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ISOLATION AND IDENTIFICATION OF ENTEROCOCCI FROM THE INTESTINAL TRACT OF THE RAT[1]

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ABSTRACT

ROGERS, C. G. (Department of National Health and Welfare, Ottawa, Ontario, Canada), AND W. B. SARLES. Isolation and identification of enterococci from the intestinal tract of the rat. J. Bacteriol. **88**:965-973. 1964.—Surface inoculation was employed in a comparison of three selective media, M-enterococcus (M) agar, thallium acetate-glucose (TITG) agar, and KF-streptococcus (KF) agar, for enumeration and isolation of enterococci from contents of the digestive tract of the rat. Similar yields of enterococcus colonies were obtained with M, TITG, and KF agar. When cecal contents were examined, species identification of 120 isolates, 40 from each medium, revealed only minor differences in selective activity. *Streptococcus faecalis* and its variety *zymogenes* represented 55 to 63% of the isolates from each medium; the remaining strains resembled *S. durans* or *S. faecium*. More than 99% of the isolates failed to survive the heat-tellurite tolerance test. Periodic analyses of intestinal contents from young rats indicated that enterococci became established in the gut from 10 to 14 days after birth. The species isolated most frequently on M agar was *S. faecalis* var. *zymogenes*. When rats were fed a purified diet with casein as the source of nitrogen, *S. faecalis* var. *zymogenes* predominated among isolates from contents of the small intestine plated on M agar. This species was largely replaced by others resembling *S. durans* or *S. faecium* when mixtures of L-amino acids provided the dietary nitrogen. Nineteen enterococcus isolates, representing all that met the Sherman criteria, required arginine, glutamic acid, histidine, isoleucine, leucine, methionine, tryptophan, and valine; of these, two strains required in addition threonine, glycine, and lysine; four required threonine and glycine; two, threonine and lysine; and one, threonine.

Enterococci can be defined as fecal streptococci of Lancefield serological group D which fulfill the criteria of Sherman (1937). This group comprises *Streptococcus faecalis* and its varieties *zymogenes* and *liquefaciens*, *S. durans*, and *S. faecium* (Orla-Jensen, 1919). Although estimates of numbers of these bacteria in the alimentary tract of the rat have been reported by many workers (Gant et al., 1943; Askalonov, Dobrier, and Shvaiko, 1957; Raibaud, 1958), few attempts have been made to identify the species that exist in this environment. Raibaud (1958) observed an increase in numbers of *S. faecalis* var. *liquefaciens* and *S. faecium* in stomach, small intestine, and cecal contents of rats receiving chlortetracycline, whereas *S. faecalis* or its variety *liguefaciens* was prevalent among 161 fecal isolates examined by Ferraro (1960).

Media containing thallium acetate (Barnes, 1956) or sodium azide (Slanetz and Bartley, 1957; Kenner, Clark, and Kabler, 1961) as the selective inhibitor have been reported to be highly selective for fecal streptococci. The present investigation was undertaken to compare these media for enumeration and isolation of enterococci from contents of the enteric tract of rats, and to identify the species that prevail in this environment. Other aspects of this study included: (i) periodic analyses of intestinal contents of young rats from birth until weanling age to determine when enterococci become established in the gut; (ii) effects of various sources of dietary nitrogen on the enterococcus flora of the small intestine; and (iii) an examination of the amino acid requirements of various enterococcus isolates.

MATERIALS AND METHODS

Animals. Rats from two sources were used in these studies: (i) an inbred strain of Wistar rats from the Food and Drug Laboratories, Department of National Health and Welfare, Ottawa, Canada; and (ii) rats of the Sprague-Dawley strain (Sprague-Dawley Inc., Madison, Wis.) and Holtzman strain (Holtzman Co., Madison, Wis.)

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from the Biochemistry Department of the University of Wisconsin. The animals were housed individually, in wire-bottom cages, and fed *ad libitum*.

Selective media. To develop a suitable method for enumeration and isolation of enterococci from the intestinal tract of the rat, three media were compared: M-enterococcus (M) agar (Slanetz and Bartley, 1957), thallium acetate-glucose (TITG) agar (Barnes, 1956), and KF-streptococcus (KF) agar (Difco; Kenner et al., 1961). To the latter medium was added a sterile, aqueous solution of 2,3,5-triphenyl tetrazolium chloride (TTC) to give a concentration of 0.01%. The pour plate method was used in preliminary experiments, but later was replaced by a surface inoculation technique. In the latter procedure, excess moisture was removed from the agar prior to inoculation by drying the plates in an incubator at 37 C for 48 hr (Hentges and Fulton, 1960).

Enumeration and isolation of enterococci. Intestinal and cecal contents were obtained for analysis from anesthetized rats as previously described (Rogers and Sarles, 1963). For plating, a 1:100 dilution was prepared by the addition of a 0.5-g sample to 49.5 ml of sterile phosphate buffer (American Public Health Association, 1955). The sample was disrupted and homogenized by shaking with glass beads, further diluted, and plated in 1-ml amounts on the predried surface of the selective medium. Two to five plates of each dilution were prepared. Colonies were counted after incubation at 37 C for 48 hr. Cells from well-isolated colonies were transferred to slants of Tryptose-yeast extract (TYE) agar (Rogers and Sarles, 1963), incubated 24 hr at 37 C, and then stored at 5 C for later study.

Identification of isolates. Membership in the enterococcus group was determined by subjecting the isolates to the criteria of Sherman (1937); i.e., growth in TYE broth at 10 and 45 C; in TYE broth at pH 9.6; in TYE broth containing 6.5% NaCl; after 30 min in TYE broth at 60 C; and in sterile skim milk containing 0.1% methylene blue. Biochemical tests were conducted at 37 C, and included: (i) reduction of litmus in skim milk (Burnett, Pelczar, and Conn, 1957; tubes were read after 24 hr and again at 7 days for acid production, reduction of litmus, clot formation, and proteolysis); (ii) hydrolysis of gelatin (Burnett et al., 1957) in a medium containing 2% Tryptose, 0.5% sodium chloride, 0.25% K_2HPO_4, 0.3% yeast extract, 1.5% agar, and 0.4% gelatin (pH 7.2); (iii) reduction of potassium tellurite, 1:2,500 in skim milk; (iv) reduction of tetrazolium chloride (Barnes, 1956) in TYE agar at pH 6.0; (v) hemolysis in TYE agar containing 5% (v/v) human blood. All strains were tested for ability to ferment L(+)-arabinose, mannitol, sorbitol, melibiose, melezitose, and glycerol, each at a concentration of 0.5%, in sterile 1% peptone broth containing bromocresol purple as an internal indicator. Tubes were read for acid production after 7 days.

Each enterococcus isolate was also subjected to the heat-tellurite tolerance test of Cooper and Ramadan (1955).

Enterococci in young rats. Experiments were undertaken to determine when enterococci become established in the intestinal tract of the rat. Litters of inbred Wistar rats were housed individually in nesting cages with fiber-chip bedding. A stock ration was supplied *ad libitum* to the adult animal in each cage. At intervals from a few hours after birth until weaning at 21 or 22 days of age, a young rat was selected from each of three litters (experiment 1) or two litters (experiment 2), anesthetized, and the small intestine removed aseptically. The intestinal segment was disrupted in phosphate buffer (pH 7.0) by shaking with glass beads, diluted, and surface-plated on M agar (Slanetz and Bartley, 1957). Samples were plated at dilutions of 10^{-3} and higher. Enterococci were enumerated, and isolates were identified as described above. The absence of typical colonies on plates inoculated with the lowest dilution was taken as evidence that the intestinal tract contained fewer than 1,000 enterococci per gram.

Amino acid requirements. The synthetic medium of Horn, Jones, and Blum (1950) was used in the determination of individual amino acid requirements. Cells for the inoculum were prepared by growing the cultures at 37 C for 18 hr in the synthetic medium with amino acids (excepting L-tryptophan and L-cystine) omitted and replaced by 10 ml per 100 ml of 10% vitamin-free acid-hydrolyzed casein (Nutritional Biochemicals Corp., Cleveland, Ohio). The cells were harvested by centrifugation (2,000 × g for 5 min), washed once in sterile saline, and resuspended in sterile saline to give a turbidity equivalent to 75 to 80% transmission of light at 540 mμ in a Bausch & Lomb Spectronic-20 colorimeter. All tests were performed in culture tubes (15 by 170 mm). One drop of the washed-cell suspension was added to

3 ml of single-strength medium in each tube. After 72 hr at 37 C, acidity in the complete medium was titrated with 0.05 N NaOH, and compared with that in the same medium from which each of 19 amino acids in turn was omitted.

Results

Effect of method of inoculation. A comparison of the effect of surface or pour plate inoculation on numbers of enterococci obtained with M agar or KF agar is shown in Table 1. Although significantly higher counts were obtained by surface inoculation ($P = 0.05$), M agar permitted growth of larger numbers of enterococcus colonies regardless of the technique employed ($P = 0.01$). Surface inoculation resulted in the development of larger colonies that were easier to count and to differentiate on the basis of reduction of tetrazolium chloride in the medium. In view of these findings, surface inoculation was used in all later experiments.

TABLE 1. *Effect of method of inoculation of diluted intestinal contents on numbers of enterococci obtained with two selective media*

Medium	Enterococci per g (dry wt) × 10⁶	
	Surface inoculation	Pour plate inoculation
M-enterococcus agar	202.1 ± 10.1*	169.2 ± 10.2†
KF-streptococcus agar	165.9 ± 9.2*	136.5 ± 18.6†

* Mean of 8 plate counts ± standard error.
† Mean of 4 plate counts ± standard error.

Comparison of selective media. M, TITG, and KF agars were compared for the enumeration of enterococci in contents from the small intestine, cecum, and colon of inbred Wistar rats. Numbers of enterococci (Table 2) were smallest in contents from the small intestine, but increased from the cecum to the colon. Although some variation in numbers of enterococcus colonies was evident among the three media, in general, the differences were not great. Enterococci with strong reducing powers, shown by the production of dark-red colonies on agar containing TTC, predominated in each region of the gut.

Identification of isolates. When cecal contents were examined, identification of isolates from M, TITG, and KF agars revealed only minor differences in selective activity (Table 3). *S. faecalis* and its variety *zymogenes* represented 55 to 63% of the isolates from each medium, followed by *S. durans* and atypical strains that resembled it (35

TABLE 2. *Enumeration with three selective media of enterococci in contents from the small intestine, cecum, and colon*[a]

Determination	Medium	Enterococci per g (dry wt)[b] × 10⁶		
		Small intestine	Cecum	Colon
Total count	M	3.7 ± 0.6[c]	55.0 ± 1.1[d]	247.0 ± 7.3[c]
	TITG	5.3 ± 0.6[c]	58.0 ± 3.2[d]	321.0 ± 12.8[c]
	KF	2.1 ± 0.3[d]	51.0 ± 2.8[d]	306.0 ± 7.6[d]
Weak reducers of TTC[e]	M	0.5 ± 0.2[c]	16.0 ± 1.5[d]	100.0 ± 5.2[c]
	TITG	0.5 ± 0.4[c]	15.0 ± 0.8[d]	105.0 ± 3.6[c]
	KF	0.2 ± 0.1[d]	13.0 ± 0.8[d]	111.0 ± 10.3[d]
Strong reducers of TTC[f]	M	3.2 ± 0.6[c]	39.0 ± 2.1[d]	147.0 ± 7.3[c]
	TITG	4.7 ± 1.0[c]	45.0 ± 2.6[d]	212.0 ± 26.8[c]
	KF	1.9 ± 0.3[d]	38.0 ± 2.1[d]	195.0 ± 8.8[d]

[a] Animals were maintained 14 days on a diet of the following composition in (%, w/w): vitamin-free casein, 20; corn starch, 69; vitamin mixture in casein (Beare et al., 1959), 1; salt mixture, USP IV, 4; alphacel (non-nutritive cellulose, Nutritional Biochemicals Corp.), 6.
[b] Figures are for pooled samples from ten rats.
[c] Mean of six plate counts ± standard error.
[d] Men of five plate counts ± standard error.
[e] Paale-pink or colorless colonies.
[f] Dark-red colonies.

TABLE 3. *Identification of enterococci isolated from cecal contents with three selective media*

Species or variety	No. (and %) of strains		
	M agar	TITG agar	KF agar
Streptococcus faecalis	20 (51.3)	16 (40.0)	18 (45.0)
S. faecalis-like*	2 (5.1)	3 (7.5)	4 (10.0)
S. faecalis var. *zymogenes*	1 (2.6)	6 (15.0)	0 (0.0)
S. faecalis var. *liquefaciens*	0 (0.0)	0 (0.0)	0 (0.0)
Subtotal	23 (59.0)	25 (62.5)	22 (55.0)
S. durans	4 (10.3)	3 (7.5)	15 (37.5)
S. *durans*-like*	10 (25.6)	11 (27.5)	3 (7.5)
Subtotal	14 (35.9)	14 (35.0)	18 (45.0)
S. faecium	0 (0.0)	0 (0.0)	0 (0.0)
S. faecium-like*	2 (5.1)	1 (2.5)	0 (0.0)
Subtotal	2 (5.1)	1 (2.5)	0 (0.0)
Total	39 (100.0)	40 (100.0)	40 (100.0)

* Differed from recognized species in one or more sugar-fermentation reactions.

TABLE 4. *Subgroups of atypical enterococci isolated from cecal contents with three selective media*

Subgroup	No. of isolates/total*		
	M agar	TITG agar	KF agar
S. durans-like			
Raffinose +	10/39 (25.6)	8/40 (20.0)	2/40 (5.0)
Sorbitol +	0/39 (0.0)	1/40 (2.5)	1/40 (2.5)
Raffinose +, melezitose +	0/39 (0.0)	2/40 (5.0)	0/40 (0.0)
S. faecalis-like			
Melezitose −	2/39 (5.1)	1/40 (2.5)	3/40 (7.5)
Melibiose +	0/39 (0.0)	2/40 (5.0)	1/40 (2.5)
S. faecium-like			
Arabinose −	2/39 (5.1)	1/40 (2.5)	0/40 (0.0)
Total	14/39 (35.8)	15/40 (37.5)	7/40 (17.5)

* Numbers in parentheses indicate the percentage of isolates in each subgroup.

to 45%). The remaining strains resembled *S. faecium* (0 to 5%), but were atypical in one or more sugar-fermentation tests. None of the isolates from any of the selective media was identified as *S. faecalis* var. *liquefaciens*.

The incidence of subgroups of atypical enterococcus isolates, designated *S. durans*-like, *S. faecalis*-like, or *S. faecium*-like, is presented in Table 4. Approximately 36% of the isolates from M and TITG agar fell into this category, whereas about 18% of the isolates from KF agar showed atypical characteristics.

Heat-tellurite tolerance. As shown in Table 5, approximately one-half of the isolates from M and KF agar, and two-thirds of those from TITG agar, survived exposure to 63 C for 30 min in TYE broth. In contrast, almost all of the isolates that survived the heat treatment were unable to grow when transferred to skim milk containing 1:2,500 potassium tellurite. The four strains which survived the complete test were identified as *S. faecalis*.

Enterococci in young rats. Numbers of enterococci isolated from young rats at various ages are

shown in Table 6. In the first experiment, enterococci were detected at low dilution (10^{-3}) in the intestinal tract of one of three animals examined 10 hr after birth, after which none was detected until the 10th day *post-partum*. On the 14th day, the bacteria were detected in the intestines from animals of all three litters. Similar results were obtained at weaning age (22 days old). Of 44 isolates examined, 26 were identified as *S. faecalis* var. *zymogenes*, 10 as *S. durans*, and 8 as *S. faecium*.

In the second experiment, the small intestines of rats from each of two litters contained large numbers of enterococci 12 hr after birth. The bacteria were again detected on the third and fifth days, after which none was detected until the 11th day. Animals from both litters contained measurable numbers of enterococci on the 14th and 21st days. Twenty-three cultures of enterococci were isolated during this experiment, and all were identified as *S. faecalis* var. *zymogenes*.

Effect of dietary nitrogen. In Table 7 are shown the identity and frequency of occurrence of enterococcus species from contents of the small intestines of Holtzman rats fed diets in which nitrogen was provided by casein or mixtures of L-amino acids. Isolates were selected from colonies that developed on M agar; all met the criteria of Sherman (1937). *S. faecalis* var. *zymogenes* predominated among isolates from rats fed purified diets with casein as the nitrogen source. In contrast, when nitrogen in purified diets was provided by mixtures of L-amino acids, the majority of isolates resembled *S. durans*, although two were identified as *S. faecium*, and one as *S. faecalis* var. *zymogenes*.

Amino acid requirements. Fourteen enterococcus isolates from rats and five from other sources (Table 8) were examined to determine their requirements for each of 19 amino acids. The group included all species and varieties of enterococci that meet the Sherman criteria. Arginine, glutamic acid, histidine, isoleucine, leucine, methionine, tryptophan, and valine were essential for growth of all species tested. Two strains of *S. durans* (11 and 12), in addition, required threonine, glycine, and lysine; three atypical strains, designated *S. faecium*-like (14, 15, and 16), and one strain of *S. faecalis* var. *zymogenes* (7) required threonine and glycine; a single strain of *S. faecalis* var. *liquefaciens* (19) and another of *S. durans* (10) each required threonine and lysine; whereas a single strain of *S. faecium* (13) required threonine. The eight amino acids for which no requirement was found were: alanine, aspartic acid, cystine, hydroxyproline, phenylalanine, proline, serine, and tyrosine.

TABLE 5. *Heat-tellurite tolerance of enterococcus isolates from cecal contents*

Medium	No. of strains tested	No. resistant to heat (63 C for 30 min)*	Heat and tellurite resistant	
			No.	Identity
M	39	18 (46.0)	1	*S. faecalis*†
KF	40	21 (52.5)	1	*S. faecalis*
TITG	40	27 (67.5)	2	*S. faecalis*

* Numbers in parentheses indicate the percentage resistant to heat.

† Did not ferment melezitose.

TABLE 6. *Numbers of enterococci in contents of the small intestines from young rats at various ages*

Expt	Age	Log10 no. of enterococci per g* in litter			No. of isolates
		1	2	3	
1	4 hr	ND†	ND	ND	0
	10 hr	3.12	ND	ND	2
	1 day	ND	ND	ND	0
	2 days	ND	ND	ND	0
	3 days	ND	ND	ND	0
	4 days	ND	ND	ND	0
	5 days	ND	ND	ND	0
	6 days	ND	ND	ND	0
	7 days	ND	ND	ND	0
	10 days	ND	8.47	ND	9
	14 days	7.45	7.26	4.54	14
	22 days‡	3.63	3.86	4.23	12
	27 days	3.93			7
	Total				44
2	12 hr	7.04	5.45		4
	3 days	5.96	4.92		4
	5 days	3.62	4.30		4
	7 days	ND	ND		0
	9 days	ND	ND		0
	10 days	ND	ND		0
	11 days	ND	3.88		1
	12 days	ND	3.01		2
	14 days	3.06	3.90		4
	21 days‡	5.62	4.34		4
	Total				23

* Wet weight of intestinal contents plus intestine.

† Enterococci not detected. Samples contained fewer than 1,000 per gram.

‡ Day on which animals were weaned.

TABLE 7. *Changes in the enterococcus flora of the small intestine in rats fed different sources of nitrogen*

Source of nitrogen in diet	Time on test	No. of samples pooled	No. of isolates examined	Identity	No.	Per cent
	days					
Casein (9%)*	14	2	12	*S. faecalis* var. *zymogenes*	12	100
Casein (12%)*	9	5	13	*S. faecalis* var. *zymogenes*	10	77
				S. durans	3	23
Casein (90%)*	14	2	12	*S. faecalis* var. *zymogenes*	12	100
L-Amino acids†	17	5	25	Atypical, resembled *S. durans*	23	92
				S. faecium	2	8
L-Amino acids†	17	4	13	*S. faecalis* var. *zymogenes*	1	8
				S. durans	12	92

* The composition of the purified basal diet to which various amounts of casein were added was the same as that of Chen et al. (1962).

† Diets containing mixtures of L-amino acids were those of Stucki (1962).

TABLE 8. *Identification and source of enterococci examined for amino acid requirements*

Strain no.	Species or variety	Source
1	*Streptococcus faecalis*	Infant stool. Originally isolated by M. E. Sharpe, University of Reading.
2	*S. faecalis* var. *zymogenes*	
3	*S. faecalis* var. *liquefaciens*	Culture collection of W. B. Sarles, Department of Bacteriology, University of Wisconsin.
4	*S. durans*	
5	*S. faecalis*	
6	*S. faecalis* var. *zymogenes*	Rat intestinal contents.
7	*S. faecalis* var. *zymogenes*	
8	*S. faecalis* var. *zymogenes*	
9	*S. faecium*	
10	*S. durans*	
11	*S. durans*	
12	*S. durans*	
13	*S. faecium*	
14	*S. faecium*-like (sorbitol +)	
15	*S. faecium*	
16	*S. faecium*-like (sorbitol +, arabinose −)	
17	*S. faecalis* var. *zymogenes*	
18	*S. faecalis*	
19	*S. faecalis* var. *liquefaciens*	

DISCUSSION

Hentges and Fulton (1960) described a surface inoculation technique which they used successfully for the differentiation of surface colonies of *Aerobacter* and *Shigella*. The present study has demonstrated that a similar procedure, in combination with a suitable selective medium, is useful for the enumeration and differentiation of enterococci from the alimentary tract of the rat.

Fecal streptococci from pigs were found by Raibaud et al. (1961) to produce larger numbers of colonies on M agar and TITG agar than on KF medium. In contrast, however, the present findings indicated that M, TITG, and KF agar compared favorably with one another in the enumeration of enterococci from cecal contents of rats. In agreement with Barnes (1959), enterococci on TITG agar were readily differentiated on the basis of colonial characteristics (reduction of tetrazolium chloride); this also applied to M agar.

It was noted that, after 48 hr at 37 C, fewer lactobacilli appeared as a background haze on the latter medium.

The three media employed were alike in selective ability. Employing different experimental conditions, Ferraro (1960) studied 161 cultures of enterococci isolated from feces of rats over a 15-day period. He identified 40.9% of the isolates as *S. faecalis*, 23.6% as *S. faecalis* var. *liquefaciens*, and 35.5% as *S. faecium*. None was identified as *S. faecalis* var. *zymogenes* or *S. durans*. Our observations agree with those of Ferraro to the extent that most isolates from rat cecal contents, regardless of the selective medium employed, resembled *S. faecalis;* in contrast to his work, 35 to 45% resembled *S. durans* and 5% or less resembled *S. faecium*.

The problem of species identification of the enterococci has been recognized by many workers, including Shattock (1955), Bartley and Slanetz (1960), Papavassiliou (1962), Medrek and Barnes (1962), and Deibel, Lake, and Niven (1963). In the present study, 18 to 37% of the isolates from rat cecal contents were designated atypical because they differed from recognized species in the ability to ferment certain sugars (Table 4). The largest number of atypical strains resembled *S. durans* or *S. faecium;* this illustrates the difficulties encountered in the classification of these species. Since *S. durans* is differentiated from *S. faecium* only by the inability to ferment L(+)-arabinose and mannitol, Medrek and Barnes (1962) concluded that the relationship between these species can be clarified only when more information is available on the serology and physiology of a large number of isolates from different sources. Recently, it has been proposed that *S. durans* be considered a variety of *S. faecium* (Deibel et al., 1963).

Cooper and Ramadan (1955) attempted to differentiate between fecal streptococci of human and of animal origin on the basis of resistance to heat and subsequent ability to grow in the presence of potassium tellurite. Only strains that survived both treatments were considered of human origin. The failure of the enterococcus isolates from rats to survive the heat-tellurite tolerance test would appear to support their conclusion.

The presence of fecal streptococci in the intestinal tract within 24 to 48 hr after birth was reported for chickens (Shapiro and Sarles, 1949), for young pigs (Smith and Crabb, 1961; Pesti, 1963), and for certain other mammalian species, e.g., calves, lambs, human babies, and a rabbit (Smith and Crabb, 1961). The present findings with rats are in agreement with these studies, in that enterococci were sometimes detected within 10 to 12 hr after birth. In recent studies by Dubos, Schaedler, and Costello (1963) on a strain of mice (NCS) with an intestinal flora containing large numbers of lactobacilli and *Bacteroides* spp., but free from coliforms, lactobacilli were present in the gut shortly after birth, reached a high level in 4 to 7 days, and remained in large numbers thereafter. In contrast, enterococci were detected somewhat later, reached a maximum after 2 weeks, and then decreased. The present findings suggest that the occurrence of enterococci in the intestinal tract of the rat in the first 7 days after birth arises primarily from chance contamination, and that establishment of the bacteria probably does not take place until the animals have consumed solid food. This appeared to occur about 2 weeks after birth, when the young animals opened their eyes and began to move about freely.

Chen, Rogers, and Harper (1962) reported that the consumption by rats of a diet in which nitrogen was provided by crystalline amino acids was associated with a depression of food intake, an increase in the amount of moisture in the stomach contents, and an alteration in the stomach-emptying pattern, compared with animals fed a diet with casein as the nitrogen source. The present findings suggest that these changes may affect the intestinal microflora. Among intestinal isolates from rats fed casein, *S. faecalis* var. *zymogenes* predominated, but was largely replaced by strains which resembled *S. durans* or *S. faecium* in rats fed mixtures of L-amino acids.

The similarity in essential amino acid requirements shown by different isolates of enterococci suggests that a homogeneous pattern of metabolism may exist among members of this group. The data presented are consistent with those of Horie (1959), who found a common pattern of amino acid requirements among 14 nonhemolytic strains of enterococci, and of Ford (1960), who worked with a strain of *S. faecalis* var. *zymogenes*. That the 19 enterococci examined here had a common requirement for six of the nine amino acids essential for the rat implies that they may compete with the host for these nutrients.

With the advent of techniques for rearing experimental animals in a germ-free environment, methods are becoming available for studying in greater detail the effects of specific microor-

ganisms on the nutrition and metabolism of the host (Levenson and Tennant, 1963). Such an approach may contribute to a further understanding of the enterococcus flora in the intestinal tract of the rat.

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