A note on the stability of conjugated diene absorption of rat liver microsomal lipids after carbon tetrachloride poisoning

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SUMMARY Liver microsomal lipid peroxidation has been observed in fatal human CCl_4 poisoning, in rats with fatty livers induced by CCl_4 or by yellow phosphorus, and in mice poisoned with 1,1,2,2-tetrachloroethane. These observations suggest the possibility that other instances of toxic liver injury may involve lipid peroxidation. Cases of acute, fatal, toxic liver injury (e.g., from halothane anesthesia) are not likely to occur at or near laboratories equipped to determine whether any lipid peroxidation might have taken place. The data presented indicate that rat livers may be stored frozen for at least 7 days with no demonstrable diminution in CCl_4-induced conjugated diene absorption of liver microsomal lipids.

SUPPLEMENTARY KEY WORDS liver injury · lipid peroxidation

The occurrence in vivo of peroxidative decomposition of hepatic microsomal lipids can be detected by application of techniques of tissue homogenization, differential centrifugation, lipid extraction, and ultraviolet spectroscopy (1). The method depends on the generation of conjugated dienes in lipids containing polyenoic fatty acids during the process of peroxidative decomposition. Such conjugated dienes are not present in normal animals. They appear in lung lipids after exposure of rats to nitrogen dioxide (2) and after exposure of mice to ozone (3) at ambient concentrations of these gases equivalent to those present in Los Angeles smog. Conjugated dienes appear very rapidly in liver microsomal lipids after carbon tetrachloride poisoning (4). They are not a nonspecific by-product of necrobiological processes (5). Conjugated dienes have been detected in liver lipids in fatal human carbon tetrachloride poisoning (5), in rats given yellow phosphorous (6), and in mice with fatty livers induced by administration of 1,1,2,2-tetrachloroethane (7). Hepatic microsomal lipid peroxidation does not occur in choline deficiency fatty liver (8).

As is well known, liver injury can occur in man from action of a variety of poisons and industrial solvents, from complex organic compounds of plant and microbiological origin, and from drug sensitivity reactions (9). Although infrequent, liver injury from halothane anesthesia can occur. It would be of general interest to determine in cases of acute fatal liver injury in man whether or not peroxidative decomposition of liver lipids is involved in the mechanism of action of the causative toxic agent. However, cases of such fatal liver injury are not likely to occur at or near laboratories equipped to determine whether any liver lipid peroxidation might have taken place. The point of this note is to indicate conditions which would permit storage and shipping of liver tissue prior to ultimate analysis.

Male Sprague-Dawley rats, weighing 140–200 g, were used. After an overnight fast, carbon tetrachloride (5 ml/kg of body weight) was administered as a mixture with mineral oil (CCl_4–mineral oil 2:1, v/v) by stomach tube. Rats serving as controls received only mineral oil. 45 min later the rats were killed by decapitation and the hepatic microsomal lipids were examined for conjugated diene absorption by methods previously described (1, 4, 10). Samples of the same livers (2 g in weight) were preserved immediately in 0.25 M sucrose, 0.003 M EDTA, pH 7.4, and were kept frozen just below 0°C for 7 days, after which they were homogenized and subjected to differential centrifugation to obtain the microsome fraction. The microsomal lipids were extracted and analyzed for conjugated dienes as in the samples of fresh liver. The difference spectrum, CCl_4-poisoned minus controls, for the livers analyzed immediately was nearly identical with the corresponding difference spectrum determined 7 days later (Fig. 1). An absorption maximum near 235 nm is indicative of conjugated dienes. The slight increase in absorption near 280 nm after 7 days storage is due to conjugated trienes. These data indicate that frozen livers can be stored up to 7 days with confidence that subsequent analysis is fully capable of revealing whether microsomal lipid peroxidation has taken place.

In another experiment, in order to simulate autopsy conditions in practice, the killed rats were left at room temperature (about 25°C) for 2 hr. Conjugated diene absorption of microsomal lipids was again determined immediately and after 7 days of freezing in the sucrose–EDTA suspension medium. In confirmation of earlier work (5), mere retention of killed normal animals for 2 hr at room temperature did not result in any detectable microsomal lipid peroxidation. Furthermore, in confirmation of the data shown in the figure, the CCl_4-induced difference spectrum when determined immediately after the 2-hr delay at room temperature was virtually identical with the difference spectrum obtained after a further 7 days of storage while frozen. After 14 days of storage of whole liver samples below 0°C, the results were variable and were considered by us to be unreliable.

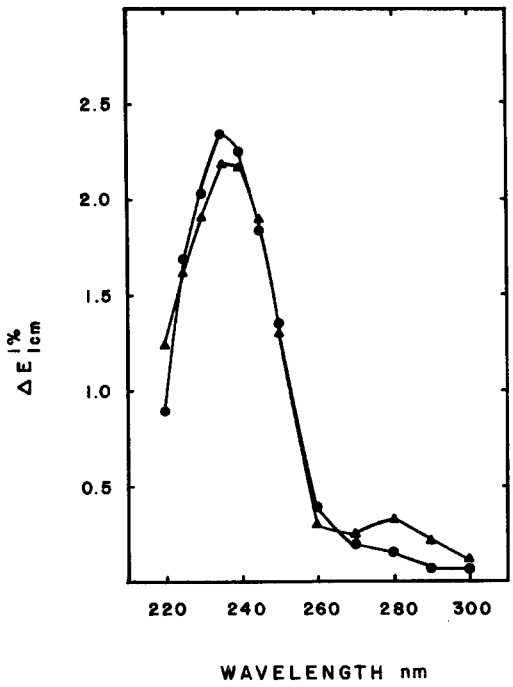

FIG. 1. Difference spectra obtained by ultraviolet spectrophotometric analysis of rat liver microsomal lipids after CCl_4 poisoning: effect of freezing and storage of the livers for 7 days. ●—●—●, livers analyzed immediately; ▲—▲—▲, livers analyzed after freezing and storage. Note: The extracted microsomal lipids were dissolved in cyclohexane for UV spectrophotometric analysis at a concentration of approximately 1 mg of lipid/ml. Immediately following UV scanning, the exact lipid concentration was determined by the method of Chiang, Gessert, and Lowry (11). The raw UV absorbance data was then rationalized to a uniform base concentration of 1 mg of lipid/ml of cyclohexane.

In practice, abnormal lipid conjugated dienes are detected by the difference spectrum obtained when the end-absorption of normal liver microsomal lipids is subtracted from the absorption values given by peroxidized lipids (1, 10). In the single study of human carbon tetrachloride poisoning on record (5), the absorption spectrum for normal human liver lipids was obtained from the liver of a traffic fatality. If lipid peroxidation is suspected, a suitable lipid sample serving as the control would have to be obtained from human liver with no history or sign of disease. Alternatively, absorption spectra for a number of normal human livers could be accumulated and averaged. Such an average spectrum could then serve as a standard of comparison.

In summary, rat livers kept at room temperature up to 2 hr, or even at body temperature up to 4 hr postmortem (5), will not exhibit any evidence of microsomal lipid peroxidation. On the other hand, once initiated by carbon tetrachloride poisoning, liver microsomal lipid peroxidation as evidenced by the appearance of conjugated dienes is fully manifested after at least 7 days of storage of frozen liver samples. The significance of these observations is that they establish realistic boundary conditions for postmortem handling of human liver tissue preparatory to ultimate work-up and analysis for possible evidence of peroxidative decomposition of lipids.

We thank Mr. Alfred Budka for expert technical assistance.

This work was supported by grant no. 5-R01-AM01489 from the National Institutes of Health, U.S. Public Health Service.

Manuscript received 21 May 1971; accepted 8 July 1971.

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