groups: one fed the steroid in citrated beef blood but not injected with tritium; one fed citrated beef blood without steroid and injected with tritium; and one fed citrated beef blood without steroid and not injected with tritium. The staging characteristics of oogenesis used in the analysis were based on those of Cummings and King (*8*).

In the day 1 labeled controls, the tritiated uridine was found in both the nucleus and cytoplasm of the nurse cells 1 hour after injection, but 6 and 24 hours after injection, it was found in the cytoplasm of the nurse cells. The pattern was similar at day 2. However, on days 3 and 4, labeling 1 hour after injection was primarily in the nucleus of the nurse cells; 6 and 24 hours after injection it was primarily in the cytoplasm, except that on day 4 no samples were taken 24 hours after injection. In flies fed 20-hydroxyecdysone, the labeling was like that in the controls except that on day 4 at 6 hours after injection the labeling was in the nucleus instead of the cytoplasm of the nurse cells. Throughout the experiment, the follicular cells of both the controls and the flies fed steroid were labeled similarly. No noticeable concentration of label was found in the yolky ooplasm of the controls.

The follicles of the treated and untreated flies developed similarly through stages 2 to 6 since the RNA was labeled first in the nucleus and later in the cytoplasm of the nurse cells. However, after day 2, the follicles in flies fed 20-hydroxyecdysone showed no further morphological changes in size or development and remained static in stage 6 (Fig. 1). In contrast, the oocyte within the ovarian follicle of the controls changed significantly from a spherical shape (follicle stage 6) to an elongated shape, which indicated that protein was synthesized in the nurse cell cytoplasm for vitellogenesis. The nurse cells degenerated, and the oocytes reached their maximum volume at day 4 of the control. The beginning development of the second follicular group in the ovaries was also observed at this time. This sequence was prevented in those flies treated with the 20-hydroxyecdysone.

The 20-hydroxyecdysone thus prevented the synthesis of the lipid materials necessary for vitellogenesis and final egg maturation. The time of activity occurred after the previtellogenic development as the egg chambers of both the treated and untreated flies grew at similar rates during this period. The egg chamber in the treated flies then abruptly stopped growth and thereby rendered the females sterile.

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Chemical Methylation of Inorganic Mercury with Methylcobalamin, a Vitamin B_{12} Analog

Abstract. *Chemical methylation of mercuric chloride with methylcobalamin has been studied. Methylated mercury was detected by gas chromatography; and analysis of the products of the reaction by thin-layer chromatography revealed that the methylation proceeded at a remarkably high rate when methylcobalamin and inorganic mercury were mixed. Dimethylmercury was an initial product of the reaction.*

Although use of organomercurial pesticides has been banned and the release of methyl- and ethylmercury into rivers and bays is restricted in Japan, relatively little attention has been paid to the risks arising from inorganic mercury pollution.

Jensen and Jernelöv have found that inorganic mercury is converted into methylmercury by some microorganisms (*1*), and that the bottom sediments of a lake and of an aquarium, as well as the homogenate of rotting fish, were capable of methylating mercury (*2*). Using an isolated strain of methanogenic bacterium (MOH), Wood *et al.* studied mercury methylation in vitro and showed that, in the presence of the crude extract of this microorganism, the methyl moiety of methylcobalamin

Table 1. Thin-layer chromatography of the reaction mixture containing methylcobalamin and varying amounts of $HgCl_2$. Methylcobalamin (2 μmole) was subjected to reaction with 1, 2, and 10 μmole of mercuric chloride, respectively, at 37°C in 1 ml of 0.2*M* potassium phosphate buffer (*p*H 7.0) in the dark. After 30 minutes, 5 hours, or 24 hours, the reaction mixture was extracted with 200 μl of benzene, and a 60-μl portion was analyzed by silica gel thin-layer chromatography with the use of solvent 1. Mercury was detected by spraying 0.05 percent dithizon-chloroform solution. The extent of the coloration of mercury dithizonate is expressed by ++ (strong), + (positive), ± (faint), and − (not detectable); D, dimethylmercury (R_F, 0.58); M, methylmercuric chloride (R_F, 0.25).

Molar ratio ($HgCl_2$/CH_3-[Co])	Detection of mercury after a reaction time of					
	30 minutes		5 hours		24 hours	
	D	M	D	M	D	M
0.5	+	−	+	−	+	−
1	+	±	+	++	±	++
5	+	++	±	++	−	++

(CH_3-[Co], a vitamin B_{12} analog) was transferred to mercury. In addition, the transfer of the methyl group occurred chemically under mild reducing conditions in the absence of the cell extract (*3*).

We now report on the kinetics of the chemical transmethylation from methylcobalamin (a known methyl donor in biological systems) to inorganic mercury under various conditions. The presence of reducing agents such as Zn · NH_4Cl (*3*), $NaBH_4$, or $SnCl_2$ · HCl in the reaction mixture interfered with the quantitative recovery of the product from the mixture. The transmethylation proceeded at an unexpectedly high rate after the methylcobalamin was mixed with inorganic mercury in neutral aqueous solution in the absence of reducing conditions.

Methylcobalamin was incubated with various amounts of mercuric chloride in phosphate buffer (*p*H 7.0) at 37°C in the dark (Table 1). The reaction mixture was extracted with benzene at termination of the incubation. A silica gel thin-layer chromatography of the benzene layer with the use of three solvent systems [solvent 1, ether–petroleum ether (30 : 70); solvent 2, acetone–*n*-hexane (15 : 85); and solvent 3, *n*-hexane–chloroform (10 : 90)] revealed that two reaction products, that is, dimethylmercury and methylmercuric chloride, were formed in different ratio, depending on the molar ratio of the reactants and the reaction times.

The initial product of this reaction is dimethylmercury, especially when equimolar or lesser amounts of mercuric chloride are used. Dimethylmercury thus synthesized seemed to be converted into methylmercuric chloride by further action of mercuric chloride (*4*).

$$2\ CH_3\text{-[Co]} + HgCl_2 \rightarrow (CH_3)_2Hg$$

$$(CH_3)_2Hg + HgCl_2 \rightarrow 2\ CH_3HgCl$$

Although more precise experiments have to be carried out for the accurate elucidation of the reaction mechanism, the scheme of the reaction described above can be tentatively proposed for the described reaction conditions. The rate of methylation reaction was estimated by quantitative gas chromatography (Fig. 1).

Because of the high volatility of dimethylmercury and because it is known to give the same retention time as methylmercuric chloride, owing to possible decomposition in the column when an electron-capture detector is used in gas chromatography (*5*), the reaction mixture was treated with hydrochloric acid (*6*) to convert the dimethylmercury formed during the reaction quantitatively into methylmercuric chloride.

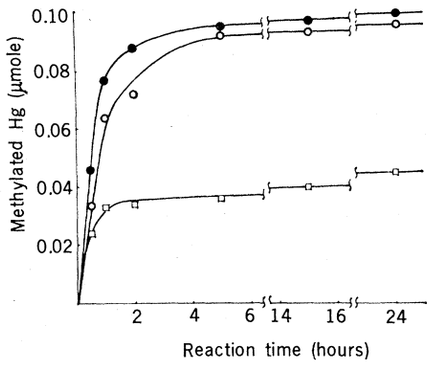

Fig. 1. Formation of methylated mercury from mercuric chloride in the presence of methylcobalamin. Reaction conditions: methylcobalamin (0.1 μmole) was treated with 0.05 (□), 0.1 (○), and 0.5 (●) μmole of mercuric chloride at 37°C in 1 ml of 0.2*M* potassium phosphate buffer (*p*H 7.0) in the dark. To the reaction mixture was added 1 ml of concentrated HCl (*9*) after the reaction, and the mixture was shaken for 90 minutes to convert dimethylmercury into methylmercuric chloride. The mixture was extracted with 1 ml of benzene after treatment with acid, and a 0.1-ml portion from the benzene extract thus obtained was diluted with 1 ml of benzene containing ethylmercuric chloride (3.3 μg/ml) as an internal standard for gas chromatography; 1 μl of the diluted solution was subjected to gas chromatography for determination of methylmercuric chloride. [The gas chromatography apparatus consisted of a Shimadzu gas chromatograph, model GC-4AIE with electron-capture detector; a glass column (0.4 by 100 cm) with 25 percent polydiethyleneglycol succinate coated on Chromosorb W AW HMDS (60 to 80 mesh); temperature, column, 160°C; injection port, 185° to 190°C; and detector, 200°C; carrier gas, 80 ml of nitrogen per minute. Under these conditions a linear calibration curve was obtained with 0 to 2.5 ng of methylmercuric chloride injected.]

The reaction proceeded at a high rate, and inorganic mercury was methylated almost quantitatively (Fig. 1).

The amount of methylcobalamin that decomposed into aquocobalamin after release of the methyl group was estimated spectrophotometrically by increase of absorbance at 352 nm, which is characteristic for aquocobalamin. After a reaction time of 5 hours, the methyl moiety was released from methylcobalamin almost quantitatively. The methyl moiety thus liberated was completely utilized for the formation of methylmercury. In the absence of mercuric chloride, methylcobalamin was quite stable and remained unchanged even after 48 hours under the same conditions as above. These results indicated that mercuric chloride was essential for the liberation of the methyl group from methylcobalamin in this reaction system.

S-Adenosylmethionine, another methyl donor in biological systems, was also treated with mercuric chloride under the same conditions as those used for methylcobalamin. The formation of methylmercury was not observed.

Our results show that highly toxic methylmercury is easily generated from inorganic mercury in the presence of methylcobalamin.

It has been reported that methylcobalamin is isolable not only from microorganisms but also from mammalian sources such as calf liver and human blood plasma (*7*). It is therefore believed to be widely distributed in nature. Among these natural sources, the methanogenic bacteria which are very numerous in sediments of rivers and in sludge of sewage beds are supposed to contain methylcobalamin in large quantities as an intermediate of methane biosynthesis (*8*).

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