TABLE I. Alkaline Phosphatase in Pancreatic Tissue and Pancreatic Juice of Various Species.

Species	Reaction of intralobular duct to stain for alkaline phosphatase	Pancreatic juice	
		No. of specimens	Alkaline phosphatase (units/100 cc)
Dog	+	124	.4 to 41
Human	+	2	2.1 to 3.8
Rabbit	+	8	2.6 to 4.4
Guinea pig	0	3	0
Cat	0	10	0

phosphatase is demonstrable histochemically in the intralobular ducts and centro-acinar cells of the pancreatic tissue and is present in the pancreatic juice. In the cat and guinea pig this enzyme is present neither in the intralobular ducts and centro-acinar cells nor in the pancreatic juice.

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Development of Resistance to 4-Amino-N^{10}-Methyl Pteroylglutamic Acid (Amethopterin) by *Streptococcus faecalis*.* (19058)

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In children with acute leukemia, whose disease initially responds well to treatment with 4-amino-N^{10}-methyl pteroylglutamic acid (amethopterin), there develops in the leukemic process, after 6 to 18 months, refractoriness to therapy(1-4). Prevention of this development of resistance would be of the greatest clinical importance, since in the absence of resistance these children could be treated again and again and the disease suppressed indefinitely. The development of resistance to amethopterin in a previously sensitive strain of mouse leukemia has been reported by us(5), and was later repeated in another strain of leukemia by Law and coworkers(6). With these two strains of amethopterin-fast leukemias, studies are in progress on the mechanisms of resistance in the hopes of finding technics for preventing its development. In order to provide another system for the study of resistance to amethopterin, attempts were made to develop an amethopterin-fast strain of *Streptococcus faecalis*, an organism ordinarily requiring folic acid, and very sensitive to the inhibitory action of amethopterin.

Method. Streptococcus faecalis (ATCC No. 8043) was grown on Difco folic acid assay broth in the presence of 5 mγ/ml of pteroyl-

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glutamic acid (PGA), and then inoculated into similar broth containing the same amount of PGA, and concentrations of amethopterin varying from 1 to 128 mγ/ml. In 24 hours there was visible growth only in the tubes containing 2 and 4 mγ/ml, but after 48 hours there was slight growth in the tube containing 32 mγ/ml. No growth occurred, however, after 5 days in the tubes containing 64 or 128 mγ/ml. The original strain was then inoculated into 4 pour plates containing 5 mγ/ml of PGA and 0, 10, 20, and 80 mγ/ml respectively of amethopterin. In 24 hours, there was heavy growth only in the plate containing no amethopterin, but after 96 hours one colony each appeared on the 10 and 20 mγ/ml plates, but none on the 80 mγ/ml plate. The colony from the 20 mγ/ml plate was inoculated into broth containing PGA and various concentrations of amethopterin. Twenty-four hours later, good growth was seen at 8, 16, and 32, but not at 64 mγ/ml. An inoculum from the highest concentration allowing growth was then transferred into pour plates containing increasing amounts of amethopterin. Colonies were picked after 72 to 120 hours from the plate containing the highest concentration of antimetabolite and inoculated into amethopterin containing liquid media. This alternation between liquid and solid media was continued for 16 transfers, after which time only liquid media was used. Media used in the first 6 transfers contained 5 mγ/ml of PGA. After that time 10 mγ/ml was used.

Studies on the ability of the resistant strain to utilize certain antimetabolites were done in Difco folic acid assay liquid media without the addition of PGA.

Results. As can be seen from Fig. 1, resistance to amethopterin appeared gradually in a stepwise fashion. On the 6th transfer, a few colonies grew on the plate containing 5 mγ/ml PGA, and 1,000 mγ/ml of amethopterin. By the 14th transfer, a few colonies grew out in the plate containing 20,000 mγ/ml. After a total of 28 transfers, good growth occurred in 20-24 hours in liquid media containing 10 mγ/ml PGA, and 40,000 mγ/ml amethopterin. It is now being maintained in media containing 50 γ/ml amethopterin.

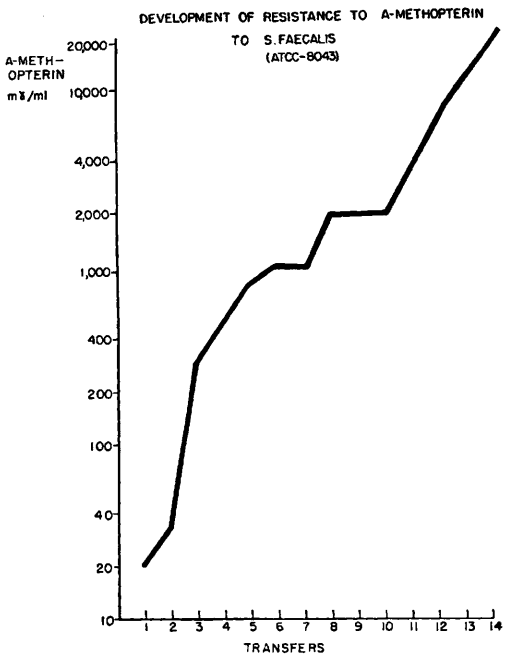

FIG. 1.

No morphologic differences between the sensitive and resistant variants could be detected in Gram stained preparations. The resistant organism had a smaller quantitative requirement of PGA (0.1 mγ/ml) for half maximum growth in the absence of amethopterin than did the parent sensitive strain (0.3-0.5 mγ/ml). In the presence of 1 to 25,000 mγ/ml of amethopterin, however, the resistant strain grew without PGA. 4-amino-PGA (aminopterin) and 4-amino-pteroylaspartic acid (amino-an-fol) could also be utilized by this organism to supply its PGA requirements.

Discussion. The development of resistance to amethopterin by *Streptococcus faecalis* is more analogous to the resistance to penicillin (7,8), chloromycetin(9), aureomycin(8,10),

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and terramycin(11-13), than to streptomycin (14), in that it occurs gradually in a stepwise fashion, and in that there has been so far no demonstration of an amethopterin-dependent strain of this organism.

This resistance is presumed to be due to a random mutation selected out by the presence of the drug as previously demonstrated for *Staphylococcus aureus* with both penicillin and streptomycin(7,14).

The fact that the resistant bacterial cell is able to utilize 4-amino PGA and 4-aminopteroyl aspartic acid, as well as amethopterin, to supply its PGA requirement is of considerable interest. The mechanisms by which this resistance and this ability to utilize amethopterin are achieved, are under study at the present time.

Summary. (1) By growing *Streptococcus faecalis* (ATCC No. 8043) in a medium containing successively higher concentrations of amethopterin, more than a thousand-fold increase in resistance has been achieved. (2) This amethopterin-fast variant has a slightly lower requirement for folic acid in the absence of amethopterin than the parent sensitive strain. In the absence of folic acid, however, amethopterin and certain other 4-amino derivatives of PGA can replace this folic acid requirement in the resistant strain.

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Evaluation of Some Iodine-Containing Organic Compounds as X-Ray Contrast Media. (19059)

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Organic compounds containing iodine have been useful aids in radiographic diagnoses. We have previously studied the physiological behavior of one of these, Urokon(1), which has proven of value in the visualization of the pelves and ureters of the kidneys of man(2). Five analogues of Urokon and a number of other iodine-containing compounds have now been investigated. Some of these which are of experimental and potential clinical interest are listed in Table I. Two approaches to the determination of their value have been made, radiographic study of the compounds after intravenous or oral administration (dogs) and analyses of the iodine content of kidney, liver, and blood after intravenous administration (rats).

Roentgenographic studies. Dogs 5 to 9 kg in weight were maintained in the laboratory over several months, so that the same animals might be used in a comparative series of investigations with various potential cholecystographic agents. A portable Picker x-ray unit was employed with instrumental settings at 50 kilovolts and 84 milliamperes and exposures of 1 to 1.75 sec, depending on the size of the animal. Roentgenograms were taken at intervals as shown in Table II. Under these conditions, it was possible to obtain clear visualization of the gall bladder and biliary ducts. For intravenous injection, 10% solutions (pH 7-9) of the sodium salts (sodium carbonate as neutralizing agent) of the compounds studied were used. After a 20 hr fast, the dog was fed the yolk of a hard boiled egg, 4 hr preceding the injection of

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