Studies on the Role of Hydrolysed Glucose Cyclo-acetoacetate on the Restoration of the Activities of Certain Enzymes in Scorbutic Guinea Pigs.

M. C. NATH, G. A. TEJWANI AND N. NATH

University Department of Biochemistry, Nagpur, India.

(Received July 20, 1969)

The activities of certain enzymes in scorbutic guinea-pigs was studied. There was a significant decrease in the activity of hepatic succinic dehydrogenase, serum alkaline phosphatase and blood catalase. The activity of adenosine triphosphatase increased markedly in scorbutic conditions. Hydrolysed glucosecycloacetoacetate (GCA) could maintain the activities of all these enzymes at their normal levels. A possible mechanism of the beneficial effect of GCA has been discussed.

That there is a marked derangement in the activities of the enzymes in the blood and tissues in scurvy has already been reported by several workers. An increase in the activity of adenosine-triphosphatase in scurvy was reported by Murray *et al.* (1). Several other investigators have shown a marked decrease in the activities of hepatic succinic dehydrogenase (2), serum alkaline phosphatase (3, 4) and blood catalase (5, 6) in scorbutic guinea pigs. Continued injection of acetoacetate to animals, which brings about the condition of hyperglycemia (7) has also been known to aggravate scorbutic condition in guinea pigs (8).

The role of hydrolysed product of glucose-cycloacetoacetate (GCA) in elevating the ascorbic acid content in the blood and tissues of animals fed with a necrogenic diet was reported by Nath and Vadalkar (9). It has recently been shown by Idrees Bhai *et al.* (10) that GCA restored the activities of hepatic succinic dehydrogenase, catalase and xanthine oxidase in acetoacetate treated rats. It was also found to normalize the changes in the levels of monoamine oxidase (11), and liver betaine homocysteine transmethylease

(BHT) in acetoacetate administered animals (12).

Recently preliminary studies from this laboratory revealed that GCA could restore the activities of hepatic tyrosine transaminase, *p*-hydroxyphenylpyruvic acid oxidase and homogenetic oxidase in scorbutic and acetoacetate injected guinea pigs.

In view of the above findings it was thought pertinent to find out the role of GCA, if any, on the activities of adenosine triphosphatase, hepatic succinic dehydrogenase, blood catalase and serum alkaline phosphatase in scorbutic guinea pigs.

MATERIALS AND METHODS

Forty eight male guinea pigs (150-200 g) were maintained on a laboratory stock diet consisting of green vegetables and wheat flour for few days. The scorbutic diet was essentially that described by Banerjee (13) and consisted of the following ingredients: Crushed barley, 64 parts; crushed gram (*Cicer arietinum*) 20 parts; casein 12 parts; CaCO_3 3 parts; NaCl 1 part. All the animals were divided equally into three groups and were housed in individual cages. They were fed on a scorbutic diet. Group 1 and Group 3 animals, in addition received orally 5 mg of ascorbic acid or 10 mg of GCA per

day by intraperitoneal injections, respectively.

Paired feeding technique was followed to obviate any results due to inanition. The animals of Group 2 served as the control for this purpose. Water was supplied *ad libitum*. The weight of each animal was taken every week.

The animals were sacrificed by decapitation after eighteen days of dietary regimen. Blood was collected and liver was separated, blotted and frozen until used.

Hydrolysed product of GCA was prepared by the method described earlier (14,15). The blood ascorbic acid was analysed by the methods of Roe and Kuether (16).

The liver mitochondria were prepared by the method of Schneider (17) and different enzymatic assays were done as follows.

1. Determination of Succinic Dehydrogenase

The tetrazolium chloride reduction method of Kuhn and Abood (18) was used for the measurement of the activity of the enzyme.

2. Determination of Serum Alkaline Phosphatase

Sodium β -glycerophosphate was used as the substrate for the enzyme. The inorganic phosphate liberated was measured by the method of Fiske and SubbaRow (19).

3. Determination of Catalase

The blood catalase was assayed by using perborate as the substrate by the method of Fienstein (20).

4. Determination of Adenosine Triphosphatase

The method was essentially that adopted by Reddi and Nath (21). Sodium salt of ATP was used as the substrate. The liberated inorganic phosphate was measured in an aliquot of the reaction mixture by the method of Fiske and SubbaRow (19).

5. Protein Determination

Mitochondrial protein content was determined by the biuret method of Jacob *et al.* (22), using deoxycholate as a solubilizing agent. Total protein in liver homogenate was estimated by the method of Lowry *et al.* (23).

RESULTS AND DISCUSSION

The beneficial role of hydrolysed GCA is evident from the studies on the gain in the body weight of guinea pigs. At the end of 18 days of giving the scorbutic diet there was a 25 % decrease in the body weight of the animals as compared to the group supplemented with ascorbic acid. When the hydrolysed compound of GCA was supplied to the scorbutic guinea pigs, there was a positive gain in the body weight. At this period, however, there was almost no ascorbic acid in the blood and tissues of the animals of Groups 2 and 3. The specific role of ascorbic acid in the animal body has not been pinpointed yet. It is suggested by Knox and Goswami (24) that very many functions of ascorbic acid could be replaced by certain reducing compounds. GCA, which has been shown to be a strongly reducing compound (25), has been found to replace vitamin C in maintaining the gain in the body weight of guinea pigs.

The activities of all the four enzymes in different experimental conditions are represented in Table I. At the end of eighteen days a decrease in the activities of succinic

TABLE 1
Alteration in the activities of enzymes in scorbutic guinea pigs and restoration by GCA

Group	No. of animals	Succinic dehydrogenase ^a	Alkaline phosphatase ^b	ATPase ^c	Catalase ^d
1. Scorbutic+ascorbic acid (5 mg per day per animal)	6 (5) ^e	1.56 $\pm$ 0.07	12.60 $\pm$ 1.50	21.0 $\pm$ 0.30	1.28 $\pm$ 0.03
2. Scorbutic	6 (22) ^e	0.86 $\pm$ 0.12	6.10 $\pm$ 1.80	28.0 $\pm$ 0.80	0.88 $\pm$ 0.05
3. Scorbutic+GCA (10 mg per day per animal)	6 (2) ^e	1.30 $\pm$ 0.09	9.80 $\pm$ 1.70	23.0 $\pm$ 0.60	1.25 $\pm$ 0.03

$\pm$ represents standard error of the mean.

^a μ g of TTC reduced per mg of wet weight of tissue (liver) in 10 min.

^b mg of P_i liberated/100 ml of serum per hr.

^c μ moles of P_i/mg of protein in 20 min.

^d meq perborate destroyed/ml blood in 5 min.

^e The figure in parenthesis represents the average loss in the body weight in g/100 g.

dehydrogenase, serum alkaline phosphatase and blood catalase was found. The decrease in the activities of these enzymes were 45, 56 and 30 %, respectively, in the scorbutic animals. However, ATPase activity was found to be elevated to an extent of 30 %. It is evident further that the hydrolysed GCA could maintain the activity of each enzyme almost to the normal level.

The decrease in the activity of serum alkaline phosphatase in scorbutic animals was suggested to have been caused by the fall in the weight of the animals due to scurvy (26). In the present experiment, GCA could maintain the weight of the animals to that of normal ones. It can be said that GCA could normalize the activity of serum alkaline phosphatase due to its action on the gain in the body weight of the scorbutic animals.

The oxidative enzymes such as succinic dehydrogenase and catalase have been shown to be sulphhydryl in nature. These enzymes require several sulphhydryl groups for their activity. Glutathione serves to protect the SH-enzymes of the cells and thus maintain them in an active state. In scorbutic conditions, when glutathione decreases in the body, there is decreased activity of the enzymes. Banerjee (27) has shown that with the occurrence of dehydroascorbic acid in the tissues of scorbutic guinea pigs the glutathione content of blood and tissues had been decreased. GCA, which has been shown to maintain the reduced glutathione level (28, 29), might possibly prevent the inhibition of these enzymes by retaining the normal level of reduced glutathione of the cell.

The present investigation shows an elevated hepatic ATPase activity in scorbutic guinea pigs, which is in confirmation with the earlier observations of Murray (1). GCA was found to normalize the elevated enzyme activity in scorbutic guinea pigs. Although the protective influence of GCA to maintain the hepatic ATPase activity has been reported by Reddi and Nath (21) in phenylhydrazine treated rats, it is premature, at this stage, to speculate the possible role of GCA on hepatic ATPase activity in scorbutic guinea pigs.

REFERENCES

1. Murray, H. C., *Proc. Soc. Expt. Biol. Med.*, **75**, 598 (1950).
2. Harrer, C. A., and King, C. G., *J. Biol. Chem.*, **138**, 111, (1941).
3. Gould, B. S. and Schwachman, H., *Am. J. Physiol.*, **135**, 485, (1942).
4. Bourne, G. H., *Quart. J. Expt. Physiol.*, **32**, 1, (1943).
5. Torok, G. and Nahfeld, L., *Klin. Wochschr.*, **13**, 1205, (1934).
6. Tria, E., *Att. reale accad. Italia, Mem. classe, Sci. fis. met. e. nat.*, **3**, 806, (1942).
7. Nath, M. C. and Brahmachari, H. D., *Nature*, **154**, 487, (1944).
8. Nath, M. C., Sahu, V. K. and Chitale, R. P., *Biochem. J.*, **53**, 684, (1953).
9. Nath, M. C. and Vadalkar, K., *Biochem. J.*, **78**, 743, (1961).
10. Bhai, I., Nath, N. and Nath, M. C., *Enzymologia*, **34**, 4, (205), (1968).
11. Nath, M. C., Sivakumar, B., *Enzymologia*, **36**, 86, (1969).
12. Nath, M. C., Nath, N., *Enzymologia*, **36**, 86, (1969).
13. Banerjee, S., *J. Biol. Chem.*, **159**, 327, (1945).
14. West, E. S., *J. Biol. Chem.*, **74**, 561, 1927.
15. Nath, M. C., Chitale, R. P. and Belavady, B., *Nature*, **170**, 545, (1952).
16. Roe, J. H. and Kuether, C. A., *J. Biol. Chem.*, **147**, 399, (1943).
17. Schneider, W. C., *J. Biol. Chem.*, **176**, 259 (1948).
18. Kuhn, E. and Abood, L. G., *Science*, **109**, 194, (1949).
19. Fiske, C. H. and SubbaRow, Y., *J. Biol. Chem.*, **66**, 375, (1925).
20. Fienstein, R. N., *J. Biochem.*, **180**, 1197, (1949).
21. Reddi, T. G., Nath, M. C., *Can. J. Biochem.*, **47**, 297, (1969).
22. Jacob, E. E., Jacob, M., Sanadi, D. R. and Bradley, L. B., *J. Biol. Chem.*, **223**, 147, (1956).
23. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, **193**, 965, (1951).
24. Knox, W. E. and Goswami, M. N. D., *J. Biochem. J.*, **235**, 2662, (1960).
25. Nath, M. C. and Behki, R. M., *Arch. Biochem. Biophys.*, **73**, 65, (1958).
26. Perkins, and Zilva, S. S., *Biochem. J.*, **47**, 306, (1950).
27. Banerjee, S., *J. Sci. Ind. Res.*, **17C**, 1-4, (1958).
28. Nath, M. C., Nath, N., *Proc. Soc. Exptl. Biol. Med.*, **210**, 124, (1966).
29. Nath, M. C., Saikia, A., *Ind. J. Med. Res.*, **48**, 3, 382, (1960).