

## Biliary Metabolism of Some Minerals

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*Introduction*—Bile has been shown to contain several minerals of established biological significance. The role of biliary excretion as a primary or intermediate factor in mineral metabolism has received little attention. A disturbed calcium balance has been reported in obstructive jaundice with a decrease of calcium in the heart muscle by Horrall and others. When there is a loss of bile through a fistula, certain effects are noted; such as spontaneous fractures with bone abnormalities, spontaneous bleeding and anemia, duodenal ulcers, and cholelithiasis, also suggesting a disturbed trace mineral metabolism.

In order to determine fundamental areas of investigation, several basic aspects of mineral metabolism related to biliary functions were investigated, i.e., the rate of excretion, quantity of trace mineral excreted in relation to the time, and the length of time necessary for the trace mineral to enter the bile.

These parameters composed phase one of the experimental design. Phase two consisted of extending the observations established in phase one and establishing the role of the adrenal gland as a direct or intermediate factor in biliary metabolism of the trace minerals.

*Materials and Methods*—Fifty female Charles Rivers rats weighing  $250 \pm 30$  g were used throughout this study. The common bile duct was exposed by an abdominal approach. The duct was cannulated using PE-10 tubing of sufficient length to allow considerable latitude in the placement of the rat relative to the collecting tube. Following the placement of the cannula, the incision was closed using uninterrupted silk suture. The rat was placed in a container approximately 12 inches above the collection vessel and restrained with tape. The cannula was positioned so that bile flowing through it would drop directly into the collecting tube. Collecting tubes were placed in a fraction collector directly beneath the restrained rat. Various time periods (6-24 hr) were chosen for the collection of bile and the fraction collector so programmed. Matched controls were sham operated with a similar procedure, with the exception that the bile duct was not cannulated. Immediately following the surgical procedure, both control and treated animals were injected subcutaneously with  $\text{Ca}^{45}$ ,  $\text{Mn}^{54}$ , or  $\text{Zn}^{65}$ .

After bile collection, all rats were sacrificed, at which time blood, bile, liver, kidney, spleen, and skeletal muscle were removed for determination of isotopic content. All tissues were dried, weighed, and counted in a gamma scintillation detector, or in the case of  $\text{Ca}^{45}$ , in a low-beta gas flow detector. The above procedure constituted phase I of this experimental design. Phase I established the normal distribution of a particular isotope in the control and cannulated rat. In this manner the intestinal resorption of a particular isotope could be studied. It was possible to

determine the effect of isotope resorption on the distribution of that isotope in various tissues of the cannulated rat.

Phase II was designed to study the effect of adrenal hormones on the absorption and distribution pattern established in phase I.

The common bile duct was cannulated and the adrenal glands removed in a single operation using an abdominal approach. The rats were injected subcutaneously with 8.61  $\mu\text{c/ml}$  of  $\text{Ca}^{45}$  immediately following surgery and bile collection. A group of rats was chosen at random and each rat was also injected with 5 cc of 2% NaCl intraperitoneally to supplement the loss of sodium.

Controls were cannulated, but not adrenalectomized, and bile collected as in phase I. The maximum collection period for this phase was 9 hr.

**Results**— $\text{Ca}^{45}$  reached a maximum output per unit volume between 40 and 60 min.  $\text{Mn}^{54}$  reached a maximum output per unit volume between 360 and 500 min. The output for  $\text{Zn}^{65}$  appears to be variable and will require further investigation.  $\text{Ca}^{45}$  was found to be significantly increased in the heart of the cannulated rat when compared to control values. This was the only tissue indicating an increased Ca uptake under these conditions. The kidney was found to contain less isotope in the cannulated rat than in the control.  $\text{Mn}^{54}$  was found to be at lower level in the skeletal muscle of cannulated rats. This was the only tissue showing a statistically significant alteration in Mn metabolism.  $\text{Zn}^{65}$  values were not altered in any tissues studied.

Cannulated-adrenalectomized rats showed a greater loss of Ca than did the cannulated controls. A great deal of variation in Ca output was noted in rats both adrenalectomized and cannulated, but the lowest values observed still remained well above control values.

The injection of the NaCl solution had no statistically significant effect on Ca output.

**Discussion**—This preliminary data would seem to indicate that  $\text{Ca}^{45}$  and  $\text{Mn}^{54}$  metabolism depends, in part, on biliary excretion and on resorption. The elevated values for  $\text{Ca}^{45}$  in cardiac tissue in the absence of bile implies a control mechanism limiting cardiac Ca levels mediated by an unknown bile constituent. The lower  $\text{Ca}^{45}$  values for kidney may indicate either (1) less  $\text{Ca}^{45}$  is available for absorption due to biliary loss or (2) an agent is present in the bile that is resorbed to facilitate the renal uptake of Ca.  $\text{Mn}^{54}$  absorption was depressed in skeletal muscle and remained normal in all other tissue measured. This may be considered similar to the circumstances for Ca in (2) above.

The lack of specific variation in  $\text{Zn}^{65}$  uptake for the tissues studied would seem to indicate that bile may offer a route of excretion that is less specific in nature than that observed for Ca and Mn. At present studies are in progress to isolate the observed effects of the adrenals on biliary metabolism. Cortical steroids are being injected in intact rats and the biliary metabolism of trace minerals studied.