TRACHEAL PHOSPHATASES AFTER A LONG TERM CO POISONING IN INDIAN PALM SQUIRRELS. A HISTOCHEMICAL STUDY

S. V. S. RANA, R. K. GAUTAM AND V. P. AGRAWAL

Department of Zoology, D.A.V. (P.G.) College, Muzaffarnagar 251 001

ABSTRACT

Effect of carbonmonoxide, on the activity of the enzymes alkaline phosphatase, acid phosphatase, 5-nucleotidase, lipase and phosphamidase, have been analysed in the trachea of common ground squirrel, *Funambulus pennanti*. Enzyme histochemical reactions have been treated as link between morphology and biochemistry. Altered enzymatic activities thus observed have been correlated with the effects of CO on cell memberanes, microenvironment of the cell, physiological condition, protein and lipid metabolism. Various causes and significance of changed enzyme levels have also been discussed.

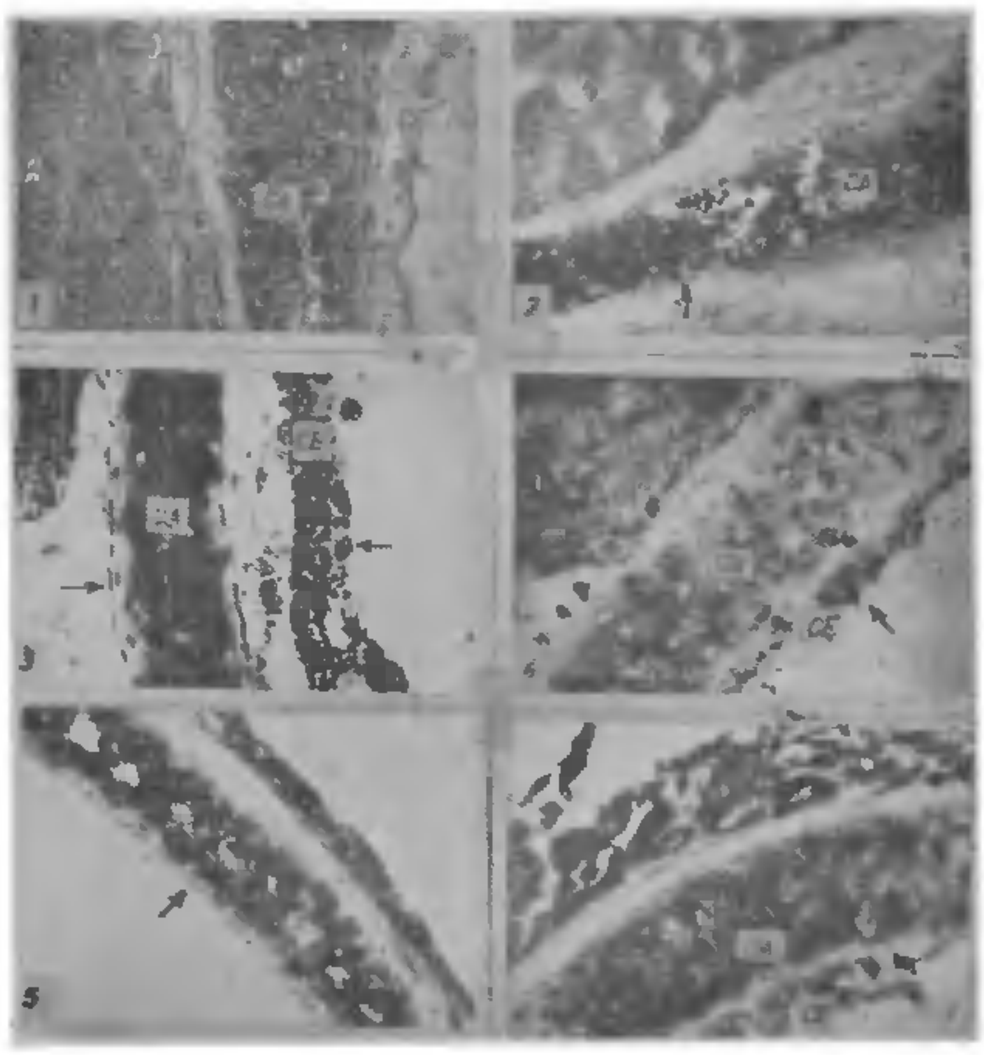
CARBONMONOXIDE, an atmsopheric pollutant, is an asphyxiant gas that poses deleterious physiological effects in exposed subjects. It binds to haemoglobin with an affinity greater than oxygen-forming carboxyhaemoglobin[1]. It causes hypoxia and inhibits cytochrome oxidase activity[2]. Further carbonmonoxide has been shown to produce lung damage and alter pulmonary functions in several animals[3–5]. Still its biochemical behaviour is very little known. Since enzyme histochemical data may give an additional dimension to biochemical results, enzyme activities are treated as markers of subcellular components and as parameters of metabolic pathways and processes. An attempt has therefore been made to analyse the enzymes alkaline phosphatase, acid phosphatase, 5-nucleotidase, lipase and phosphamidase histochemically in the trachea of Indian palm squirrels, *Funambulus pennanti*, after CO poisoning. Cause and significance of altered enzyme levels in exposed subjects have also been discussed.

MATERIALS AND METHODS

Twenty common ground squirrels weighing 80–100 gm were selected. Each squirrel of the first group of 10 squirrels was exposed to 0·05% of carbonmonoxide in the environmental chamber for four minutes. Squirrels of the second group inspired filtered air. This treatment continued for fifteen days on each alternate day, animals were provided lab. chow and water *ad libitum*. After two weeks, all the squirrels were sacrificed by decapitation. Tracheae were removed, fixed in absolute acetone at 4° C, paraffined and fixed frozen sections thus prepared were processed for alkaline phosphatase[6], acid phosphatase[6], 5-nucleotidase[7], lipase[8] and phosphamidase[9].

RESULTS

Effect of CO on alkaline phosphatase was mainly centered at mucosa, that showed no reaction after CO poisoning. Cartilage and ciliated epithelium were positive in both the subjects (Figs. 1 and 2).

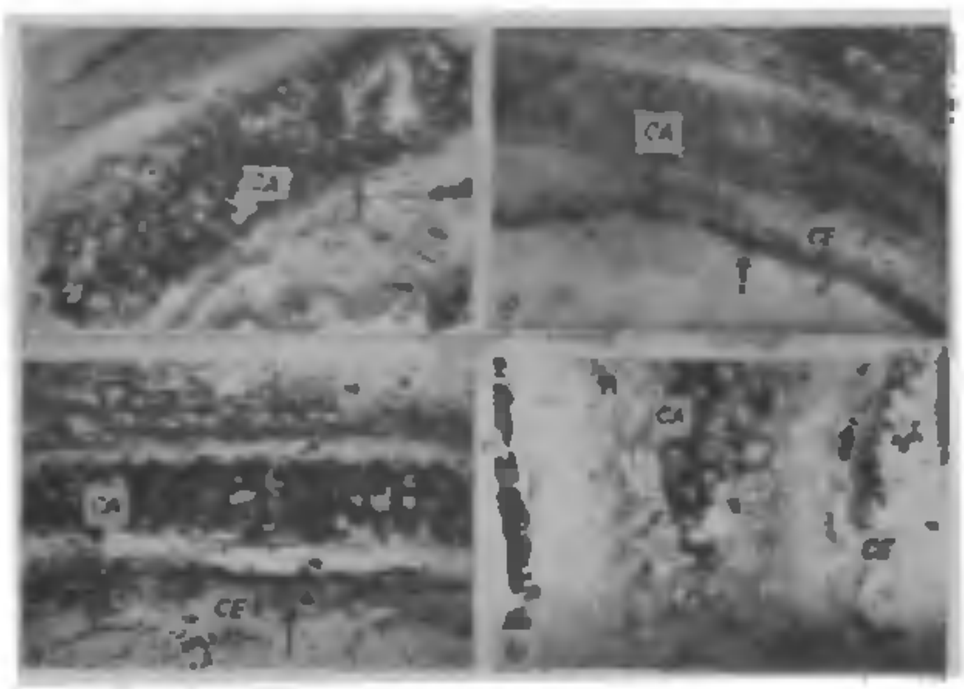


FIGS. 1–6. Fig. 1. Trachea from control squirrels show a uniform and strong positive reaction in the cartilage (CA) and a dull activity in mucosa (MU) and ciliated epithelium (CE), × 80. Fig. 2. A diffused activity in the cartilage (CA) alone is observed after CO poisoning, X 80. Fig. 3. In normal squirrels, a strong positive reaction for acid phosphatase occurs in cartilage (CA) as well as in ciliated epithelium (CE), × 125. Fig. 4. Topography of this enzyme is not affected by CO poisoning, however a dull reaction in cartilage (CA) as well as ciliated epithelium are noteworthy, × 125. Fig. 5. A strong positive reaction for 5-nucleotidase in all the layers is evident from control trachea, × 80. Fig. 6. Inhibited reaction product was formed in the cartilage (CA) and ciliated epithelium (CE) after CO treatment.

CO effect did not alter the topography of the enzyme acid phosphatase; however, in comparison to controls a dull reaction in the cartilage was visualized (Figs. 3 and 4).

All tracheal layers in controls including perichondrium, cartilage, mucosa and ciliated epithelium exhibited strong positive reaction for 5-nucleotidase. In CO poisoned squirrels, a dull enzyme reaction was traced in cartilage and ciliated epithelium only (Figs. 5 and 6). Lipase restricted its activity only in the cartilage portion of trachea (Fig. 7). After CO poisoning a dull reaction could be traced in the cartilage only (Fig. 8).

Loss of phosphamidase activity from ciliated epithelium is evident from the present studies. However, a poor activity in cartilage could be restored even after CO treatment (Figs. 9 and 10).



FIGS. 7–10. Fig. 7. In control trachea, lipase also remain confined to the cartilage (CA), other parts showed a nil activity, × 80. Fig. 8. Very dull activity is observed in the cartilage (CA) after CO poisoning, × 80. Fig. 9. Remarkable strong reaction occurs for phosphamidase in the cartilage (CA) and ciliated epithelium (CE) of control trachea, × 80. Fig. 10. Comparatively poor activity for phosphamidase could be restored in the cartilage (CA) even after CO treatment, × 80.

DISCUSSION

As already pointed out, biochemical behaviour of CO is poorly known. Data on its effects on tissue enzyme levels are also not available. Present observations, being a link between morphology and biochemistry are important. To establish a relationship between CO poisoning and enzyme levels, a discussion on the role of a particular enzyme needs prior consideration. Though alkaline phosphatase is present in every organ, yet its physiological role in different localizations is still uncertain. The preferred localization in the plasma-membrane[10] has led to the conclusion that it plays a role in the transport of phosphate through cellular membranes. *In vivo*, its active site may be influenced by high local concentration of different ions resulting in a change in alkaline pH.

CO may change the pH of the mucosa unabling enzyme to work on substrate. Heterogeneity of the plasma membrane of mucosal cells may be another factor. However, on the relationship that exists between alkaline phosphatase and plasma membrane, a damage to it in CO poisoning may be speculated.

Acid phosphatase has pre-eminently been regarded as marker enzyme for lysosomes. Recent evidence shows that acid phosphatase has not been restricted to lysosomal fraction, but is also found in golgi cisternae, specialized regions of endoplasmic reticulum known as GERL[11]. Further, it has been suggested[12] that the lysosomal enzymes undergo metabolic transformations *in vivo* resulting in changes of substrate specificity. A dull activity under CO influence exhibits its interference with lysosomes.

The enzyme 5-nucleotidase is located primarily in the plasma membrane of most cells and is widely used as marker enzyme for these membranes[13,14]. These reports suggest that enzyme is also associated with membranes of endoplasmic reticulum. Several suggestions have been made for the physiological function of the enzyme. It has been assumed that 5-nucleotidase is only a degradative enzymes[15]. Klaushofer and Bock[16] suggested that it is also associated with proliferating cell types. Loss of its activity from perichondrium and mucosal lining after CO treatment suggests that their cells are more susceptible to CO, hence nucleotidase catabolism and transport of nucleotides across membranes in mucosa and perichondrium is blocked by carbonmonoxide.

Inhibited lipase reaction observed in the cartilage after CO poisoning reflects its interference with lipid metabolism that has been further confirmed by the present authors[17].

Enzyme, phosphamidase is known to hydrolyse P–N bond of the amides of phosphoric acid[18]. Though little is known about its physiological function in mammalian tissues, it is present in trachea. Loss of its activity from the ciliated epithelium and poor reaction in cartilage signify its susceptibility to carbonmonoxide.

Regulation of enzyme activity by synthesis or degradation of an enzyme causes the simultaneous expression or loss of all catalytic functions. As stressed by Holzer and Duntze[19] the enzymes regulated by chemical modification are the key enzymes of metabolic pathway. Such key enzymes will have to respond to a great number of controlling effects and their regulation will, therefore, require a multitude of different controlling sites. However, it can be concluded that regulation of the membrane bound enzyme activities may be altered by changes in the microenvironment of the cell.

Presumably, the observed change in enzyme activity is the result of variation in the level of enzyme protein

with the consequent consideration of cellular organelles as highly dynamic structures. Enzymes discussed here are the markers of cellular organelles. Ultra-structural study of cellular membranes is needed to confirm this hypothesis. However, results are expected to be helpful in manifesting the biochemical behaviour of carbonmonoxide.

ACKNOWLEDGEMENT

The authors are highly thankful to University Grants Commission, New Delhi, for the financial assistance.

1. Agrawal, V. P., Rana, S. V. S. and Gautam, R. K., *Ind. J. Exp. Biol.*, 1977, **15**, 673.
2. Ball, E. G., Strittmatter, C. F. and Cooper, O., *J. Biol. Chem.*, 1951, **193**, 635.
3. House, W. B., Air Force Tech. Rep. No. ASD-TR-61-519 III, 1964.
4. Bushtueva, K. A., *Gig. Sanit.*, 1957, **22**, 17.
5. Prokhorov, Y. D. and Rogov, A. A., *Ibid.* 1959, **24**, 22.
6. Gomori, G., *Microscopic Histochemistry*, Univ. Chicago Press, Chicago, Illinois, 1952.
7. Pearse, A. G. E., *Histochemistry, Theoretical and Applied*, J. and A. Churchill Ltd., London, 1961.
8. Gomori, G., *Proc. Soc. Exper. Biol. and Med.*, 1945, **58**, 362.
9. Holter, H. and Si-Oh-Li, *Compt. Vend. Lab. Carlsberg. Ser. Chim.*, 1952, **27**, 393.
10. De Duve, C., "General principles,"—In D. B. Roodyn (ed.), *Enzyme Cytology*, Academic Press, London, 1967, pp. 26.
11. Farquhar, M. G., Bergeron, J. J. M. and Palade, G. E., *J. Cell. Biol.*, 1974, **60**, 8.
12. Ide, H. and Fishman, W. H., *Histochemie*, 1969, **20**, 300.
13. De Duve, C., *J. Cell. Biol.*, 1971, **50**, 20 D.
14. Evans, W. H. nad Gurd, J. W., *Biochem., J.*, 1973, **133**, 189.
15. Golderg, D. M., *Digestion*, 1973, **8**, 87.
16. Klaushofer, K. and Bock, P., *Histochemistry*, 1974, **40**, 39.
17. Rana, S. V. S., Agrawal, V. P. and Gautam, R. K., *Morphol. Jahrb.*, 1978 (In press),
18. Holzer, H. and Duntze, W., *Ann. Rev. Biochem.*, 1971, **40**, 345.

EFFECT OF *IN VIVO* MUSCULAR STIMULATIONS : V. SOME ASPECTS OF CARBOHYDRATE METABOLISM OF AMPHIBIAN BRAIN DURING SHORT TERM AND PROLONGED MUSCULAR STIMULATIONS

P. REDDANNA AND S. GOVINDAPPA

Department of Zoology, Sri Venkateswara University, Tirupati 517 502, *Andhra Pradesh*

ABSTRACT

The total carbohydrate level of brain tissue decreased in response to one day *in vivo* muscular stimulations. However the carbohydrate content returned to the normal level during prolonged stimulations. It appears that the tissue is involved in the carbohydrate sparing process, through inhibition of citric acid cycle enzymes and active uptake of lactic acid and amino acids towards the formation of carbohydrates. The tissue adaptability in carbohydrate metabolism during prolonged stimulations has been discussed.

INTRODUCTION

ELECTRICAL stimulation of muscle tissue or heavy exercise is known to alter the metabolism of many tissues in the animal[1-10]. However the information on brain metabolism is scanty. Hence an attempt has been made to understand some aspects of carbohydrate metabolism of brain in response to short term and prolonged muscular stimulations in intact animal.

MATERIALS AND METHODS

Frogs belonging to the species *Rana hexadactyla* (Lesson) were employed for the present study. The right gastrocnemius muscles of the animals were stimulated with electronic stimulaor (INCO/CSIO Research Stimulator-Ambala) as described earlier[9,10] for one day for one batch of experimental animals and for ten successive days for the other.

The brain tissue was isolated from control and experimebtal animals after pithing them, and placed in amphibian Ringer for recovering from shock effects and then employed for the studies.

The activity levels of succinate, malate and lactate dehydrogenases (SDH, MDH and LDH) and of glutamate dehydrogenase (GDH) were estimated by