as those employed in earlier studies concerned with the metabolism of the amino acids (5). These involve the feeding of the substrate and sacrificing the animal after varying time intervals for the

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determination of the glycogen content in the liver, and also a study of the ketolytic properties of the amino acid as measured by its effect on the ketonuria.

For the glycogen studies rats ranging from 140 to 200 gm. were used. They were subjected to a 48 hour fast, during which time they had access to filter paper (6). Following this fast the animals were placed in individual cages and fed by stomach tube hourly an amount of the amino acid greater than could be absorbed during this time interval. The controls were fed a 7.5 per cent sodium chloride solution in place of the valine solution. At the end of 3, 6, 9, and 12 hours the various groups of rats were anesthetized with sodium amyntal and the livers removed and analyzed for glycogen according to the method of Good, Kramer, and Somogyi (7). The contents of the gastrointestinal tract were analyzed to ascertain that an excess of the acid was present and therefore maximum absorption was occurring at all times.

In the ketosis studies two different types of experimentation were employed. In the first case the female rats ranging from 187 to 224 gm. were fed sodium butyrate, 15 gm. (calculated as acetone) per sq.m. of surface area, and the effect of feeding the amino acid was superimposed on this ketosis. As in the glycogen studies, a 7 per cent aqueous solution of the amino acid was used. The dl-valine was fed in an amount equal to 12.50 gm. per sq.m. per day. Lee's method for the calculation of the body surface was used (8). The urines were collected daily and analyzed for acetone bodies by the Van Slyke method and for nitrogen by the Kjeldahl procedure.

In the second type of experimentation the animals were fed only the amino acid, dl-valine, while the controls received 1 cc. of a 7.5 per cent sodium chloride solution per 100 sq. cm. of surface area. The ketonuria which developed is a type of endogenous ketonuria. The development of this ketonuria we attribute to the incorporation of liver in the stock diet of our animals (9) and is a condition which we are at present investigating. The procedure was exactly the same as that described in the first type of experimentation, except that sodium butyrate was not fed.

Since it was found that the dl-isovaline was being excreted in the urine in an amount great enough to interfere with a quantitative determination of the acetone bodies, this acid was not in-
cluded in this study. Although valine also reacts with Denigès' reagent, not enough was being excreted to interfere with our determination.

The \textit{dl}-valine was a Hoffmann-La Roche product, while the \textit{dl}-isovaline was from Eastman. According to the Van Slyke amino nitrogen determination both compounds were of a high degree of purity.

\begin{table}
\centering
\caption{Glycogen Content of Liver of Rats Fed Either \textit{dl}-Isovaline or \textit{dl}-Valine in 7 Per Cent Aqueous Solution}
\begin{tabular}{l|cccccc}
\hline
Material fed & 3 hrs. & 6 hrs. & 9 hrs.* & 12 hrs. & \\
 & per cent & per cent & per cent & standard deviation of mean & per cent & standard deviation of mean \\
\hline
\textit{dl}-Valine & 0.07 (4)† & 0.05 (4) & 0.35 (18)‡ & ±0.04 & 0.39 (8)‡ & ±0.08 \\
Control & 0.06 (4) & 0.07 (4) & 0.05 (12) & ±0.03 & 0.03 (7) & ±0.01 \\
\textit{dl}-Isovaline & 0.08 (4) & 0.09 (4) & 0.03 (4) & ±0.02 & 0.04 (4) & ±0.03 \\
\hline
\end{tabular}
\end{table}

* The 9 hour group includes an experiment in which ten males were fed \textit{dl}-valine and six were used as controls. Statistically there was no difference in the levels of liver glycogen of the two groups. In all other experiments female rats were used.

† The figures in parentheses refer to the number of animals used.

‡ Statistically significant by the \textit{t} test of Fisher when compared to the controls.

\textbf{Results}

After the \textit{dl}-valine was fed, there was no evidence of glycogen formation until the 9 and 12 hour periods. After this period the values, while small, were definite. The percentages of glycogen for these periods were 0.35 and 0.39 as compared with 0.05 and 0.03 for the control groups. There was no indication of glycogen formation after isovaline was fed. These results are listed in Table I.

In the two types of ketosis experiments, essentially the same results were obtained. Although the level of ketonuria was much higher after the sodium butyrate was fed than when the acidosis
was allowed to develop spontaneously, qualitatively the results are the same. After dl-valine was fed, the total acetone body excretion was lower than the corresponding control group. Table II lists the results of the exogenous type of ketonuria, in which

**TABLE II**

*Ketonuria in Female Rats Fed 15 Gm. of Sodium Butyrate (Calculated As Acetone) per Sq.m. of Body Surface per Day*

One group received in addition 12.50 gm. of dl-valine per unit area per day, fed in 7 per cent aqueous solution. The control group received sodium chloride solution. The animals were fasted the preceding 48 hours; five animals in each group for each day.

<table>
<thead>
<tr>
<th>Material fed</th>
<th>Daily excretion of acetone bodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day</td>
</tr>
<tr>
<td></td>
<td>gm. per sq.m.</td>
</tr>
<tr>
<td>dl-Valine</td>
<td>6.90</td>
</tr>
<tr>
<td>Control</td>
<td>14.48</td>
</tr>
</tbody>
</table>

All results are statistically significant by the *t* test of Fisher.

**TABLE III**

*Effect of Feeding 12.50 Gm. of dl-Valine per Sq.m. of Body Surface per Day on Ketonuria Developed from Endogenous Stores in Female Rat*

For the first 2 days only, 7.5 per cent sodium chloride solution was fed to each group. *dl*-Valine was fed in 7 per cent aqueous solution; eight animals in each group for all days.

<table>
<thead>
<tr>
<th>Material fed</th>
<th>Daily total acetone body excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day</td>
</tr>
<tr>
<td></td>
<td>gm. per sq.m.</td>
</tr>
<tr>
<td>dl-Valine</td>
<td>0.09</td>
</tr>
<tr>
<td>Control</td>
<td>0.10</td>
</tr>
</tbody>
</table>

The 3rd, 4th, and 5th days are highly significant statistically by the *t* test of Fisher, when compared to the controls.

sodium butyrate was fed, while Table III contains the data from the endogenous type of experiments.

Since there was considerable variation between the individual animals, the data were subjected to statistical evaluation by the *t* test of Fisher (10). The foot-note below each table shows significance. In all cases a high degree of significance was observed.
DISCUSSION

Although the values for liver glycogen after dl-valine feeding are not great, they are significant. If one considers the increase over the control, the values are of about the same order as for glycine (11), which is usually considered as a sugar-forming amino acid. MacKay et al. (12) have lately reported that glycine, after a latent period, is quite good as a glycogenic agent. Some 14 hours after a single dose of this amino acid was fed, the liver glycogen rose to a rather high level. Similar experiments, although not reported in detail, were carried out after a single dose of dl-valine. The experimental periods were of 16, 20, and 24 hours. Under these conditions insignificant amounts of liver glycogen were formed, thus indicating that valine does not have a latent period which characterizes glycine.

These results are in sharp contrast to those found after dl-isovaline is fed. In these experiments no glycogen was found in any of the groups.

The effect of feeding dl-valine to animals suffering from a ketosis is what one would predict from the glycogen studies; namely, a lowering of the excretion of the acetone bodies. This is shown both by the experiments in which a high ketonuria was produced by the feeding of sodium butyrate and in those in which an acetanuric resulted from endogenous sources. In the latter study it is believed that this condition is traceable to the inclusion of fresh liver in the diet of our stock animals. McHenry and Gavin (13) have reported that a fraction prepared from fresh liver can cause a deposition of large amounts of fat in the liver. This is possibly the cause of the rather high level of acetone body excretion. In the experiments in which the sodium butyrate was superimposed on the already existing ketosis, a very high ketonuria resulted. In fact, we were recovering in the urine of the control animals almost as much material as we were feeding. The animals were receiving 15 gm. of sodium butyrate (calculated as acetone) and excreting from 12.84 to 14.48 gm. of total acetone bodies per sq.m. of surface area per day. However, regardless of the height of the ketonuria, feeding valine did cause a distinct lowering of the acetone body excretion.

In the experiments reported in Table III in which the ketonuria was developed from endogenous stores, during the first 2 days of
the experiment, in order to insure that we were dealing with comparable groups of animals, only a solution of sodium chloride was fed. This was given to insure a large urine volume. The animals were arranged so that the level of ketonuria was as nearly comparable between the two groups as possible. Again a distinct lowering of the acetonuria followed the feeding of the valine on the last 3 days of the experiment.

In all of the various techniques used each demonstrates that valine may be classified as a sugar-forming amino acid, although this property is not great. We have no information as to whether only one isomer has the ability to give rise to glycogen or whether both forms may be active. One must await the resolution of the racemic mixture to answer this question.

SUMMARY

1. dl-Valine, when fed to rats, has been shown to give rise to a small but significant amount of liver glycogen.

2. dl-Isvaline is devoid of any glycogenic properties.

3. When dl-valine is fed to a rat suffering from a ketosis, the excretion of acetone bodies is markedly decreased. This is true both in a ketonuria arising from endogenous stores and when the feeding of sodium butyrate increases the already existing acidosis.

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