

THE IMPLANTATION OF ORAL AND INTESTINAL STRAINS OF *L. ACIDOPHILUS* IN THE ALBINO RAT¹

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During the past few years some investigators have ascribed to lactobacilli a definite rôle in the production of dental caries. A few have concluded from their studies that these lactobacilli were *Lactobacillus acidophilus* species, identical with those of intestinal origin, for example, Bunting (1937) states: "... dental caries is a specific bacterial disease and the specific organism involved is *L. acidophilus*." The principal exponents of these views include Howe and Hatch (1917), Rodriguez (1922), Bunting and Palmerlee (1925), Rosebury, Linton, and Buchbinder (1929), Howitt and van Meter (1930), Hadley (1933), Johnston, Williams, Anderson, Tisadall and Kaake (1936), Bunting (1937), and Jay (1937).

Other investigators are diametrically opposed to the foregoing claims as evidenced by many publications. Morishita (1929), Rettger (1932), Weinstein, Anderson and Rettger (1933), and Rettger, Levy, Weinstein and Weiss (1935), have concluded from their studies that the oral and intestinal strains of lactobacilli are distinctly separate groups of organisms, and they have not been able to demonstrate a striking correlation between the occurrence of caries and the presence of *L. acidophilus*.

In view of these contradictory opinions and since reported

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results indicate that cultural, morphological, biochemical and serological characteristics afford a poor basis for differentiation of these lactobacilli, the present investigation was undertaken to determine whether the organisms of either oral or intestinal origin differed in their ability to become implanted in the intestinal tract of white rats.

EXPERIMENTAL

Methods

Cultures—type and source. A. *Oral or dental strains:* Nos. 14, 18, and 19 were obtained from L. F. Rettger, labelled S₁, Flynn, and Rosebury respectively. Nos. 60, 61, 94, and 97 were obtained from H. R. Curran.³ All of these cultures were of the smooth (Y) type. B. *Intestinal strains:* No. 64 was obtained from Curran, originally isolated from acidophilus milk, nos. 15 and 20 from Rettger,⁴ and nos. 1 and 9 from R. P. Meyers. All of these were rough (X), filamentous colony types. Nos. 10 and 12 were received from J. M. Sherman, K from C. W. England, and K4y from N. Kopeloff. These were smooth (Y) intestinal types. C. *Lactobacillus bulgaricus:* Only one culture was used. This was obtained from Sherman, originally from Rettger.

Preparation of milk culture. The milk culture used for feeding was prepared by the inoculation of skimmed milk, sterilized by autoclaving. Cultures used for inoculum in the preparation of the fermented milk and for daily feeding were forty-eight hours old. Plate counts were made of each fermented milk several times during the course of this work and with but two exceptions these indicated that the viable organisms in each preparation were several hundred million per cc.

Animals and diet. The implantation of the organisms was determined by feeding experiments conducted with male and female albino rats. Before use in the experiments the rats were maintained on a ration of commercial dog pellets which kept them

³ Originally from Enright, his nos. 144 and 42, the Morishita collection no. 13, and Kulp, his S b, respectively.

⁴ The Wickerham and Cohen starter strains respectively.

in an apparently healthy condition. Two weeks previous to the administration of a culture, each rat was placed in a separate cage and fed solely upon 10 grams of ground beef daily in addition to water. At the end of the two-week period, the intestinal flora, as determined by microscopic examination of gram-stained smears and plate cultures from fecal specimens, was composed almost entirely of gram-negative bacteria. After this period, the rats were fed approximately 5 cc. of the lactobacilli preparations daily (one strain for each rat), in addition to the ground beef. As controls one rat was fed meat only, and another rat 5 cc. of sterile skimmed milk daily in addition to meat. The feeding continued for three weeks, after which five of seventeen rats were sacrificed for bacteriological examination of intestinal contents (table 2). The remaining rats were then fed a diet of meat (10 grams), sterile skimmed milk (5 cc.), and lactose (1 gram) for two weeks during which time fecal examinations were made.

Collection of feces and intestinal contents. Fecal specimens were collected regularly at three-day intervals while feeding cultures, and after 3, 6 and 14 days during lactose feeding. Specimens were collected as nearly as possible under aseptic conditions into sterile petri dishes.

The entire intestinal tract was removed from each rat sacrificed, sections selected from the duodenum, ileum, caecum, and colon, and the contents ejected into a sterile 9 cc. physiological saline dilution blank.

Bacteriological examination. A portion of each fecal specimen, approximately one-fourth of a gram, was disintegrated into a uniform suspension in 9 cc. of sterile physiological saline. The collection of intestinal specimens in 9 cc. blanks sufficed for the initial dilution. Smears were made from these original suspensions and gram-stained, and further dilutions of 1:1,000, 1:10,000, and 1:1,000,000 were prepared for plating. While the dilutions were not exactly quantitative (since the original sample was approximate) this did not interfere with the results inasmuch as determination of the number of bacteria per gram of feces was not attempted. Identification of *L. acidophilus*, and determination of its approximate percentage of the total bacterial flora were the primary objectives.

The plating medium used was that suggested by Rettger *et al.* (1935) composed of tomato juice, peptonized milk, neopeptone, and yeast extract. All plates were incubated in an atmosphere of 10 per cent carbon dioxide at 37°C. for 3–5 days. Following incubation, the plates containing the most suitable number of colonies for examination were selected for further study. By means of a dissecting microscope the various colony types were examined and their description recorded, together with the approximate percentage of each type. Gram stains were made of each colony type, and tubes of litmus milk were inoculated from colonies resembling those of lactobacilli. Litmus milk tubes showing reactions characteristic of the lactobacilli were held for identification studies. Sometimes it required several transfers before a typical reaction occurred. Tubes in which curdling and reduction of litmus milk did not occur were discarded.

Identification of cultures. It was not our aim to make a detailed study of the cultural and biochemical characteristics of all strains employed. Our primary objective was to determine a few characteristics of each strain before implantation and then repeat these same tests upon *acidophilus-like* organisms which were isolated from the feces. In this manner we could determine whether the recovered organism was the same one which was being administered orally. For this purpose the morphology, colonial characteristics, ability to ferment maltose, mannitol, sucrose and raffinose, and the phenol resistance of each original strain, as well as of all isolated strains, were recorded. Lipolytic activity, although not determined in this study, has been suggested for differentiation of closely related strains of lactobacilli, and Sabine (1937) indicated that this was valuable in clinical work to demonstrate that recovered strains were identical to administered strains.

Fermentation studies were carried out in precisely the same manner described by Curran, Rogers, and Whittier (1933) except that a proteose-peptone yeast-extract broth was used as a basic medium instead of casein-digest broth. In preliminary experiments this proteose-peptone yeast-extract broth was shown capable of supporting good growth of both oral and intestinal strains of

lactobacilli. Its use was advantageous, both in simplicity of preparation and in uniformity of composition of different batches of medium, which may be variable with casein-digest broth. Phenol tolerance was determined as described by Kulp (1929) and employed by Curran, Rogers and Whittier, except that agar shake cultures were substituted for plate cultures.

Results

An example of the detailed manner in which all fecal specimens were examined is given in table 1, such results being recorded for each rat used. Although the examination of the stained smears of fecal specimens along with plating and macroscopic and microscopic examination of resulting colonies were useful in following the change in fecal flora, we were interested primarily only in identification of lactobacilli strains. Table 1 also illustrates the detailed plan used in partially identifying lactobacilli recovered after implantation, some additional characteristics used for this purpose being listed in table 3. This is mentioned, particularly, since it is our opinion that the process of identifying lactobacilli in some earlier studies of implantation may have been done hastily and perhaps inadequately.

A summary of the percentages of lactobacilli present in each specimen examined by the above-mentioned procedure is given in table 2. It will be noted that two figures are given for each analysis, the first indicating the *entire Lactobacillus* content of the feces irrespective of type, while the second percentage refers to the *type administered* to that particular animal, as determined by certain characteristics apparently of value in differentiation as summarized in table 3.

Controls. The rat (no. 2) subsisting on the basic diet (10 grams of ground beef) had a fecal flora in which gram-negative organisms predominated. Lactobacilli could not be isolated by cultural methods. Rat 1 which received 5 cc. of sterile skimmed milk in addition to ground beef yielded lactobacilli in the feces after two weeks, and later, when the animal received lactose, these organisms were predominant, almost to the exclusion of all other types.

Oral strains. Rats fed oral strains of lactobacilli and the one

TABLE 1
Typical results of microscopic and cultural examination of fecal specimens from a white rat receiving L. acidophilus culture
 Rat 13. Culture 1 (intestinal (X) strain). Diet 10 grams beef + 5 cc. of milk culture

DAYS ON DIET	FECAL SUSPENSION		COLONIES		ORGANISMS		L.A. per cent
	Stain, shape	Description	Description	Per cent	Stain, Shape	Description	
3	Gram + rod	Long-single, pairs	Subsurface filamentous	80	Gram + rod	Long, thin-single, short chains	80
	+ rod	Short-single, chains	Subsurface	15	+ rod	Short, single-pairs, chains	
	- rod	Short, single	Elliptical smooth	5	- rod	Short, single	
	+ cocci	Single and pairs	Surface circular, large				
6	+ rod	Long-single, pairs, chains	Subsurface, very filamentous	95	+ rod	Long, thin-single, short and long chains	95
	+ rod	Short-single, pairs, chains	Subsurface, circular and elliptical	5	+ rod	Short-single, chains	
	+ cocci	Single, pairs (large)					
	- rod	Single, long and short					
9	+ rod	Long-single, chains	Subsurface, extremely filamentous	99	+ rod	Long, thin-single, chains	99
	+ rod	Short-single, chains	Subsurface, circular and elliptical	1	+ rod	Short-single, chains	
	+ cocci	Single-pairs, chains					
12	+ rod	Long, thin-single chains	Subsurface, extremely filamentous	99	+ rod	Long, thin-single, chains	99
	+ rod	Short-single, chains	Subsurface, circular and elliptical	1	+ rod	Short, single-pairs, chains	
	+ cocci	Single and pairs					
	- rod	Single, short					
15	+ rod	Long, thin-single, chains	Subsurface, extremely filamentous	90	+ rod	Long, thin, single and chains	90
	+ rod	Short-single, chains	Subsurface, circular and elliptical	10	+ rod	Short-single, chains	
	+ cocci	Single, pairs					
	- rod	Short, single					

18	+ rod + rod + cocci - rod	Long, thin-single, pairs, chains Short-single, chains Single, pairs (large) Short-single	Subsurface, extremely filamentous Subsurface, circular, elliptical	99 1	+ rod + rod	Long, thin-single, chains Short-single, chains	99
21*	+ rod + rod + cocci	Long, thin-single, pairs, chains Short-single, chains Single, pairs	Subsurface, extremely filamentous Subsurface, circular and elliptical	99 1	+ rod + rod	Long, thin-single, chains Short-single, chains	99
24	+ rod + rod + cocci	Long, thin-single, short chains Short-single, short chains Large-single, pairs	Subsurface filamentous Surface and subsurface circular, elliptical	80 20	+ rod + rod	Long, thin-single, chains Short-single, pairs	100
27	+ rod + rod + cocci	Short-single and short chains Long, thin-single, short chains Large-single, pairs, short-single (very few)	Surface and subsurface circular, elliptical Subsurface filamentous; surface, circular, large	50 45 5	+ rod + rod - rod	Short-single, short chains Long, thin-single, chains Short-single	45
35	+ rod + rod + cocci - rod	Short-single, short chain Long, thin-single, pairs Large-single, pairs Short-single	Surface and subsurface circular and elliptical Surface, circular, moist; subsurface flamen- tous	80 10 10	+ rod - rod + rod	Short-single, pairs and short chains Short-single Long thin-single and pairs	10

L. A. Percentage of *L. acidophilus* of the strain present in the milk culture administered.

* Last day of administration of milk culture—*L. acidophilus* feeding replaced by 5 cc. of sterile skimmed milk and one gram of lactose.

TABLE 2
Percentage of *Lactobacilli* in the feces of albino rats and the percentage of the total comprised of organisms administered orally in milk cultures

DIET: GROUND BEEF PLUS	RAT NO.	DAYS ON DIET												(Per cent <i>Lactobacilli</i> as determined from tomato agar plates)		
		3	6	9	12	15	18	21*	24	27	35					
		A/A'	A/A'	A/A'	A/A'	A/A'	A/A'	A/A'	A/A'	A/A'	A/A'	A/A'				
Controls:	2	0/0	0/0	0/0	0/0	0/0	0/0	0/0†								
Sterile milk	1	0/0	0/0	0/0	0/0	40/0	40/0	40/0	80/0	80/0	80/0	80/0	80/0	80/0	80/0	80/0
Dental strains:	8	100/0	85/0	90/0	95/0	95/0	85/0	90/0†								
14	9	0/0	80/0	90/0	90/0	90/0	90/0	80/0	90/0	90/0	90/0	90/0	90/0	90/0	90/0	90/0
18	10	0/0	40/0	90/0	95/0	95/0	95/0	95/0†								
19	6	0/0	20/0	90/0	80/0	90/0	90/0	90/0	90/0	90/0	90/0	90/0	90/0	90/0	90/0	90/0
60	3	0/0	20/0	90/0	90/0	90/0	90/0	85/0	70/0	80/0	80/0	80/0	80/0	80/0	80/0	80/0
61	7	0/0	90/0	90/0	100/0	100/0	100/0	100/0†								
94	4	80/0	90/0	80/0	80/0	90/0	90/0	80/0	80/0	80/0	80/0	80/0	80/0	80/0	80/0	80/0
97																
L. bulgaricus:	5	10/0	80/0	85/0	80/0	85/0	90/0	90/0†								
Intestinal strains																
(rough):	13	95/80	100/95	100/99	100/99	100/90	100/99	100/99	100/80	95/45	90/10	95/45	90/10	95/45	90/10	90/10
1	19	60/10	95/25	100/60	100/80	100/80	100/80	100/80	100/25	95/40	70/30	95/40	70/30	95/40	70/30	70/30
9	18	75/25	100/95	100/98	100/100	100/100	100/100	100/100	100/100	100/45	65/5	100/45	65/5	100/45	65/5	65/5
15	15	90/30	100/95	100/90	100/90	100/100	100/100	100/100	100/80	100/25	90/15	100/25	90/15	100/25	90/15	90/15
20	12	95/0	95/0	100/0	100/0	100/0	100/0	100/0	100/0	90/0	90/0	90/0	90/0	90/0	90/0	90/0
64																
Intestinal strains																
(smooth):	16	70/20	70/20	90/30	100/40	100/70	100/90	100/90	100/60	80/30	70/0	80/30	70/0	80/30	70/0	70/0
10	14	30/0	90/40	95/35	100/40	100/55	100/60	100/50	80/20	50/0	60/0	50/0	60/0	50/0	60/0	60/0
12	17	90/30	95/45	95/35	95/35	95/45	95/35	95/35	100/20	95/5	80/0	95/5	80/0	95/5	80/0	80/0
K																
K4y	11	60/30	60/30	90/70	90/80	100/90	95/85	100/80	80/30	75/5	90/0	75/5	90/0	75/5	90/0	90/0

* Last day of administration of milk culture—diet changed to sterile skimmed milk (5 cc.) and ground beef (10 grams), plus lactose (1 gram).

A—Percentage of total fecal flora composed of *Lactobacilli*.

A'—Percentage of total fecal flora present representing the strain administered in the milk culture.

† Sacrificed for bacteriological examination of intestinal contents

rat receiving a culture of *L. bulgaricus* exhibited the same characteristic trend with respect to intestinal transformation. In each case, without exception, lactobacilli predominated after the first few days of feeding. However, the lactobacilli which were isolated from these rats differed slightly in their colonial characteristics from the strains which were being fed. In addition, the same organism appeared in the feces of the rat receiving *L. bulgaricus* and a control rat receiving sterile skimmed milk. This made it evident that the recovered organisms were not the oral strains being fed to the rats, but instead, some other lactobacilli, apparently a rat strain initially present in the intestinal tract and stimulated by milk in the diet. As will be shown later, by comparing the cultural and physiological characteristics of the organisms fed and isolated, we were able to conclude that the oral strains were not present in the feces. Similarly *L. bulgaricus*, was never recovered from the feces of the rat receiving this culture. At the end of three weeks, when lactobacilli culture feeding ceased, and the diet of five rats of this group consisted of ground beef, sterile skimmed milk, and one gram of lactose, there was practically no change in the intestinal flora of those rats which had previously received lactobacilli cultures. The control rat (no. 1) which had been fed meat and sterile skimmed milk, and now received lactose in addition, yielded a marked increase of lactobacilli.

The remaining five rats of this group, the one fed ground beef, the one fed *L. bulgaricus*, and three which had received oral strains of lactobacilli were sacrificed. Upon examination of specimens from the duodenum, ileum, caecum and colon of each animal, organisms of the original strains could not be isolated although lactobacilli, apparently rat strains and identical with those found in previous examinations of the feces were recovered. With the exception of duodenal specimens where organisms were relatively scarce and in one case absent, bacteriological examinations of sections of the intestinal tract of sacrificed animals yielded the same results as those reported from examinations of the feces of live animals.

Intestinal strains. With one exception (culture 64) the rough

intestinal organisms were recovered from the corresponding rats to which they were fed. Apart from the one exception, the rough strains appeared in feces in great numbers after the first few days of feeding almost to the exclusion of all other bacteria. The smooth strains did not show such predominance, although in most cases after two weeks of feeding they constituted the majority of the bacteria present. Lactobacilli of the type isolated from rats receiving oral strains were also isolated. As can be seen from table 2, the intestinal flora of each rat in the group receiving intestinal strains of *L. acidophilus* was comprised almost entirely of lactobacilli, including both the administered intestinal strain and the rat strain apparently initially present in the intestinal tract. When *L. acidophilus* culture feeding was replaced by lactose and sterile skimmed milk there was a gradual disappearance of the strain which had been administered, with a gradual increase of the rat intestinal type. After two weeks without culture feeding the smooth intestinal strains which had been fed were no longer present in the feces, while the rough strains, with the exception mentioned previously (culture 64), were present to a very small extent. In each instance after cessation of culture feeding the rat intestinal type of lactobacilli eventually predominated over the strains fed.

Cultural characteristics. The morphology of all organisms varied considerably, which is characteristic of the *Lactobacillus* genus. Some of the rods were extremely short having the appearance of ovoid cells while others were slender and long, appearing single, in pairs and in chains. The rat strains of lactobacilli isolated were nearly always short to medium sized rods. Colonies of smooth intestinal and smooth oral types could not be differentiated. The rat strains also produced colonies which were smooth, but more dense, white, smaller, and always had an entire edge, while colonies of the rough intestinal strains were extremely filamentous. All strains reduced and curdled litmus milk after 24 or 48 hours. All strains of the oral and intestinal groups fermented maltose, sucrose (with one exception) and mannitol (with two exceptions). These exceptions occurred among the oral strains. The reaction on raffinose was variable. Those

TABLE 3

Certain cultural and biochemical characteristics of strains of Lactobacilli fed and strains isolated from the feces of albino rats

CULTURE NUMBER	RAT NUMBER	ORIGINAL CULTURES FED					CULTURES ISOLATED				
		Colony type	Fermentation of				Colony type	Fermentation of			
			Sucrose	Maltose	Mannitol	Raffinose		Sucrose	Maltose	Mannitol	Raffinose
Controls	2	(Basic diet)					(No lactobacilli isolated)				
	1	(Basic diet plus skimmed milk)					S	+	-	+	-
<i>L. bulgaricus</i>	5	R	+	-	-	-	S	+	+	+	-
B											
Oral strains											
(<i>L. acidophilus</i>):											
14	8	S	+	+	+	+	S	+	-	+	-
18	9	S	+	+	+	+	S	+	-	+	-
19	10	S	+	+	+	+	S	+	-	+	-
60	6	S	+	+	+	-	S	+	-	+	-
61	3	S	+	+	+	+	S	+	+	+	-
94	7	S	-	+	-	-	S	+	-	+	-
97	4	S	+	+	-	-	S	+	-	+	+
Intestinal strains											
(<i>L. acidophilus</i>):											
1	13	R	+	+	+	+	R*	+	+	+	+
							S	+	-	+	+
9	19	R	+	+	+	-	R*	+	+	+	-
							S	+	-	+	-
15	18	R	+	+	+	+	R*	+	+	+	+
							S	-	-	+	+
20	15	R	+	+	+	+	R*	+	+	+	+
							S	+	-	+	-
64	12	R	+	+	+	-	S	+	-	+	-
10	16	S	+	+	+	-	S*	+	+	+	-
							S	+	-	+	+
12	14	S	+	+	+	+	S*	+	+	+	+
							S	+	-	+	-
K	17	S	+	+	+	+	S*	+	+	+	+
							S	+	-	+	-
K4y	11	S	+	+	+	+	S*	+	+	+	+
							S	+	-	+	-

* Has the same characteristics as the culture originally fed, indicating implantation of the original strain.

strains isolated which were of rat origin fermented sucrose and mannitol with only one exception while the reaction on maltose and raffinose was variable. Intestinal strains were much more tolerant to phenol than the oral strains, being able to initiate growth in concentrations of 1:400 or greater. Strains of rat origin, although not as resistant to phenol as the stock intestinal strains, were slightly more tolerant than the oral strains used.

These cultural characteristics are presented in detail in table 3, wherein the characteristics of the strain administered orally is compared to those of *acidophilus*-like organisms found in the feces of the same rat. In this way it could be ascertained whether the organism isolated from the feces of a particular rat was the same one which had been fed to that rat.

As already shown, fermentation reactions alone were inadequate for classification of the strains but they were of real value, along with cultural and other characters, in determining the characteristics of a known strain before implantation. By repetition of these tests upon organisms isolated after culture feeding we could determine with considerable certainty whether or not the recovered organisms were identical with those administered. Colonies of the rat strains (always smooth) did differ in size, color and contour from the smooth human types and we believe the constancy of these characteristics and the appearance of the organisms in control rats, along with the fermentation reactions described above, warrant designation of these lactobacilli as rat strains.

DISCUSSION

The implantation of *L. acidophilus* in the intestinal tract of man and rats has been repeatedly demonstrated. Since results of the present investigation show that none of the oral strains used were implantable it seems that some differences must exist between the lactobacilli of oral and intestinal origin. This is in accord with the results of Rettger and associates, Curran, Rogers and Whittier, and Ulicny, who have reported differences between these two groups with respect to cultural characteristics, type of lactic acid produced, and the quantitative utilization

of lactose, respectively, but different from the conclusions of those who maintain that both groups are comprised of the same organisms. Considering the many similarities which exist between the oral and intestinal lactobacilli and the fact that many of the differences are quantitative in nature, we would be inclined to class the oral strains as atypical species and not the true central type, *L. acidophilus*. While no studies were made of these organisms from the standpoint of the etiology of dental caries, we are inclined to be of the same opinion as Rettger, Levy, Weinstein, and Weiss who maintain that there is still a lack of sufficient evidence to show that the intestinal types are of significance in dental caries.

The failure to implant one of the intestinal strains (64) is significant but not necessarily surprising. Except for the inability of this organism to become established in the intestinal tract of rats, its characteristics conformed to those of other strains of *L. acidophilus* which were implanted. This is significant in view of the similarity between the two species, *L. acidophilus* and *L. bulgaricus*, the latter considered not implantable. Although some have separated these species on the basis of maltose fermentation, the recent work of Curran and associates hardly substantiates such a separation. Recently Kopeloff and Kopeloff (1937) reported that R forms of both *L. acidophilus* and *L. bulgaricus* produce inactive lactic acid, while the smooth types of both produce dextro-rotary acid. Admitting the intimate relationship between these organisms, it is questionable whether our culture no. 64 should be considered an atypical strain of *L. acidophilus* or whether it belongs to the *L. bulgaricus* species. Perhaps organisms described as *L. bulgaricus* may be in reality unimplantable *L. acidophilus*. We are of the opinion that culture no. 64 is an atypical strain of *L. acidophilus* inasmuch as with other bacteria it is not unusual to find one species of a group deviate so from the central or typical type.

The appearance in the feces of lactobacilli of a type other than those fed was to be expected since they may be normal inhabitants of the intestinal tract, their predominance depending upon the composition of the diet. Porter, Weinstein, and Rettger (1938)

in an investigation to ascertain the bacterial flora of the stomach, segments of the small intestine and cecum of white rats, found lactobacilli present in large numbers throughout. Eppright, Valley and Smith (1937) have shown that salts of calcium and phosphorus, in addition to carbohydrates, were essential for the maintenance of an aciduric flora. Since milk contains all of these we would expect it to favor the development of those aciduric organisms already present as well as those which were being fed, providing the organisms were implantable, and this appeared to occur in our experiments. However, acidophilus milk was much more effective than sterile skimmed milk in increasing the number of those aciduric organisms originally present in the intestinal tract.

Whether the aciduric organisms isolated from rats should be classified as *Lactobacillus acidophilus* or *Lactobacillus bifidus* is another debatable question. Rettger and others have reported isolation and identification of both *L. acidophilus* and *L. bifidus* from rat feces. Other than differences in carbohydrate reactions which are of questionable value for separating members of this genus, the organisms isolated in our study were very much like the smooth (Y) type of *L. acidophilus* and hence we have designated them as such. Possibly some could have been placed in the so called *L. bifidus* groups, except that results already published may not warrant the establishment of two distinct species for organisms so closely related. Weiss and Rettger (1934) (1938) have emphasized the similarity of these organisms and suggest that *L. bifidus* from breast-fed infants and rats be considered a variant of the species *L. acidophilus*.

Another point of interest is the possible significance of aciduric organisms normally present in the intestinal tract. Since these species may be made to predominate by regulation of the diet it would seem that when they are present it might be superfluous to administer other foreign aciduric bacteria. However, in these studies the administration of *L. acidophilus* milk possessed a distinct advantage over sterile milk in stimulating the normal aciduric flora.

Finally, this investigation emphasizes the need for a method of

examining commercial acidophilus preparations beyond mere identification of organisms. Our results indicate that there may exist rough (X) types of *L. acidophilus* which are not implantable in white rats, although they are typical in all other respects. This is in agreement with other investigations which have indicated that some strains of *L. acidophilus* vary in their ability to become acclimated to the intestinal tract. Hence, it seems that the important features of an analytical procedure for acidophilus preparations should be to establish the identity of the organisms and to determine their implantation.

SUMMARY AND CONCLUSIONS

Of the seven oral or dental strains of lactobacilli employed, none were implantable in the intestinal tract of white rats by the procedure used. On the other hand, four of five rough intestinal strains and all of the five smooth intestinal strains were implantable.

Rough intestinal *Lactobacillus acidophilus* organisms (with the exception of one strain) were more readily implanted and persisted for a longer time following the cessation of feeding the organisms than did smooth strains. The one culture of *Lactobacillus bulgaricus* used was not implantable which is in accord with previous studies.

With the exception of the duodenal contents, which contained relatively few organisms, other specimens from sections of the intestinal tract of the animals sacrificed gave results upon microscopic examination and cultivation similar to those obtained with fecal samples.

Fermentation of sucrose, maltose and raffinose by intestinal strains, sucrose and maltose by dental strains and sucrose and mannitol by rat strains occurred with few exceptions. The remaining fermentation reactions were irregular and, thus, fermentation reactions alone could not be used for separation of strains into groups, although in individual instances fermentation results aided in identification of a given strain. The intestinal strains exhibited a slightly higher tolerance to phenol than did

the dental strains, and this was the only biochemical characteristic used which correlated with the results on implantation.

Lactobacilli, apparently rat strains of *Lactobacillus acidophilus*, were isolated from each rat receiving lactobacilli milk cultures as well as from a control rat receiving sterile skimmed milk. This emphasizes the necessity of controlling implantation experiments adequately to insure that the strain isolated is actually the strain administered, especially when smooth strains of lactobacilli are employed.

The results emphasize the errors that are likely to occur in identifying aciduric organisms from the oral cavity as *Lactobacillus acidophilus*. Biochemical differences between intestinal and oral strains have been shown to exist by other investigations, while this work points out the inability of the oral strains studied to become implanted in the intestinal tract of rats. Such evidence may not justify designation of oral strains of aciduric organisms as *Lactobacillus acidophilus* since implantation is a generally accepted characteristic of this species.

The results also indicate the need for improved methods of examining *Lactobacillus acidophilus* preparations to include not only identification of the organisms but in addition whether or not they can be implanted.

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