veronal-acetate buffers were similar to those previously obtained. In the range tested, from about pH 4.0 to pH 6.0, physiologic saline had an effect similar to that noted with veronal-acetate buffer solutions. On the other hand, the survival of virus in distilled water over the same range was apparently greater. Material diluted to 10^{-6} after exposure for 24 hours in a 10^{-4} dilution at about pH 4.0 was lethal for mice. After exposure for one week virus had survived in the 10^{-4} dilution close to pH 4.0 (Table III).

Summary. In various buffers, MM virus was stable at room temperature only at neutral or alkaline reactions. When diluted virus was exposed at approximately pH 1.0 to neutrality the infectivity was diminished within twenty-four hours. There was a slower decrease in virus titer at pH 9 to 10. At pH 7 to 9, the virus was found to be stable for more than seven days. The deleterious effect of unfavorable pH was less for concentrated virus suspensions. MM virus was apparently more stable in distilled water acidified with hydrochloric or acetic acid than in acidified physiologic saline or in veronal-acetate buffer solutions of approximately the same pH value.

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Experimental Enterococcal Food Poisoning in Man.

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Several outbreaks of food poisoning of a mild type in which enterococci were the predominant organisms isolated from the suspected foods, are described in a previous paper.[1] The literature on food poisoning caused by non-hemolytic streptococci is also discussed and it is pointed out that, in several instances in which the inculpated organisms were closely identified, they were found to be enterococci.

The relatively small number of outbreaks reported as caused by *Streptococcus faecalis* is in sharp contrast to the widespread occurrence of this organism in nature.[1] Accordingly the studies reported in this paper were initiated in an attempt to determine whether *Streptococcus faecalis* can act as a food poisoning agent. They consisted of feeding experiments with *Streptococcus faecalis* employing kittens and human volunteers.

The literature contains the following references to experimental streptococcal food poisoning in man. Cary, Dack, and Meyers[2] reported that a volunteer who had eaten parts of several sausages similar to others which had been implicated in an outbreak of mild gastroenteritis, developed nausea which increased for 24 hours, with two periods of vomiting and epigastric pain, followed by marked exhaustion and constipation. A green "pleomorphic" streptococcus whose species designation is not determinable from the description given was isolated from the sausage. Later Cary, Dack, and Davison[3] reported abdominal distress, cramps, and diarrhea, in 5 of 7 volunteers who ingested "reasonable" doses of several strains of alpha streptococci which had been implicated in 2 outbreaks of food poisoning. On the other hand, Dolman[4] reported failure to produce food poisoning in any of 4 volunteers each of whom had eaten a meat pie heavily infected with *Streptococcus "viridans"* which had been isolated from

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[2] Cary, W. E., Dack, G. M., and Meyers, E., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 214.

[3] Cary, W. E., Dack, G. M., and Davison, E., *J. Inf. Dis.*, 1938, **62**, 88.

[4] Dolman, C. E., *Canad. J. P. H.*, 1943, **34**, 97.

rissoles implicated in a food poisoning outbreak.

Some success in producing experimental *Streptococcus faecalis* food poisoning in man is reported in the present paper.

Experimental. Studies with Kittens. Studies were carried out with 70 kittens in which evaporated milk containing 20-hour cultures (test animals) or evaporated milk alone (control animals) was fed. This line of investigation was found to be unsatisfactory and abandoned because it was noted that the controls occasionally developed diarrhea.

Studies with Human Subjects. All persons who served as subjects for these studies had undergone physical examination and gave no history of recent gastro-intestinal disturbance. Volunteers were given food which had been experimentally contaminated with *Streptococcus faecalis.*

Feeding with 20-hour Cultures. Attempts to produce experimental gastroenteritis with 20-hour cultures of *Streptococcus faecalis* were not fruitful. Twenty-one volunteers were fed 2.5 to 50 ml of 20-hour cultures of this streptococcus grown in milk or infusion broth. In no instance was a definite food poisoning syndrome provoked although 4 subjects complained of slight nausea which persisted in one individual for 24 hours. There were no such complaints among 11 volunteers who drank the vehicle without organisms. The reason for the failure of these 20-hour cultures to produce symptoms is not apparent.

Feeding with 5-hour Cultures. Four strains of *Streptococcus faecalis* were used. Three of these (A4, B3, JA2) had been isolated from human feces within 2 months of their use; the fourth, A34.1, had been isolated about one year previously from a can of evaporated milk implicated in an outbreak of gastroenteritis.

Three different foods were used; they were egg salad, custard, and sterile milk. Each food was prepared in average sized individual portions. The portions were inoculated with 5.0 ml of a 20-hour infusion broth culture, incubated at 37°C for 5 hours and then served. The egg salad portion consisted of the amount needed for one sandwich, the custard portion of one cup, and the milk of 50 ml which was diluted with sterile milk to make up one glassful. Foods used for control purposes were prepared and incubated in the same manner as were the test foods except that no cultures were added to them.

All feedings were given shortly before noon. There was no marked difference in taste between the treated and untreated foods and the volunteers were unaware of the status of the food which each consumed. The volunteers limited their subsequent luncheon to lettuce and tomato sandwiches, tea or black coffee, and fruit.

When symptoms occurred after the experimental feeding the diagnosis was made by a physician who did not know the test status of the individual concerned.

Results and Discussion. The findings are recorded in Table I. It is noted that 6 of the subjects who ingested food contaminated with cultures of *Streptococcus faecalis* had definite symptoms of food poisoning, while a seventh had questionable ones. Nineteen volunteers who ate the same contaminated foods developed no symptoms, while 18 others who received the same foods but without addition of the specific bacteria, likewise, remained negative. Only 2 of the 4 strains used provoked symptoms of food poisoning. One of these was a recently isolated fecal strain and the other had been recovered about one year previously from food implicated in an outbreak. Thus 6, possibly 7, of 17 persons who ingested these strains became ill. Some of those who showed no symptoms ingested larger doses than others who did become ill. The production of symptoms in only a fraction of the subjects is paralleled by somewhat similar findings in some naturally occurring outbreaks of food poisoning apparently induced by *Streptococcus faecalis.*[1] The 2 strains which failed to produce symptoms were tested on a total of 9 persons.

The use of 5-hour cultures in this experiment was based on observations of the growth rates of these organisms in broth culture which indicated that good growth had occurred in that time and also, perhaps more

TABLE I.

Food Poisoning in Human Volunteers.

Ingestion of Milk, Custard, and Egg Salad Inoculated with *Streptococcus faecalis* and Incubated at 37°C for 5 hr.

	Cultures	Quantity of food	No. of organisms per g ($\times 10^6$)	Test			Control (food without culture)	
				No. of persons fed	No. of persons ill	Case	No. of persons fed	No. of persons ill
Milk	A4, A34.1, B3	50 ml	454-990	3	1	A	2	0
Custard	A4, A34.1, B3, JA2	92-94 g	168-1,050	12	2	B, C	8	0
Egg Salad	A4, A34.1, B3, JA2	41-87 g	740-2,100	11	4 (1?)	D, E, F, G(?)	11	0

	Case	Culture	Dose	Symptoms
Milk	A	A4	22.7×10^9	Nausea and regurgitation which began after 2½ hours and lasted for 24 hours. Slight diarrhea after 9 hours.
Custard	B	A34.1	45×10^9	Nausea, diarrhea and repeated vomiting which began 2 hours after feeding and persisted for 32 hours.
	C	A34.1	79.2×10^9	Nausea, eruction and mild diarrhea. Symptoms began after 2 hours and persisted for 37 hours.
Egg Salad	D	A4	35.7×10^9	Nausea and vomiting, dizziness and abdominal cramps which began after 2 hours and lasted for 30 hours.
	E	A34.1	107×10^9	Nausea, abdominal cramps and one bout of vomiting which began after 10 hours and lasted for 2 hours.
	F	A4	148×10^9	Diarrhea after 8 hours.
	G (?)	A34.1	99.6×10^9	Nausea which began after 2 hours and lasted for 12 hours.

significantly, on the knowledge that incubation periods of such duration of suspected foods are frequently found in food poisoning outbreaks. It was found that 10 to 25 fold increases of initially large inoculums had taken place in the vehicle foods prior to ingestion.

It is of interest that the number of organisms which were found to produce experimental symptoms in this study is of the same order as that which Kelly and Dack[5] found able to produce symptoms of gastroenteritis when a strain of *Staphylococcus aureus* was fed. These authors produced illness in one volunteer with 48×10^9 organisms and in another with 69.3×10^9. In the present study the symptom producing dose of *Streptococcus faecalis* ranged from 22.7×10^9 to 148×10^9 organisms. This comparison is noteworthy since staphylococcal food poisoning is believed to be caused by an "enterotoxin" while Sherman, Gunsalus, and Bellamy[6] suggest the possibility that the toxic principle of *Streptococcus faecalis* poisoning may be tyramine formed by the decarboxylation of tyrosine by this organism. Also perhaps worthy of comment is the fact that like staphylococcal food poisoning, streptococcal food poisoning usually has a short incubation period.

Summary. Symptoms of acute gastric or intestinal disturbance or of both were produced in 6, or possibly 7, of 26 human volunteers who ate foods in which strains of *Streptococcus faecalis* had grown for 5 hours.

Attempts to produce similar symptoms in man with 20-hour cultures were unsuccessful. Kittens were found to be unsatisfactory test animals in feeding experiments with inoculated condensed milk.

Conclusion. The experimental production of acute mild intestinal or gastric disturbance or both in man, tends to confirm the etiological role of *Streptococcus faecalis* in naturally occurring outbreaks of food poisoning.

We are indebted to Drs. William Birnkrant and Morris Greenberg of the Bureau of Preventable Diseases who conducted the physical examinations and diagnosed the illnesses which occurred.

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Distribution and Nature of the "Antigen" Responsible for Experimental Meningoencephalomyelitis in the Guinea Pig.*

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Previous work has shown that a meningoencephalomyelitis, sometimes with foci of demyelinization, may occur in monkeys, guinea pigs, and rabbits 2 to 9 weeks following the subcutaneous or intramuscular injection of brain tissue mixed with acid-fast bacilli in a water-in-oil emulsion.[1-6] In con-

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