Effect of Alteration in Body Temperature on the Biliary Excretion of Copper[1] (37515)

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The biliary excretion of copper has been extensively investigated because it is probably a defect in this process that results in Wilson's disease or hepatolenticular degeneration. When copper is administered to an animal it is rapidly taken up by the liver (1) and excreted into the bile (2, 3) or integrated into the ceruloplasmin molecule and reenters the plasma (4). Once the copper is excreted into the bile it does not appear to undergo an extensive enterohepatic circulation (5, 6), due in part to the fact that some of the copper in bile is bound to macromolecules (7, 8).

The excretion of compounds into the bile has been divided by Brauer (9) into three classes on the basis of bile/plasma concentration ratios during their excretion. Class A substances have a ratio of nearly one, class B substances have a range from 10 to 1000, and class C substances have a ratio less than 1. We have recently demonstrated that lead is excreted into bile against a large bile/plasma concentration ratio and thus can be included in the class B group (10). The excretion of lead into the bile was demonstrated to be highly temperature dependent (10). Therefore, it was of interest to determine if copper is also excreted into the bile against a large bile/plasma concentration gradient and if its excretion is as highly temperature dependent as that of lead.

Methods. Simonsen Sprague–Dawley male rats (250–350 g) were used throughout. Urethane (900 mg/kg, ip) was used as the anesthetic. Rectal temperature of the anesthetized rats was maintained at 37° with a heat lamp, except where stated otherwise, to prevent hypothermic alteration in hepatic function (11).

The femoral vein and artery was cannulated with PE-50 tubing for administering copper and obtaining the blood samples respectively. Cupric acetate was mixed with various amounts of ^{64}Cu (International Chemical and Nuclear Corp., Irvine, California) to give dosages of 0.03, 0.1, 0.3, 1.0, and 3.0 mg Cu/kg and administered iv (0.2 ml/kg) to the rats. Bile was collected at timed intervals thereafter. The bile volume was measured with a graduated pipet. Blood was collected into heparinized tubes. The ^{64}Cu content in plasma and bile were determined in a Packard Auto-Gamma spectrometer (La Grange, Ill.). At the end of the two-hour experiment a portion of the liver (approx. 1.5 g) was placed in a test tube for ^{64}Cu quantitation.

The effect of lead on the biliary excretion of copper was determined by administering lead acetate (3 mg Pb/kg, iv) and followed 15 min later by ^{64}cupric acetate (1.0 mg Cu/kg, iv). The amount of ^{64}Cu excreted into the bile over a two hour period was quantitated and compared to the group of rats receiving only the copper.

The *in vitro* binding of ^{64}Cu to various dilutions of plasma, liver and bile was determined by ultrafiltration (12). The liver was homogenized in a volume of 0.25 *M* sucrose equal to its weight (in ml) with a motor driven Potter–Elvehjem homogenizer (Teflon pestle). Various dilutions of the plasma, liver

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and bile were made with 0.25 *M* sucrose. ^{64}Cu acetate was added to each solution to give a final concentration of 12 μg Cu/ml. The Visking tubing (Union Carbide, Chicago, Ill.) was soaked overnight in a solution of cupric acetate and rinsed with water before usage to decrease the binding of ^{64}Cu to the bags.

In the experiments in which the effect of alteration in body temperature on the biliary excretion of copper was examined, the rectal temperatures of the rats were maintained at 30, 35, and 40°. Body temperature of the 30° group was lowered by contact with a plastic bag filled with ice water, and that of the 40° group was raised by being placed under an incandescent lamp. This experiment was performed similarly to the dose-response studies except that only 1 mg of copper/kg was administered.

Results. The disappearance of ^{64}Cu from the plasma, its rate of excretion into the bile and its concentration in the bile at various times after the intravenous administration of 0.03, 0.1, 0.3, 1.0, and 3.0 mg Cu/kg is shown in Fig. 1. Following the three lower doses, the copper disappears from the plasma at a similar rate, but at the two higher doses it disappears at a slower rate.

The excretion of copper into the bile increases as the dose administered increases, except after the highest dose. This suggests that a transport maximum for copper has been reached before this dose. However, since the highest dose is nearly lethal in this species, the excretion may be limited by its toxicity.

The concentration of copper in the bile at the various times after administration is usually much higher than in the plasma. Thus copper can be considered a class B compound. In an attempt to determine if the major concentration gradient is from plasma to liver or from liver to bile, the concentration of ^{64}Cu was also measured in the liver 120 min after administration (Table I). The major concentration gradient was from the plasma to the liver while the liver to bile gradient was quite small.

In an attempt to determine if the higher concentration of copper in the liver and bile

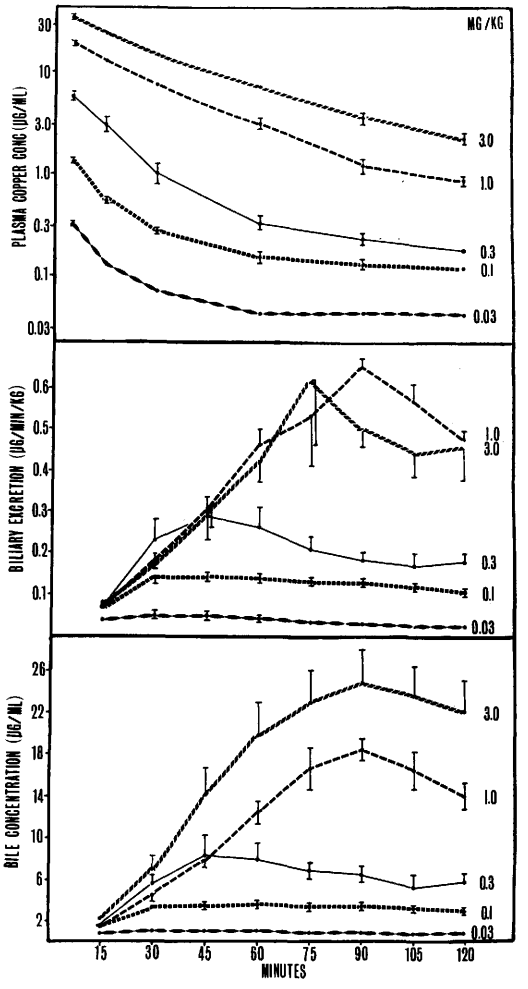

FIG. 1. Concentration of ^{64}Cu in plasma and bile and its excretion into bile after various doses of copper were administered iv to rats. Each value represents the mean ± SE of 4–6 rats.

than in the plasma might be due to binding of the copper to various components of the hepatocyte or bile, the percent of copper bound (determined by ultrafiltration) to various dilutions of bile, plasma and liver when 12 μg Cu/ml was added *in vitro* was determined (Fig. 2). The percentage of copper bound to bile increased up to a ¼ dilution. With further dilution, the binding of copper to bile markedly decreased. Plasma and liver bind essentially all of the copper at the lower dilutions, but as they are diluted

TABLE I. Dose (mg/kg).[a]

	0.03	0.1	0.3	1.0	3.0
Plasma concn (μg/ml)	0.039 ± 0.002	0.123 ± 0.010	0.177 ± 0.018	0.882 ± 0.135	2.27 ± 0.25
Liver concn (μg/g)	0.349 ± 0.034	1.32 ± 0.15	4.56 ± 0.29	11.8 ± 0.91	22.37 ± 1.59
Bile concn (μg/ml)	0.806 ± 0.099	2.90 ± 0.32	5.68 ± 1.09	13.92 ± 1.38	22.09 ± 2.96
Bile/plasma	18.5 ± 2.5	25.1 ± 4.1	34.9 ± 8.2	16.6 ± 2.1	11.5 ± 1.3
Liver/plasma	7.09 ± 1.63	11.4 ± 1.7	26.4 ± 1.3	14.3 ± 2.0	11.3 ± 2.2
Bile/liver	2.08 ± 0.15	2.34 ± 0.28	1.30 ± 0.28	1.24 ± 0.02	1.09 ± 0.13

[a] Rats were injected with copper 120 min before plasma and liver samples were taken. Bile sample was collected from 105–120 min after administration. Each value represents the mean ± SE of 4–6 rats.

further, the percentage of copper bound to plasma decreases, but a greater dilution is necessary than for a corresponding decrease using bile, and the greatest dilution is necessary to decrease the binding of copper to liver. Thus it appears that liver has the highest affinity for copper, plasma is intermediate, and bile the lowest. This suggests that copper does not pass from the liver to bile because of a higher affinity for bile than liver.

Since copper is excreted against a concentration gradient such as we recently observed for lead (10), it was of interest to determine if lead would decrease the excretion of copper. Lead did not appear to have a marked effect on the biliary excretion of copper because after 1 mg Cu/kg, 3.8–4.0% of the dose was excreted into the bile within two hours and a similar amount was excreted into the bile of rats that also received lead (3.7–4.4, range of 2 rats each).

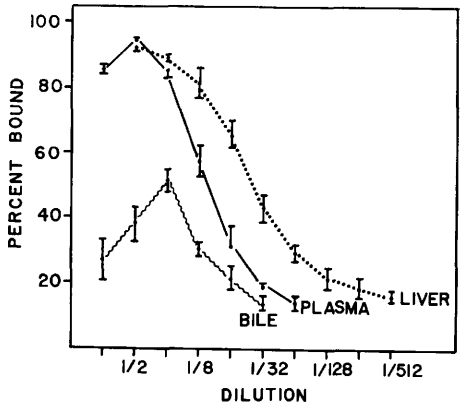

FIG. 2. Copper not ultrafilterable from solutions containing 12 μg/ml copper and various dilutions of plasma, bile and liver homogenate. Each value represents the mean ± SE of 4–5 rats.

Alteration of the rectal temperature of rats had a marked effect on the plasma disappearance and biliary excretion of copper in the rat (Fig. 3). The plasma concentration of copper at the three temperatures was similar in the 5-min samples. However, a difference in plasma concentration of copper became apparent soon thereafter and by 2 hr the concentration of copper in plasma of rats maintained at 30° was approximately 10 times higher than the rats at 40°. Differences in body temperature also had a marked effect on the biliary excretion of copper. Rats maintained at 40° reached their maximal excretory rate at the end of one hour while the excretory rate in the other group increased throughout the two hour period. Rats at 30° had a maximum excretory rate of 0.08 μg/min/kg, 0.46 at 35°, and 1.1 at 40°. That is, as the temperature was decreased from 40 to 30°, about a fourteenfold decrease in the excretory rate of copper was observed. This decrease in biliary excretion is largely due to the lower concentration of copper in the bile of the warmer rats but partially due to the lower rate of bile production. A decrease in temperature also decreased the concentration of copper in the liver measured two hours following administration; the concentration in the

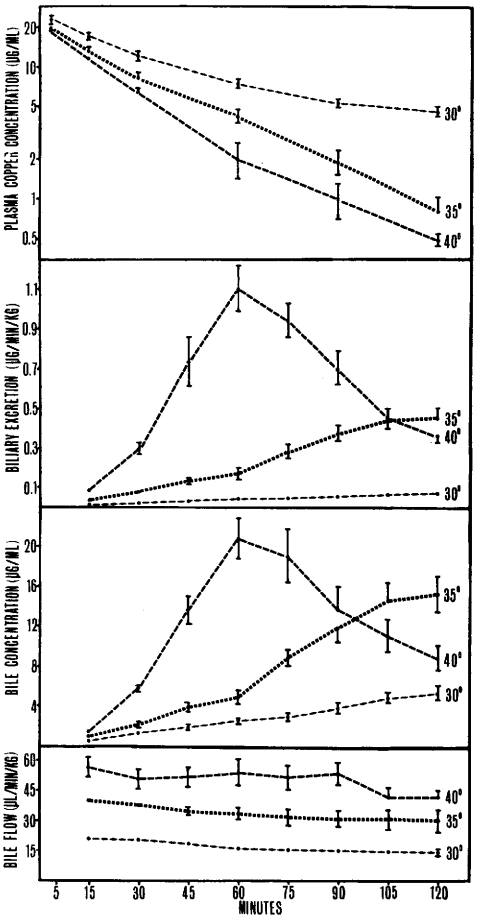

FIG. 3. Effect of alteration of rectal temperature of rats on the plasma disappearance and biliary excretion of copper. Each value represents the mean ± SE of 5 rats.

livers of the rats was 2.7 ± 0.2 μg/g (mean ± SE of 5 rats) at 30°, 7.9 ± 0.6 at 35°, and 13.0 ± 0.9 μg/g liver at 40°.

Discussion. The excretion of lead and copper into the bile can be characterized as class B that their concentration in bile exceeds that in plasma. The mechanism by which these metals are excreted into the bile is not completely understood, but since lead is maintained at a much higher concentration in the bile than the plasma and an apparent transport mechanism for its excretion exists, an active transport mechanism has been implicated (11). It is not known if copper is excreted by the same or similar mechanisms, but a number of similarities between the biliary excretion of these two metals have been demonstrated. For example, the overall concentration gradient of both metals from plasma to bile is large and when this gradient is dissected, the plasma to liver gradient is always much larger than the liver to bile gradient. Alterations in body temperature have a very marked effect on the biliary excretion of both these metals. However, data were also obtained suggesting that copper and lead are excreted by different mechanisms, in that the dose of lead that produces the maximal rate of lead excretion into the bile had no effect on the simultaneous excretion of copper.

Changing the body temperature of rats from 40 to 30° decreased the biliary excretion of copper fourteenfold. This is similar to that observed with lead, in that a twentyfold decrease was observed over the same temperature change. This is a very marked temperature dependence in contrast to bilirubin and sulfobromophthalein for which excretion is only halved between 40 and 30° (11). The exact mechanism that produces the increased excretion of these metals as the body temperature is increased is not known, but this increase in biliary excretion is largely due to the higher concentration of metals in the bile of the warmer rats and partially due to the higher rate of bile production.

Summary. Copper is excreted into the bile of rats against a large bile/plasma concentration ratio. The biliary excretion of copper is very temperature dependent in that a fourteenfold decrease in excretion was observed when the temperature of the rats was decreased from 40 to 30°.

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1. Goresky, C. A., Holmes, T. H., and Saas-Koktasak, A., Can. J. Physiol. Pharmacol. **46**, 771 (1968).

2. Owen, C. A., Amer. J. Physiol. **207**, 1203 (1964).

3. Cikrt, M., Brit. J. Ind. Med. **29**, 74 (1972).

4. Owen, C. A., and Hazelrig, J. B., Amer. J. Physiol. **210**, 1059 (1966).

5. Farrer, P., and Mistilis, S. P., Nature (London) **213**, 291 (1966).

6. Mistilis, S. P., and Farrer, P. A., Scand. J. Gast. **3**, 586 (1968).

7. Evans, G. W., and Cornatzer, W. E., Proc. Soc. Exp. Biol. Med. **136**, 719 (1971).

8. Frommer, D., Clin. Sci. **41**, 485 (1971).

9. Brauer, R. W., J. Amer. Med. Assoc. **169**, 1462 (1959).

10. Klaassen, C. D., and Shoeman, D. W., Proc. Fifth Inter. Cong. Pharmacol. (abstract), 757 (1972).

11. Roberts, R. J., Klaassen, C. D., and Plaa, G. L., Proc. Soc. Exp. Biol. Med. **125**, 313 (1967).

12. Anton, A. H., J. Pharmacol. Exp. Ther. **129**, 282 (1959).

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