Distribution of glucose and lactate in the brain parts we investigated is simmilar to that of glycogen (Table II). These findings suggest that there is more intensive glycolysis in medulla oblongata, compared with the other parts of brain.

Résumé. Le cervelet contient des quantités plus grandes d'ATP, d'ADP et de nucléotides totaux que le lobe frontal du cerveau ou la moelle cervicale. Ces différences ne sont pas trouvées avec l'AMP. Le contenu en glycogène, en glucose et en lactate est plus grand dans la moelle cervicale que dans le cervelet et dans le lobe frontal.

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Methyl Mercury Effect on Rat Liver Mitochondrial Deshydrogenases

Much attention has been paid to mercury and its organic derivates as they accumulate in the food chain[1]. Although there are many observations on biochemical alterations induced by organomercury compounds[2], the way methyl mercury exerts its toxicity on target organs is still subject to controversial interpretation: methyl-mercury reacts with sulfhydryl groups present at the active sites of several enzymes as other mercurials do[3,4]. According to SEGALL and WOOD[5], it can also catalyze the hydrolysis of a group of phospholipids.

Liver tissue has been selected because it accumulates methyl mercury and participates in the entero-hepatic reabsorption of the compound. Furthermore, recent ultrastructural foetal liver electron microscopy has shown mitochondrial swelling after low exposures which did not produce maternal neurological symptoms[6]. Therefore, besides its already recognized detoxification role, liver has a degenerative response to methyl mercury.

We have investigated the effect of methyl mercury on isolated inner membranes of rat liver mitochondria and its inhibitory action on several enzymes of the oxydative chain. Work has been focused on 3 hydroxybutyrate deshydrogenase, which requires lecithin as a cofactor[7] and has thiol residues in the active site[8]. A protective effect was obtained after a pre-incubation with the phospholipid and a sulphydryl reagent.

Material and methods. Male Wistar rats (180–200 g) were sacrificed. Livers were removed and mitochondria were extracted in 0.25 *M* sucrose. Inner membrane matrix preparation was obtained by digitonin treatment and disrupted by sonication according to the method of LEVY et al.[9]. Respiration rate and enzymatic activities: succinate deshydrogenase, 3-hydroxybutyrate deshydrogenase, cytochrome oxydase were assayed according to several methods detailed in a previous publication[10]. The protein content of the enzymatic preparation was determined by the method of LOWRY[11].

Liposomes of egg lecithin were prepared according to the method of SINGLETON et al.[12]: 2 mercaptoethanol was diluted to 0.5 *M* in phosphate buffer 0.2 *M* pH 7.4. Methyl mercuric chloride solutions in aq. 5% (v/v) ethanol were prepared just before use. The amount of ethanol added with the inhibitor has no significant effect on the activities assayed. Inner membranes were incubated for 5 min with the inhibitor before measuring the enzymatic activity.

Results and discussion. Some results of the inhibitory effect of methyl mercury on oxidase and deshydrogenase activities are shown in the Figure. A decrease in oxygen consumption is observed on both substrates. However, methyl mercury concentrations should not be considered as absolute values, since manometric operations require an incubation temperature (30 °C) at which methyl mercury vaporization is significant. Enzymatic assays show that 50% inhibition of 3 hydroxybutyrate activity is

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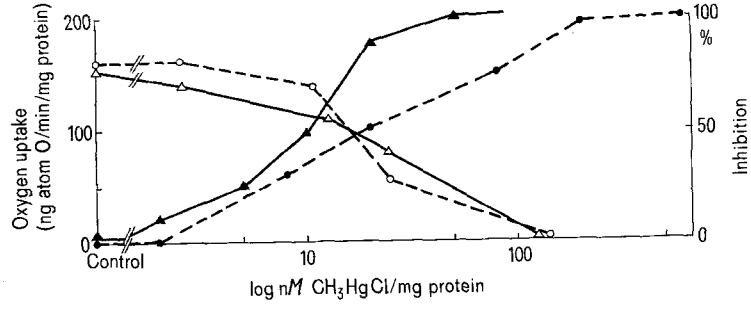

Methyl mercury effect on oxydase and deshydrogenase activities. ○—○, succinate oxydation; △—△, hydroxybutyrate oxydation; ●—●, succinate deshydrogenase activity (% inhibition); ▲—▲, D-3-hydroxybutyrate deshydrogenase activity (% inhibition).

Effect of pretreatment of the inner membrane with lecithin and mercaptoethanol on methyl mercury inhibition

Enzyme preparation	CH_3HgCl nM/mg protein	D-3-hydroxybutyrate activity nM substrat/min/mg protein
Inner membrane	–	460
Inner membrane	10	240
Inner membrane + lecithin	10	300
Inner membrane + 2 mercaptoethanol	10	420
Inner membrane + lecithin + 2 mercaptoethanol	10	460

obtained for methyl mercury concentration of 10 nM/mg protein. The enzyme is more sensitive than succinate deshydrogenase (20 nM/mg protein). Cytochrome oxydase is not inhibited in this range of concentrations, (17% inhibition for 4 mM/mg protein). The subsequent addition of pure lecithin and 2 mercaptoethanol (0.67 mM/mg protein) in the medium could not reverse the activity of 3 hydroxybutyrate deshydrogenase incubated with 10 nM/mg protein inhibitor. The effect of a pretreatment of the inner mitochondrial matrix with pure lecithin (0.67 mg/mg protein) and 2 mercaptoethanol (0.22 mM/mg protein) on the inhibitory action of methyl mercury on 3 hydroxybutyrate deshydrogenase is reproduced in the Table. The enzymatic activity of the control is not significantly stimulated by the addition of the reagents.

D-3-hydroxybutyrate deshydrogenase activity is partially protected by pretreatment with lecithin, however higher concentrations of the phospholipid do not produce a better action. Addition of an-SH reagent associated with the phospholipid prevents an inhibitory action of methyl mercury. The inhibition of methyl mercury on D-3-hydroxybutyrate deshydrogenase seems to imply both phospholipids and thiol groups as possible sites of action. Further studies are under way to elucidate the possible interaction of methyl mercury with lecithin and the relative stability of the binding to sulfhydryl groups of the enzyme[13].

Résumé. L'inhibition par le méthyl mercure de certains enzymes de la membrane interne de mitochondries de foie de rat, montre que la D-3-hydroxybutyrate deshydrogenase est très sensible. L'enzyme pré-incubée avec de la lecithine et du 2 mercaptoethanol est protégé.

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Demonstration of Methanol Dehydrogenase in Methanol Assimilating Yeasts

Methanol oxidation in bacteria has been investigated by several workers[1–3], and no evidence of a pyridine nucleotide-linked methanol dehydrogenase has been reported. OGATA et al.[4] described a methanol oxidation in yeast via an oxidase, but they did not detect a pyridine nucleotide-linked dehydrogenase. Their data suggests that a flavine nucleotide cofactor was involved in the oxidation. The present note describes the oxidation of methanol by cellular extracts of *Pichia pinus* and *Kloeckera sp.* 2201 utilizing pyridine nucleotide (NAD) or dichlorophenol indophenol (DCPIP) as electron acceptors.

Materials and methods. A 5-day-old slant culture of *Pichia pinus* NRRL YB-4025 and *Kloeckera sp.* 2201[4] was inoculated into respective flasks containing a sterile mineral salts medium composed of 1% NH_4NO_3, 0.1% K_2HPO_4, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, vitamin mixture[4] and 2% methanol. Cultures were propagated for 4 days on a rotary shaker at 25C (250 RPM with 2″ throw). Cells were centrifuged and washed twice with 0.01 M potassium phosphate buffer, pH 7.0. Washed cells were resuspended in buffer and sonicated at 4 °C in ice-bath for 5 min (ten 30 sec intervals) with Branson Ultra Sonified (Branson Instruments, Conn. USA). Cell debris was removed by centrifugation at 20,000 $\times g$ for 10 min. The clear supernatant was used for enzyme assay.

Methanol dehydrogenase activity was assayed spectrophotometrically using a Zeiss Gilford instrument with an automatic chart recorder. The assay system (NAD-linked) contained potassium phosphate or *Tris*-HCl buffer, pH 7.0 and 8.5, 70 µmoles, NAD-1 µmole, enzyme (1 mg protein), and substrate, 20 µmoles. NAD reduction was measured at 340 nm. Specific activity was expressed as nanomoles of NAD or DCPIP[3] reduced per min per mg protein.

Results and discussion. Cell-free extracts of both yeast cultures grown on a methanol containing medium oxidized methanol via NAD-linked methanol dehydrogenase. The Table shows the activity of methanol dehydrogenase at pH 7.0 and at pH 8.5. In the case of *Pichia pinus*, the methanol oxidation rate utilizing NAD or DCPIP at pH 8.5 was greater than that at pH 7.0, whereas, in *Kloeckera sp.* 2201, NAD-linked activity was found to be greater at pH 7.0. OGATA et al.[4] described methanol oxidation via methanol oxidase in *Kloeckera sp.* 2201, but no dehydrogenase activity using NAD, NADP, or DCPIP was reported. Lack of NAD or DCPIP-linked activity in the *Kloeckera sp.* 2201 cell-extract of OGATA

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