EFFECT OF THE ISOMERIC CONFIGURATION OF THE SOURCE OF NITROGEN ON CHANGES IN POPULATION AND METABOLISM IN CULTURES OF BRUCELLA

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The accumulation of alanine as a metabolite of most smooth strains of *Brucella* growing under nonaerated conditions in Gerhardt-Wilson synthetic medium has been shown to exert an inhibitory effect on smooth cells and to create an environment selectively favoring the establishment of nonsmooth mutants or variants with increased alanine resistance (Goodlow, Mika, and Braun, 1950). Subsequently, it was found that these effects can also be produced in young cultures with low alanine levels by adding D-alanine to the medium, but not by the addition of L-alanine (Goodlow, Braun, and Mika, 1951).

The earlier work on these problems utilized DL-asparagine as the sole source of nitrogen in the synthetic medium. When either D- or L-asparagine was substituted for DL-asparagine, some striking differences in results became apparent. The manner in which the isomeric configuration of the asparagine affected growth, production of metabolites, and changes in the population is described in this paper.

MATERIALS AND METHODS

Four strains of *Brucella* were employed, *Brucella abortus*, strain 6232 (CO_2-requiring), *Brucella abortus*, strain 19, *Brucella suis*, strain PSIII, and *Brucella melitensis*, strain 4247. Smooth clones of these strains were inoculated into flasks containing Gerhardt-Wilson medium (Gerhardt and Wilson, 1948), in which the source of nitrogen consisted of 1.5 g of D-asparagine per liter, 1.5 g of L-asparagine per liter, or 3.0 g DL-asparagine per liter. After various periods of incubation at 37 C, quantitative plate counts, determinations of the percentage of nonsmooth variants, and chromatograms for amino acids were made.

RESULTS

In cultures of *B. abortus*, strain 6232, containing L-asparagine as the sole source of nitrogen, chromatograms showed the accumulation of glutamic acid but no alanine, whereas media containing D-asparagine showed accumulation of alanine but no glutamic acid (table 1). Associated with the accumulation of alanine in the D-asparagine medium were the appearance and establishment of nonsmooth (rough and mucoid) variants (table 2). In contrast, no significant population changes occurred in the L-asparagine medium in which no accumulation of alanine was detectable. As expected, alanine and nonsmooth variants appeared in the DL-asparagine medium but to a lesser extent than in medium

with D-asparagine as the sole source of nitrogen. In agreement with past experience, it was expected that increased population changes would be associated with increased ultimate suppression of growth of the parent smooth cells. That this was actually the case is shown in figure 1. Inhibition of growth was most marked in D-asparagine medium, significantly less in DL-asparagine medium, and far less in L-asparagine medium.

The strains differed in the manner in which the accumulation of metabolites was affected by the isomeric form of asparagine. In cultures of *B. abortus*, strain 19, alanine accumulated in D-asparagine media but not in L-asparagine media,

TABLE 1

Ninhydrin positive metabolites detectable by paper partition chromatography in cultures of different strains of Brucella following growth in media containing D- *or* L-*asparagine**

CONFIGURATION OF ASPARAGINE	*B. abortus*, STRAIN 6232		*B. abortus*, STRAIN 19		*B. melitensis*, STRAIN 4247		*B. suis*, STRAIN PSIII		
	Glutamic acid	Alanine	Glutamic acid	Alanine	Glutamic acid	Alanine	Glutamic acid	Alanine	Valine
L	+	−	−	−	+	−	−	−	+
D	−	+	−	+	no growth	no growth	−	+	−

* In addition to the amino acids listed above, aspartic acid was found in all culture filtrates.

TABLE 2

Alanine production and variation in cultures of smooth Brucella abortus grown in a synthetic medium with D-, L-, *or* DL-*asparagine as the source of nitrogen*

DAY OF GROWTH	L-ASPARAGINE		D-ASPARAGINE		DL-ASPARAGINE	
	Per cent variants	Alanine	Per cent variants	Alanine	Per cent variants	Alanine
0	0	−	0	−	0	−
5	<1	−	<1	−	<1	−
11	<1	−	2	+	<1	+
15	0	−	18	+	4	+
20	<1	−	74	+	6	+

and glutamic acid, which was detected in L-asparagine cultures of strain 6232, did not appear in cultures with strain 19. Species differences were further indicated by the fact that a smooth clone of *B. melitensis*, strain 4247, grew very poorly in D-asparagine media but showed excellent growth, no accumulation of alanine, and lack of population changes in L-asparagine media. Following prolonged cultivation of this *B. melitensis* strain in D-asparagine, a mutant capable of utilizing D-asparagine was isolated, and this particular mutant showed accumulation of alanine in D-asparagine media.

Particularly interesting differences were observed when a smooth clone of *B. suis*, strain PSIII, was grown in either D- or L-asparagine medium (table 3). Mucoid variants established themselves in cultures grown in L-asparagine media,

whereas an entirely different, rough type of variant established itself in cultures containing D-asparagine. L-Asparagine cultures contained approximately 50 per cent mucoid variants after 12 days of incubation, and D-asparagine cultures

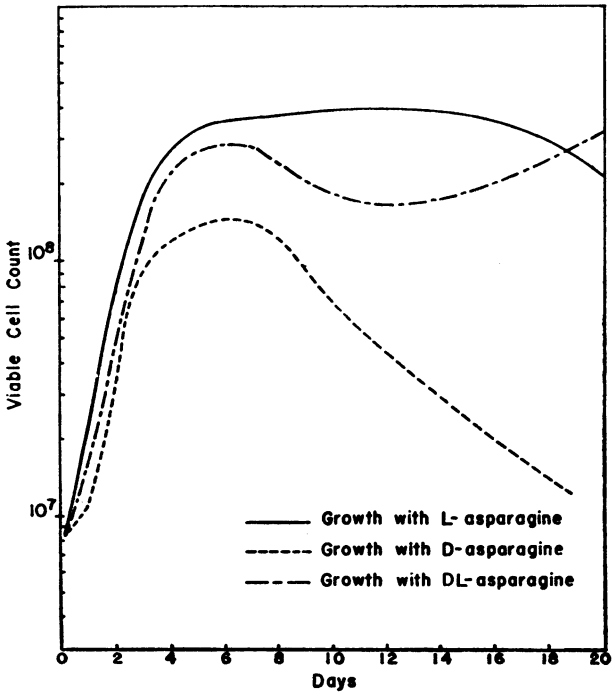

Figure 1. Growth of smooth strain of *Brucella abortus* in a synthetic medium with D-, L-, or DL-asparagine as the sole source of nitrogen.

TABLE 3

Metabolism and variation of Brucella suis in a synthetic medium with D- *or* L-*asparagine as the sole source of nitrogen*

CONFIGURATION OF ASPARAGINE	PERCENTAGE OF VARIANTS ON THE FOLLOWING DAYS					AMINO ACID METABOLITES
	5th	8th	12th	16th	20th	
L	0	0	42M*	52M	41M	Valine
D	16R†	55R	84R	90R	97R	Alanine

* M—Mucoid.
† R—Rough.

contained approximately 90 per cent rough variants after a similar period. Filtrates from the D-asparagine cultures showed accumulation of alanine, whereas L-asparagine cultures showed no evidence of alanine but yielded a spot on chromatograms with the Rf value of valine, leucine, isoleucine, or methionine. In smooth cultures and in cultures inoculated with a known mixture of smooth

and mucoid cells, the addition of valine caused a rapid establishment of mucoid cells, whereas the addition of leucine, isoleucine, or methionine failed to affect the establishment of mucoid variants. These data indicate that valine was the responsible compound and may act selectively in a manner similar to that previously described for alanine, inhibiting the growth of smooth parent cells and favoring the establishment of presumably valine-resistant mucoid variants in cultures containing L-asparagine as the source of nitrogen. As might be expected, *B. suis* grown in DL-asparagine media showed the establishment of both mucoid and rough types and accumulation of both alanine and valine in the culture liquid.

DISCUSSION

These data show that differences in the isomeric configuration of the nitrogen source in the culture medium can have a significant effect not only on the extent of population changes but also on the direction in which population changes may proceed. The finding that such differences in population changes are associated with differences in alanine or valine accumulation in the medium illustrates again that the parent type through its metabolism has a controlling effect on future population changes, even though this effect is entirely indirect. Within a closed environment the parent type can be responsible for setting up the specific environmental conditions that favor the establishment of certain specific mutants, selecting from among the presumably great variety of mutants that may occur spontaneously and undirected. During the progress of these investigations, it also became evident that apart from the effects of the nitrogen source, cultural conditions can significantly modify metabolite accumulation, growth, and population changes. Different results were obtained when identical inocula were used for cultures maintained in tubes, flasks, or bottles and when static or shaken (aerated) cultures were used. The reasons for such differences are still somewhat obscure but may be associated with the ratio of quantity of media to the surface area, which controls the available oxygen. Besides confirming the critical role of metabolites in creating conditions favoring establishment of metabolite-resistant antigenic variants, these data emphasize the necessity for rigid control of cultural conditions in studies on variation and metabolism.

SUMMARY

In initially smooth cultures of *Brucella*, alanine accumulation and population changes differ greatly depending on whether D- or L-asparagine is employed as the sole source of nitrogen in the synthetic medium employed. Thus, in contrast to results obtained previously with D- or DL-asparagine, smooth cultures of *Brucella abortus*, strain 6232, showed neither establishment of nonsmooth types when grown in L-asparagine nor significant amounts of alanine in filtrates from such cultures incubated for prolonged periods. The establishment of a non-smooth (mucoid) mutant differing from the rough mutant observed after growth in D- or DL-asparagine was noted in initially smooth cultures of *Brucella suis* grown in L-asparagine. In the latter medium no alanine was detectable, but an

amino acid that appears to be identical with valine accumulated at the time of population change. Preliminary tests indicate that, for this strain of *B. suis*, valine accumulating during growth in L-asparagine may act in a manner similar to the action of alanine accumulating during growth in D-asparagine.

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