

The Effect of Surgery and Ether Anesthesia on Excretion of Biliary Copper by the Rat (40803)¹

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When an external biliary fistula is created in a rat under ether anesthesia, the concentration of copper in the bile is higher the first day than later (1). The assumption might be made that the initial biliary copper reflects true steady state values and that for some reason the prolonged loss of bile alters copper excretion. Attempts at replacing bile by a gastric tube have not resulted in increased biliary copper excretion in our laboratory, however.

Recently, Caple and Heath (2) observed that the stress of halothane or pentothal anesthesia, with or without a surgical procedure, increased the excretion of biliary copper in sheep. If this were applicable to the etherized rat, then the initially elevated biliary copper might be a stress artifact and the later, lower concentration of biliary copper might reflect the true *in vivo* state. To test this possibility with our rat system, the concentration of copper in biliary fistula bile was measured after anesthesia (ether) and operation (biliary fistula) and compared with that when anesthesia alone was carried out 1 to 4 days after the surgical procedure.

Methods and materials. Adult, male Sprague-Dawley rats, weighing about 300 g were used. Ether was administered by inhalation in a glass jar and continued by the drip method while an external biliary fistula was being created. If no operation was carried out, the anesthetic agent was administered just as if an operation were being performed. A polyethylene PE No. 50 tube was threaded into the common bile duct, toward the liver, through a small incision in the duct. The catheter was ligated in place and the common duct was severed distally. The catheter was brought out through the skin dorsolaterally and bile was collected

for 24-hr periods while the rat was moderately immobilized in a Bollman restraining cage (3). The rats ate and drank *ad lib*.

Twenty-four hours after the operation 8 rats were again anesthetized with ether, another 8 rats at 48 hr, 7 other rats at 72 hr, and 10 other rats at 96 hr. Biliary flow rates (ml/hr) and biliary copper ($\mu\text{g/ml}$) were compared with the comparable values of the operated rats.

Copper was measured by atomic absorption spectrometry after the samples of bile were digested with nitric and sulfuric acids. All glassware was scrupulously cleansed to render it copper free.

Results. When a rat was anesthetized with ether and a biliary fistula was created, the hourly flow of bile diminished modestly after the first day (Table I). The amount of copper excreted fell significantly, 0.35 $\mu\text{g/hr}$ the second 24 hr ($P < 0.01$) and 0.40 ($P = 0.03$) the third 24 hr, compared with 0.56 $\mu\text{g/hr}$ the first 24 hr. Eight rats were studied longer and even on the sixth day biliary copper averaged 0.36 $\mu\text{g/hr}$.

When ether was administered 1, 2, 3, or 4 days after the operation, there was only a slight reduction in biliary flow rates. However, changes in biliary copper were significant. Rats anesthetized 1 day after the surgical procedure had depressed copper excretion for the entire 48-hr collection period. When anesthetized 2 days after the operation, biliary copper was suppressed only the first 24 hr. Anesthesia at 3 days depressed copper excretion less and at 4 days copper excretion resembled the original postoperative values.

Discussion. The reduction of copper in bile after creation of a biliary fistula in rats (1) has not been explained. The higher initial concentration could reflect the *in vivo* state with the subsequent reduction being the result of continued biliary loss. Or, the

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TABLE I. BILIARY FLOW RATES AND BILIARY COPPER

Days after operation that ether was administered		Hours of bile collection after anesthesia		
		0-24	24-48	48-72
0	Flow rate (ml/hr)	0.76 ± 0.15 ^a	0.66 ± 0.16	0.67 ± 0.08
	Copper (μg/hr)	0.56 ± 0.15	0.35 ± 0.15	0.40 ± 0.12 ^c
1	Flow rate	0.67 ± 0.10	0.62 ± 0.11	
	Copper	0.26 ± 0.13 ^b	0.15 ± 0.04 ^b	
2	Flow rate	0.66 ± 0.10	0.63 ± 0.15	
	Copper	0.22 ± 0.11 ^c	0.34 ± 0.15	
3	Flow rate	0.52 ± 0.15 ^d	0.59 ± 0.19	
	Copper	0.33 ± 0.15 ^d	0.39 ± 0.09	
4	Flow rate	0.57 ± 0.08	0.55 ± 0.08	
	Copper	0.57 ± 0.22	0.36 ± 0.25	

^a SD.

^b $P < 0.01$ from Day 0 value and <0.05 from values 1-6 days later.

^c $P < 0.01$ from Day 0 value and values 1-6 days later.

^d $P < 0.05$ from Day 0 value.

^e Eight rats were studied 4, 5, or 6 days after operation; biliary copper continued virtually unchanged, averaging 0.36 μg/hr on the sixth day.

later, lower concentrations could be true of the *in vivo* state with the higher immediate postoperative concentration representing the surgical-anesthetic stress. When Cable and Heath (2) reported that halothane or pentothal anesthesia increased the excretion of biliary copper in sheep, it was decided to study the effect of our anesthetic agent (ether) in rats. No attempt was made to compare the rat with sheep or to compare ether with halothane or pentothal. Simply, does ether anesthetization alter biliary copper in the rat? Since administration of ether to rats 4 days or longer after creation of a biliary fistula did not affect biliary excretion of copper, the answer seems to be no.

There was a radical reduction in the amount of copper in the bile when biliary fistula rats were ether anesthetized 24 or 48 hr after the initial surgical procedure. By 72 hr the reduction was limited and 4 days after the operation the anesthesia had no obvious effect. Thus, the operation seems to be responsible for the anesthetic-induced changes occurring the first 3 days post-operatively.

Unfortunately, the question as to the normal concentration of copper in rat bile is unanswered.

Summary. Biliary fistulas were created in rats under ether anesthesia. Biliary excretion of copper diminished after the first day. When the rats were reanesthetized with ether 1 to 4 days after the operation, more dramatic changes in biliary copper excretion occurred. If the second anesthetic was administered 1 to 2 days after the operation, biliary copper fell about one-half. The reduction was modest on the third day and 4 days after the operation biliary excretion of copper was little affected by ether anesthetization.

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- Owen, C. A., Jr., Mayo Clin. Proc. 50, 412 (1975).
- Cable, I. W., and Heath, T. J., Comp. Biochem. Physiol. A. 61, 503 (1978).
- Bollman, J. L., and van Hook, E., J. Lab. Clin. Med. 13, 1348 (1948).

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