THE BIOCHEMISTRY OF METHANOL POISONING

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Enough evidence has accumulated from many laboratories, including ours, to show clearly that the toxic agent responsible for the blindness in methanol poisoning is not methanol *per se*, but its oxidation product, formaldehyde. It is also now fairly well established that the primary site of damage is in the retina, rather than in other portions of the optic system.

Our initial working hypothesis related this problem to energetics, i.e., it was postulated that formaldehyde interferes with the generation of adenosine triphosphate (ATP) and that the resultant deficiency in ATP ultimately causes retinal degeneration leading to blindness. After working for two years on this problem, we have not encountered any results which would negate this thesis; indeed, a considerable amount of evidence in support of it has been adduced.

In the usual clinical cases of methanol poisoning, the concentration of methanol in the body tissues may be calculated to approximate 0·04 molar; thus, the maximum concentration of free formaldehyde that could be expected in the retina would be 0·04 molar. In all the experiments carried out *in vitro*, it has been clear that enzymatic inhibitions occur with formaldehyde at concentrations far below this figure, e.g., in the range of 0·0005 to 0·001 molar. In experiments *in vivo*, in which formaldehyde was injected directly into the eye, it was necessary (for reasons discussed below) to use a larger concentration of formaldehyde, 0·01–0·02 molar, but these concentrations are still reasonable pharmacologically.

Some of the results obtained have been summarized in Table I. Other than experiments involving oxidative phosphorylation or electron transport, which were performed with bovine retinal mitochondria, the findings presented in this table were obtained with intact bovine retina. As shown in the table, the very high anaerobic glycolysis of the retina is quite sensitive to formaldehyde; thus, an inhibition of 50% occurs at a concentration of formaldehyde of 0·0005 molar. It is difficult to assess the significance of this finding, however, since the retina normally is

dependent upon a continuous supply of oxygen and it might be argued that, *in vivo*, anaerobic glycolysis may not operate. In marked contrast to the effects of formaldehyde on anaerobic glycolysis, this agent has either no effect or even stimulates aerobic glycolysis slightly. Respiration, as measured by either oxygen-uptake or the conversion of ^{14}C-glucose to $^{14}CO_2$, is not very sensitive to formaldehyde. Similarly, electron transport in retinal mitochondrial preparations is scarcely affected by formaldehyde at low concentrations. Accordingly, the most significant

TABLE I

Enzymatic activities	Molar concentration of formaldehyde which causes 50% inhibition
Anaerobic glycolysis	0.5×10^{-3}
Respiration	3×10^{-3}
Incorporation of ^{32}P-phosphate into phospholipids	1×10^{-3}
Oxidative phosphorylation	$0.5\text{-}1 \times 10^{-3}$
Electron transport	3×10^{-3}

finding obtained is the marked sensitivity of oxidative phosphorylation of the retina to this toxic agent. Formaldehyde, at a concentration of 0·0005–0·001 molar, diminished the efficiency of oxidative phosphorylation by about 50%, when pyruvate, α-ketoglutarate and succinate were used as substrates. By way of control, acetaldehyde had no effects, even when used at a concentration as high as 0·005 molar. Since there is no experimental approach to the direct measurement of oxidative phosphorylation in *intact* mammalian cells, the indirect approach which we have used in whole retina has been the uptake of ^{32}P-phosphate into phospholipids, a process known to be dependent on the maintenance of conditions optimal for oxidative phosphorylation. This device has permitted the demonstration that formaldehyde, at a level of 0·001 molar, inhibits by 50% the incorporation of ^{32}P-phosphate into phospholipids. To test whether this depression in the labeling of phospholipids can occur *in vivo*, formaldehyde and ^{32}P-phosphate have been injected intraocularly into rabbits, in order to achieve a final concentration of formaldehyde of approximately 0·01 molar; when the rabbits were sacrificed 24 hr later, and the retinas were removed and assayed for ^{32}P-phospholipids, an inhibition to the extent of 50% in the incorporation of the isotope was observed, as compared to a control eye injected only with ^{32}P-phosphate.

Correlative studies also were performed in order to assess the effect of the inoculation of formaldehyde on the histology of the rabbit retina. The toxic agent, at concentrations of 0·02 molar or higher, caused degenerative changes and loss of ganglion cells, cyst formation in inner

nuclear and inner plexiform layers, blurring of the layer of rods and cones, and swelling and edema of the optic nerve fibers. These histological changes, which could be seen on occasion with formaldehyde in a concentration of 0·01 molar, are generally similar to those changes seen in retinal sections of cases of human methanol poisoning which were terminated by death.

In these experiments *in vivo*, it was observed that the injection of methanol, sodium formate or acetaldehyde, when tested in concentrations as high as 0·05 molar, had no effect either on the incorporation of ^{32}P-phosphate into phospholipids or on the histology of the retina. An explanation for the higher concentrations of formaldehyde required *in vivo*, as compared to those which were found necessary *in vitro*, may be that this very reactive agent is bound to many substances within the eye, and that the actual concentration in the retina is considerably lower than that estimated without taking such a factor into consideration. Furthermore, it is possible that some of the formaldehyde injected into the eye is eliminated, either by diffusion or enzymatic oxidation, with a resultant diminished concentration in the retina.

Another finding of interest was related to the fact although formaldehyde uncouples oxidative phosphorylation in retinal mitochondria, as mentioned above, in liver mitochondria formaldehyde, in concentrations up to 0·001 molar, is a substrate for oxidative phosphorylation and gives a P/O ratio which is close to 2.

With respect to the enzyme system in the body which catalyzes the oxidation of methanol to formaldehyde there are many conflicting reports in the literature. Some investigators have stated that the catalase-peroxide system is the responsible agent, while others have maintained that alcohol dehydrogenase is involved. An attempt to resolve this problem has involved the use of a crude homogenate of monkey liver for the oxidation of methanol, with purification of the enzyme system concerned. Monkey tissue was used in these experiments because the signs of experimental poisoning with methanol in the monkey parallel those seen in human subjects. This approach soon indicated that alcohol dehydrogenase is the responsible enzyme system. The ratio of activity, with either ethanol or methanol as the substrate, did not change as the crude homogenate of liver was carried through procedures which led to a 90-fold purification of the activity. In addition, enzyme activity with both substrates was inhibited to the same degree by either *p*-chloromercuribenzoate or o-phenanthroline. Finally, the enzyme exhibited only one peak in the ultracentrifuge. When methanol was administered to monkeys and the rate of disappearance of the alcohol from the plasma was followed, it was calculated that, without even considering the elimination of methanol from the lungs or its metabolism by extrahepatic tissues, there is sufficient alcohol dehydrogenase in the liver

to account for the disappearance of methanol *in vivo*. Also, it was found that alcohol dehydrogenase prepared from human liver effects the oxidation of methanol to formaldehyde; thus, this observation strongly supports the concept that the physiological mechanism responsible for the formation of formaldehyde from methanol involves catalysis by alcohol dehydrogenase. However, it must be pointed out that there are at least six different enzyme systems in the body which are capable of metabolizing formaldehyde, and it cannot be concluded from these studies that the formaldehyde which causes the blindness comes from the oxidation of methanol by the liver; thus, formaldehyde formed in the liver may not reach the retina. Since alcohol dehydrogenase, the identity of which with retinene reductase is now well established, is present in retinal extracts, it is suggested that it is the formaldehyde formed *in situ* which causes ocular toxicity.

In summary, it is belived that the ingestion of methanol results in a uniform distribution of the alcohol in the various tissues of the body, including the retina. In the retina, the methanol which is oxidized to formaldehyde uncouples oxidative phosphorylation and perhaps inhibits anaerobic glycolysis, the end result being a deficiency of ATP which probably is required for visual processes; this deprivation of ATP within the retinal cells, even though temporary in nature, could lead to blindness.

With respect to the species differences observed in methanol poisoning, the biochemical studies have not revealed any differences between the retinas of primates and those of non-primate species. Accordingly, it is suggested that the blindness caused by poisoning with methanol is a disease which is observed only in primates primarily because this species either synthesizes or destroys formaldehyde at rates leading to a prolonged accumulation of the toxic agent in the retina. An alternative explanation might be that the morphology of the primate eye differs markedly from that of non-primate species in such manner that the retinal cells of primates are more freely available to formaldehyde. The answer to this peculiar species difference must await further studies with primate material.