Biliary Copper Excretion in the Rat (35349)

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Although biliary excretion is known to be the major pathway for hepatic copper removal (1, 2), very little is known regarding the factors regulating biliary copper excretion or the nature of copper secreted into bile. Earlier experiments demonstrated that corticosterone induces precocious removal of hepatic copper in the neonatal rat (3). Recently, Mearrick and Mistilis (4) confirmed these results and demonstrated that corticosteroids increase copper secretion into the intestine.

In the adult rat, hepatic copper is elevated following either adrenalectomy (5, 6) or hypophysectomy (6, 7). The elevated hepatic copper following adrenalectomy or hypophysectomy is a result of decreased biliary copper excretion (6). In view of the anabolic action of adrenal steroids, Mearrick and Mistilis (4) and Gregoriadis and Sourkes (5) suggested that these hormones may promote biliary copper excretion by inducing synthesis of a specific protein required for copper removal. Therefore, attempting to further explain the mechanism of biliary copper excretion, we have characterized copper-binding components in bile.

Methods. Adult male Sprague-Dawley rats were used in all experiments. Adrenalectomized rats (adx) and hypophysectomized rats (hypox) were purchased from Badger Research Corp., Madison, Wis. Experiments were conducted 4 weeks after surgery.

The nitrate form of ^{64}Cu (International Chemical and Nuclear Corp.; sp act = 11.8 mCi/mg) was adjusted to pH 7.0 with sodium hydroxide and diluted with physiological saline for injection.

To analyze biliary ^{64}Cu and bile flow rate, 10 control animals, 10 adrenalectomized, and 10 hypophysectomized animals were anesthetized with sodium pentobarbital after which the hepatic bile duct was cannulated with polyethylene tubing. Following cannulation, 10 μg of ^{64}Cu (124 μCi) was injected into the inferior vena cava. Following surgery, the animals were placed in plastic restraining cages and bile was collected into graduated glass tubes over a period of 4 hr.

Bile samples were chromatographed on Sephadex G-150 which has a fractionation range of 4000–150,000 molecular weight for globular proteins. A 2.5 $\times$ 40-cm column was packed with gel and equilibrated with buffer. The buffer solution, pH 7.4, contained 0.025 *M* potassium phosphate and 0.05 *M* potassium chloride. Bile from the cannulated control animals described above was pooled, and a 5.0-ml sample was applied to the column. Gel filtration was carried out at 22° with a flow rate of 0.5 ml/min using the buffer described above. Fractions were collected in 3.0-ml volumes and analyzed for radioactivity in a gamma well scintillation counter (Tracerlab).

Following chromatography on Sephadex G-150, the fraction which contained the highest ^{64}Cu content was transferred to a quartz cuvette and the ultraviolet absorbance spectrum was determined on a Cary 15 recording spectrophotometer. The reference cuvette contained the same buffer used to elute the sample from the column. Following determination of the absorbance spectrum, 0.1 m*M* biscyclohexanone oxaldihydrazone (Cuprizone; G. F. Smith Chemical Co.) in 0.3 ml was added to each cuvette, mixed and incubated at room temperature for 30 min. Thereafter, a second absorbance spectrum was determined and corrected for volume change.

Results. When rat bile containing ^{64}Cu was analyzed on Sephadex G-150, radioactivity

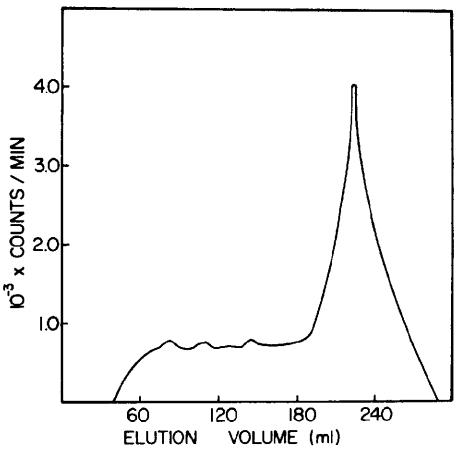

FIG. 1. Elution of ^{64}Cu in rat bile from a Sephadex G-150 column. Bile samples from normal rats injected with ^{64}Cu were chromatographed on a Sephadex G-150 column. For details, see text.

was observed nonspecifically throughout the fractions and a ^{64}Cu peak was eluted at 216 ml (Fig. 1). The elution volume of the single ^{64}Cu peak corresponded with the total volume of the column used in these experiments. Since the lower exclusion limit of Sephadex G-150 is 5000, molecules of 5000 molecular weight or less are eluted at the total volume. These results suggested that a large portion of biliary copper is associated with components of low molecular weight.

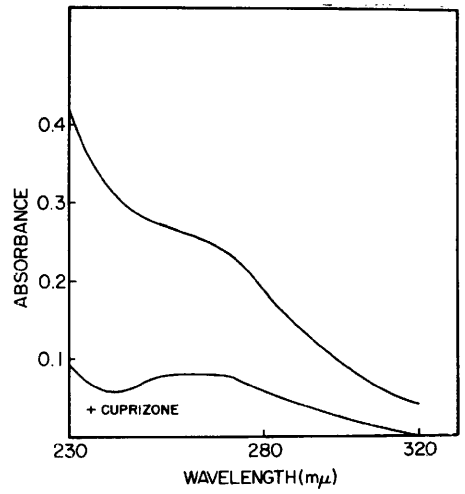

FIG. 2. Absorbance spectra of copper-containing fractions from rat bile. Following chromatography of ^{64}Cu-labeled rat bile on Sephadex G-150, the fraction which contained the highest ^{64}Cu content (216-ml fraction, see Fig. 1) was analyzed on a Cary 15 recording spectrophotometer. The same fraction was analyzed after the addition of Cuprizone. For details, see text.

To further examine the nature of biliary copper complexes, the copper-containing fractions from bile were analyzed by ultraviolet spectrophotometry (Fig. 2). The ultraviolet absorption spectrum shown in Fig. 2 is similar to that observed by Spies (8) with copper–amino acid complexes and copper–peptide complexes. Furthermore, addition of the copper chelating agent, Cuprizone, abolished the characteristic absorbance spectrum of the copper-containing fractions from bile. These experiments suggest that a large fraction of biliary copper is associated with amino acids and small peptides.

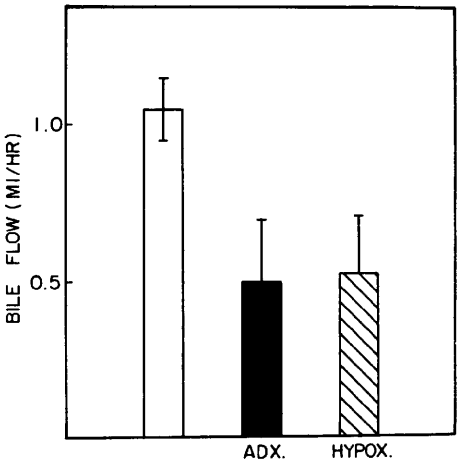

FIG. 3. The effect of adrenalectomy or hypophysectomy on bile flow in the adult rat. Bile flow rate was determined in 10 adrenalectomized rats (adx), 10 hypophysectomized rats (hypox), and 10 control rats. For details, see text. Values are expressed as mean ± standard deviation.

As illustrated in Fig. 3, the bile flow rate of both adrenalectomized and hypophysectomized rats was significantly less ($p<.01$) than the flow rate of the control animals. Since biliary copper excretion is apparently non-dependent upon a specific protein, these results indicate that the decreased biliary copper excretion following adrenalectomy or hypophysectomy results from diminished bile flow.

Discussion. Characterization of biliary copper-binding components provides no evidence of a specific copper-binding protein in bile. Regarding the low molecular weight fraction of biliary copper, a copper–amino acid complex may represent the transport form of copper across the bile canaliculus. Since amino acids facilitate transport of copper across cell membranes (9, 10), perhaps a copper–amino acid complex is involved in the excretory mechanism.

As described previously, adrenalectomy or hypophysectomy results in decreased biliary copper excretion. Previous experiments have demonstrated that bile flow is diminished following adrenalectomy or hypophysectomy (11, 12). The experiments described here demonstrate that the decreased biliary copper excretion accompanying adrenalectomy or hypophysectomy is a secondary result of decreased bile flow. Since bile is the major pathway for hepatic copper excretion, these results indicate that corticosteroids maintain hepatic copper homeostasis by choleretic action.

Summary. Gel filtration chromatography and spectral analysis of copper-containing fractions from rat bile suggest that the major fraction of biliary copper is associated with amino acids and small peptides.

Following adrenalectomy or hypophysectomy, bile flow was significantly decreased suggesting that adrenal steroids regulate biliary copper excretion by choleretic action.

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