5. Elks, J., Hey, D. H., *J. Chem. Soc.*, 1943, 441.
6. LeFevre, R. J. W., Liddicot, T. H., *ibid.*, 1951, 2743.
7. Bass, A. D., Feigelson, M., PROC. SOC. EXP. BIOL. AND MED., 1949, v69, 339.
8. Cochran, K. W., DuBois, K. P., *J. Pharmacol. and Exp. Therap.*, 1949, v97, 105.
9. Nelson, N., *J. Biol. Chem.*, 1944, v153, 375.

Received June 19, 1958. P.S.E.B.M., 1958, v99.

Copper Metabolism. XXVIII. Influence of Biliary Duct Ligation on Serum and Tissue Copper.* (24249)

H. DEMPSEY,† G. E. CARTWRIGHT AND M. M. WINTROBE
Department of Medicine, University of Utah College of Medicine, Salt Lake City

In both man(1,2) and dogs(3), the most important excretory route for copper is through the biliary system. However, no detailed studies have been performed in animals to determine the influence of ligation of bile ducts on tissue copper. The purpose of this paper is to present the results of studies on tissue and serum copper in rats with ligated bile ducts. Although normal serum copper values have been observed in swine and dogs with obstruction to outflow of bile(3), hypercupremia has been observed in patients with biliary cirrhosis(4). Copper accumulates in livers of patients with cirrhosis of the liver and associated cholangiolitis, but not in livers of most patients with uncomplicated cirrhosis (5). In one patient with primary biliary cirrhosis, an amount of copper (426 mg) was found in the liver, which was in excess even of that usually observed in livers of patients with Wilson's disease(5). Several investigators(2,6) have observed that a smaller proportion of intravenously administered radioactive copper is excreted in stools of patients with Wilson's disease than in stools of normal subjects. It has been suggested that the excessive deposition of copper in tissues may be due to impaired biliary excretion of copper (6). Therefore, it seemed of interest to determine if impairment of the outflow of bile in animals would be accompanied by excessive deposition of copper in the liver, brain and kidneys.

Methods. Adult male rats of Sprague-Dawley strain, 150 to 200 g in weight, were used. The animals were grouped into units of 3 of the same weight. One animal in each unit, designated as "bile duct-ligated," was anesthetized with sodium "Nembutal" solution given intraperitoneally. The common bile duct was located through a paramedian incision, ligated in 2 places, and sectioned between the ligatures. A careful search was made for accessory ducts. When these were found, they were also ligated and sectioned. The second animal in each unit, designated as "sham-operated," was treated in a similar manner except that the bile duct was not ligated or sectioned. The third animal in each unit, designated as "control," served as a dietary control. Bile duct ligation impaired the appetite of rats. To maintain dietary intake of copper at as constant a level as possible, the "bile duct-ligated" animal in each unit was offered 25 g of diet daily. On the following day, the uneaten portion of the diet was weighed and an amount equal to that consumed by the rat was fed to the "sham-operated" and "control" animal of that unit. Composition of diet was as follows (g/kg): casein, 200; sucrose, 640; lard, 110; sodium chloride, 6.75; magnesium carbonate, 4.25; monopotassium phosphate, 2.85; calcium phosphate, 23.15; potassium chloride, 3.35; potassium iodide, 0.1135 calcium carbonate, 8.45; ferric pyrophosphate, 1.05; copper sulfate, 0.011; manganese chloride, 0.01; zinc oxide, 0.0008; cobalt chloride, 0.008; and choline

* This investigation was supported by research grant (C-2231) from Nat. Cancer Inst., N.I.H., U.S.P.H.S.

† Medical Research Fellow, Canadian Nat. Research Council.

TABLE I. Mean Values for Serum, Liver, Kidney and Brain Copper in "Control," "Bile Duct-Ligated" and "Sham-Operated" Rats.

Group	Length of observation, wk	No. of animals	Serum copper, μg/100 ml	Liver copper	Kidney copper	Brain copper
				μg/total organ		
Control	2 & 4	25	118 ± 27	28.0 ± 10.0	12.1 ± 5.7	4.2 ±1.14
Sham-operated	2	12	128 ± 25	29.0 ± 10.4	8.5 ± 2.0	4.6 ± .32
Idem	4	12	143 ± 31	37.0 ± 9.6	15.0 ± 6.0	4.1 ± .64
Bile duct-ligated	2	14	195*	23.3 ± 10.2	9.0 ± 6.2	4.0 ± .43
Idem	4	11	273 ±100	82.0 ± 29.2	29.0 ± 7.5	4.3 ± .22

Figures refer to mean ± one stand. dev. * Mean of 7 sera which were pooled.

chloride, 2.3. Vitamins were added to the diet as follows (mg/kg): thiamine hydrochloride, 12.5; riboflavin, 6; nicotinic acid, 60; pyridoxine hydrochloride, 10; calcium pantothenate, 25; inositol, 5; para-amino benzoic acid, 5; pteroylglutamic acid, 15; Vit. E, 7.6; and Vit. K, 7.6. In addition, 23,000 units of Vit. A and 4250 units of Vit. D were added to 1 kg of diet. Following successful ligation of bile ducts, progressively deepening icterus of the ears, nose, and skin of animals was noted within 4 days. Animals which did not show a significant degree of jaundice in this period of time were excluded. Severely ill animals together with their controls were sacrificed during the 2nd week of the experiment. The remainder of the animals were killed during the fourth week. The animals were anesthetized with ether and exsanguinated *via* the abdominal aorta. The liver, kidneys and brain were removed, rinsed with copper-free water, weighed and stored in the frozen state. Methods for estimation of serum(7) and tissue copper(5) have been described previously.

Results. The results are summarized in Table I. Values for serum, liver, kidney and brain copper in the "control" group 2 weeks after beginning of experiment were the same as after 4 weeks. Therefore, all of animals in the "control" group, regardless of time of sacrifice, were analyzed as one group.

Serum copper values in the "bile duct-ligated" group increased significantly after 2 weeks as compared with "control" and "sham-operated" groups. However, liver, kidney and brain copper values were not significantly different at this time from mean values in the 2 other groups. ($P = 0.1$). Nevertheless, by the fourth week, the amount of copper in liver and kidney was markedly increased as compared with both "control" and "sham-operated" rats ($P = <0.01$). At no time was a significant increase in brain copper observed.

Discussion. Under conditions of these experiments, obstruction to the outflow of bile was associated with excessive deposition of copper in serum, liver, and kidneys. Since the biliary system is the main excretory route for copper(1-3), the most logical explanation for these changes is that copper was retained because excretion of this element was impaired. It has been shown in dogs that when excretion of copper *via* the bile is impaired, excretion of copper by the kidneys increases (3). Therefore, it is not surprising that deposition of copper occurred in the kidneys.

In rats and man(4,5), but not in dogs and swine(3), obstruction to outflow of bile is associated with hypercupremia. The reason for these differences is not apparent. It would be helpful to know if hypercupremia in rats and man is due to increase in ceruloplasmin or due to increase in direct-reacting fraction of serum copper(8). Unfortunately, the direct-reacting copper fraction cannot be measured in the presence of jaundice.

It is interesting that although copper was deposited in liver and kidneys of rats with ligated bile ducts, no deposition of copper was observed in the brain. Thus, distribution of deposited copper differed from that in Wilson's disease(9). It would have been desirable to maintain animals for a longer period of time with the bile ducts ligated. However, this was not possible since the animals would not have survived under these conditions for more than one month.

It has been suggested that excessive deposition of copper in tissues of patients with Wil-

son's disease is due to formation of abnormal proteins in tissues which have a high avidity for copper(10,11). Such a mechanism does not appear to be necessary for excessive deposition of copper in the liver and kidneys of animals with bile ducts ligated or in animals fed a high copper diet(12).

Summary. Ligation of the bile ducts of adult male rats was followed by hypercupremia and deposition of copper in liver and kidneys, but not in the brain.

1. Van Ravesteyn, A. H., *Acta Med. Scandinav.*, 1944, v118, 163.

2. Bush, J. A., Mahoney, J. P., Markowitz, H., Gubler, C. J., Cartwright, G. E., Wintrobe, M. M., *J. Clin. Invest.*, 1955, v34, 1766.

3. Mahoney, J. P., Bush, J. A., Gubler, C. J., Moretz, W. H., Cartwright, G. E., Wintrobe, M. M., *J. Lab. and Clin. Med.*, 1955, v46, 702.

4. Bearn, A. G., Kunkel, H. G., *J. Clin. Invest.*, 1954, v33, 400.

5. Gubler, C. J., Brown, H., Markowitz, H., Cartwright, G. E., Wintrobe, M. M., *ibid.*, 1957, v36, 1208.

6. Bearn, A. G., Kunkel, H. G., *J. Lab. and Clin. Med.*, 1955, v45, 623.

7. Gubler, C. J., Lahey, M. E., Ashenbrucker, H., Cartwright, G. E., Wintrobe, M. M., *J. Biol. Chem.*, 1952, v196, 209.

8. Gubler, C. J., Lahey, M. E., Cartwright, G. E., Wintrobe, M. M., *J. Clin. Invest.*, 1953, v32, 405.

9. Cartwright, G. E., Hodges, R. E., Gubler, C. J., Mahoney, J. P., Daum, K., Wintrobe, M. M., Bean, W B., *ibid.*, 1954, v33, 1487.

10. Uzman, L. L., Iber, F. L., Chalmers, T. C., *Am. J. Med. Sci.*, 1956, v231, 511.

11. Uzman, L. L., *A.M.A. Arch Path.*, 1957, v64, 464.

12. Dempsey, H., Cartwright, G. E., Wintrobe, M. M., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 520.

Received June 20, 1958. P.S.E.B.M., 1958, v99.

Relation Between Structure of Sulfonylurea Compounds and their Effect on Frog Muscle Metabolism.* (24250)

D. R. H. GOURLEY

Department of Pharmacology, University of Virginia Medical School, Charlottesville

Two sulfonylurea compounds, carbutamide and tolbutamide,† have been studied extensively as hypoglycemic agents. Although in normal dogs, rats and toads the 2 compounds are equally effective in reducing concentration of glucose in blood(1), they differ markedly from each other and from insulin in their effect on *in vitro* metabolism of intact frog muscle(2). Carbutamide (R = NH_2 in Fig. 1) inhibits oxygen consumption of frog muscle to a small but significant degree, as does the same concentration of sulfanilamide. Tolbutamide (R = CH_3 in Fig. 1), on the other hand, greatly stimulates oxygen consumption of muscle due to increased production of lactate from muscle glycogen. The fact that these compounds differ only in part of molecule labeled R in Fig. 1, suggested that the difference in effect on muscle was due to the type of substitution on the R position of the molecule. To investigate this possibility, similar experiments were done with a series of sulfonylbutylurea compounds which differ only in the R position in Fig. 1.

Methods. Oxygen consumption of intact

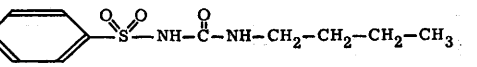

FIG. 1. Structural formula of the *para*-substituted sulfonylbutylurea compounds.

* This investigation was supported in part by grant from Division of Biology and Medicine, Atomic Energy Com.

† Tolbutamide (1-butyl-3-*p*-tolylsulfonylurea) was supplied by Upjohn Co. through the courtesy of Dr. C. J. O'Donovan; carbutamide (1-butyl-3-sulfanilylurea) was supplied by Lilly Research Labs. through the courtesy of Dr. W. R. Kirtley; 1-butyl-3-*p*-carboxyphenylsulfonylurea was a gift of Dr. L. H. Louis, Univ. of Michigan; all other sulfonylbutylurea compounds were supplied by Farbwerke Hoechst Ag. (Germany) through the courtesy of Dr. W. Meier.