heated under reflux for 1 hr. The solvent was then removed under reduced pressure, and the solid was recrystallized from ethanol to give 0.84 g. (81% from I) of nicotinoyl glycine hydrazide, m.p. 177–180°. Recrystallization gave an analytical sample from ethanol, m.p. 179–180° [lit. (2) m.p. 178.5°]; $\nu_{max.}^{mineral\ oil}$ 3300, 1640, 1600, and 1550 cm.$^{-1}$.

Anal.—Calc. for $C_8H_{10}N_4O_2$: C, 49.50; H, 5.14. Found: C, 49.69; H, 5.39.

Nicotinoyl Glycine (IV)—Compound I (1.14 g., 0.005 mole) and methyl glycinate hydrochloride (0.63 g., 0.005 mole) were suspended in 50 ml. of dry benzene, the mixture was heated under reflux for 20 hr., and any undissolved material was separated from the hot solution. The filtrate was evaporated under reduced pressure, and the residue was treated with 6 ml. of 1 *N* NaOH. After acidification to pH 2 with 2 *N* HCl, followed by evaporation to dryness under reduced pressure, the residual solid was recrystallized from water to give 0.69 g. of IV, m.p. 245–246° (3). This weight represented a yield of 75% from I.

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COMMUNICATIONS

A New Antibacterial Mode of Action for Sulfonamides

Keyphrases ☐ Sulfonamides—antibacterial mechanism ☐ Antibacterial agents—sulfonamides, mode of action ☐ Streptococci, oral—mechanism of antibacterial sulfonamides ☐ Sulfadiazine-containing agar medium—growth of oral streptococci

Sir:

The several species of common human oral streptococci differ in their use of the sucrose molecule. *Streptococcus salivarius* forms a levan-type extracellular polysaccharide from the fructose portion of sucrose, *Streptococcus mutans* forms a dextran-type extracellular polysaccharide from the glucose portion of sucrose, and *Streptococcus mitis* is incapable of forming any extracellular polysaccharide from sucrose. Of these streptococci, only *Strep. mutans* is resistant to the inhibitory effect of sulfonamides. *Streptococcus faecalis*, which is found transitorily in the mouth, is also resistant to sulfonamides but is incapable of forming any extracellular polysaccharide from sucrose.

During a study on the metabolic relationships of the oral streptococci, we grew the organisms on an agar medium composed of: 1.5% trypticase peptone[1], 0.5% NaCl, 0.2% sulfadiazine[2], 1% total sugar(s), 0.002% phenol red, and 0.8% Oxoid Ionagar No. 2[3]. The sugars used as energy sources were: sucrose alone, glucose alone, fructose alone, and an equimolar mixture of glucose and fructose. The inoculated plates were observed after 24 hr. of incubation at 37°. The results are shown in Table I.

Surprisingly, the sulfadiazine-sensitive streptococci *Strep. salivarius* and *Strep. mitis*, were able to grow on the sulfadiazine-containing agar medium supplemented with fructose. Subsequently, a wider variety of bacteria was inoculated into liquid media containing: 1.5% trypticase peptone, 0.5% NaCl, 0.2% sulfadiazine, 1% methionine, either 1% glucose or 1% fructose, and 0.002% phenol red. The inoculated tubes were observed after 24 hr. of incubation at 37° (or at room temperature for *Leuconostoc*). The results are shown in Table II.

From the data in Tables I and II, two basic observations were made concerning the growth of the organisms on the sulfadiazine-containing media: (*a*) all the sulfadiazine-resistant bacteria typically grew and formed acid from fructose and from glucose; and (*b*) with the exception of two species, all the sulfadiazine-sensitive bacteria typically grew and formed acid from fructose but did not grow in the presence of glucose. Each of the typical organisms, whether resistant or sensitive to

Table I—Growth of *Strep. faecalis* and Common Human Oral Streptococci on Sulfadiazine-Containing Agar Medium Supplemented with Sucrose or Glucose and/or Fructose

	Energy Source			
Organism[a]	Sucrose	Glucose	Fructose	Glucose and Fructose
Strep. faecalis F24	+[b]	+	+	+
Strep. mutans BHT	+	+	+	+
Strep. salivarius HHT[c]	+	−	+	+
Strep. mitis 903[c]	−	−	+	+

[a] Obtained from Dr. Allan L. Delisle, Department of Microbiology, School of Dentistry, University of Maryland. [b] + = growth *with acidity*; − = no growth. [c] Sensitive to sulfonamides.

[1] BBL (Baltimore Biological Laboratories), Cockeysville, Md.
[2] Eli Lilly and Co., Indianapolis, Ind.
[3] Colab Laboratories, Inc., Glenwood, Ill.

Table II—Growth of Various Bacteria in Sulfadiazine-Containing Broth Medium Supplemented with Either Glucose or Fructose

Organism[a]	Energy Source: Glucose	Energy Source: Fructose
Staphylococcus aureus	+[b]	+
Streptococcus bovis 9809	+	+
Escherichia coli	+	+
Klebsiella pneumoniae	+	+
Salmonella typhi	+	+
Proteus vulgaris[c]	+	/+/
Leuconostoc mesenteroides C 33[c]	+	−
Staphylococcus epidermidis[c]	−	+
Micrococcus lysodeikticus[c]	−	+
Lactobacillus casei 4646[c]	−	+
Corynebacterium diphtheriae[c]	−	+

[a] *Streptococcus bovis* 9809, *Leuconostoc mesenteroides* C 33, and *Lactobacillus casei* 4646 were obtained from Dr. Allan L. Delisle; all other organisms were obtained from the culture collection maintained by the Department of Microbiology, School of Dentistry, University of Maryland. [b] + = growth *with acidity;* /+/ = growth without acidity; − = no growth. [c] Sensitive to sulfonamides.

sulfadiazine, possesses *all* of the enzymes of the glycolytic pathway. The two exceptions, however, differ in these ways: (*a*) the organism *Proteus* does not obtain energy by means of the glycolytic pathway but uses the pentose phosphate pathway; and (*b*) the organism *Leuconostoc* obtains its energy from the heterolactic pathway. *Proteus*, therefore, uses glucose as a starting point for the production of energy by means of the pentose phosphate pathway—fructose is used only as an intermediate in the molecular interconversions in the pentose phosphate pathway. And *Leuconostoc* uses glucose as a starting point for the production of energy by means of the heterolactic pathway; fructose is not used (Table II).

The trypticase peptone used in the preparation of the media contained high levels of both methionine (2.4%) and *p*-aminobenzoic acid (0.21 mcg./g.) (1). An even higher level of methionine was added to the medium used in the second experiment (represented by Table II), thereby minimizing the effect of the sulfa drug on the folic acid cycle. Since the typical sulfa-sensitive bacteria were able to grow on the sulfa-containing media supplemented with fructose but were unable to grow on the sulfa-containing media supplemented with glucose, it is evident that sulfadiazine exerted a second and major inhibitory action at the level of glycolysis. Interestingly, in 1937, Barron and Jacobs (2) found that sulfanilamide, at a concentration of 0.2%, slightly inhibited the oxidation of glucose by hemolytic streptococci and by *Klebsiella pneumoniae*. And, in 1938, Chu and Hastings (3) reported that sulfanilamide, at concentrations of 0.660 g. %, invariably reduced oxygen consumption for both bacteria (β-hemolytic streptococci, gonococci, meningococci, and types I and II pneumococci) and mammalian cells (obtained from rat liver, diaphragm, and blood). All of these were glucose-containing systems.

This second inhibitory action of sulfonamides does not appear to be directed against the requisite phosphorylation of hexoses, because all of the sugar substrates added to our media were nonphosphorylated. Rather, the inhibition appears to occur at the point where glucose-6-phosphate is isomerized to fructose-6-phosphate, since the glycolytically competent, sulfadiazine-sensitive bacteria were able to grow in the presence of fructose but not glucose. (However, when an organism possesses a phosphogluconate oxidative pathway, glucose is used in preference to fructose, unless fructose is an intermediate of the pathway.) Additional support for this thesis is derived from our finding that, of all the bacteria named in Tables I and II, *only* the sulfa-resistant organisms were able to use mannitol when it was present as the sole carbohydrate in sulfadiazine-containing media. Mannitol, as well as sorbitol, is converted to fructose in the process of metabolism; hexoses other than mannitol and sorbitol are converted to glucose before utilization.

Further proof of this inhibitory mechanism of action of sulfanomides is dependent upon the use of an *in vitro*, cell-free system. Such a system has already been tested with promising results and will be the topic of a subsequent communication.

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Biliary Excretion of Riboflavin in Man

Keyphrases □ Biliary excretion—riboflavin absorption in man □ Riboflavin, absorption—biliary excretion, man

Sir:

The time course of urinary excretion of riboflavin after oral administration of high doses of riboflavin-5′-phosphate to normal human subjects frequently shows two excretion rate maxima, with the second maximum usually following a meal (1). This finding and various observations in animals (reviewed in *Reference 1*) suggested that riboflavin may be subject to enterohepatic cycling in man. Subsequently, it was found that oral administration of bile salts enhances the absorption of riboflavin in normal subjects (2) while the absence of bile due to biliary obstruction decreases riboflavin absorption in children (3). Since bile flow into the human intestine is intermittent and is stimulated by food (4, 5), the possibility had to be considered that the secondary excretion maximum of riboflavin may be due to bile-mediated enhanced absorption of riboflavin rather than to enterohepatic cycling.