L-arginine is an efficient means of detoxifying ammonium acetate; whereas the present study suggests that arginine is not adequately effective with INH. Possibly amino acids may counteract the inhibitory effect of INH on such enzymes as diphosphopyridinenuclease, or on the diphosphopyridine nucleotide itself.

One clinical report has indicated that use of simultaneous glycine and sodium glucuronate with INH did not control peripheral neuritis, but in 2 tuberculous patients markedly reduced frequency of convulsive seizures induced by the prolonged INH therapy and thus permitted uninterrupted treatment(8). Since L-cysteine-HCl may be effective due to a different mechanism than glycine and sodium glucuronate, it is conceivable that its use might benefit those INH-treated patients in whom toxic reactions are not counteracted by glycine and sodium glucuronate.

Summary and conclusions. Twenty-six amino acids have been tested for their ability to reduce the toxic effect of isoniazid (INH) in mice. Eleven of these—L-cysteine-HCl, L-glutamic acid-HCl, L-histidine-HCl, L-aspartic acid, hydroxy-L-proline, L-ornithine-diHCl, acetyl-DL-methionine, L-arginine-HCl, DL-aspartic acid, L-serine, and DL-ornithine-HCl—in appropriate concentration (a 5:1 molar ratio) permitted at least 50% survival of DBA mice given an 8 mg lethal dose of INH per 20-g mouse (400 mg/kg). Optimal results were obtained with single or repeated administration of 58 mg of L-cysteine-HCl (2900 mg/kg) with 8 mg INH. This dosage yielded a maximum blood plasma level of 7.8 mg% 1 hour after administration, as contrasted with 6.5 mg% following a 5 mg dose of INH alone. No interference by 4 amino acids, L-cysteine-HCl, L-glutamic acid-HCl, DL-serine, or acetyl-DL-methionine, was noted in the INH bacteriostatic test on one strain of avirulent *Mycobacterium* B103. From this study it would appear that L-cysteine-HCl, and perhaps other amino acids, are as effective as glycine and sodium glucuronate, previously reported, in increasing the tolerated dose of INH in DBA mice.

1. Prescott, B., Kauffmann, G., and James, W. D., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 682.
2. ———, *ibid.*, 1957, v94, 94.
3. ———, *ibid.*, 1957, v94, 272.
4. ———, *ibid.*, 1953, v84, 704.
5. (a) Martin, G. J., Thompson, M. R., and Accousti, N. J., *Am. J. Hyg.*, 1941, v34, 23.
(b) Martin, G. J., Fisher, C. V., and Thompson, M. R., *Arch. Int. Med.*, 1942, v69, 662.
6. Ragno, I., Preziosi, P., and Porcellati, G., *Riforma med. (Naples)*, 1953, v67, 1022; *abstr. J.A.M.A.*, 1954, v154, 95.
7. Greenstein, J. P., Winitz, M., Gullino, P., Birnbaum, S. M., and Otey, M. C., *Arch. Biochem. Biophys.*, 1956, v64, 342.
8. Katz, S, Gimble, A., McCormick, G., and Prescott, B., *Annal. Int. Med.*, 1956, v44, 576.

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Bile Constituents of the Opossum *Didelphys marsupialis virginiana.** (23331)

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In the study of gallstone disease the usual laboratory animal is not satisfactory because of low concentration of cholesterol in bile of the animals (Table I). It would be advantageous to find an animal which excretes a large amount of cholesterol in the bile and in high concentration. Wibler(6) reported significantly high cholesterol content in opossum gallbladder bile. This report is based on the several constituents of the gallbladder and hepatic bile of the opossum. Bile acids in opossum gallbladder bile were first studied by Haslewood and Wootton(7), and cholic acid

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TABLE I. Cholesterol Content of Gallbladder Bile* of Different Species.

Species	Cholesterol conc.	Literature
Human	623	Isaksson(1)
Ox	29 (57)†	" (2)
Dog	120 (79)†	" (2)
Hog	100–350 (165)†	Doyon and Dufourt(3)
Rabbit	100–120 (70)†	Parhon and Werner(4)
Rat‡	13.5	Byers and Friedman(5)

* Variable on account of concentrating function of gallbladder.
† ( ) are authors'.
‡ Hepatic.

was found as its major bile acid. In the present study, chenodeoxycholic acid was identified as another major bile acid beside cholic acid by paper chromatography according to Sjövall(3). It was found that the bile acids in opossum bile are conjugated with taurine. No glycine conjugated bile acid was found.

Methods. The opossum† was anaesthetized with ether and laparotomy was performed. Gallbladder bile was aspirated by syringe before cholecystectomy was done. The small size polyethylene tube was inserted in the common duct and the bile drained continuously for 24 to 48 hours. Cholesterol in bile was determined according to the method of Foldes (9), and Foldes and Wilson(10). Cholic acid was determined by the method of Irvin, Johnston and Kopola(11). Chenodeoxycholic acid was determined according to the method of Isaksson(12). Deoxycholic acid content was determined according to Szalkowski and Mader(13) from Isaksson's hydrolyzed extracts of the bile mentioned above. The content of phospholipids was obtained by multiplying the value of organic phosphorus by 25. Organic phosphorus was determined by the method of King(14). For extraction preparatory to the phosphorous determination, a mixture of 3 parts ether and one part ethanol was used. Bile salts used for paper chromatography were extracted and purified according to the method of Haslewood and Wootton (7). Unhydrolyzed and hydrolyzed bile acid extracts were subjected to paper chromatography according to Sjövall(8). Bile acids on the paper chromatogram were located with the use of antimony trichloride reagent under an ultraviolet lamp according to the method of Carey and Bloch(15). Identical samples of several bile acids used in paper chromatography were prepared as follows. Cholic acid (Matheson-Coleman) was used after one recrystallization from ethanol (m.p. 198°). Because of the difficulty of obtaining the pure deoxycholic acid, chenodeoxycholic acid, glycocholic acid and taurocholic acid, they were synthesized. Deoxycholic acid was synthesized from cholic acid by the method of Fieser and Rajagopalan(16) (m.p. 172°). Chenodeoxycholic acid was synthesized from cholic acid according to the method of Fieser and Rajagopalan(17) (m.p. 142°). Glycocholic acid and taurocholic acid were synthesized by the method of Cortese and Bauman (18).

TABLE II. Cholesterol, Bile Acids and Phospholipids Content of Opossum Bile.

	Cholesterol, mg %	Bile acids, mg/ml			Phospholipids, mg/ml
		Cholic	Chenodeoxycholic	Deoxycholic	
Hepatic bile					
1	41.1	1.9	2.0	.48	13.6
2	19.2	1.6	2.9	.55	9.6
3	8.9	1.3	1.9	.46	4.2
Mean	23.1	1.6	2.4	.50	9.1
Gallbladder bile					
1	259				
2	50.8	28.0			33.8
3	104	22.9	13.2	5.1	21.1
Mean	137.9	25.5	13.2	5.1	27.5
Wibler(6)	635				1.42

† These were supplied by Michigan Department of Conservation.

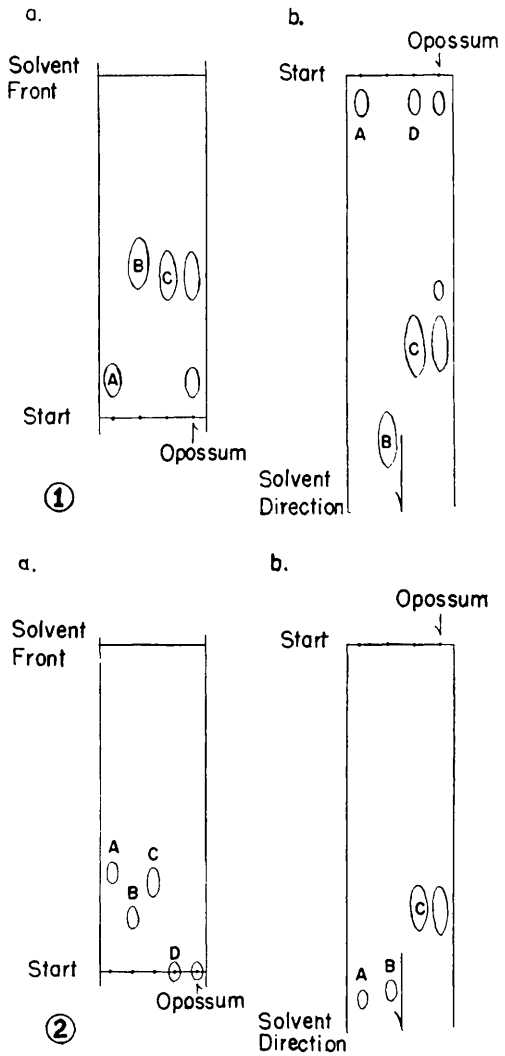

FIG. 1. Paper chromatograms of hydrolyzed bile acids of opossum. a. Ascending, 4 hr, moving phase: Isopropyl ether: heptane 60:40 v/v. Stationary phase: 70% acetic acid. Acids: Cholic (A), deoxycholic (B), chenodeoxycholic (C). b. Descending, 18 hr. Moving phase: Isopropyl ether: heptane 20:80 v/v. Stationary phase: As 1a. Acids: Cholic (A), deoxycholic (B), chenodeoxycholic (C), impurity in chenodeoxycholic (D).

FIG. 2. Paper chromatograms of unhydrolyzed bile acids of opossum. a. Ascending, 4 hr. Moving phase: Isopropyl ether: heptane 80:20 v/v. Stationary phase: 70% acetic acid. Acids: Cholic (A), glycocholic (B), glycodeoxycholic (C), taurocholic (D). b. Descending, 12 hr. Moving phase: n-butanol saturated with 3% acetic acid-water v/v. Stationary phase: As 2a. Acids: Cholic (A), glycocholic (B), taurocholic (C).

Results of determinations of several constituents of opossum bile are summarized in Table II. Cholesterol content of both gallbladder and hepatic bile is not as high as reported by Wibler(4), but is as low as that of usual laboratory animals. The relatively high concentration of chenodeoxycholic acid is remarkable. Isaksson's hydrolyzed extract(10) was also subjected to the determination of deoxycholic acid according to the method of Szalkowski and Mader(11). It has to be mentioned that the result of the amount of deoxycholic acid determined by this method is influenced by the existence of the large amount of other bile acids, *i.e.*, cholic acid, et cetera(11).

The method of paper chromatography according to Sjövall has been used to trace the bile acids. Fig. 1 shows the spots corresponding to cholic acid (Fig. 1a) and chenodeoxycholic acid followed by one unidentified spot (Fig. 1b). No spot corresponding to deoxycholic acid was found. In the system used in Fig. 2a, free and glycine conjugated acids run faster than taurine conjugated acids which are practically on the starting line, whereas bile acids of the opossum remain on the starting line. No spot corresponding to free and glycine conjugated acid was found. Fig. 2b shows the spot corresponding to taurine conjugated acid, *i.e.*, taurocholic acid. Free and glycine conjugated acids all run faster and practically with the front.

As mentioned above, chenodeoxycholic acid was identified as another major bile acid besides cholic acid which was isolated by Haslewood and Wootton from opossum bile(5). It was found that opossum bile contains taurine conjugated bile acids.

Summary. Several components of opossum bile were analyzed. Cholesterol content is comparable to that of other laboratory animals. Chenodeoxycholic acid was identified as another major bile acid besides cholic acid. Opossum bile contains taurine conjugated bile acids. No free and glycine conjugates were found.

1. Isaksson, B., On Lipids and Bile Acids in Normal and Pathological Bladder Bile. A Study of the Main Cholesterol Dissolving Components of Human Bile, Thesis, Lund, Sweden, 1952.

2. ———, *Acta. Soc. Med. Upsal.*, 1952, v56, 177.

3. Doyon and Dufourt, E., *Compt. rend. Soc.*

Chim. Biol., 1896, v48, 486.
4. Parhou, L., and Werner, G., *ibid.*, 1931, v107, 401.
5. Byers, S. O., and Friedman, M., *J. Physiol.*, 1952, v168, 297.
6. Wibler, C. G., *J. Mammalogy*, 1952, v33, 105.
7. Haslewood, G. A. D., and Wootton, V., *Biochem. J.*, 1950, v47, 584.
8. Sjövall, J., *Acta Chem. Scand.*, 1954, v8, 339.
9. Foldes, F. F., *J. Lab. and Clin. Med.*, 1942-1943, v28, 1889.
10. Foldes, F. F., and Wilson, B. C., *Anal. Chem.*, 1950, v20, 1210.
11. Irvin, J. D., Johnston, C. G., and Kopola, J., *J. Biol. Chem.*, 1944, v153, 439.
12. Isaksson, B., *Acta Chem. Scand.*, 1954, v8, 889.
13. Szalkowski, C. R., and Mader, W. J., *Anal. Chem.*, 1954, v24, 1602.
14. Carey, J. B., and Bloch, H. S., *J. Lab. and Clin. Med.*, 1954, v44, 486.
15. King, S. J., *Biochem. J.*, 1932, v26, 292.
16. Fieser, L. F., and Rajagopalan, S., *J. Am. Chem. Soc.*, 1949, v71, 3935.
17. ———, *ibid.*, 1950, v72, 5530.
18. Cortese, F., and Bauman, L., *ibid.*, 1937, v59, 2532.

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Carbohydrate Metabolism in Experimental Nephrosis (23332)

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It has been suggested that the nephrotic syndrome may be considered as "protein diabetes," the primary defect being loss of protein in the urine, and other biochemical abnormalities occurring as a consequence of this (1). In support of this view, Drabkin and Marsh(2) reported decreased liver glycogen levels and marked hypoglycemia on fasting in experimental nephrosis, changes interpreted as evidence of decreased gluconeogenesis consequent on the proteinuria. However, these observations were made under conditions which make interpretation difficult: (a) food intake was not controlled, (b) the animals were acutely ill and would have died within a few days(3). To investigate these results, a study has been made of carbohydrate metabolism in experimentally nephrotic animals after the acute stage, while on a fixed food intake.

Materials and methods. Adult male Sprague-Dawley rats weighing 380-420 g were used. They were fed twice daily by gavage with a diet providing a daily allowance of 55 calories, and containing 57% carbohydrate, 24% protein and 15% fat. There was free access to water. Animals were housed in individual metabolism cages in a room maintained at 26 ± 1°C and 40 ± 2% relative humidity. Urine was collected under toluene. Post-absorptive blood glucose, 24-hour urine glucose, glucose tolerance and insulin sensitivity were measured before and at intervals after induction of nephrosis. Each animal thus served as its own control. Blood sugar measurements were made on 5 animals subjected to a 48-hour fast after 28 days of nephrosis. Since the animals had not been subjected to such a procedure during the control period, a new group of 6 normal rats of the same weight range were adapted to tube feeding and then fasted for 48 hours. Nephrosis was produced with anti-kidney serum by the method of Heymann and Lund(4). The daily protein excretion was 400-700 mg. Intravenous glucose tolerance tests were performed by injecting 1.5 g of glucose/kg body weight in 50% solution; tail tip blood was sampled 5, 10, 15, 20 and 30 minutes later. A plot of the log of blood sugar concentration against time gave a straight line from which the half-life of the injected glucose was obtained. To test insulin sensitivity 0.1 unit of commercial insulin/kg body weight was injected intravenously; blood sugar was measured at 0, 15, 30, 45, 60, 90 and 150 minutes after injection and the values plotted against time. Two