

## The biochemistry of methanol poisoning

### I. Phosphorylation coupled to the mitochondrial oxidation of formaldehyde

It is now generally accepted that the retinal degeneration associated with methanol ingestion is caused by its metabolic derivative, formaldehyde, and that only primates exhibit this ocular toxicity<sup>1</sup>. During the course of comparative studies on the metabolism of formaldehyde by monkey and beef liver and retinal preparations, it was observed that liver mitochondria are able to catalyze the oxidation of formaldehyde and that this oxidation is accompanied by the esterification of inorganic phosphate. Previously, BERNHEIM<sup>2</sup> had described the oxidation of formaldehyde by rat-liver homogenates and WALKENSTEIN AND WEINHOUSE<sup>3</sup> had demonstrated that various aldehydes, including formaldehyde, are oxidized by rat-liver mitochondria, but none of these investigators studied concomitant phosphate esterification. This communication describes an examination of phosphorylation efficiencies during the oxidation of formaldehyde by mitochondrial fractions of liver and retina.

Thrice-washed mitochondria were isolated from fresh liver tissue of the monkey (*Macaca mulatta*) and the cow using ice-cold 0.25 *M* sucrose, according to the technique of SCHNEIDER AND HOGEBOOM<sup>4</sup>. Beef and monkey retinal mitochondria were isolated in 0.25 *M* sucrose as described by KINI AND COOPER<sup>5</sup>.

In accord with the results of earlier workers, we observed that a concentration of formaldehyde higher than 1 *mM* inhibited the respiration of liver mitochondria. However, at concentrations of 1 *mM* or less, incubation of formaldehyde with liver mitochondria resulted in a rapid oxidation of the compound, with a concomitant esterification of inorganic phosphate, giving a P/O ratio of approx. 2 (Table I). From the figures indicating the amount of formaldehyde converted to CO<sub>2</sub>, it is apparent that most of the added formaldehyde was oxidized only to formate.

This esterification of inorganic phosphate coupled to formaldehyde oxidation was sensitive to the presence of inhibitors or uncoupling agents such as antimycin A and dinitrophenol. The addition of 1 *mM* amobarbital, an inhibitor at the DPNH-cytochrome *c* reductase level<sup>6</sup>, reduced both oxygen uptake and the P/O ratio by about 50 %.

Both DPN and GSH are coenzymes for the formaldehyde dehydrogenase described by STRITTMATTER AND BALL<sup>7</sup>. However, addition of these compounds, either singly or in combination, to liver mitochondria had no significant effect on either the oxygen uptake or the esterification of phosphate.

The electron-transport chain involved in formaldehyde oxidation by liver mitochondria is not clear. From the figures on P/O ratios obtained experimentally, which point to a value of 3, and from the sensitivity of the system to amobarbital, it would appear that the enzyme system involved may be the particulate DPN-linked formaldehyde dehydrogenase. Although the apparent lack of stimulation upon addition of DPN and GSH might appear to oppose this hypothesis, it is known that liver mitochondria can carry DPN-linked oxidations without the necessity of adding coenzyme<sup>8</sup>.

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Abbreviations: DPN, diphosphopyridine nucleotide; GSH, reduced glutathione; ATP, adenosine triphosphate; DPNH, reduced diphosphopyridine nucleotide.

Mitochondria isolated from the liver of both monkey and cow exhibited the same ability to oxidize formaldehyde through a phosphorylating pathway (Table I). However, beef retinal mitochondria, in contrast to liver mitochondria, were unable

TABLE I

## OXIDATION OF FORMALDEHYDE BY LIVER MITOCHONDRIA

Each Warburg flask contained the following basic medium in a final volume of 3.0 ml: sodium phosphate buffer (pH, 7.4), 0.01 *M*; KF, 0.01 *M*; ATP, 0.001 *M*; H<sup>14</sup>CHO, 0.001 *M* (91,000 counts/min/ $\mu$ mole); MgCl<sub>2</sub>, 0.067 *M*; KCl, 0.033 *M*; cytochrome *c*,  $1.3 \cdot 10^{-5}$  *M*; D-glucose, 0.02 *M*; yeast hexokinase, 7.1 units, assayed by the procedure of CRANE AND SOLS<sup>9</sup>; suspension of liver mitochondria containing 6.4 mg protein, estimated according to LOWRY *et al.*<sup>10</sup>. Incubation temperature, 25°. The reaction was stopped after 45 min by addition of 1.0 ml 10 % trichloroacetic acid. Phosphate uptake was measured by the method of FISKE AND SUBBAROW<sup>11</sup>. Formaldehyde disappearance was estimated by the method of TANENBAUM AND BRICKER<sup>12</sup>. Labeled CO<sub>2</sub> in the alkali of the center well was determined by a modification of the method of VILLEE AND HASTINGS<sup>13</sup>. Endogenous oxygen uptake amounted to 1.1  $\mu$ atoms and was not subtracted from the figures quoted below.

Special additions	Oxygen uptake ( $\mu$ atoms)	Phosphate esterified ( $\mu$ moles)	P/O ratio	HCHO utilized ( $\mu$ moles)	HCHO oxidized to CO <sub>2</sub> ( $\mu$ moles)
<i>Monkey liver</i>					
None	4.1	7.9	1.95	2.42	0.0083
Antimycin A (1 $\mu$ g)	0	0	0	0	0.0076
Dinitrophenol (0.1 mM)	2.60	— 1.4	0	2.33	0.0102
Amobarbital (1 mM)	1.91	1.9	1.0	2.25	0.0072
GSH (0.67 mM)	2.75	5.9	2.14	2.14	0.0100
GSH (0.67 mM) + DPN (0.67 mM)	3.6	7.9	2.17	2.17	0.0092
<i>Beef liver</i>					
None	3.75	5.44	1.44	2.35	0.0142

TABLE II

## OXIDATION OF FORMALDEHYDE BY RETINAL MITOCHONDRIA

The experimental conditions were identical to those given in Table I, except that variable amounts of H<sup>14</sup>CHO (91,000 counts/min/ $\mu$ mole) were added to the medium containing either beef retinal mitochondria (3.4 mg protein) or monkey retinal mitochondria (2.1 mg protein).

Additions	Oxygen uptake ( $\mu$ atoms)	Phosphate esterified ( $\mu$ moles)	P/O	HCHO utilized ( $\mu$ moles)
<i>Beef retina</i>				
	0	0	0	0.00
HCHO (0.1 mM)				
HCHO (0.2 mM)	0	0	0	— 0.01
HCHO (0.5 mM)	0	0	0	— 0.05
HCHO (1 mM)	0.25	0	0	— 0.21
HCHO (1 mM) + DPN (0.67 mM)	0.38	0	0	0.063
HCHO (1 mM) + GSH (0.67 mM)	0.38	0	0	0.25
HCHO (1 mM) + DPNH (0.67 mM) + GSH (0.67 mM)	0.88	0	0	0.94
<i>Monkey retina</i>				
HCHO (1 mM) + DPN (0.67 mM) + GSH (0.67 mM)	0	0	0	1.04
Pyruvate (10 mM) + DPN (0.67 mM) + fumarate (0.67 mM)	5.46	13.2	2.42	—

to oxidize exogenous formaldehyde, added at concentrations ranging from 0.1 to 1 mM (Table II). The same preparations, with pyruvate as substrate and fumarate as "sparker", gave a P/O ratio approaching 3, while with succinate as substrate the ratio was 2. Although it could be shown by sensitive isotopic methods that very small amounts of added formaldehyde are oxidized to CO<sub>2</sub>, conventional manometric and chemical methods failed to indicate any oxidation of formaldehyde; indeed, after incubation, all the formaldehyde could be recovered quantitatively, within the limits of experimental error.

In order to obtain maximal oxidation rates with a substrate such as pyruvate, DPN must be added to the mitochondria of retina, as with those of brain and certain tumors<sup>5,9</sup>. The addition of DPN or GSH had a small and variable effect on formaldehyde utilization, but a combination of these two compounds definitely stimulated the metabolism of formaldehyde. However, the formaldehyde was oxidized by a non-phosphorylating pathway, since no esterification of inorganic phosphate could be demonstrated (Table II).

To determine whether the retina of a primate exhibited any difference from that of the cow in its capacity to utilize formaldehyde, similar experiments were carried out with monkey retina. In the isolation of monkey retinal mitochondria, no attempt was made to exclude choroidal tissues. As seen from Table II, these mitochondria gave essentially the same results as was obtained with those of the cow.

In a forthcoming paper<sup>5</sup> it will be shown that formaldehyde is an extremely potent uncoupler of oxidative phosphorylation, in both beef and monkey retinal mitochondria. Thus, it may be inferred that the ocular toxicity resulting from methanol ingestion, which is noted only in primates, is not based on a difference between primates and non-primates with respect to the retinal metabolism of formaldehyde. Rather, the ocular toxicity may reflect a difference in the rate of production of formaldehyde from methanol and perhaps its subsequent elimination.

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