THE GROWTH EFFECT OF D-VALINE*

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More than 20 years ago, experiments were conducted in this laboratory for the purpose of ascertaining whether D-valine can be utilized by the rat for growth. The results thereby obtained were mentioned on several occasions (cf. (1, 2)), but the experimental data were never published in full. It may now be stated for the record that the animals which received D-valine experienced a mean loss in body weight of 8.5 gm. in 28 days, while the positive controls which consumed an identical ration, except for the presence of L-valine, showed a mean gain in weight of 87.0 gm. Litter mates on a valine-free diet suffered a mean loss of 19.0 gm. On the basis of these findings, the conclusion was drawn that only the natural enantiomorph of valine is effective for growth purposes.

It was realized at the time that the findings, though apparently unequivocal, were based upon the use of a relatively small number of animals. Of greater concern, however, was the problem of the nutritive quality of the experimental rations. Crystalline vitamins had not become available commercially when the valine investigation was undertaken, and several of the vitamins which presently are well known had not yet been discovered. This situation necessitated the use of vitamin concentrates derived from milk, yeast, or the polishings of rice as dietary supplements. Furthermore, shortly after the completion of the valine experiments, systematic investigations were inaugurated in this laboratory with the intent of establishing the most favorable conditions for the growth of the rat on diets containing mixtures of amino acids. The findings in these studies revealed the desirability of altering our rations in the direction of lowering their fat content and of furnishing a better source of inorganic salts (3).

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† On leave from the Human Nutrition Research Branch, Agricultural Research Service, United States Department of Agriculture, Washington 25, D. C.
‡ Some of the experimental data in this paper are taken from a thesis submitted by Barbara B. Snyder in partial fulfilment of the requirements for the degree of Master of Science in Biochemistry in the Graduate College of the University of Illinois.

Eppstein, S. H., and Rose, W. C., unpublished experiments.
In the light of these observations, publication of the data obtained in the valine tests, and in many other investigations which had been carried out during this transition period, was postponed until additional data could be acquired under more ideal dietary conditions (cf. (4)).

The problem of the growth effect of D-valine has been brought into focus anew by the papers of White, Fones, and Sober (5) and Wretlind (6). According to White et al., D-valine supports the growth of rats at approximately half the rate induced by L-valine. Furthermore, following the administration of D-valine labeled in the methyl groups with C¹⁴, L-valine similarly labeled was isolated from the tissues. Wretlind reports that the presence of D,L-leucine in the food inhibits the utilization of D-valine for growth purposes, possibly through competition of D-leucine and D-valine for the enzyme system involved in the deamination of the latter amino acid. He suggests (personal communication) that this may account for the difference between our findings and those of White et al., since we employed the racemic compound and the latter investigators used the L isomer. Wretlind makes the additional observation that, even with a ration containing L-leucine, 1 per cent of D-valine is an insufficient level to permit growth, and that relatively large quantities must be supplied to produce maximal utilization.

In a straightforward problem of this nature, it appears unlikely that the observations made in any of the three investigations outlined above could be erroneous. It seems much more reasonable to assume that the divergence in findings is to be accounted for by differences in the dietary or other conditions under which the growth tests were conducted. To clarify these aspects of the question, the experiments recorded below were undertaken.

**EXPERIMENTAL**

Four preparations of D-valine were used in the course of the experiments. Two of these were made in this laboratory by the resolution of the racemic amino acid. For this purpose, a slight modification of the procedure of Price, Gilbert, and Greenstein (7) was employed. The other two samples were commercial products. Each had a total nitrogen content which was in close agreement with the theoretical value. The specific rotations of the four products in 6 N hydrochloric acid were −27.2°, −27.6°, −28.2°, and −28.3°, respectively. The values in the literature range from −27.1° (7) to −28.8° (8).

In order to exclude the possibility that the D-valine preparations might...
contain significant amounts of the L isomer, each was subjected to a microbiological assay. One of the samples prepared in this laboratory was assayed by the method of Henderson and Snell (9). The test organisms were Lactobacillus arabinosus 17-5 and Leuconostoc mesenteroides P-60. Two types of culture media were employed. One was valine-free, while the other contained 5 γ of L-valine per tube, thereby to permit very slow growth of the microorganism. Graded additions of D-valine up to 1000 γ per tube failed to reveal any significant difference in acid production. The slight fluctuations in titration values occasionally observed, if due to contamination rather than to inherent errors of the method, would be equivalent to less than 0.1 per cent of L-valine.

The other three preparations were assayed by the method of Horn, Jones, and Blum (10), in which Streptococcus faecalis 9790 was used as the test organism. The second sample, which was also made in this laboratory, was devoid of measurable amounts of L-valine, but the two commercial products yielded titration values corresponding to 0.12 and 0.15 per cent, respectively, of the L isomer. If one assumes that the values are real and, on the basis of a contamination of 0.15 per cent, calculates the L-valine intake of the animal that consumed the largest amount of D-valine in any 4 day period, the figure obtained is 0.27 mg. per day. The average intake of L-valine by the rats which received 2.0 per cent of n-valine, as described below, could not have exceeded 0.17 mg. per day. White et al. (5) report that the addition to a valine-free basal ration of an amount of L-valine corresponding to 1 per cent of the D-valine employed in their experiments failed to diminish the loss in weight of the subjects. It seems obvious, therefore, that no measurable effect could have resulted from the apparent contamination of our preparations. Indeed, on several occasions, one of our products was substituted in the diet for another without inducing the slightest alteration in the response of the animals.

The amino acids other than D-valine were invariably purified until they yielded correct analytical values. They were also tested microbiologically for L-valine (10), except in the first series of animal experiments, and were found to be devoid of detectable amounts of this amino acid.

Weanling rats served as the experimental subjects save in one series of tests in which, for reasons to be explained later, heavier animals were employed. Males only were used. Each was housed in a separate cage and permitted to consume food and water ad libitum.

All of the animal tests involved the use of a single basal diet, the composition of which is shown in Table I. The amino acid mixture (Mixture XXIII-f) was identical in make-up with Mixture XXIII (which we regard

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3 The authors are indebted to Dr. L. M. Henderson of this department for much valuable aid and advice in making these tests.
as our standard (12)), except that the former contained 0.2 gm. of \( L \)-aspartic acid instead of 0.4 gm. of the racemic acid and was devoid of leucine and valine. The latter two amino acids were added directly \( \text{to the basal ration} \), in the quantities desired, at the expense of like weights of dextrin. When thus formulated, the diets furnished nineteen amino acids. In addition to the vitamins present in the basal ration, those ordinarily used in this laboratory and listed elsewhere ((13), Table II) were incorporated in each kilo of food.

The results of the experiments are summarized in Table II. Series I

<table>
<thead>
<tr>
<th>Table I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composition of Basal Diet</strong></td>
</tr>
<tr>
<td>Amount</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td><strong>gm.</strong></td>
</tr>
<tr>
<td>Amino acid Mixture XXIII-( f )</td>
</tr>
<tr>
<td>Sucrose</td>
</tr>
<tr>
<td>Dextrin</td>
</tr>
<tr>
<td>Cellu flour</td>
</tr>
<tr>
<td>Salt mixture*</td>
</tr>
<tr>
<td>Corn oil</td>
</tr>
<tr>
<td>Vitamin A and D concentrate( \dagger )</td>
</tr>
<tr>
<td>Inositol</td>
</tr>
<tr>
<td>Choline chloride</td>
</tr>
<tr>
<td>Liver extract( \ddagger )</td>
</tr>
<tr>
<td>100.00</td>
</tr>
</tbody>
</table>

* Jones and Foster (11).
| \( \dagger \) This contained 60,000 U. S. P. units of vitamin A and 8500 U. S. P. units of vitamin D per gm.
| \( \ddagger \) Wilson’s liver concentrate N. F.

was carried out about 3 years ago and is a repetition, under better dietary conditions, of the tests made in 1936. For the purpose in question, twenty-nine rats from ten litters were divided, as equitably as possible with respect to their initial body weights, into three groups. One group received a diet containing 1 per cent of \( L \)-valine, the second an identical diet but containing 1 per cent of the \( D \) isomer, and the third a ration which was devoid of both. \( L \) Leucine, at a level of 1.2 per cent, was used throughout. The proportion of valine was based on an earlier observation that 0.7 per cent of the \( L \) enantiomorph represents the minimal requirement of the rat for optimal growth (1). It was assumed that 1 per cent of \( D \)-valine would be a liberal allowance, provided this amino acid can be inverted by the animal organism.
The data show that, under the conditions specified, L-valine permitted a mean gain in 28 days of 115.4 ± 1.5 gm., while D-valine resulted in a mean loss in body weight of 4.7 ± 1.4 gm. The negative controls (valine-free)

**Table II**

Comparative Growth Response of Rats to L- and D-Valine

Males only. All animals had initial body weights of 48 to 62 gm. except those of Series III as indicated below.

<table>
<thead>
<tr>
<th>Series No.</th>
<th>No. of animals</th>
<th>Duration of test</th>
<th>Mean gain or loss in weight and probable error of mean</th>
<th>Nature of diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>days</td>
<td>gm.</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>10</td>
<td>28</td>
<td>+115.4 ± 1.5</td>
<td>1.0% L-valine + 1.2% L-leucine</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>28</td>
<td>−4.7 ± 1.4</td>
<td>1.0% D-valine + 1.2% “</td>
</tr>
<tr>
<td></td>
<td>9*</td>
<td>28</td>
<td>−15.0 ± 0.9</td>
<td>No valine + 1.2% L-leucine</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>28</td>
<td>+104.3 ± 4.6</td>
<td>1.0% L-valine + 1.2% L-leucine</td>
</tr>
<tr>
<td></td>
<td>10†</td>
<td>28</td>
<td>−12.4 ± 0.8</td>
<td>1.0% D-valine + 1.2% “</td>
</tr>
<tr>
<td></td>
<td>10‡</td>
<td>28</td>
<td>−20.2 ± 0.9</td>
<td>No valine + 1.2% L-leucine</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>16</td>
<td>−0.5 ± 0.7</td>
<td>1.0% D-valine + 1.2% “</td>
</tr>
<tr>
<td>IV</td>
<td>5¶</td>
<td>28</td>
<td>−9.8 ± 1.4</td>
<td>1.0% “ + 1.2% “</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>28</td>
<td>+13.2 ± 2.5</td>
<td>2.0% “ + 1.2% “</td>
</tr>
<tr>
<td>V</td>
<td>5</td>
<td>28</td>
<td>+25.6 ± 1.8</td>
<td>2.0% “ + 1.2% “</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>28</td>
<td>−1.2 ± 0.8</td>
<td>2.0% “ + 2.4% DL-leucine</td>
</tr>
</tbody>
</table>

* Two of these animals died after 26 and 24 days, with losses in body weight of 14 and 13 gm., respectively.
† Two of these animals died after 24 and 26 days, with losses in body weight of 12 and 19 gm., respectively.
‡ All of these rats succumbed during the course of the experiment, the period of survival varying between 16 and 27 days.
§ Seven of these subjects died. The period of survival varied from 19 to 27 days, and the weight losses ranged from 11 to 21 gm.
|| The animals of this series had initial weights of 100 to 120 gm. and received a valine-free diet for 12 days. During this period, they experienced a mean loss in weight of 32.6 ± 0.6 gm. They were then transferred to rations containing L- or D-valine and were thus maintained for 16 days. Two of the rats which received D-valine died on the 3rd day following the transfer from the depletion ration and consequently are not included in the calculation of the mean loss in body weight.
¶ One of these animals died after 24 days, having experienced a loss in body weight of 18 gm.

ration) made an even poorer showing in that they experienced a mean loss of 15.0 ± 0.9 gm. It is evident, therefore, that the more recent data confirm the earlier findings except that, under the improved dietary conditions now used, all subjects, particularly those receiving L-valine, made a more satisfactory showing.
No definite benefit attributable to L-leucine could be detected by comparing the findings in Series I with those obtained many years earlier with diets containing D,L-leucine. However, in an attempt to obtain more convincing evidence concerning this point, a second experiment was undertaken, the results of which are summarized as Series II in Table II. In this experiment, forty rats from nine litters were divided into four groups. Three groups received diets containing, respectively, 1 per cent of L-valine, 1 per cent of D-valine, and no valine. Each of these carried 1.2 per cent of L-leucine. The fourth group received a like ration containing 1 per cent of D-valine and 2.4 per cent of D,L-leucine. The animals used in Series II were of a different strain from those employed in Series I and yielded results which were much more variable, as shown by the probable errors of the means. Furthermore, the mortality among the rats which were deprived of valine, or which received the D isomer, was much higher. Despite these unfortunate circumstances, the data demonstrate clearly that D-valine at a 1 per cent level, though superior to no valine, is incapable of supporting growth or of even maintaining body weight. On the other hand, the response of the subjects to D-valine when D,L-leucine was included in the food appears to have been less satisfactory than when the ration contained L-leucine. Statistical analysis of the data is complicated by the high mortality of the group which consumed the racemic compound. Nevertheless, it seems reasonable to assume that, had the animals survived for 28 days, the mean loss in weight would have been greater than the 17.1 ± 0.8 gm. shown in Table II. Thus, in so far as one may judge, the findings with respect to D,L-leucine appear to confirm the observation of Wretlind (6). Further information concerning this point will be cited later.

The experiments of Series I and II demonstrate that D-valine, at a level equal to an excess of the L isomer, is incapable of supporting growth. On the other hand, in both experiments, as in the older tests, the animals which received D-valine lost less weight than did their litter mates that consumed the valine-free ration. The most probable explanation of this is that some inversion of D-valine actually occurs. Thus, D-valine behaves like D-leucine, which also fails to support growth when supplied at a level corresponding to an excess of the L isomer (1, 2). That it can be inverted in part in the animal organism has been shown also by labeling it with isotopes (14). With both amino acids, the rate of the inversion may be one of the significant factors in preventing greater utilization.

An important difference in the technique used in our experiments and in those of White et al. (5) is that the latter investigators depleted their rats by placing them on a valine-free ration for 28 days prior to the administration of the D isomer. Our experience with depletion has led us to believe that it renders animals more responsive to any substance which can
be transformed into the dietary component of which they have been de-priv ed. It seemed possible that this difference in procedure might account for the divergence in findings. In an experiment designed to test this point, sixteen rats having initial weights of 100 to 120 gm. each were placed on a valine-free ration containing L-leucine. It soon became apparent that our strain of animals would not survive as prolonged a depletion period as had been used by White et al. (5). By the end of 12 days, the mean loss in body weight amounted to 32.6 ± 0.6 gm. Consequently, the subjects were divided into two groups, one of which received 1 per cent of n-valine and the other a like amount of the D isomer. In making the division, litter mates were distributed equally between the two groups. The feeding trials were then continued for 16 days.

The results of the experiment are summarized as Series III in Table II. Two of the animals which received n-valine died on the 3rd day following the transfer from the depletion ration and, hence, are not included in the calculation of the mean change in body weight, which amounted to −0.5 ± 0.7 gm. In this case, the individual behavior of the subjects is more revealing than the mean. Of the six animals that survived, three showed losses of 2, 3, and 3 gm., respectively, and three managed to gain 1, 1, and 3 gm., respectively. Thus, depletion did render the rats somewhat more sensitive to the effect of n-valine. The positive controls also were rendered more responsive. The mean daily gain of these subjects was slightly less than 5.0 gm., while the figures for the corresponding animals of Series I and II were 4.1 and 3.7 gm., respectively. However, depletion alone, at least for a 12 day period, is not sufficient to account for the gains observed by White et al. (5) following the administration of the D-amino acid.

Although, as pointed out above, 0.7 per cent of L-valine supports optimal growth, Wretlind (6) reports that levels of n-valine in excess of 1 per cent are necessary to permit growth. Both he and White et al. (5) used 2 per cent in most of their tests. We have confirmed Wretlind's observation in an experiment initiated to test this point. Of ten weanling rats from five litters, one animal from each litter was placed upon each of the two rations. The results are summarized as Series IV in Table II. The data demonstrate clearly that the subjects responded much better on the higher intake. During the 28 day period, the animals receiving 1 per cent of D-valine experienced a mean loss in body weight of 9.8 ± 1.4 gm., while those ingesting 2 per cent showed a mean gain of 13.2 ± 2.5 gm. The latter figure represents much slower growth than that reported by White et al. (5), but compares favorably with most of the gains observed by Wretlind (6) under similar conditions.

No entirely satisfactory explanation is available to account for the fact that 2 per cent of D-valine is necessary, even for slow gains, when half this
amount of the L isomer is more than enough to permit optimal growth. It is known that D-amino acids are less rapidly absorbed from the intestine (15) and are less efficiently retained by the kidneys (16) than are L-amino acids. Perhaps these factors are responsible for the unexpectedly high level of D-valine which is required for growth. If so, the utilization of other D-amino acids may be more dependent upon dosage than has hitherto been assumed.

The final experiment had for its objective the acquisition of more convincing information concerning the effect of DL-leucine. For this purpose, ten rats were separated into two groups. One group received a diet containing 1.2 per cent of L-leucine, and the other, composed of litter mates, consumed a ration containing 2.4 per cent of the racemic compound. Both diets carried 2 per cent of D-valine. The results are summarized as Series V in Table II.4 As will be observed, the animals which received L-leucine showed in 28 days a mean gain in weight of 25.6 ± 1.8 gm., while those which consumed DL-leucine suffered a mean loss of 1.2 ± 0.8 gm. These data confirm those of Wretlind (6) and leave no doubt regarding the inhibitory effect of the racemic amino acid when administered at the 2.4 per cent level. Whether this effect is due to a competitive inhibition of D-valine inversion, as proposed by Wretlind, or is to be explained on some other basis, must await further investigation. Suggestive are the results of certain short term experiments which are not included in Table II. At the expiration of the tests of Series V, the L-leucine content of the first diet was raised from 1.2 to 2.4 per cent, and the DL-leucine content of the other diet was lowered from 2.4 to 1.2 per cent. In the latter case, the effective leucine content of the food was reduced to a level which was below the minimal requirement for optimal growth (cf. (1, 17)). During the subsequent 8 days, the mean daily gain in weight of the first group dropped from 0.91 gm. to 0.48 gm. During the same interval, the rats which had been losing slightly on the higher percentage of DL-leucine now manifested a mean daily gain of 0.80 gm. In other words, the mean growth rates were reversed by the dietary alterations. No definite conclusions can be drawn from these short tests, but the findings suggest that an amino acid imbalance may be responsible for the growth-inhibitory action of DL-leucine at the 2.4 per cent level.

The experiments outlined above confirm the findings of White et al. (5) and of Wretlind (6) that growth can be induced by D-valine if sufficient quantities are included in the food. At best, however, the gains are very poor. The greatest mean daily increase in weight observed in our experiments with 2

4 The rats of Series V were of a different strain from those used in the other experiments. Doubtless this accounts for the divergence between the mean gains of the animals receiving D-valine and L-leucine in Series IV and V.
per cent of d-valine was 0.91 gm. Under similar conditions, the largest daily gains reported by White et al. (5) and Wretlind (6) were 1.16 and 1.40 gm., respectively. Even with a diet containing 4 per cent of d-valine, Wretlind obtained a daily increase of only 1.90 gm. These figures are to be compared with mean daily gains of 3.72 to 4.96 gm. (Series I to III) when the ration contained 1 per cent of L-valine. Certainly, the growth effect of the D isomer is of little practical significance when the racemic amino acid is used as a dietary component.

Attention has been called to the occurrence of quantitative differences in the growth response of different strains of rats following the administration of d-valine. Apparently, qualitative variations also may arise in the physiological symptoms resulting from L-valine deprivation. Many years ago, Rose and Eppstein (18) described a peculiar syndrome which develops in rats on valine-free rations. The most characteristic features of this condition are a marked sensitivity to touch and a profound lack of coordination in movement. In the present investigation, the animals which were deprived of valine (Series I and II) manifested these symptoms, though as would be anticipated, in varying degrees and after different intervals of time. White et al. (5) report that they likewise observed them after their subjects had experienced a valine deficiency for 28 days. On the contrary, Wretlind’s (6) rats exhibited signs of cachexia only, with none of the neurological traits which, in our experience, are so generally associated with this type of dietary inadequacy.

SUMMARY

Diets containing 1 per cent of d-valine are incapable of supporting the growth of weanling rats despite the fact that this intake level of L-valine provides more than enough of the amino acid to permit optimal gains in weight. When the d-valine content of the food is raised to 2 per cent, slow growth occurs. However, even at the higher level, the gains are extremely poor as contrasted with those induced by half as much of the L isomer. Either the inversion of d-valine is difficult for the organism to accomplish, or the slow absorption of the amino acid from the intestine and its rapid excretion by the kidneys interfere with its utilization.

As observed by Wretlind (6), the moderate effectiveness of d-valine in promoting growth at the 2 per cent level is suppressed when the L-leucine content of the diet is replaced by twice the amount of D.L-leucine. Certain factors which may account for this phenomenon are discussed.

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