Effect of Carbon Tetrachloride Inhalation on Rat Serum Enzymes. (23680)

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Ball and Kay(1) showed that exposure of rats to carbon tetrachloride vapor results in a decrease in serum esterase activity. Subcutaneous injection of carbon tetrachloride into rats was found by Affonso *et al.*(2) to produce an elevation in serum xanthine oxidase activity. Increases in serum glutamic-oxaloacetic-transaminase (GOT) have been reported in humans following severe accidental exposure to carbon tetrachloride(3).

Since these investigations were carried out under widely varying conditions, it seemed advisable to study these 3 enzymes simultaneously under controlled conditions of exposure to carbon tetrachloride vapor. This preliminary report deals with the effect of acute inhalation of varying concentrations of carbon tetrachloride on rat serum esterase, xanthine oxidase, and glutamic-oxaloacetic-transaminase (GOT).

Methods. Male rats of the Sprague-Dawley strain, 150-200 g in weight, were used in this investigation. The animals were maintained on an *ad libitum* diet of Purina Laboratory Chow. The animals were exposed to known concentrations of carbon tetrachloride vapor using a dynamic exposure chamber, according to the procedure described by Ball and Kay (1). Chamber concentrations of carbon tetrachloride were checked during the course of the experiment by a modification of the method of Peterson *et al.*(4), and were found to be within $\pm$ 10% of calculated values. Three concentrations were selected for study: 40 rats were exposed to 1500 ppm of CCl_4 vapor, 40 rats to 1000 ppm, and 40 rats to 250 ppm. In this series of acute experiments, exposure was limited to a single 4 hour period. Immediately following the exposure, 4 rats in each group were killed by exsanguination, and enzyme activities determined on the serum from each animal. On each subsequent day for a period of 9 days, 4 additional animals from each group were sacrificed. Thirty unexposed rats (150-200 g in weight) were used for the determination of control levels of these enzymes in rat serum. *Serum esterase activity* was determined by the Gomori colorimetric technic(5), xanthine oxidase activity by the method of Westerfeld *et al.*(6), and glutamic-oxaloacetic-transaminase (GOT) activity by the procedure of Steinberg *et al.*(7).

Results. Control values and the standard deviations for the activity of these enzyme systems in rat serum were: for transaminase 171 $\pm$ 41 units/ml serum at 25°C; for xanthine oxidase, 191 $\pm$ 45 mg xanthine/ml serum/hr; and for esterase, 8.0 $\pm$ 1.8 mg phenol/ml serum/hr.

Fig. 1A shows the changes in serum enzyme activity resulting from exposure of the rats to 1500 ppm of carbon tetrachloride vapor. In this figure, the control value is taken as 100%. Changes in enzyme activity resulting from the exposure are expressed as percentage of the control value. It can be seen from Fig. 1A that the most striking change occurred in serum GOT activity. In 24 hours, the activity of this enzyme was 765% of control levels. On the second day following exposure, GOT activity was 570% of control values. By the fourth day this value had returned to the control level.

Xanthine oxidase activity (Fig. 1A) was also increased as a result of exposure to 1500 ppm of carbon tetrachloride. This effect is greatest on the second day following exposure, when activity was 265% of control levels. Xanthine oxidase levels had returned to control values within 4 days following the exposure.

Serum esterase activity (Fig. 1A) decreased as a result of the 4 hr exposure to carbon tetrachloride. On the second day following exposure, serum esterase values were only 54% of control levels. Within 4 days, this value had returned to essentially the control level.

Fig. 1B shows the results obtained after the exposure of rats to 1000 ppm of carbon tetrachloride. Changes in enzymatic activity were

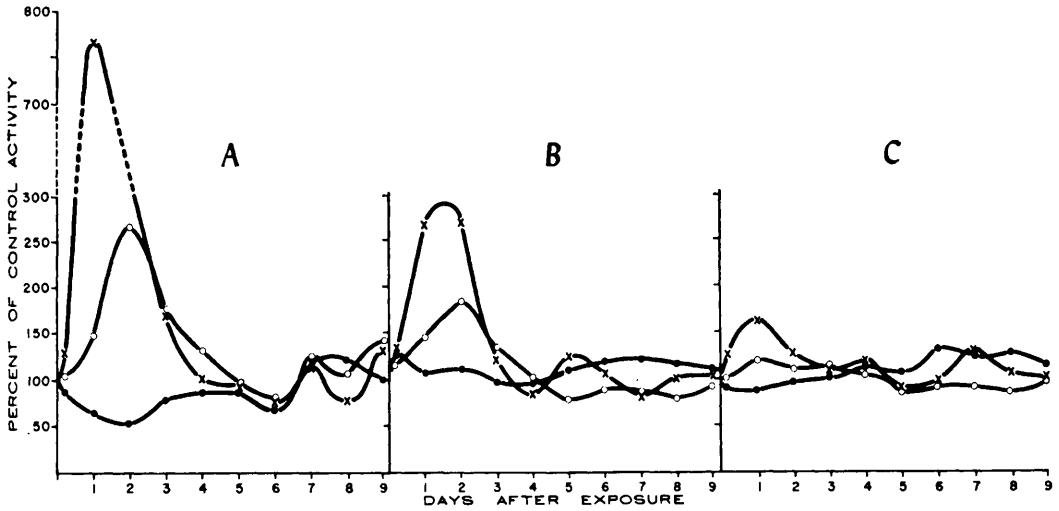

FIG. 1. The effect of a single 4-hour inhalation of carbon tetrachloride vapor on rat serum esterase (●—●), xanthine oxidase (○—○), and glutamic oxaloacetic-transaminase (GOT) (×—×). 1500 ppm in air (A), 1000 ppm in air (B), 250 ppm in air (C).

less marked at this concentration than at 1500 ppm. Serum GOT reached its peak activity (288% of normal) on the second day following exposure. By the third day this value had returned to control levels. Xanthine oxidase activity rose to 186% of control levels on the second day following exposure and returned to control levels by the fourth day. Serum esterase activity was apparently unaffected by a single 4 hour exposure at this concentration.

Fig. 1C shows the results obtained after a 4 hr exposure of rats to 250 ppm of carbon tetrachloride. The only enzyme significantly affected by this concentration of vapor was serum GOT, whose activity rose to 163% of control levels within 24 hours. Serum xanthine oxidase and esterase activities were essentially unaltered following this level of exposure.

Discussion. Exposure of male rats to 1500 ppm of carbon tetrachloride vapor brought about increases in serum glutamic-oxaloacetic-transaminase (GOT) and xanthine oxidase activities and a decrease in serum esterase activity. Serum GOT appears to be the most sensitive in its response, showing a marked rise in activity following a 4 hr exposure of rats to 250 ppm of carbon tetrachloride vapor. These studies are based on a single acute exposure and do not necessarily reflect the response to chronic exposure at low vapor concentrations. Ball and Kay(1) found that lowered serum esterase activities tended to return to control levels even though the animals were exposed daily to low concentrations of carbon tetrachloride vapor. The effect of repeated low concentrations of carbon tetrachloride vapor on serum GOT and xanthine oxidase activity will be reported later.

It remains to be demonstrated whether any or all of these enzymatic changes are directly related to the production of liver damage. Bruns and Neuhaus(8) have shown that, in mice, following the intraperitoneal injection of carbon tetrachloride, increases in serum aldolase and phosphohexose isomerase activity parallel decreases in the activity of these enzymes in the liver. The authors(8) suggest that injured liver cells may lose enzymes to the serum. Affonso *et al.*(2) found an increased xanthine oxidase activity in both liver and serum following the subcutaneous injection of carbon tetrachloride into rats. These workers(2) postulated that this increase might be due to the release of enzyme from lipoprotein.

Although either hypothesis could explain the increased activity of serum GOT and xanthine oxidase following carbon tetrachloride exposure, the decrease in serum esterase activity cannot be attributed to either of these mechanisms.

The practical advantage of serum enzyme

determinations as a sensitive measure of the biological response to inhalation of toxic vapors encourages further investigation into the nature, extent, and significance of these enzyme changes.

Summary. Serum esterase, xanthine oxidase, and glutamic-oxaloacetic-transaminase (GOT) activities were followed for 9 days after a single 4 hr exposure of male rats to carbon tetrachloride vapors. 1500 ppm of carbon tetrachloride in air produced marked changes in all 3 enzyme systems. 1000 ppm affected only serum GOT and xanthine oxidase activities. At 250 ppm, only serum GOT activity was significantly affected.

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Bentonite Flocculation Test for Rheumatoid Arthritis.* (23681)

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The various serological tests for rheumatoid arthritis have attracted increasing attention and interest in recent years, largely because they have been widely recognized as useful diagnostic measures. As an objective diagnostic criterion, the test is helpful in segregating an homogeneous type of rheumatoid arthritis from a general and heterogeneous group of polyarthritides. In addition, the test makes it possible for workers in different parts of the world to agree on the definition of this disease entity. Some basis for such agreement is especially important in determining the prevalence and incidence of rheumatoid arthritis in diverse and widely separated populations and also in critically evaluating therapeutic measures used in the management of this disease. A variety of serological tests for rheumatoid arthritis have been described since the first one was reported(1). A comprehensive review of the methods, principles, relative merits, and clinical correlation of the different tests used to date has been published recently by Ziff(2). All serological tests for rheumatoid arthritis depend on clumping of particulate matter by addition of serum from patients. Some of the tests employ sheep cells; in this type of test, the rbc are either mixed with a subagglutinating amount of immune rabbit serum or with human gamma globulin (HGG); later several dilutions of patient's serum are added to rbc. Other tests used collodion or latex particles coated with HGG. In the present study, bentonite particles were coated with HGG. These particles were employed by Bozicevich *et al.*(3) to adsorb antigenic material from trichina in a serologic test for the presence of antibodies in the serum of patients infected with trichina. The technic used in the present study is a slight modification of that published in 1951. Although useful, most of these tests are complicated, time consuming, and require specialized technics. The bentonite flocculation test, described here, is simple and can be done in 20 minutes once the stock solutions are prepared.

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