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.

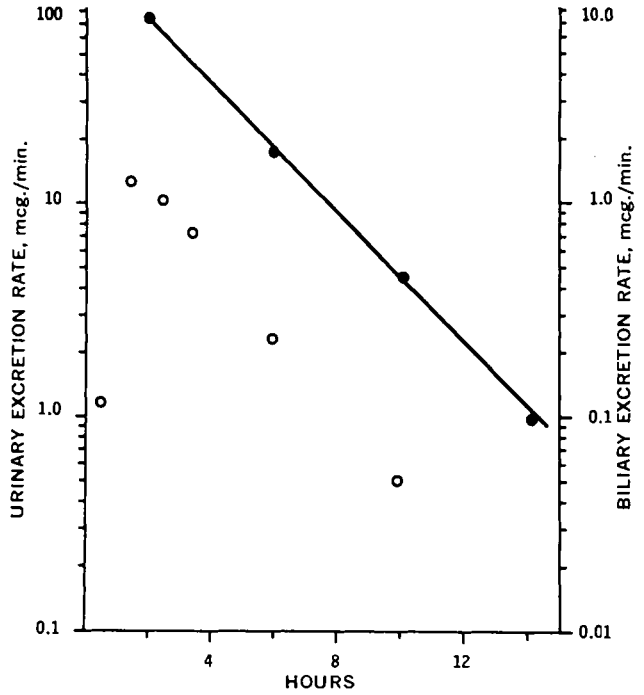

Figure 1—*Rates of urinary (●) and biliary (○) excretion of riboflavin by an 11-year-old girl after intramuscular injection of 17.5 mg./m.² riboflavin as the phosphate.*

We recently sampled the bile of a 3.5-month-old boy with refractory diarrhea who received only parenteral alimentation; the diarrhea was under control at the time of the study. Duodenal fluid was obtained by a continuous siphon through a Levin tube placed in the duodenum. Hourly collections started 1 hr. before and extended to 4 hr. after intramuscular injection of 10 mg. riboflavin as the phosphate. Gallbladder contraction was stimulated by the hourly administration of corn oil. Analysis of these samples (6) yielded a total of only 0.042 mg. riboflavin, while 9.54 mg. was recovered in the urine over 36 hr. Since the intestinal intubation procedure does not assure complete collection of bile and since the patient was quite young, we could not conclude with certainty that biliary excretion of riboflavin is negligible.

However, a subsequent study was carried out in an 11-year-old girl with complete obstruction of the common bile duct who had external biliary drainage through a temporary common bile duct cannulation. This patient was also receiving only parenteral alimentation at the time of the study. She was given 17.5 mg. riboflavin as the phosphate per m.² body surface area intramuscularly, urine and bile being collected completely at intervals from 2 hr. before to 24 hr. after injection. Preinjection excretion rates of apparent riboflavin by fluorometric assay (6) were 0.54 and 0.049 mcg./min. for urine and bile, respectively. Total recovery of injected riboflavin was 88.7% of the dose from the urine and only 0.97% from the bile. Known amounts of riboflavin added to bile obtained before injection of the vitamin were recovered quantitatively.

The time courses of urinary and biliary excretion of riboflavin by the girl are shown in Fig. 1. The kinetics of urinary excretion are similar to those of normal children given the same dose of the vitamin by the same route (data to be published). Urinary and biliary excretion rates of riboflavin were essentially parallel.

It appears that biliary excretion of riboflavin is negligible in man and that the secondary excretion rate maxima observed in man after large oral doses of the vitamin are due to further absorption of riboflavin. This is probably related to the discharge of bile into the intestine, but the mechanism of the apparent absorption-enhancing effect of bile is still uncertain (3). Future studies on patients whose bile is completely diverted from the intestine, and who are given riboflavin orally with and without bile, may establish more definitively the role of bile in riboflavin absorption.

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Biological and Chemical Evaluation of a 43-Year-Old Sample of *Cannabis* Fluidextract

Keyphrases □ *Cannabis* fluidextract—stability of 43-year-old sample □ Cannabinoids—presence, activity in 43-year-old *Cannabis* fluidextract

Sir:

The instability of *Cannabis sativa* L. preparations has been generally accepted as fact because of numerous observations of loss in biological activity with time.