We have attempted to determine the total amount of circulating cholic acid by an isotope dilution in vivo. By determining the fall of specific activity with time information has been gained on the rate of formation of this compound. 3-4 μC of cholic acid-24.14C was given orally as sodium salt to healthy male medical students. At different time intervals bile was obtained through a duodenal tube after administration of 1-3 mg of a purified pancreozymin-cholecystochochloine preparation (kindly supplied by Professor Erik Jorpes, Stockholm). Cholic acid was isolated and the specific activity determined. A bile sample was used for a quantitative determination with a paperchromatographic technique of the concentration of various bile acids. These determinations were kindly carried out by Dr. J. Sjövall. By the use of the figures thus obtained the total amount of circulating acids was calculated. In the table the results obtained in three subjects are given:

<table>
<thead>
<tr>
<th>Subject</th>
<th>Cholic acid g</th>
<th>Total amount of bile acids g</th>
<th>&quot;Half-life&quot; of cholic acid in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>0.575</td>
<td>1.85</td>
<td>1.2</td>
</tr>
<tr>
<td>P</td>
<td>0.92</td>
<td>3.65</td>
<td>2.5</td>
</tr>
<tr>
<td>S</td>
<td>1.29</td>
<td>3.55</td>
<td>3</td>
</tr>
</tbody>
</table>

1. Josephson, B. Physiol. Rev. 21 (1941) 463.

On the Metabolism of Bile Acids in Germ-free Rats

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The bile of rats contains taurocholic and taurochenodeoxycholic acids. With the aid of 24.14C-labelled acids it has been shown that these acids are extensively modified during the passage down the intestinal tract. When the fecal acids were chromatographed these modifications were found to include:
1) a splitting of the peptide bond,
2) an attack on the bile acid moiety leading to a variety of mostly less hydrophilic compounds.

It has been found that in rats treated with intestinal chemotherapeutics no such modifications occur making it highly probable that microbial enzymes are responsible for the reactions. Furthermore several strains of bacteria that can split the conjugated bile acid in vitro have been isolated from the rat intestine. We have earlier reported on the turnover of bile acids in normal rats and in animals treated with chemotherapeutics. The half-lives were found to be about 2.5 and 6-7 days, respectively. In order to study the importance of intestinal microorganism for the elimination of bile acids we have administered 24.14C-labelled cholic acid to germ-free rats and to a rat that had been infected with Aspergillus niger and then further with Clostridium perfringens type E. The rats were reared with slight modifications in the technique of Gustafsson. The time course of excretion was followed and the acids in the feces fractionated with chromatography. One rat was finally transferred from the apparatus to normal environments and the elimination rate investigated.

The germ-free rats were found to excrete unchanged taurocholic acid in their feces. This was also the case in the rat infected with the mould. When the same rat was also infected with the Clostridium strain, however, about 25% of the labelled acids appeared in the feces in the free form, mainly as cholic acid. The chromatographic pattern was in this case similar to that found after in vitro incubation of taurocholic acid with the same strain of bacterium.

In all rats studied the excretion rate was quite slow with a half life-time of about 7 days. It may be concluded that the state of the intestinal flora greatly influences the rate of elimination from the enterohepatic circulation. As, however, neither the mould nor the peptide bond-splitting Clostridium increases the excretion rate it seems probable that other microorganisms are of importance for the rate of elimination of bile acids.