EFFECT OF D-AMINO ACIDS ON GROWTH OF LACTOBACILLI[1]

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It has been shown (Hier *et al.*, 1944; Graham *et al.*, 1950; Russell *et al.*, 1952; Teeri and Josselyn, 1953) that an unusually high concentration of a single amino acid in the diet or medium may cause growth repression, even though it may be an essential amino acid. Suggestions have been made that such repression may be due to amino acid imbalance (Hier *et al.*, 1944) or that it may be a characteristic property of the specific amino acid (Russell *et al.*, 1952). It is interesting to note that this effect has occurred often with D- (or DL-) amino acids, a fact which invites speculation that it may be due to the less commonly utilized D-isomer. For example, it has been shown (Hegsted, 1945) that in the absence of L-leucine, D-leucine allows some growth of *Lactobacillus arabinosus* although the rate is much below normal. Also using this same organism (Hegsted, 1944) it was concluded that D-valine is completely inactive and furthermore does not inhibit the response of DL-valine. Preliminary experiments in connection with the study reported herein indicated that D-valine does inhibit the response of DL-valine. This difference in results probably is explained by the fact that a much higher concentration of the D-amino acid was used in the latter case. Other studies (Fox *et al.*, 1944; Fling and Fox, 1945; Kobayashi *et al.*, 1949; Yaw and Kakavas, 1952) have shown inhibitory effects by some D-amino acids on the growth of *L. arabinosus*, *Escherichia coli*, and *Brucella abortus*.

Although, as indicated above, there have been reports indicating the lack of availability of one of the two enantiomorphs of an amino acid for growth, there are no examples which demonstrate whether or not a true competitive inhibition is involved. This investigation has been undertaken, therefore, to study the effects of D-amino acids on various microorganisms and to determine whether growth repression, when it occurs, might be due to a true competitive inhibition or to some other factor not explainable on the basis of a simple metabolite-antimetabolite relationship.

EXPERIMENTAL METHODS

The microorganisms used in this study were *Lactobacillus arabinosus*, strain 17-5 (ATCC no. 8014), *Lactobacillus fermenti*, strain 36 (no. 9338), *Lactobacillus casei* (no. 7469), and *Lactobacillus leichmannii* (no. 7830). Since the primary concern was to learn whether D-amino acids can inhibit growth in a complete medium, and not whether any particular D- or L-amino acid is an essential nutrient, the complete media previously described (Teeri and Josselyn, 1953) were used. Growth was determined by photoelectric turbidity measurements after sixteen to eighteen hours' incubation at 35 C.

RESULTS AND DISCUSSION

The data of table 1 show that both D-leucine and D-valine, if present in high concentration, inhibit growth of *L. arabinosus* in a complete medium. These data further show that while the extent of growth repression is of approximately the same order for the two D-amino acids at a concentration of twenty mg per tube (10 ml of medium), D-valine exerts a somewhat stronger inhibitory effect at lower concentrations.

Since failure to show reversal by a metabolite rules out the substance in question as a competitive antagonist for that metabolite (Roblin, 1949), the data of table 2 indicate that D-valine is not a true antimetabolite for L-valine. In fact, excess of the L-form appears to have an inhibitory effect also, though not so pronounced as the D-compound. Similar results were obtained with D- and L-leucine and serine.

Table 3 presents additional evidence that both isomers of leucine or valine repress the growth of

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TABLE 1

Effect of concentration on the growth inhibitions of Lactobacillus arabinosus caused by D-leucine and D-valine

D-LEUCINE OR D-VALINE ADDED PER TUBE	TURBIDITY READINGS	
	D-Leucine	D-Valine
mg		
0.0	100	100
1.0	100	100
2.0	100	100
4.0	100	100
6.0	100	90
8.0	100	85
10.0	90	60
15.0	35	50
20.0	35	30

TABLE 2

Growth repression of Lactobacillus arabinosus by D-valine in presence of graded amounts of L-valine

D-VALINE ADDED PER TUBE	L-VALINE ADDED PER TUBE	TURBIDITY READINGS
mg	*mg*	
0	0	150
20	0	81
20	1	80
20	2	82
20	4	80
20	6	75
20	8	77
20	10	68
20	15	61
20	20	61

TABLE 3

Growth of Lactobacillus arabinosus in presence of D-, L-, and DL-leucine and valine

LEUCINE ADDED	TURBIDITY READINGS	VALINE ADDED	TURBIDITY READINGS
None	314	None	314
20 mg D-	121	20 mg D-	122
20 mg L-	225	20 mg L-	195
20 mg DL-	160	20 mg DL-	155
40 mg DL-	86	40 mg DL-	66

L. arabinosus, and that the D- is considerably more repressive than the L-form.

Table 4 presents similar data with respect to *L. leichmannii*. With this organism D-leucine

TABLE 4

Growth of Lactobacillus leichmannii in presence of various D-, L-, and DL-amino acids

The recorded values are turbidity readings.

AMINO ACID ADDED PER TUBE	LEUCINE	VALINE	METHIONINE	SERINE
None	270	270	270	272
20 mg D-	160	230	246	208
20 mg L-	247	233	244	270
20 mg DL-	178	228	249	233
40 mg DL-	117	189	215	208

represses growth to a considerably greater extent than does the L-form. The repressive effects of the D- and L-forms of both valine and methionine, however, are equal. With serine it appears that only the D-isomer inhibits growth, but there is no reversal of this inhibition by the L-form.

Other amino acids studied and found lacking in any growth repressive effect were alanine, isoleucine, phenylalanine, threonine, and tryptophan. With all of the amino acids tested, no growth repressions of *L. fermenti* or *L. casei* were found which were attributable specifically to the D- or the L-form.

The results of this study suggest that, in some cases, either form of an amino acid (D- or L-) may be converted to some intermediate before being utilized for protein synthesis. Presumably the D- form of such an acid is changed to the intermediate with considerably greater difficulty than is the L-isomer. Furthermore, it is possible that a large excess of the D-amino acid in some cases represses conversion of the L-compound. That such a repression may not be permanent is indicated by the fact that these results were obtained only when growth measurements were made after a relatively short (16 to 18 hours) incubation period, which is consistent with the suggestion of Camien (1952) that a conversion of the D- to the L-form may precede utilization.

It appears that growth repression by a D-amino acid, when it occurs, probably is not due to a true competitive inhibition of the L-form.

SUMMARY

Some D-amino acids, when present in relatively high concentration, repress growth of various lactobacilli (*Lactobacillus arabinosus*, *L. fermenti*,

L. casei, L. leichmannii). In some cases, the L-forms of these amino acids cause a similar, but more moderate, repression, indicating that these D-amino acids do not act as antimetabolites for the L-forms. That the effect is not permanent is indicated by the fact that repressions were obtained only when the incubation period was relatively short. This suggests a possible conversion of the D- to the L-form before utilization.

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