CM-Sephadex chromatography (phosphate buffer, 10 mM, pH 7.4) resolved the lithocholic acid-binding mixture obtained from DEAE-Sephadex into two fractions, both able to bind lithocholic acid and both having glutathione S-transferase activity. Agarose and polyacrylamide-gel electrophoresis of the fractions obtained from CM-Sephadex chromatography demonstrated the presence of only a single protein-containing band in each fraction. These fractions were further investigated for glutathione S-transferase activity by measuring the conjugation of ethacrynic acid [2,3-dichloro-4-(2-ethylacyroyl)phenoxyacetic acid] with glutathione. Both fractions had this activity, suggesting that one of them is the anion-binding protein ligandin (Habig et al., 1974).

The physiological relevance of these findings is not clear. The value of the dissociation constant for binding of lithocholic acid to hepatic supernatants suggests a transport function. Ligandin can bind many different anions which are transported into bile by the liver (Levi et al., 1969). Phylogenetic and clinical studies have led to the proposal that ligandin is involved in the hepatic transport of bromosulphophthalein and bilirubin. It seems unlikely, however, that ligandin is involved in the transport of bile acids as well as bilirubin and bromosulphophthalein, since studies have suggested that bile acid transport is mediated by a mechanism different from the other anions.


The Effect of Elimination of the Gastrointestinal Flora on the Accumulation of Methylmercuric Chloride by the Rat

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When methylmercuric chloride labelled with $^{203}$Hg (CH$_3^{203}$HgCl) was incubated in vitro with the contents of the small intestine or caecum of the rat, the radioactivity was volatilized over a period of 3–4 days (Fig. 1). To determine whether the intestinal metabolism of CH$_3^{203}$HgCl has any significance in vivo, we have compared the accumulation of CH$_3^{203}$HgCl by organs of conventional rats and of rats treated with antibiotics to eliminate their intestinal microflora.

Of two groups of seven male Wistar rats (135–150 g), obtained from a specific-pathogen-free breeding colony, one group (control) was given water and autoclaved Spillers Laboratory Small Animal Diet ad lib., and the other group the autoclaved diet and water containing bacitracin, neomycin sulphate and streptomycin sulphate, each at 2 mg/ml. Faecal samples were taken each day so that the degree of bacterial colonization of the intestinal tract could be determined. The samples were inoculated on to plates of blood agar and incubated aerobically and anaerobically. The faeces were found to be free of bacteria after 3 days of antibiotic treatment. On days 4–8 and 11–14 inclusive, the rats were given, by stomach tube, 0.5 ml of CH$_3^{203}$HgCl in 0.9% NaCl, such that each rat received nine doses of 13 $\mu$g (0.03 $\mu$Ci of $^{203}$Hg) as CH$_3$HgCl.

At 18 h after the last treatment, the rats were anaesthetized with diethyl ether and killed by exsanguination. The radioactivity in the kidneys, liver, lung, brain, skeletal muscle, blood and faeces was measured and hence the mercury concentration in the tissues could be calculated (Table 1).

The amount of mercury accumulated by the blood, kidneys, brain, muscle and lung was significantly greater in the animals treated with antibiotics than in the control rats, indicating that the intestinal flora influences the absorption of mercury from
Fig. 1. Metabolism of CH$_3^{203}$HgCl by the caecum or small intestine of the rat

The contents of the caecum (▲) or small intestine (●) were suspended at 0.2 g/ml in 0.1 M-KH$_2$PO$_4$/NaOH buffer, pH 7.0, containing peptone, tryptone, yeast extract and D-glucose, each at 0.5% (w/v). The suspensions were incubated at 37°C with CH$_3^{203}$HgCl (2 μg/ml) in screw-cap bottles, and samples were removed at the times shown and counted for radioactivity. Controls with autoclaved caecal (▲) or small-intestinal (●) contents were included.

Table 1. Mercury concentration in tissues and faeces of control and antibiotic-treated rats given methylmercuric chloride

Control and antibiotic-treated rats were given, by stomach tube, nine doses of 13 μg of CH$_3^{203}$HgCl over a period of 11 days. The amount of $^{203}$Hg in the tissues and faeces was estimated by γ-scintillation spectrometry. Samples of the tissues were homogenized in distilled water, acidified with conc. HCl, treated with NaCl (0.2 g/ml of sample) and extracted with benzene to estimate the percentage of organic mercury present. The values are means±s.e.m. for seven rats and those marked with an asterisk differ significantly from the control (Wilcoxon rank sum test, $P \leq 0.01$).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>μg of Hg/g of tissue</th>
<th>Percentage extracted by benzene</th>
<th>μg of Hg/g of tissue</th>
<th>Percentage extracted by benzene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>Antibiotic-treated</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>1.53 ± 0.07</td>
<td>53.0 ± 2.2</td>
<td>2.06* ± 0.18</td>
<td>51.5 ± 1.7</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.72 ± 0.07</td>
<td>58.6 ± 1.3</td>
<td>2.45* ± 0.11</td>
<td>66.4* ± 1.0</td>
</tr>
<tr>
<td>Liver</td>
<td>0.43 ± 0.03</td>
<td>61.8 ± 2.5</td>
<td>0.41 ± 0.03</td>
<td>54.8 ± 1.9</td>
</tr>
<tr>
<td>Brain</td>
<td>0.11 ± 0.01</td>
<td>82.8 ± 1.0</td>
<td>0.14* ± 0.01</td>
<td>83.2 ± 1.0</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.32 ± 0.03</td>
<td>85.9 ± 3.0</td>
<td>0.45* ± 0.02</td>
<td>83.8 ± 1.5</td>
</tr>
<tr>
<td>Lung</td>
<td>0.36 ± 0.02</td>
<td>77.9 ± 1.4</td>
<td>0.48* ± 0.04</td>
<td>82.8 ± 3.5</td>
</tr>
<tr>
<td>Faeces</td>
<td>0.27 ± 0.04</td>
<td>13.9 ± 1.5</td>
<td>0.09* ± 0.01</td>
<td>26.1* ± 1.2</td>
</tr>
</tbody>
</table>

the gut. The weight of the rats was slightly higher in the control group (245 ± 8.9 g) than in the antibiotic-treated group (213 ± 7.7) and this difference was also reflected in the weights of the organs, which were higher in the former group. However, the differences in organ weights were not sufficient to account for the differences in mercury concentration in any of the organs.

To determine the form of the mercury present in the tissues, we extracted samples of the homogenized tissues with benzene, which extracts methylmercury, but not inorganic mercury, from aqueous solution (Westöö, 1966). Samples of the benzene
extracts were chromatographed on plates of silica gel G, with chloroform/n-hexane (9:1, v/v) as developer (Imura et al., 1971), to show that all the extracted radioactivity migrated at the same rate as a CH$_3$HgCl standard.

The amount of methylmercury extracted from the tissues by this method varied from 13.9 to 85.9% (Table 1). In model experiments to determine the degree of extraction of CH$_3$HgCl from tissue homogenates, between 81 and 86% of added CH$_3$HgCl could be extracted by benzene. Therefore it appears that in brain and skeletal muscle virtually all of the accumulated Hg is in the form of CH$_3$HgCl, whereas in blood, kidney, liver and faeces a substantial part of the Hg is present in other forms, presumably inorganic (Table 1; Norseth & Clarkson, 1971). In the kidney, although not in any other organ tested, the percentage of Hg present as methylmercury was significantly higher in the rats without a gut flora than in the control rats (Table 1), which again suggests that the intestinal flora plays some part in the metabolism of CH$_3$HgCl. It must be noted that, although the concentration of Hg in the faeces was much lower in the antibiotic-treated rats than in the controls, the total weight of faeces produced by the former was much greater and hence, in terms of the total amount of Hg excreted, the two groups may not differ significantly. Nevertheless, the percentage of total Hg present as methylmercury in the faeces of the antibiotic-treated animals was almost twice that in the faeces of the controls (Table 1), thus providing further evidence for the role of the gut flora in metabolizing CH$_3$HgCl.

It appears from our results therefore that members of the gastrointestinal flora can metabolize CH$_3$HgCl and convert it into a form that is absorbed from the intestine, or accumulated by tissues, less readily than CH$_3$HgCl itself.


Copper- and Zinc-Binding Protein Fractions in the Soluble Cytoplasm of Rat Tissues

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Copper and zinc occur in the soft tissues of several mammalian species associated with soluble proteins having mol.wts. of about 10000 and 35000 (Evans et al., 1970; Bremner & Marshall, 1974; Bremner, 1976). When the supernatant from rat liver is separated by gel filtration on Sephadex G-75 two corresponding fractions are obtained, but copper and zinc are also found associated with protein-containing fractions having mol.wts. of approx. 65000 and over 75000 (Bremner et al., 1973; Alfaro & Heaton, 1974). The present investigation was therefore undertaken to examine the distribution of copper and zinc among proteins in the supernatants of several rat tissues under conditions that would permit the separation of materials over a wider range of molecular weight.

Studies were conducted on weanling (50g) and adult (300–500g) Wistar albino rats of both sexes. The liver, small intestine and thigh muscle were homogenized separately in a Potter–Elvehjem homogenizer with a Teflon pestle, in ice-cold 0.075 M-potassium phosphate buffer, pH 7.0, to give 50% (w/v) homogenates. The homogenates were centrifuged at 105000g for 60 min and the supernatants stored at −20°C if they could not be fractionated immediately. A portion (4ml) of each supernatant was applied to a column (62 cm × 2.6 cm) of Sephadex G-150, and elution was carried out at 4°C with