ANTIBACTERIAL ACTIVITY ASSOCIATED WITH *LACTOBACILLUS ACIDOPHILUS*[1]

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In earlier studies, evidence was obtained for the existence of antagonism *in vivo* between intestinal lactobacilli and certain of the enteric bacteria responsible for postirradiation infections in the rat and mouse (Vincent *et al.*, 1955; Haley *et al.*, 1957). In experiments *in vitro*, definite inhibition of growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* was observed when these organisms were streaked on liver veal agar plates heavily grown with lactobacilli isolated from rat intestine (Vincent *et al.*, 1955).

This was not surprising as antibacterial activity associated with cultures of lactobacilli had been noted previously for a lactobacillus (Grossowics *et al.*, 1947), *Lactobacillus acidophilus* (White and Hill, 1949; Polonskaya, 1952), *Lactobacillus lactis* (Wheater *et al.*, 1951; Ritter, 1951), and for *Lactobacillus helveticus* (Ritter, 1951). It was found, however, that the antibacterial activity associated with the lactobacilli isolated from the intestine of laboratory animals appeared to differ from the substances reported previously.

In the present communication we are reporting studies on the assay of this antibacterial activity, the purification of the substance which appears to be involved, and some of the characteristics of intestinal lactobacilli of man and several common laboratory animals which produce it. For convenience, we will refer to the active substance as lactocidin.

MATERIALS AND METHODS

Cultures. Stock laboratory cultures of *Salmonella enteritidis*, *Escherichia coli*, *Corynebacterium xerosis*, *Gaffkya tetragena*, *Staphylococcus epidermidis*, and *Streptococcus faecalis* were furnished by the Department of Infectious Diseases, and *P. aeruginosa* (ATCC 7700), *Mycobacterium smegmatis* (ATCC 10143), *Mycobacterium phlei* (ATCC 10142), and *L. acidophilus* (ATCC 4357) were obtained from the American Type Culture Collection. Intestinal lactobacilli from species of laboratory animals were isolated by procedures described earlier (Vincent *et al.*, 1955). *Trichophyton gypseum*, *Microsporum lanosum*, and *Penicillium notatum* were strains of unknown origin carried in the laboratory.

Culture media. Lactobacilli were maintained in a semisolid medium composed of tryptose phosphate broth plus 10 per cent liver veal agar (Difco). Rogosa's medium (Rogosa *et al.*, 1951), tomato juice agar (Difco), and the medium of Harrison and Hansen (1950) were employed occasionally. For production of lactocidin, the lactobacilli were grown in liver veal agar plus an additional 0.5 per cent of agar. The medium employed for turbidimetric assay of lactocidin was tryptose phosphate broth (Difco) which had been adjusted to pH 4.7 with HCl then boiled and filtered prior to final autoclaving. Other organisms utilized in the study were maintained in tryptose phosphate broth (Difco).

Other materials. Silicic acid employed for chromatographic fractionation was Baker's reagent grade, lot no. 9094. It was employed without further treatment.

Characterization of L. acidophilus. Lactobacilli isolated from intestine were identified on the basis of comparisons of their cultural characteristics with those assigned to *L. acidophilus* from studies of Curran *et al.* (1933), Rogosa *et al.* (1953), and Wheater (1955). The lactic acid produced by one strain of rabbits' lactobacilli was isolated and characterized by the method of Brin *et al.* (1952).

Production and purification of lactocidin. An inoculum of lactobacilli known to produce good activity was seeded throughout 200 ml of liver veal agar contained in a flat, rectangular, screw-cap quart bottle in sufficient amount to give

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heavy growth. A series of such cultures was incubated 5 to 6 days aerobically at 37 C, then capped and incubated for 2 to 52 weeks.

When streak tests on replicate bottles indicated the presence of appreciable activity, 400 ml of a solution 0.005 M with respect to both lactic and acetic acids ("acid solution") was added to each of the remaining cultures. The contents were shaken vigorously to disrupt the agar mass, transferred to a blender, homogenized briefly, and allowed to stand overnight at 37 C. The supernatant obtained on centrifuging 1 hr in the cold at 4500 rpm was then lyophilized to provide a crude stable active fraction. In some instances the centrifuged agar mass was reextracted with "acid solution" to improve the over-all recovery of activity from the agar, though more than two extractions were deemed unprofitable.

Ten grams of the crude lyophilized material dissolved in 60 ml of "acid solution" was added to 60 g of silicic acid, and after 30 min, 400 ml of distilled water was added. The pH was then adjusted to 7.4 with 12 N sodium hydroxide and after standing 2 hr at 37 C, silicic acid was removed by centrifuging. The clear supernatant was adjusted to pH 4.5 with acetic acid, dialyzed against 0.01 M acetic acid, and the dialyzed solution centrifuged if necessary and lyophilized.

A 10 by 4.5 cm chromatograph column was formed by pouring a slurry of silicic acid in distilled water. Three to 4 g of the above fraction were dissolved in 15 to 20 ml of "acid solution," the pH adjusted to 3.5 to 4, the mixture introduced on to the column, and elution started with 0.01 M sodium hydroxide. Eight to 10 fractions of 100 ml each were collected, the concentration of base in the solution raised to 0.05 M, 7 to 8 fractions collected and the base strength raised again to 0.1 M at which point 2 or 3 final fractions of 100 ml were collected. Fractions were collected at a column flow rate of about 0.3 ml/min/sq cm.

All fractions eluted from the column were adjusted to a pH of 4.5 to 5.0 with acetic acid, dialyzed against 0.01 M acetic acid and lyophilized, redissolved in 5 ml of 0.005 M acetic acid, and centrifuged to remove silicic acid. Samples of the supernatants were assayed turbidimetrically, and the remainder lyophilized in order to determine their dry weight. The above operations were carried out aseptically as far as practical.

Turbidimetric assay of lactocidin. To each of a series of tubes were added known weights of material to be assayed, dissolved in 1 ml of 0.005 M acetic acid. Specially prepared tryptose phosphate broth, pH 4.7 was inoculated at room temperature with a 16-hr culture of *S. faecalis* which had been grown previously on tryptose phosphate broth at pH 7.3, and immediately 9 ml of the inoculated medium were added to each assay tube. The inoculum of *S. faecalis* was chosen so that each assay tube contained 1 ml of the 16-hr culture. Control tubes containing 1 ml of 0.005 M acid but no test material and those containing fractions for test were incubated 3½ hr at 37 C. Turbidity was measured on a photronreflectometer (Libby, 1938).

Under our test conditions, the logarithm of growth rate as measured by increasing turbidity, was a linear function of time for at least 3½ hr in the presence of various amounts of lactocidin. The amount of inhibition of growth at 3½ hr was directly proportional to the amount of active material present in the experimental tubes, as shown in figure 1, and tubes were read after this interval. The assay was sufficiently reproducible to meet the needs of an assay for following purification of the active substance.

For convenience, one unit of lactocidin was defined as that amount of active material necessary to limit the growth of *S. faecalis* to half

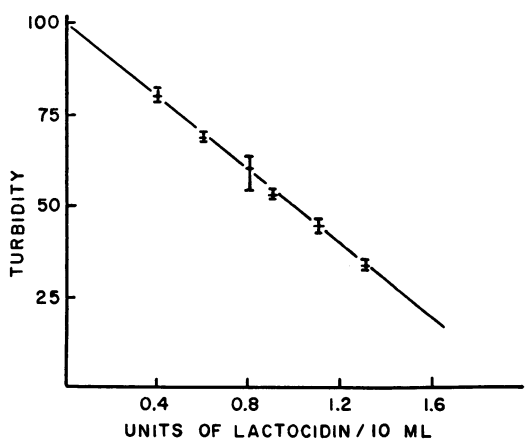

Figure 1. Growth inhibition curve of *Streptococcus faecalis* after 3.5 hr in various concentrations of lactocidin. Turbidity is expressed in arbitrary units, the 3½-hr control (no lactocidin) being taken as 100. Each point represents the mean of triplicate determinations. Two different samples, assayed 6 months apart, are represented in the figure.

the value obtained in control tubes under the conditions described above.

The requirement of solid media for production of lactocidin imposes troublesome problems in equating the total lactocidin activity actually present in solid cultures to activity of partial extracts of the agar. Although it is not practical to quantitatively extract all activity prior to assay, it was found that a second extract of once extracted agar contained roughly the number of turbidimetric units expected from the units in the first extract, if all extractable activity had partitioned between agar and extract in proportion to their volumes.

By accepting such an approximation, we have estimated the total units of extractable activity per volume of agar from the turbidimetric assay of the first extract and the volume of agar and extract.

Streak test for lactocidin in agar cultures of lactobacilli. Procedures involving extraction were cumbersome in routine use and a more convenient estimate of activity in solid cultures was derived from the results of a streak test.

A series of replicate cultures of lactobacilli were prepared. When their pH had dropped to 4.5, one of each of the replicates was extracted and the extract assayed turbidimetrically and the total units calculated to be present in the agar, estimated as indicated above. Each of the other replicates were streaked with 2-day cultures of *M. smegmatis* and *M. phlei* and 18-hr cultures of *S. enteritidis, P. aeruginosa, E. coli, C. xerosis, G. tetragena, S. epidermidis,* and *S. faecalis.* Streaks showing visible growth were recorded and streaks showing no apparent growth were subcultured after 24, 48, or 72 hr into 10 ml of tryptose phosphate broth. The subcultures showing visible growth after 18 hr or 2 days in the case of the mycobacteria were tabulated.

It was found that these test organisms survived in relation to the amount of calculated extractable activity present, as shown in table 1. Thereafter, a rough estimate of activity present in an unknown was estimated by results of streak test referred to the tabulation.

TABLE 1

Results of streak tests on agar cultures containing various amounts of lactocidin activity

Estimated Activity in Agar†	Time of Subculture after Streaking	Test Organisms*								
		Salmonella enteritidis	*Pseudomonas aeruginosa*	*Escherichia coli*	*Mycobacterium smegmatis*	*Mycobacterium phlei*	*Corynebacterium xerosis*	*Gaffkya tetragena*	*Staphylococcus epidermidis*	*Streptococcus faecalis*
units/ml	*hr*									
>6	24	−	−	−	−	−	−	−	−	−
5–6	24	−	−	−	−	−	−	−	−	+
	48–72	−	−	−	−	−	−	−	−	−
4–5	48–72	−	−	−	−	−	−	−	−	+
3–4	48–72	−	−	−	−	−	−	−	+	+
2–3	48–72	−	−	−	−	−	±	+	+	+
1–2	48–72	−	−	±	+	+	+	+	+	+
0–1	48–72	±	+	+	+	+	+	+	+	+

* No growth is indicated by −, growth on the agar plate or on subculture by +.

† The units/ml estimated to be in the agar were calculated as indicated in the text.

It was found that these test organisms survived in relation to the amount of calculated extractable activity present, as shown in table 1. Thereafter, a rough estimate of activity present in an unknown was estimated by results of streak test referred to the tabulation.

RESULTS

Characteristics of intestinal lactobacilli. Strains of lactobacilli isolated from hamster, mouse, and human sources showed the same cultural characteristics as those previously reported for lactobacilli isolated from intestine of the rat (Vincent *et al.*, 1955). They showed no growth at 20 C but vigorous growth at 45 C, and fermented salicin and cellobiose but not mannitol (Wheater, 1955).

L. acidophilus strains from rats and from mice shared the greatest number of common characteristics, grew best on liver veal agar, and appeared most closely related to our three *L. acidophilus* strains of human origin. The strains of lactobacilli isolated from hamster intestine grew best on Rogosa's medium, which in turn was unsatisfactory for culturing lactobacilli from either rabbits or rats. Rabbits' lactobacilli grew poorly on all media tested. Those which successfully subcultured did so on liver veal agar.

Lactobacilli isolated from rabbit small intestine differed from the other strains in that they failed to ferment salicin and cellobiose (Curran *et al.*, 1933; Rogosa *et al.*, 1953). Further, a 2.5 per cent aqueous solution of zinc lactate prepared from a culture of rabbit organisms gave $[\alpha]_D -3.6 \pm 0.8°$. The $[\alpha]_D$ reported for pure L(+) zinc lactate (2.5 per cent in water) is $-8.0°$, and apparently these organisms produce some L(+) lactic acid rather than solely D(−) lactic acid.

The filterable or L variant of *L. acidophilus* was isolated from homogenates of the small intestine from the rabbit and rat as colonies on

liver veal agar plates. The appearance and behavior of these forms were much the same as those reported by Kanunnikova (1954) for filterable variants of *Lactobacillus delbrueckii*. In at least two instances the original normal lactobacilli were cultured from filtrates of the L cultures of *L. acidophilus*.

Lactocidin production in vitro by various strains of L. acidophilus. Numerous attempts to obtain lactocidin activity by growing strains of *L. acidophilus* in liquid media of various types, with and without additions of enriching factors, were unsuccessful. In no instance was significant lactocidin production noted in the absence of agar, and the production of lactocidin was, therefore, carried out on liver veal agar medium.

Strains of *L. acidophilus*, isolated from initial cultures of homogenized intestine of different species of animals, were subcultured. Those which successfully subcultured through several transfers were grown in 200 ml of liver veal agar. The amount of lactocidin produced was estimated by streak test. The activities produced by various strains and substrains from various species of animals are shown in table 2.

Best activity was most consistently found with strains isolated from rabbits and one hamster. Intermediate activity was generally found with rat and human strains, whereas mouse strains were generally poor producers. However, the variability in activity produced was large in all cases except the rabbit. An attempt to improve lactocidin production by a strain isolated from rabbits by passage through chick embryos was unsuccessful. Filterable forms of rabbit lactobacilli produced little activity.

The rapidity with which appreciable activity appeared in the cultures varied considerably. The rabbit strains producing the greatest activity did so more rapidly than any other strains tested. Consequently they were the strains of choice for production and isolation studies. Strains from the hamster, although producing good activity, did so exceedingly slowly. Strains from the rat, mouse, and human were intermediate in this regard.

In conjunction with the present study, it was noted that the numbers of intestinal lactobacilli isolated from homogenates of entire small intestine were within the range of 0.5 to 7 $\times$ 10^8/g in rat, mouse, and hamster gut, whereas the numbers in rabbit gut were only 0 to 500. The predominant, nonlactic species noted were the usual enteric types, and ranged from 10^5 to 10^8/g in mice, 10^4/g in hamster and rat, and about 10^3/g in rabbit intestine.

TABLE 2

*Lactocidin production by intestinal lactobacilli of various animals**

Species	No. Animals Sampled	No. Stable Cultures Lactobacilli Examined	Incubation Time in Weeks	Lactocidin Units/Ml Agar
Rat	18	3	3–8	2–3
		2	4	3–4
		3	4–8	4–5
Mouse	9	7	8	0–1
		3	8–10	1–2
	5	3	4–10	2–3
		3	4	3–4
Rabbit	7	1	6	5–6
		3	2–3	>6
Hamster	1	5	52	5–6
Human	3	ATCC 4357	6–8	2–3
		ATCC 4962		3–4
		ATCC 9857		4–5

* Lactocidin was estimated from streak tests at the time the pH of the culture had dropped to 4.5.

TABLE 3

*Summary of silicic acid fractionation of crude lactocidin**

Fraction No.	Weight	Total Activity		Total Activity in Fractions
	mg	*units*	*units/mg*	%
1	1031	619	0.60	41
2–3	417	334	0.80	22
4–8	136	190	1.40	13
9–12	44.5	80	1.80	5
13–19	11.3	215	19.0	14
20	0.8	80	100.0	5

* A total 2.92 g of crude active material assaying 0.50 units/mg was chromatographed on a 10 by 4.5 cm column of silicic acid.

Purification of lactocidin. The antibacterial activity associated with aged agar cultures of lactobacilli appears to be firmly bound at neutrality to the agar but was found to dissociate readily below pH 5 or above pH 7.8. Activity so solubilized was nondialyzable, and stable indefinitely at room temperature or somewhat higher at pH 4.7. Purified samples were con-

TABLE 4

*Sensitivity of microorganisms to graded concentrations of crude lactocidin**

Conc of Lactocidin at which Tests Were Made	Organisms Failing to Survive at the Designated and Higher Conc	
	Test medium	
	Tryptose broth, pH 4.7	Broth-50% serum, pH 7.0
units/ml		
1.0	*Proteus vulgaris* (ATCC 9484) *P. vulgaris* (ATCC 9920)	*P. vulgaris* (ATCC 9484)
1.5	*P. vulgaris* (rat intestine) *Salmonella enteritidis* *Pasteurella multocida*	*P. vulgaris* (rat intestine) *S. enteritidis* *P. multocida* *Neisseria catarrhalis* *Escherichia coli* (rat intestine)
2.0	*Pseudomonas aeruginosa* (rat intestine) *P. aeruginosa* (ATCC 7700) Paracolobactrum (rat intestine) *E. coli* (rat intestine)	*P. aeruginosa* (rat intestine) *P. aeruginosa* (ATCC 7700) Paracolobactrum (rat intestine)
2.5	*E. coli* *Mycobacterium smegmatis* (ATCC 10143)	*E. coli* *M. smegmatis* (ATCC 10143)
3.0	*Mycobacterium phlei* (ATCC 10142) *Trichophyton gypseum* *Microsporum lanosum* *Staphylococcus aureus* (ATCC 9664)	
3.5	*Corynebacterium xerosis* *Gaffkya tetragena*	*S. aureus* (ATCC 9144)
4.0	*S. aureus* (ATCC 9144) *S. epidermidis* *Bacillus subtilis*	*Staphylococcus epidermidis*
5.0	*Streptococcus faecalis* (assay strain) *Penicillium notatum* *Lactobacillus acidophilus* (rat intestine) *L. acidophilus* (mouse intestine) *L. acidophilus* (ATCC 4357)	
6.0	*L. acidophilus* (rat intestine)	
Not killed by 6 units	*Bacillus megaterium* *Aspergillus fumigatus*	

* Crude lactocidin preparations were dialyzed, lyophilized samples prepared from acidic extracts of agar cultures.

siderably more labile. Activity was not extractable by lipophylic solvents, rather activity was destroyed in the presence of ether. Addition of acetone to neutral solutions destroyed activity as did ethanol.

Attempts to purify the active substance employing absorption on charcoal, calcium tartrate, ion exchange resins (Amberlite 120, IR-4, and BioRad AG50X) were either unsuccessful or accompanied by very poor recovery of

activity. Some purification was achieved by absorption and elution from silicic acid by the procedure described under Materials and Methods.

In a typical experiment, 800 ml of media were distributed in 4 1-L bottles, inoculated with rabbit lactobacilli, and incubated 2 weeks or more to the appearance of good activity, determined by streak test on 1 bottle. Growths in the 3 remaining bottles were harvested and extracted giving 30.0 g of lyophilized crude active material assaying 0.05 unit/mg which corresponded to about 60 per cent of the extractable activity. Batch absorption on and elution from silicic acid, dialysis, and lyophilization gave 2.92 g of hygroscopic material assaying 0.5 unit/mg, thus representing a 10-fold purification without significant loss of activity. Appreciable pigment was lost during this step. This material was then chromatographed on silicic acid as described under Materials and Methods. The fractionation achieved is shown in table 3.

The weight of material recovered was 56 per cent of that put on the column and the recovery of activity was 103 per cent. The bulk of the activity was recovered as fractions containing 0.5 to 2.0 units/mg, and small amounts with activities ranging from 19 to 100 units/mg. The total activity recovered was approximately half the calculated extractable activity in the agar. The most active fractions invariably lost most of their activity on lyophilization, or on storage in solution under conditions which did not affect the crude material.

Bactericidal spectrum of lactocidin. The bactericidal action of lactocidin was tested on 27 gram-negative, gram-positive, and acid-fast bacteria and 4 molds, employing several batches of crude lactocidin assaying 0.04 to 0.4 unit/mg. Within the limits imposed by possible variations between batches and rather low activity lactocidin used, the order of sensitivity to bactericidal effect in tryptose broth at pH 4.7, and in some instances in broth-50 per cent serum at pH 7 was that shown in table 4.

It is noted that the parasitic microorganisms are more susceptible to lactocidin than the naturally saprophytic types, and that the gram-negative are more susceptible than the gram-positive bacteria. It was observed that the rough phenotypes of a number of the bacteria which have been tested are more resistant than their smooth phenotypes for which data are given in table 4. It is worth noting that various strains of *L. acidophilus* are sensitive to higher concentrations of their own antibacterial product.

Where the comparison was made, the spectrum of activity determined in broth-50 per cent serum at pH 7 was essentially identical with that determined in tryptose broth at pH 4.7. The spectrum in serum-containing medium was determined on crude, dialyzed lactocidin preparations prior to fractionation on silicic acid. Purification through steps involving silicic acid resulted in material which maintained its activity in tryptose broth at pH 4.7 but was inactive in serum-containing media.

DISCUSSION

The cultural characteristics displayed by the lactobacilli isolated in this study support the conclusion that they all are *L. acidophilus*. It was noted that the former variety of *Lactobacillus para bifidus* Weiss and Rettger, differing from *Lactobacillus bifidus* in its production of dextro rather than racemic lactic acid, is now classified as *L. bifidus* in the 7th edition of *Bergey's Manual of Determinative Bacteriology* (Breed *et al.*, 1957). Although the rabbit strains produced L(+)-lactic acid, and might be named as a variant, in the interest of uniformity, they, too, are considered *L. acidophilus*. It would seem quite likely that the production of antibacterial activity with the particular properties of lactocidin will be found to be a common characteristic of *L. acidophilus*.

We believe that lactocidin has not been previously recognized as an antibacterial product of lactobacilli. Grossowics *et al.* (1947) reported that antibiotic activity appeared in cultures of an unidentified lactobacillus grown in nutrient broth. Their material differed from lactocidin in that it was extractable with ether. White and Hill (1949) reported antibacterial activity associated with cultures of *L. acidophilus* grown on broth, and Polonskaya (1952) found antibacterial activity in cultures on whey of *L. acidophilus* isolated from cattle feces. Ritter (1951) obtained active filtrates from cultures of *L. lactis* and *L. helveticus*. These groups of investigators did not supply sufficient criteria for an entirely satisfactory comparison of their active substances and lactocidin; however, activity appeared in cultures devoid of agar and appeared considerably more rapidly than observed in this study. Wheater

et al. (1951) found that broth cultures of *L. lactis* were strongly bacteriostatic but later Wheater *et al.* (1952) showed the activity was probably due to hydrogen peroxide. The activity noted by White and Hill, by Polonskaya, and by Ritter could also have been due to hydrogen peroxide.

It is evident that lactocidin is not hydrogen peroxide or lactic acid from its method of purification. Indeed, this had been well demonstrated prior to attempts at purification (Vincent *et al.*, 1955). For example, it was demonstrated that treatment of active agar flats with catalase prior to streak assay did not alter assay results. That activity was not due to lactic acid or other low molecular weight metabolic products was demonstrated by the nonvolatile, nondialyzable nature of the activity (table 4) eluted from active agar cultures.

The loss of activity in serum after treatment with silicic acid is most likely due to removal of inactive substances which, when present, preferentially bind serum proteins permitting display of activity, but when absent, permit binding or inactivation of lactocidin. This seems likely since 100 per cent of the activity displayed in tryptose broth is recovered on silicic acid treatment and because the spectrum of crude lactocidin is the same in tryptose broth and in medium containing serum. Although the inactivity of purified lactocidin in serum would preclude its usefulness in parenteral therapy, we believe that its activity is normally displayed *in vivo* in the gastrointestinal tract in the maintenance of the ecological relationships between populations of various microorganisms.

The existence of a large number of diverse microbial antagonisms is now recognized. A limited number of these no doubt operate within the gastrointestinal tract of mammals, and reciprocal relationships between the geographical distribution of various microorganisms in the gastrointestinal tract, and their production of antibacterial substances against their neighbors appear to exist. For example: colicine production by *E. coli* probably determines in part the interstrain relationships of enteric *E. coli* (Fredericq, 1957); Halbert *et al.* (1953) have shown that enteric microorganisms produce a number of distinct, antibacterial activities against Enterobacteriaceae, but not against gram-positive organisms; and Miller *et al.* (1956–1957) have reported an unidentified anaerobic bacterium in feces which produces a substance antagonistic to salmonella. Finally, lactobacilli have been implicated in these relationships since the pioneer work of Metchnikoff with *Lactobacillus bulgaricus*, and that of Rettger and his associates, with *L. acidophilus*.

It is unnecessary to review the voluminous early literature of the lactobacillus controversy here. Rather, it may suffice to note that peroral preparations of viable *L. acidophilus* have again attained some measure of utility in recent years in the treatment of functional gastrointestinal disturbances (Rafsky and Rafsky, 1955) and colitis of antibiotic origin (Winkelstein, 1956). Most recently Gordon *et al.* (1957) have shown that administration of a strain of *L. acidophilus* resistant to several antibiotics, as an adjuvant to oral antibiotic therapy, checked the development of antibiotic-resistant enteric staphylococci of the type leading to superinfections. These workers also noted that in a few instances, individuals on antibiotic therapy alone maintained their normal flora of lactobacilli. These individuals showed no increase in numbers of intestinal staphylococci. Vincent *et al.* (1955) noted that rats which maintained their normal number of lactobacilli after midlethal doses of X-irradiation were less susceptible to bacteremic complications than their counterparts in which the numbers of lactobacilli decreased.

These observations suggest that maintenance of the normal gastrointestinal population of *L. acidophilus* controls potential pathogenic bacteria normally present in the gut in moderate numbers. The finding that *L. acidophilus* isolated from a number of species all produce antibacterial activity with a broad spectrum against common enteric bacteria suggests that *L. acidophilus* may occupy an important position in the maintenance of normal population levels of enteric bacteria by production *in vivo* of lactocidin.

If these views are correct, it is suggested that the most effective oral preparations of viable *L. acidophilus* would be good producers of lactocidin, and further, that it would be desirable to examine the effect of oral lactobacillus therapy on the development of postirradiation bacteremias.

SUMMARY

Strains of *Lactobacillus acidophilus* obtained from mice, rats, rabbits, hamsters, and man were found to produce an antimicrobial agent in cultures grown in liver veal agar. The substance responsible has been called lactocidin. Two

methods of assay of lactocidin were devised and these were employed to select the best strains of *L. acidophilus* for production of lactocidin, and for following the purification of this material. A 2500-fold purification of lactocidin was achieved. Crude lactocidin had a broad antibacterial spectrum and was active in the presence of serum. Purified lactocidin was unstable and was inactive in serum.

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