degradation of the proteoglycan. In the rat direct filtration by the kidneys of smaller components of the heterogeneous proteoglycan may account for these urinary components. Truly macromolecular proteoglycan is fully desulphated, presumably in the liver, leading to excretion of its sulphate groups in the form of inorganic sulphate.

K. M. W. is grateful to the Science Research Council for a research studentship.


The Methylation of Mercury by the Gastro-Intestinal Contents of the Rat

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Bacteria in the sediments of lakes and rivers, and in soil, have been shown to convert inorganic mercury salts into the more toxic methylmercury and dimethylmercury, which are absorbed from the human gastrointestinal tract much more rapidly than their inorganic counterparts (Jensen & Jernelöv, 1969; Matsumura *et al.*, 1972; Beckert *et al.*, 1974; Clarkson, 1971). Abdulla *et al.* (1973) demonstrated the methylation of HgCl$_2$ in rats with jejunal blind-loops, which suggests that small amounts of methylmercury may be formed by the microbial flora of the gut. In the present communication, the ability of suspensions of rat caecal and small intestinal contents to synthesize methylmercury is demonstrated.

Table 1. *Synthesis of methylmercuric chloride by gut contents in vitro*

Suspensions of caecal or intestinal contents (0.2g/ml) were incubated for 20h at 37°C in the presence of $^{203}$HgCl$_2$ (2µg/ml). The methylmercuric chloride formed was extracted with redistilled benzene and identified by t.l.c. The results of two experiments are shown.

<table>
<thead>
<tr>
<th>Region of gut</th>
<th>Atmosphere</th>
<th>Treatment</th>
<th>Methylmercuric chloride formed (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small intestine</td>
<td>Air</td>
<td>None</td>
<td>1.2, 0.8</td>
</tr>
<tr>
<td></td>
<td>N$_2$</td>
<td>None</td>
<td>0.4, 0.2</td>
</tr>
<tr>
<td>Caecum</td>
<td>Air</td>
<td>None</td>
<td>4.8, 4.2</td>
</tr>
<tr>
<td></td>
<td>N$_2$</td>
<td>None</td>
<td>3.2, 2.8</td>
</tr>
<tr>
<td></td>
<td>Air</td>
<td>+vitamin B$_{12}$ (5µg/ml)</td>
<td>2.8, 2.8</td>
</tr>
<tr>
<td></td>
<td>Air</td>
<td>+antibiotics*</td>
<td>0.4, 1.2</td>
</tr>
<tr>
<td></td>
<td>Air</td>
<td>Autoclaved†</td>
<td>0.2, 0.0</td>
</tr>
<tr>
<td></td>
<td>Air</td>
<td>Filtered‡</td>
<td>0.2, 0.8</td>
</tr>
</tbody>
</table>

* Chloramphenicol, neomycin sulphate and tetracycline hydrochloride, each at 1mg/ml.
† The suspension of caecal contents was autoclaved [100kPa (15lb/in$^2$) for 15 min] before incubation.
‡ The suspension of caecal contents was centrifuged (10000g for 15 min) and the supernatant passed through a Millipore membrane filter, pore size 0.45µm.
$^{203}$HgCl$_2$ was incubated at a final concn. of 2$\mu$g/ml with suspensions of caecal (●) or small-intestinal (○) contents and samples were removed at the times shown and assayed for methylmercuric chloride as described in the text.

$^{203}$HgCl$_2$ was incubated for 20h at 2$\mu$g/ml final concentration, with a suspension (0.2g/ml) of caecal or small-intestinal contents from male Wistar rats. The suspensions were prepared as described by Rowland (1974) except that the medium used contained 0.1m-KH$_2$PO$_4$-NaOH buffer, pH 7.0, Bacto-peptone, Bacto-tryptone, yeast extract and D-glucose, each at 0.5 % (w/v). At the end of the incubation, the suspensions were acidified to pH1 with conc. HCl and methylmercuric chloride was extracted with redistilled benzene as described by Westöö (1966). The benzene extracts were chromatographed on plates of silica gel G by using chloroform-n-hexane (9: 1, v/v) as developer (Imura et al., 1971). The position of methylmercuric chloride was detected by spraying with 0.04% dithizone in chloroform. This zone was scraped into a vial and counted for radioactivity. The identity of methylmercuric chloride was confirmed by chromatography on plates of silica gel G developed with n-hexane-acetate (Yamada & Tonomura, 1972).

When HgCl$_2$ was incubated with caecal contents, small amounts (up to 26ng/g) of methylmercuric chloride were synthesized (Table 1). The rate of methylation of mercury was proportional to the weight of caecal contents in the incubation mixture and also depended on the initial concentration of HgCl$_2$ at concentrations below 2$\mu$g/ml.

The methylation reaction obviously requires a heat-labile component, since autoclaved caecal contents were unable to synthesize methylmercury (Table 1), so it seems likely that the process is enzyme-catalysed. Methylation of mercury was severely curtailed by filtration of the caecal suspension through a Millipore filter before incubation with HgCl$_2$, which suggests that the enzyme is associated with a particulate or cellular fraction of the gut contents. Since the presence of antibiotics (chloramphenicol, tetracycline hydrochloride and neomycin sulphate, each at 1mg/ml) also inhibited the synthesis of methylmercury (Table 1), it appears that bacteria participate in the methylation reaction. It has been shown that the presence of vitamin B$_{12}$ can enhance the methylation of HgCl$_2$ by certain bacteria (Yamada & Tonomura, 1972; Vonk & Sijpsteijn, 1973) but the vitamin had no stimulatory effect in our experiments (Table 1); however, sufficient vitamin B$_{12}$ to stimulate the reaction fully may have been already present in the caecal suspension. The atmospheric conditions did not appear to influence the rate of formation of methylmercury by caecal or intestinal contents to any great extent, since only slightly more methylmercury was formed under aerobic conditions than under anaerobic conditions.

The contents of the small intestine methylated HgCl$_2$ much more slowly than did the caecal contents (Table 1), which may be due in part to a higher rate of breakdown of methylmercury by the small intestine. The difference in methylating capacity of caecal...
and intestinal contents is also apparent from a time-course graph (Fig. 1). The amount of methylmercuric chloride produced by the caecal contents is not directly proportional to the time of incubation, but instead rises rapidly to a peak at 20–70h and then falls with further incubation. In the presence of the intestinal contents, the peak (at 48h) is sharper, and virtually no HgCl₂ is methylated at incubation times longer than 60h. It seems possible that the enzymes involved in the biosynthesis of methylmercury are unstable in vitro.

The above results provide evidence that the biosynthesis of methylmercury from HgCl₂ is possible in the intestinal tract, particularly in the caecum, and, although only small amounts may be formed, over long periods the absorption of methylmercury synthesized in the gut may contribute significantly to the body burden of mercury in man.


**Respiration Rates and Adenosine Triphosphate Synthesis in Rat Liver Mitochondria: State 4–3–4 Transition Experiments**

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In previous communications (Archbold *et al.*, 1974, 1975), we have described experiments in which the addition of a Dowex cation-exchange resin in the Na⁺, K⁺ or Ca²⁺ form (DNn, Dn, Dcn) to mitochondrial incubations has given a new insight into the nature of proton movements associated with oxidative phosphorylation. While supporting the basic chemiosmotic postulate that the primary phase of energy transduction is the translocation of protons across the inner coupling membrane, our results were not in accord with the classical presentation of the chemiosmotic hypothesis (Mitchell, 1976). In particular they showed that this presentation failed to make an essential distributive distinction between the electroneutral (charge-compensated) and electrogenic (charge-uncompensated) proton movements which are associated in experimental systems with the translocation process. Our results indicated a strictly localized electrogenic movement and received support from the growing evidence for a fixed negative charge on the outer surface of the coupling membrane; from the effect of proton translocation on this fixed charge (Azzi, 1969), and from a mathematical analysis of the influence of such a fixed charge on ion distributions and electrical potentials in the space-charge areas characteristic of such membranes (Coster, 1973).

A new equation for the protonmotive force was proposed in which all the parameters represent differentials across the membrane depletion layer:

\[ \Delta p^\circ = \Delta \psi^\circ - Z \Delta pH^\circ \]

Alternatively

\[ \Delta \mu^\circ_H = F \Delta \psi^\circ - 2.3RT \Delta pH^\circ \]