Effect of Ascorbic Acid on Cysteine Requirement of Lactobacilli.* (22063)

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In a study of amino acid requirements of a collection of oral lactobacilli it was found that, in addition to other amino acids required for growth, cysteine was needed by 23 of 28 oral cultures and also by 3 of 5 American Type Culture Collection lactobacilli(1). This result was obtained with a chemically defined medium containing 18 amino acids including methionine, needed B vitamins, purines and pyrimidines, glucose, acetate, and salts. The proportion of lactobacilli requiring cysteine under these conditions was, in general, similar to the previous result of Dunn *et al.*(2) who found that 8 of 12 lactobacilli (exclusive of other lactic acid bacteria studied) needed cysteine for growth. These lactobacilli were mostly ATCC strains obtained originally from dairy products or fermentations. Other workers also have studied the needs of certain individual strains which, for the most part, were included in the study of Dunn *et al.*(2) and need not be cited in detail here.

TABLE I. Effect of Ascorbic Acid on Cysteine Requirement of Lactobacilli.

Cultures	No. of cultures growing in chemically defined medium*		
	Complete amino acid medium	On omission of cysteine	Ascorbic acid substituted for cysteine
L. casei	12	1	2†
" , lactose neg.	4	0	0
L. plantarum	7	3	3
L. acidophilus	4	0	0
L. fermenti	8	2	6
L. brevis	1	0	0
	36	6‡	11

* The chemically defined medium contained 200 mg DL-methionine and 100 mg L-cysteine • HCl per 1000 ml. The detailed composition has been given elsewhere(1).

† Slow growth of 1 of the 2 cultures.

‡ The 6 cultures which grew on omission of cysteine were: 1 oral *L. casei*, *L. plantarum* ATCC 8014 (*i.e.*, *L. arabinosus* 17-5), 2 oral *L. plantarum*, *L. fermenti* ATCC 9338, and one oral *L. fermenti*. Growth of most of these cultures was stimulated to some extent by added cysteine though cysteine was not essential for growth.

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TABLE II. Response of Certain Lactobacilli to Ascorbic Acid and Cysteine.

Culture designation and strain No.	Additions to medium*		Growth,† days			
	Ascorbic acid, .3 mg/ml	Cysteine• HCl, 100 μg/ml	2	3	4	5-7
L. casei 1006	0	0	3	8	11	13
	0	+	78	83		
	+	0	15	27	49	51
	+	+	62	82		
L. fermenti 55	0	0	1	2	2	1
	0	+	86	88		
	+	0	73	87		
	+	+	86	89		
" 1007	0	0	2	2	1	2
	0	+	4	5	6	76
	+	0	11	54	66	
	+	+	9	17		63
L. casei 1009‡ (lactose neg.)	0	0	11		14	15
	0	+	80	82		
	+	0	6	10		9
	+	+	78	82		

* The usual synthetic medium minus cysteine was used. + = ascorbic acid or cysteine • HCl added in amount indicated, 0 = not added. Solutions of compounds were sterilized by filtration and added to the medium after autoclaving and just before inoculation.

† Figures for growth represent turbidity. Lumetron readings of % light transmission with a 650 mμ filter were subtracted from 100. This was done for the purpose of showing increasing turbidities by higher figures. 0 = clear tube, 66 to 87 = heavy turbidity, near maximal or maximal growth. Incubation was at 37°C.

‡ Culture 1009 is included for comparison with the others. It failed to grow in the absence of cysteine whether or not ascorbic acid was supplied. It is representative of the 25 strains of lactobacilli which required cysteine whether or not ascorbic acid was present.

Recently, in continuing our study of cysteine requirement it was found that on addition of ascorbic acid to the chemically defined medium, cysteine was no longer required by certain lactobacilli. Others, though still requiring cysteine for growth, showed a more rapid growth rate in the presence of ascorbic acid. These findings are presented here.

Results. Table I gives the result of tests of cysteine requirement and of the substitu-

TABLE III. Effect of Different Amounts of Ascorbic Acid as Substitute for Cysteine in Growth of *L. fermenti*.

	L. fermenti strain numbers:													
	6					55			1002			1007		
Ascorbic acid,† mg/ml	Growth at days													
	2	3	4	7	10	2	3	7	2	3	7	3	4	5
0	0	0	0	0	0	0	0	0	0	0	0*	0	0	0
.01						0	0	0				0	0	0
.1	0	0	0	0	0	11	81		0	0	51	0	0	0
.3	0	0	0	0	75	63	85		6	59	67	0	19	64
.5									36	58	60	5	38	40
1.0	0	0	36	81		75	87		55	60	55	0	39	60
2.0	55	85				81	84		43	46	40	25	26	23

Turbidity is stated as in Table II.
* Occasional slow growth (40 to 50) appeared in from 7 to 10 days, but more often negative.
† Solutions were sterilized by filtration and added to the medium just before inoculation.

tion of ascorbic acid for cysteine as determined for our collection of lactobacilli. Of 36 lactobacilli which grew in a chemically defined medium containing both cysteine and methionine (but not ascorbic acid), only 6 were able to grow when cysteine was omitted; 30 failed to grow. The distribution of these cultures according to species, as determined in other work(1), is given in the table. In the last column it is seen that when ascorbic acid was supplied 11 cultures (the same 6 plus 5 more) were able to grow without cysteine. That is, in the presence of ascorbic acid a cysteine requirement was no longer exhibited by 5 strains: 1 *L. casei* and 4 *L. fermenti*.

Table II shows the individual responses to cysteine and ascorbic acid of 3 of the foregoing 5 lactobacilli and also, for comparison, of 1 other lactobacillus. All failed to grow when cysteine was omitted from the usual synthetic medium, but grew when 100 μg per ml of cysteine • HCl was supplied, though one of them (1007) always grew slowly. When ascorbic acid was supplied in place of cysteine growth of the first 3 organisms occurred. No. 1006 grew more slowly and not as fully as in the presence of cysteine, 55 responded to ascorbic acid as readily as to cysteine, and 1007 grew slowly when either cysteine or ascorbic acid, or both, was supplied. The needs of this strain for prompt growth are not known. The remaining 2 strains, not shown in Table II, grew promptly when sufficient ascorbic acid was supplied in place of cysteine. For all 5 strains there was an apparent substitution of

TABLE IV. Effect of Some Sulfur-Containing Compounds When Substituted for Cysteine.

S-containing compound	Amt, mg/ml	*L. casei* 1006	*L. fermenti* 55	*L. fermenti* 1007
0	0	2	0	0
Na thioglycolate	.1	75	85	69 (9)
	.3	74	74	73 (9)
	1.0	77 (3)	84 (6)	72 (7)
Mercaptosuccinic acid	.1	75	85 (3)	0
	.3	79	78	41 (6)
	1.0	79 (3)	77	50 (4)
2-Mercaptoethanol	.1	46	0	0
	.3	48	0	0
	1.0	52 (6)	0	0
Na thiosulfate pentahydrate	.1	75	83	0
	.3	74	84	0
	1.0	74 (3)	87 (7)	0

Results are for 48 hr at 37°C unless otherwise noted. Slower growth, when it appeared, is indicated by the day in parenthesis following the turbidity figure.
All tubes were held for 10 to 14 days before discarding.

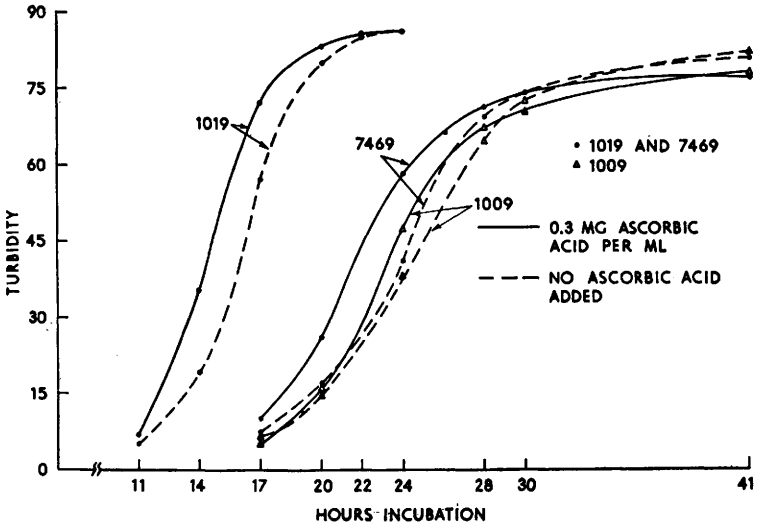

FIG. 1. Growth response of 3 lactobacilli to cysteine and to cysteine plus ascorbic acid. Cysteine • HCl, 100 μg/ml, was added to previously autoclaved medium. The cultures are *L. plantarum* 1019, *L. casei* ATCC 7469, and lactose negative *L. casei* 1009; 1019 and 1009 are of oral origin. These cultures all require cysteine irrespective of presence of ascorbic acid.

ascorbic acid for cysteine, though only partial in one case. These results were verified by repeated tests.

The effect of different amounts of ascorbic acid is shown in Table III. One strain regularly required as much as 2.0 mg per ml of medium for prompt growth while 2 others grew readily in the presence of 0.3 to 0.5 mg per ml. In certain instances there was evidence of some inhibition at the 2.0 mg level.

Other reducing substances were tested as substitutes for cysteine. In a medium lacking both cysteine and ascorbic acid, the cultures responded to additions of sodium thioglycolate or to mercaptosuccinic acid (Table IV). 2-Mercaptoethanol supported moderate growth of only 1 of 3, and sodium thiosulfate supported good growth of 2 of 3 cultures. Evidently ascorbic acid *per se* is not needed and the growth promoting effect can be secured by appropriate amounts of certain other reducing agents, though not all of the compounds are effective for all cultures. The apparent requirement for cysteine on the part of these lactobacilli is evidently a need for a reducing agent and not for cysteine, or ascorbic acid, as a structural entity.

Lactobacilli which require cysteine in the presence of ascorbic acid. Of the 36 lactobacilli studied, there remained 25 cultures which continued to exhibit a cysteine requirement in the presence of ascorbic acid, in contrast to the above cultures. For these 25 cultures cysteine apparently is needed for purposes other than the reducing effect. To determine what effect, if any, ascorbic acid might exert on growth of these lactobacilli in the presence of cysteine, observations of growth were made at different intervals with ascorbic acid added both to optimum and suboptimum amounts of cysteine. Addition of ascorbic acid to a medium containing sufficient cysteine for maximal growth produced a somewhat accelerated rate of growth, but not heavier final growth (Fig. 1). Similar results were secured with all other cultures tested in this manner: 1 *L. plantarum,* 4 *L. casei* of oral origin, and 1 *L. fermenti* which required cysteine in the presence of ascorbic acid.

For experiments with suboptimal amounts of cysteine 4 strains of lactobacilli were tested in the presence of 0, 1, 4, and 10 μg of cysteine • HCl per ml of medium. The last amount is sufficient for maximal growth of some lactobacilli but not enough for others. One set of tubes received 0.3 mg ascorbic acid per ml, the other received no ascorbic acid. It was found that the addition of ascorbic acid

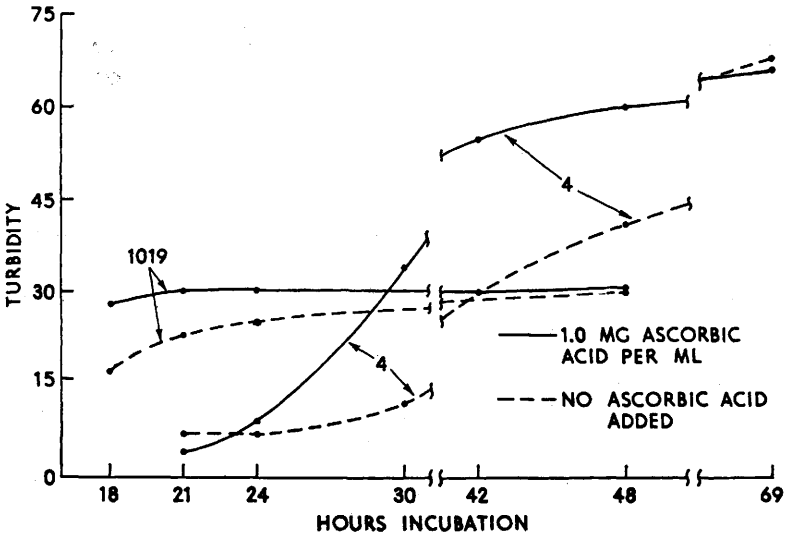

FIG. 2. Growth response of 2 lactobacilli to smaller amounts of cysteine • HCl, with and without ascorbic acid. Strain 4, a representative of lactose-negative *L. casei*, was grown in the presence of 4 μg cysteine • HCl/ml. Growth of *L. plantarum* is distinctly limited by the small amount of cysteine • HCl, 1 μg/ml, but in the presence of ascorbic acid growth is distinctly greater at the earlier data points.

accelerated the rate of growth in most instances, but there was little difference in the final amount of growth if the tubes were incubated a sufficient length of time (Fig. 2). Often several days were required to demonstrate this because growth was somewhat slower in the presence of the smaller amounts of cysteine • HCl.

Discussion. Four of the 5 cultures which do not need cysteine in the presence of appropriate reducing agents belong to the heterofermentative *L. fermenti* species. This seems significant from the standpoint of characteristics of this organism and its distinction from homofermentative lactobacilli. It suggests that a cysteine requirement is actually not as common among *L. fermenti* strains as former results had indicated and that *L. fermenti* differs from most homofermentative lactobacilli in this respect. In our collection of cultures, 6 of a total of 8 *L. fermenti* strains grew without cysteine. Two of the 6 (including the well-known ATCC 9338 strain) were able to grow in the usual chemically defined experimental media without additional reducing agents, but the other 4 grew without cysteine only in the presence of certain reducing agents. The remaining 2 strains required cysteine under all conditions tested. Whether other species of heterofermentative lactobacilli might exhibit deportment similar to the majority of *L. fermenti* cultures is not known since a sufficient number of strains has not been examined.

The ability of *L. fermenti* to dispense with preformed cysteine is in contrast to homofermentative lactobacilli, most of which exhibit a definite requirement for cysteine. In our collection of cultures only *L. plantarum* was somewhat of an exception to this trend in that 3 of 7 strains did not need cysteine. This difference in amino acid requirement is of interest in connection with another such difference recently noted. In previous work(1) it was found that threonine was either a definite requirement or stimulated growth of 5 *L. fermenti* strains, but was neither essential nor notably stimulatory for homofermentative lactobacilli: *L. casei*, lactose-negative *L. casei* and *L. plantarum* (including cultures designated *L. arabinosus* and *L. pentosus*). The present results indicate that the unessentiality of cysteine for most *L. fermenti* cultures under appropriate reducing conditions may be considered as another characteristic of this species, thus bringing to light one more

distinction between *L. fermenti* and homofermentative lactobacilli with respect to amino acid needs. A summary of other differentiating metabolic and cultural characteristics and their significance with respect to classification of the genus *Lactobacillus* has been given by Rogosa *et al.*(3).

The results have a possible bearing, too, on assay of cystine by microbiological methods. Not only is the rate of growth of many different lactobacilli accelerated by ascorbic acid in the presence of both suboptimum and optimum amounts of cysteine, but an apparent cysteine requirement of some cultures, mostly *L. fermenti,* disappears in the presence of ascorbic acid or certain other reducing agents.

Summary. 1. The requirement of some lactobacilli for cysteine, as determined in growth tests with usual chemically defined media, disappears when ascorbic acid in appropriate amounts, or other reducing substance, is incorporated in the medium. Of 5 strains of lactobacilli in this category encountered in a collection of 36 cultures, one was *L. casei,* the other 4 were heterofermentative *L. fermenti.* For these organisms the apparent cysteine requirement appears to be a need for a reducing substance and not for cysteine *per se.* Many *L. fermenti* strains evidently do not require cysteine though this may not always be apparent with some of the commonly-used synthetic media. 2. In contrast to *L. fermenti,* other lactobacilli—most *L. casei,* lactose-negative *L. casei, L. acidophilus,* and the majority of *L. plantarum*—require cysteine for growth in the presence of ascorbic acid. The rate of growth of these lactobacilli often is accelerated by ascorbic acid in the presence of either suboptimal or optimal amounts of cysteine. 3. Some implications of these findings concerning microbiological assay of cysteine and the distinguishing characteristics of heterofermentative *L. fermenti* and homofermentative lactobacilli are discussed.

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Comparative Study of Orosomucoid Preparations from Sera of Six Species of Mammals.* (22064)

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The presence of an electrophoretically and ultracentrifugally homogeneous acidic protein of very high carbohydrate content has been demonstrated in normal human plasma(1-4). This glycoprotein, designated orosomucoid (5,6), is one of the major components(5) of the "mucoprotein fraction" of human serum (6,7). "Mucoprotein" fractions have been demonstrated in the serum of many animal species(8-16). It appeared of some interest to determine whether proteins with characteristics of orosomucoid are also present in the blood of other species. In the present studies orosomucoid has been prepared from normal, pooled beef, guinea pig, horse, rabbit, and rat serum, and has been compared with that isolated from human plasma.

Materials and methods. Serum orosomucoid was separated by a procedure previously reported(1). To 500 ml of pooled serum from normal animals was added 500 ml of 0.1 M sodium acetate and 360 g of ammonium sulfate.† The precipitated proteins were

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† Erroneously reported previously(1) as 2.73 Molar.