Biochemical Pharmacology, 1962, Vol. 11, pp. 669–676. Pergamon Press Ltd., Printed in Great Britain.

SHORT COMMUNICATIONS

Inhibition of xanthine dehydrogenase by semicarbazide

(*Received* 4 *March* 1962; *accepted* 18 *April* 1962)

IT HAS been shown that semicarbide (SC) is able to block the aerobic oxidation of hypoxanthine by milk and rat liver xanthine oxidase (XO). However, the drug has no *in vitro* effect on the dehydrogenase (XD) activity of these enzymes.[1]

Semicarbazide presents a great pharmacological and biochemical interest, since it produces seizures in animals which simulate human *grand mal* epilepsy. The seizures appear only after a latent period following administration of the drug.

In the present communication it is shown that XD activity of rat blood serum is strongly inhibited *in vivo* by SC. This inhibition was observed during the latent period that precedes the seizures produced by SC.

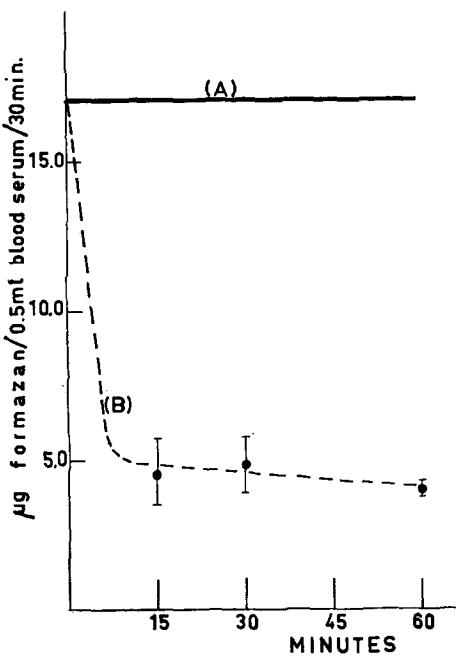

FIG.1. Effect of semicarbazide on xanthine dehydrogenase activity of rat blood serum. (A) XD activity (mean value) of control rats; (B) XD activity of rats injected with 50 mg of semicarbazide per 100 g body weight (mean ± standard deviation).

Male Wistar white rats weighing 100—150 g were used in the present investigation. The animals were kept on a well balanced diet throughout the experiments. Semicarbazide was injected intraperitoneally at the dose of 50 mg per 100 g body weight. At this dosage, seizures were produced 45—60 min after the administration of SC.

The animals were bled by heart puncture under light ether anaesthesia 15, 30 and 60 min after the injection of SC. XD activity was measured in the clear separated serum by measuring the formazan produced by the triphenyltetrazolium chloride reduction in evacuated Thunberg tubes.[2] Figures given represent the mean ± standard deviation found for each particular case, and are expressed as μg formazan/0·5 ml serum/30 min, at 37 °C.

Control rats (16 animals) showed average values of 17·00 ± 0·78. For the injected animals, the following average values were found: 15 min after the injection (7 rats): 4·59 ± 1·03; 30 min after the injection (7 rats): 4·10 ± 0·22.

These results, represented in Fig. 1, clearly indicate that SC inhibits *in vivo* the XD activity of rat serum during the latent period that precedes seizures. After this period, rats died and no more assays were made.

As has been pointed out by Westerfeld *et al.*,[1] SC inhibits the XO activity *in vitro*, but has no effect on the XD activity of milk and of the rat liver enzyme. The *in vivo* XD inhibition found in the present investigation suggests that SC also exerts its effect on the dehydrogenase portion of the enzyme.

Previous findings on the activation of the enzyme by CCl_4[2] and on the electrophoretic distribution of enzyme activity in serum and soluble proteins of rat liver[3] suggested the identity of liver and blood enzymes. The present report furnishes additional evidence on the identity of these enzymes, since XD activity of rat liver is also inhibited *in vivo* by SC, as shown by Westerfeld *et al.*[1]

Acknowledgements—We wish to thank Dr. Gilberto G. Villela for advice and interest in this work and to Conselho Nacional de Pesquisas for financial support.

Biochemical Laboratory,
Instituto Oswaldo Cruz,
Rio de Janeiro, Brazil.

E. Mitidieri
O. R. Affonso
L. P. Ribeiro

REFERENCES

1. W. W. Westerfeld, D. A. Richert and R. J. Bloom, *J. Biol. Chem.* **234**, 1889 (1959).
2. O. R. Affonso, E. Mitidieri, L. P. Ribeiro and G. G. Villela, *Proc. Soc. exp. Biol. Med.* **90**, 527 (1955).
3. E. Mitidieri, O. R. Affonso, L. P. Ribeiro and G. G. Villela, *Nature, Lond.* **178**, 492 (1956).

Action of hepatocatalase on experimental hypercholesterolaemia

(*Received* 16 *April* 1962; *accepted* 22 *April* 1962)

Previous studies on the mechanism of peroxide and lipoperoxide formation in animals have lead us to prepare the enzymes lipoxidase and catalase, and to study their pharmacological actions. As previously reported,[1] hepatocatalase (HC) was obtained from calf liver, following the technique of Sumner and Dounce,[2] and standardized by the method of Beers and Syzer.[3] We accepted as one Pevya unit the minimum quantity of enzyme able to decompose 10 mg of H_2O_2 in 1 min.

The present investigations were made possible through an original method of stabilizing the HC which consisted essentially of lyophylizing (freeze-drying) it from a solution of disacharide, in which it can be kept indefinitely in siliconized bottles. HC obtained under these conditions had such a low general toxity in experimental animals that it was difficult to establish the LD_{50}, and no lesions were observed in the viscera of rats treated for two months with daily subcutaneous injections of up to 25 000 I.U./kg of the product. Previous experimental and clinical studies show that HC decreases the urinary excretion of 17-hydroxycorticosteroids,[4] and the blood level of uric acid.[5] A decrease of blood cholesterol has also been observed in gouty patients threated with HC.

The present study is on the possible influence of HC on experimental hypercholesterolaemia. The results obtained in hypercholesterolaemia induced by the administration of a surface-active agent, Tween 80, are presented.