Blood Serum Xanthine Oxidase of Rats Poisoned with Carbon Tetrachloride. (22087)

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In previous studies(1,2) it has been shown that carbon tetrachloride when injected subcutaneously increases the activity of rat liver xanthine oxidase. A strong activation was also observed when this drug was added *in vitro* to the liver homogenate. We were therefore interested to know if the same activation could be detected in the blood serum. Few reports have been published on presence of xanthine oxidase in blood. Older literature dealing with distribution of this enzyme in tissues, is deficient in quantitative data on blood xanthine oxidase(3,4). Blauch *et al.* studying uric acid of rat blood observed that when rat blood is incubated with xanthine an increase of uric acid is obtained due to presence of xanthine oxidase(5). Using a spectrophotometric technic Westerfeld *et al.* determined xanthine oxidase activity of rat blood serum in experimental cancer of the liver and reported values of 50-60 μg of xanthine oxidized/1 ml/hour(6).

Due to the lack of quantitative values for xanthine oxidase and xanthine dehydrogenase of blood serum measured by manometric and colorimetric methods we undertook a series of determinations in normal rats which served for comparison with the injected ones.

Methods. Three hundred male Wistar white rats weighing between 100 to 150 g and maintained on well balanced diet were used. Blood samples were withdrawn by

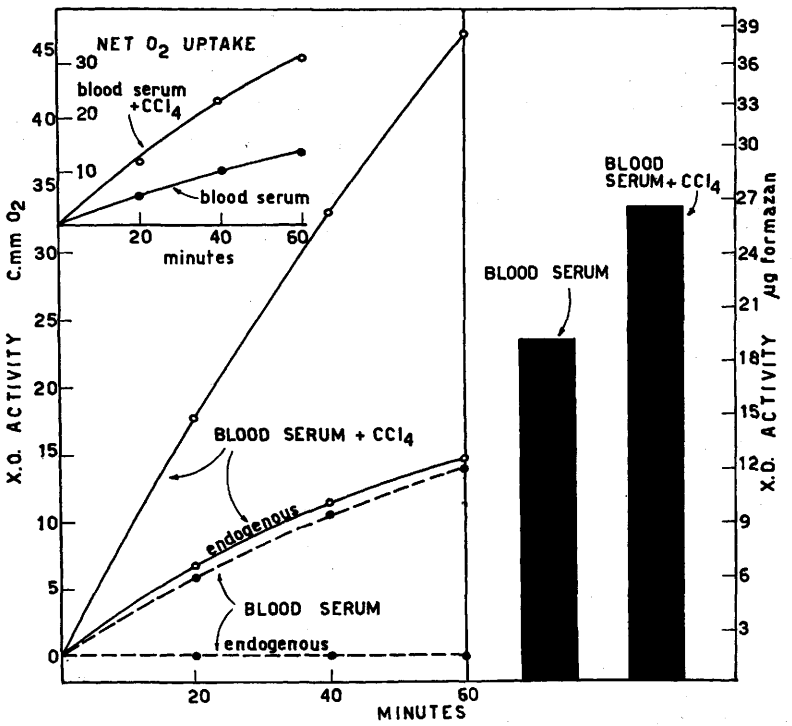

FIG. 1. *In vitro* effect of CCl_4 on xanthine oxidase and xanthine dehydrogenase activity of rat blood serum. Left: oxygen uptake of blood serum in mm^3 measured in Warburg manometers before and after addition of CCl_4. Net oxygen consumption due to xanthine is shown in upper left-hand corner. Right: xanthine dehydrogenase expressed in μg formazan produced/1 ml serum/30 min. before and after activation by CCl_4.

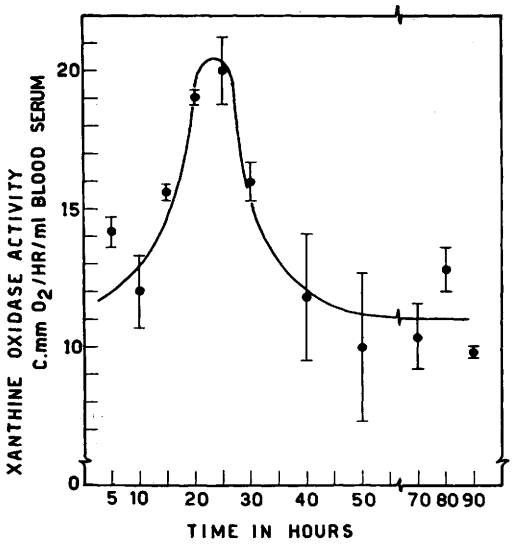

FIG. 2. Blood serum xanthine oxidase activity of rats poisoned with CCl_4. Each point in the graph represents determinations on pooled nonhemolyzed serum from 15 rats (3 groups of 5 rats). Vertical bars indicate avg values $\pm$ stand. dev.

heart puncture and each determination was carried out on pooled nonhemolyzed serum from 5 rats. The animals were injected subcutaneously with 0.1 ml CCl_4/100 g body weight and the blood taken at different intervals (5, 10, 15, 20, 25, 30, 40, 50, 70, 80 and 90 hours) after injection. The coagulated blood was centrifuged, the serum separated and used immediately for analysis. Hemolyzed samples were discarded since it was observed that hemolysis interferes with reduction of the triphenyltetrazolium dye. Xanthine oxidase activity was measured manometrically as previously described(1) using 1 ml of blood serum, 0.2 ml of 0.05 M xanthine solution in 0.05 M NaOH and enough pyrophosphate buffer pH 8.6 to give a final volume of 2.0 ml. Xanthine dehydrogenase activity was measured colorimetrically by the method of Villela(7) using 1 ml of blood serum, 0.1 ml of 0.05 M xanthine solution, 0.3 ml of 0.1% triphenyltetrazolium chloride and 2.0 ml of pyrophosphate buffer pH 8.6. *In vitro* experiments were carried out by adding 0.1 ml CCl_4 to 10 ml blood serum, mixing well for 10 minutes and then centrifuging. Determinations were made in the CCl_4 free supernatant layer.

Results. Normal values were obtained from the blood serum of 55 rats. The average values together with the range plus or minus the standard deviation showed values of 12.9 ± 0.86 cmm O_2/ml serum/60 minutes for xanthine oxidase activity and 17.81 ± 3.00 μg formazan/ml serum/30 minutes at 37°C for xanthine dehydrogenase activity. Each determination was carried out on pooled serum from 5 rats.

In vitro activation of rat blood serum xanthine oxidase and xanthine dehydrogenase by CCl_4 is shown in Fig. 1. Values of 120% activation for xanthine oxidase and 38.5% for xanthine dehydrogenase activity were observed.

The enzyme activities increased in animals injected with CCl_4, the highest values were found 20-25 hours after injection of the drug (Fig. 2). Thereafter, the enzyme activity for xanthine oxidase and xanthine dehydrogenase returned to the normal level.

Discussion. Bruns and Neuhans showed that aldolase and phosphohexose isomerase increased in serum of mice poisoned by intraperitoneal administration of carbon tetrachloride(8). The highest activity was reached 18 to 20 hours after injection of the drug. However, the same enzymes decreased in liver, reaching lowest values 50 hours after injection. These authors suggested that a loss of these enzymes from liver tissues could be the cause of the sharp increase presented in the serum.

It was demonstrated that CCl_4 activates strongly the xanthine oxidase of the rat liver a few hours after subcutaneous injection of the drug(1). Xanthine oxidase was also activated in the blood serum 20 hours after the single subcutaneous injection of carbon tetrachloride as shown in Fig. 2. The simultaneous increased activity of this enzyme in the liver and blood serum indicate that a different mechanism than that proposed for aldolase and phosphohexose isomerase must be responsible.

The distribution of xanthine dehydrogenase was recently studied in the serum of normal rats. It was found that enzyme activity is confined to the lipoprotein fractions(9), sug-

gesting that the activation by CCl_4 could be attributed to the split of the linkages between the enzyme and the lipids as observed when milk(10) and liver xanthine oxidase(2) are treated by other organic solvents.

Summary. Quantitative determinations of xanthine oxidase and dehydrogenase activity in blood serum of normal rats were made. It was shown that a single injection of 0.1 ml CCl_4/100 g body weight is able to activate the blood xanthine oxidase and dehydrogenase, reaching the highest values 20-25 hours after injection. However, after 35 hours the values returned to normal. Activation of the enzyme was also obtained *in vitro* by adding CCl_4 to a sample of serum of known enzyme content.

1. Villela, G. G., and Mitidieri, E., *Nature,* 1955, v175, 208.
2. ———, *Rev. Bras. Biol.,* 1955, v15, 285.
3. Morgan, E. J., *Biochem. J.,* 1926, v20, 1282.
4. Dixon, M., *Enzymologia,* 1938, v5, 198.
5. Blauch, M. B., and Koch, F. C., *J. Biol. Chem.,* 1939, v130, 455.
6. Westerfeld, W. W., Richert, D. A., and Hilfinger, M. F., *Cancer Res.,* 1950, v10, 486.
7. Villela, G. G., *Rev. Bras. Biol.,* 1954, v14, 455.
8. Bruns, F., and Neuhaus, J., *Arch. Biochem. Biophys.,* 1955, v55, 588.
9. Mitidieri, E., Ribeiro, L. P., Affonso, O. R., and Villela, G. G., *Biochim. et Biophys. Acta,* 1955, v17, 587.
10. Polonovsky, M., Baudu, L., and Neuzil, E., *Bull. Soc. Chim. Biol.,* 1947, v29, 958.

Received October 13, 1955. P.S.E.B.M., 1955, v90.

Recently Classified Types of Coxsackie Virus, Group A. Behavior in Tissue Culture. (22088)

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In addition to the 10 previously described types of Coxsackie virus, Group A(1,2), 9 new types have now been assigned numbers. The representative strain of Type 11 is the Belgium 1 strain of Godenne and Curnen (3), that of Type 12, the Texas 12 of Contreras, Barnett, and Melnick(4). Type 13 was isolated in this laboratory from fecal specimens from Mexico. Type 19 was received from Dr. R. J. Heubner, and the remainder from Dr. J. H. S. Gear of the South African Institute for Medical Research(5). All are viruses which induce flaccid paralysis in suckling mice but no apparent disease in older mice and are characterized by the production of marked muscle degeneration but no significant lesions in the central nervous system in the experimental animal. With the exception of Types 12 and 14 all resemble Types 1, 7, and 9 in low infectivity of brain suspensions for suckling mice. Passage and testing with muscle suspension are necessary. In neutralization tests in suckling mice there is definite, although moderate, reciprocal cross reactivity of A-11 with A-15 and of A-13 with A-18. As noted by Contreras(4), A-12 cross reacts with A-5. Regular cross reactions may also be found with A-8 and A-12, more observable when brain tissue is used in neutralization tests. Slight cross reactivity of A-14 in A-7 serum, A-15 in A-17 serum, and A-17 in A-11 serum has been seen.

Tissue culture. Methods. Human uterine tissue and trypsinized monkey kidney tissue cultures were prepared as previously described (6). Trypsinized monkey testicular cultures were made similarly to the kidney cells. HeLa cell cultures were supplied by the Laboratories for Virology under the direction of Dr. Irving Gordon. On receipt, the nutrient fluid containing human serum was removed and replaced by 1.5 ml of the maintenance fluid of Scherer, Syverton, and Gey(7) without added serum. The tubes were rotated so that the entire inside surface was washed and were then allowed to stand upright for a minimum of one-half hour. The washings were removed and replaced by 1.0 ml of mainten-