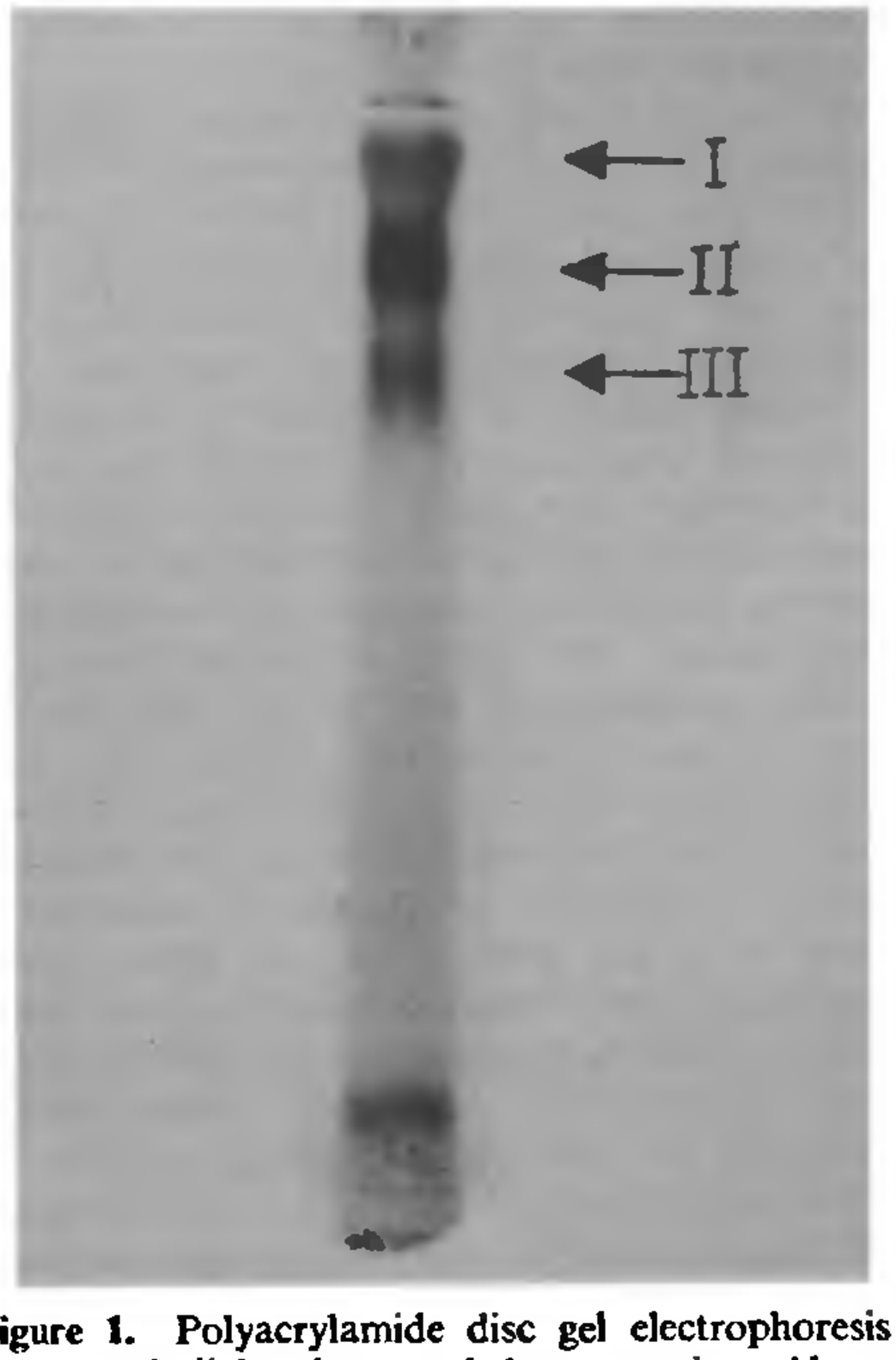

Figure 1. Polyacrylamide disc gel electrophoresis pattern of dialysed normal human urine. About 150 μg protein was electrophoresed and transpeptidase activity was localized by the method of Selvaraj and Balasubramanian[2].

isozyme is lower and that of the bound isozyme forms considerably higher to give an elevated total urinary GGTP activity in leukaemia patients[5]. However, other cancer patients (except those under treatment) showed no change in urinary transpeptidase activity.

Tsuji *et al.*[6] have reported that liver GGTP presumably leaks into serum, because many properties of liver and serum GGTP are comparable. Hence the level of GGTP in the serum can be used as an index of hepatic function.

GGTP in the human kidney is located on the extrinsic surface of the brush border membrane and, to some extent, elsewhere in the kidney[7]. Although the molecular weights of GGTP from urine and kidney were found to be different[8], Scherberich *et al.*[9] have shown immunological identity between urinary GGTP and GGTP of proximal tubule cells. Based on these similarities (immunological and electrophoretic properties), it can be presumed that urinary GGTP is leaked from the kidney membranes into urine, and that any change in the proportions of the urinary transpeptidase isozymes can be used to assess the dynamic status of the brush border membrane. Further, this type of study might be of diagnostic importance in differentiating between non-renal and renal conditions. However, more studies are required to establish the actual clinical use of estimating the isozymes of urinary enzymes like GGTP, alanine aminopeptidase, alkaline phosphatase, lysozyme, etc.

The authors (KBA and SBH) thank ICMR, New Delhi and UGC, New Delhi respectively for financial assistance.

7 November 1988

1. Davis, B. J., *Ann. N.Y. Acad. Sci.*, 1964, **121**, 404.
2. Selvaraj, P. and Balasubramanian, K. A., *Clin. Chim. Acta*, 1982, **121**, 291.
3. Miller, S. P., Awasthi, Y. C. and Srivastava, S. K., *J. Biol. Chem.*, 1976, **251**, 2271.
4. Rambabu, K., Jacob, R. T. and Pattabiraman, T. N., *Clin. Chim. Acta*, 1981, **109**, 257.
5. Basheer Ahamad, K., Hinchigeri, S. B. and Datta, K. S., (Manuscript under preparation).
6. Tsuji, A., Matsuda, Y. and Katunuma, N., *Clin. Chim. Acta*, 1980, **104**, 361.
7. Meister, A., *Trends Biochem. Sci.*, 1981, **6**, 231.
8. Scherberich, J. E., Falkenberg, F., Mondorf, W., Muller, H. and Pfleiderer, G., *Clih. Chim. Acta*, 1974, **55**, 179.
9. Scherberich, J. E., Kleemann, B. and Mondorf, W., *Clin. Chim. Acta*, 1979, **93**, 35.

INDUCTION OF LYSOSOMAL ENZYMES IN EHRLICH ASCITES TUMOUR CELLS BY BROMOPHOS

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IN mammals xenobiotics are detoxified mainly in liver cells by enzymes located predominantly in the endoplasmic reticulum. These enzymes are known as

'microsomal detoxification enzymes'[1]. They have been studied extensively in safety evaluation of drugs and environmental chemicals. However, very little is known about the role of lysosomal enzymes in the detoxification of xenobiotics. Although lysosomes have been implicated in the metabolism of xenobiotics, a direct demonstration of the involvement of lysosomal enzymes has not been made. Hence the induction of lysosomal enzymes in cultured Ehrlich ascites tumour (EAT) cells by bromophos, a non-systemic organophosphorus insecticide, was studied.

EAT cells were cultured in the peritoneal cavity of Swiss mice and harvested 10–12 days after transplantation. The aspirated cells were suspended in saline. Contaminating erythrocytes, if any, were lysed by a brief hypotonic shock. Viability of the cells was determined by trypan blue dye exclusion[2]. Cells (5×10^6/ml) were then suspended in saline and incubated without bromophos or with 50 or 100 nmol of bromophos (*O,O*-dimethyl-*O*-(2,5-dichloro-4-bromophenyl)-phosphorothioate) at 37°C for 1 h. After the incubation the cells were pelleted by centrifugation at 800 *g* for 5 min. The cell pellet was homogenized in a Potter–Elvehjem homogenizer in saline containing 0.01% Triton X-100. The cell homogenate and cell supernatant were used for enzyme assays. Hexosaminidase (EC 3.2.1.52) was assayed using 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide as substrate[3]. β-Glucuronidase (EC 3.2.1.31) was assayed using phenolphthalein glucuronide as the substrate[4]. Aryl ester hydrolase (EC 3.1.1.2) was assayed using α-naphthyl acetate as substrate[5]. Glucose-6-phosphatase was assayed by measuring the inorganic phosphate[6] released from glucose-6-phosphate[7]. Protein was determined by Lowry's method[8].

More than 90% of freshly harvested cells were viable, by trypan blue dye exclusion. Activities of the four enzymes assayed in cell pellet and cell supernatant are shown in figure 1. In the cell pellet activities of hexosaminidase, glucuronidase and aryl ester hydrolase increased with increasing concentration of bromophos. In the cell supernatant, activity of hexosaminidase increased with increasing concentration of bromophos, whereas increase in aryl ester hydrolase activity was marginal and glucuronidase activity did not increase. Glusose-6-phosphatase activity in both cell pellet and the cell supernatant decreased with increasing concentration of bromophos.

The study of induction of enzymes involved in xenobiotic metabolism in experimental animals has

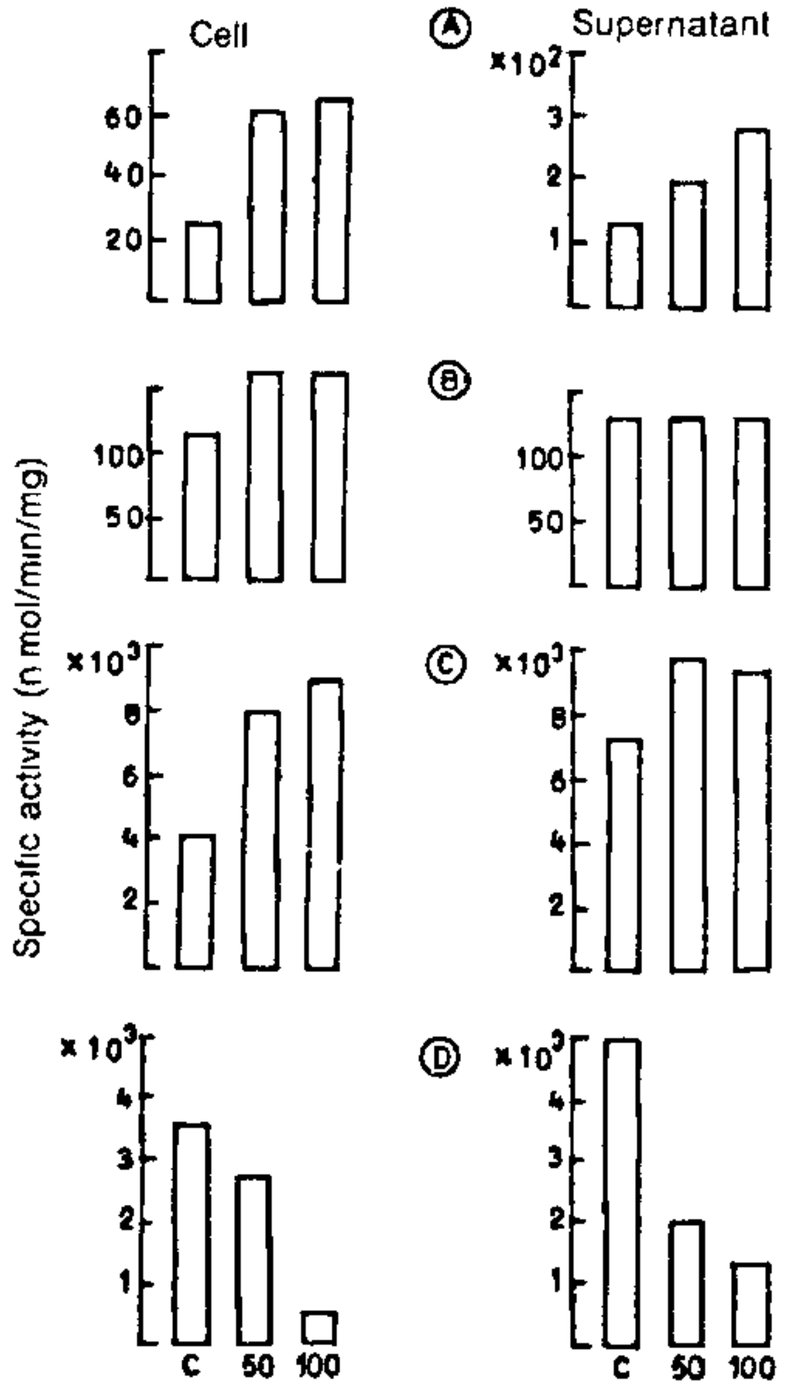

Figure 1. Effect of bromophos (C, control; 50, 50 nmol; 100, 100 nmol bromophos) on activities of (A) hexosaminidase, (B) glucuronidase, (C) aryl ester hydrolase and (D) glucose-6-phosphatase in EAT cells and cell supernatants. Each result is the mean of two independent determinations.

many inherent problems. First of all the distribution of xenobiotic in the different tissues must be known. Secondly the response of different tissues to the same xenobiotic may be different owing to factors such as hormonal interactions and immunological responses. Hence in our study we used cultured EAT cells since they provide a homogeneous population of rapidly dividing cells. Our results show that bromophos stimulates the activity of lysosomal enzymes and that at least some of these enzymes are released into the medium. Glucose-6-phosphatase, an enzyme located primarily in the endoplasmic reticulum, is not normally induced by xenobiotics. Hepatotoxic chemicals have been shown to decrease its activity in liver microsomes[9]. Xenobiotics like phenobarbital have been shown to mask the activity of the enzyme by inducing a change in the endoplasmic reticulum[10]. In our study, although the cause of inhibition of glucose-6-phosphatase is not known, bromophos, or some metabolic product of bromo-

phos, acting as an inhibitor of the enzyme cannot be ruled out. Such inhibition by organophosphorus compounds is well known. Although the significance of the increase of lysosomal enzymes on the one hand and the selective release of some of them on the other in EAT cells is not known, lysosomal enzymes may be important in xenobiotic detoxification reactions.

One of the authors (CJMD) thanks CSIR, New Delhi, for financial assistance.

5 December 1988

1. Armstrong, R. N., *Crit. Rev. Biochem.*, 1987, **22**, 39.
2. Phillips, H. J., *Tissue Culture Methods and Applications*, Academic Press, New York, 1973, pp. 406.
3. Stirling, J. L., *Methods of Enzymatic Analysis*, vol. 4, Verlag Chemie, 3rd edn, 1984, pp. 269.
4. Stahl, P. D. and Fishman, W. H., *Methods of Enzymatic Analysis*, vol. 4, Verlag Chemie, 3rd edn, 1984, pp. 246.
5. Junge, W. and Klees, H., *Methods of Enzymatic Analysis*, vol. 4, Verlag Chemie, 3rd edn, 1984, pp. 8.
6. Seaman, E., *Methods Enzymol.*, 1968, **12**, 219.
7. Baginski, E. S., *Methods of Enzymatic Analysis*, vol. 3, Verlag Chemie, 2nd edn, 1974, pp. 876.
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
9. Parke, D. V. *Enzyme Induction*, Plenum Press, New York 1975, pp. 207.
10. Pandhi, P. N. and Baum, H., *Life Sci.*, 1970, **9**, 87.

TRACE METALS IN CANCER TISSUES

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CURIOSITY about the trace metal composition of cancer tissues was aroused by the accumulation of copper and certain other trace elements that one of us (TRD) had observed in the affected skin of cases of leprosy, leucoderma and eczema some time ago (unpublished). Reference to the literature gave very little information on the mineral profiles of cancerous tissues although several metals are implicated in carcinogenesis and allergy in humans and experimental animals[1]. We have analysed a number of cancerous and the corresponding normal tissues, procured through the courtesy of local hospitals, for certain physiologically important trace elements—Ca, Cd, Co, Cu, Fe, Mg, Mn and Zn. The results are presented in this communication.

The analyses have been completed in six cancer cases and an equal number of normals. In the cancer patients diagnosis had been confirmed on the basis of clinical and histological criteria. These include two cases each of cervical, oesophageal and gastric cancer. The normal cases were adjudged to be so on the basis of freedom from all overt clinical signs and symptoms of cancer or other diseases and the normal histological picture of the biopsy specimens of the specific tissues taken for analysis.

The tissues were excised by biopsy guided by endoscopy, parallel specimens being taken from the same area for histological examination. Tissues were preserved in absolute alcohol until analysis. They were dried, digested in a mixture of sulphuric and nitric acids[2], and made up to volume, and aliquots taken for estimation of the various mineral elements by atomic absorption spectrometry[2] (Perkin–Elmer Model 403 instrument). The same wet-digestion and subsequent analytical steps were followed with blood sera also.

The data are presented in table 1. The affected tissues in three cases of cancer of the colon and two cases of rectal cancer were also analysed, but as the corresponding normal tissues were not available, the data are not included here. There were no significant differences in the pattern of minerals assayed in blood sera between nine normal and six cancer cases and so they are also not reported here.

In spite of the wide individual variations, the trace metal spectra of the cancerous tissues examined, compared with those of the corresponding normal tissues (table 1), reveal several interesting features. The general trend of the results indicates that, in any given type of cancerous tissue, the sense of departure from the normal is the same for all the metals examined. Both cancerous cervix and cancerous oesophagus show decreases ranging from 25 to 80%, reckoned on the basis of the mean values. On the other hand, in cancerous gastric mucosa the concentration of the metals increases by two to seven times. That the former two tissues are ectodermal and the latter endodermal in origin may be a