1. Praaken, R., *Hereditas*, 1943, **29**, 475.
2. Gottschalk, W. and Baquar, S. R., *Can. J. Genet. Cytol.*, 1971, **13**, 138.
3. Sahu, G. R., Tewari, V. and Singh, R. B., *J. Cytol. Genet.*, 1981, **16**, 71.
4. Bozzini, A. and Martini, G., *Caryologia*, 1971, **24**, 307.

ENZYME ADAPTATION IN THE KIDNEY OF COBALT FED RATS.

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THE phenomenon of enzyme adaptation has been studied in the kidney of cobalt fed rats through histochemical parameters. Disappearance of alkaline phosphatase, acid phosphatase, 5-nucleotidase and cholinesterase from kidney after a longer exposure to cobalt and then appearance after still longer treatment has been discussed in terms of adaptation to the changed environment of cell and its organelle.

Enzyme regulation, denaturation, turnover, cycling, modification by drugs, sex, nutrition and hormonal status are well known events in cellular biochemistry[1]. In addition, influences of circadian rhythms on enzyme levels have also been recorded[2]. Most interesting and important are the spontaneous processes involved in enzyme reversal after cell injury[3]. Occurrence of such a phenomenon warrant confirmation after diverse experiments in biology and medicine. Since heavy metals are enzyme poisons, it was found appropriate to gather more information on such mechanisms during investigations on heavy metal toxicity in the laboratory animals. Present communication describes reversal of a few important enzymes *viz.* alkaline phosphatase, 5-nucleotidase and cholinesterase in the kidney of rats (*Rattus rattus* albino) fed on cobalt for considerably longer periods.

Cobalt as cobalt acetate (BDH, mol. wt. 145) was fed for 30 days by gavage (50 mg/kg body weight) each day to a group of 10 laboratory bred, 3 months old (100 ± 10 g) rats maintained on laboratory chow (Hindustan Lever Ltd., Bombay) and tap water *ad lib* under standard laboratory conditions after making primary toxicological observations[4] like LD_{60}. Another group of ten rats was subjected to the same treatment but for 60 days. Whereas twenty rats, ten with each group, provided with a cobalt free diet but same environment served as controls.

Ten rats were sacrificed by decapitation on 30 days and the rest ten after 60 days. Kidneys were carefully removed and fixed frozen in 10% neutral formaline. Paraffin and frozen sections were processed for histochemical assay of alkaline phosphatase[5], acid phosphatase[6], 5-nucleotidase[7] and cholinesterase[8]. Histopathological observations were made after staining sections with hematoxylin and eosin. Suitable reactions controls were also run simultaneously.

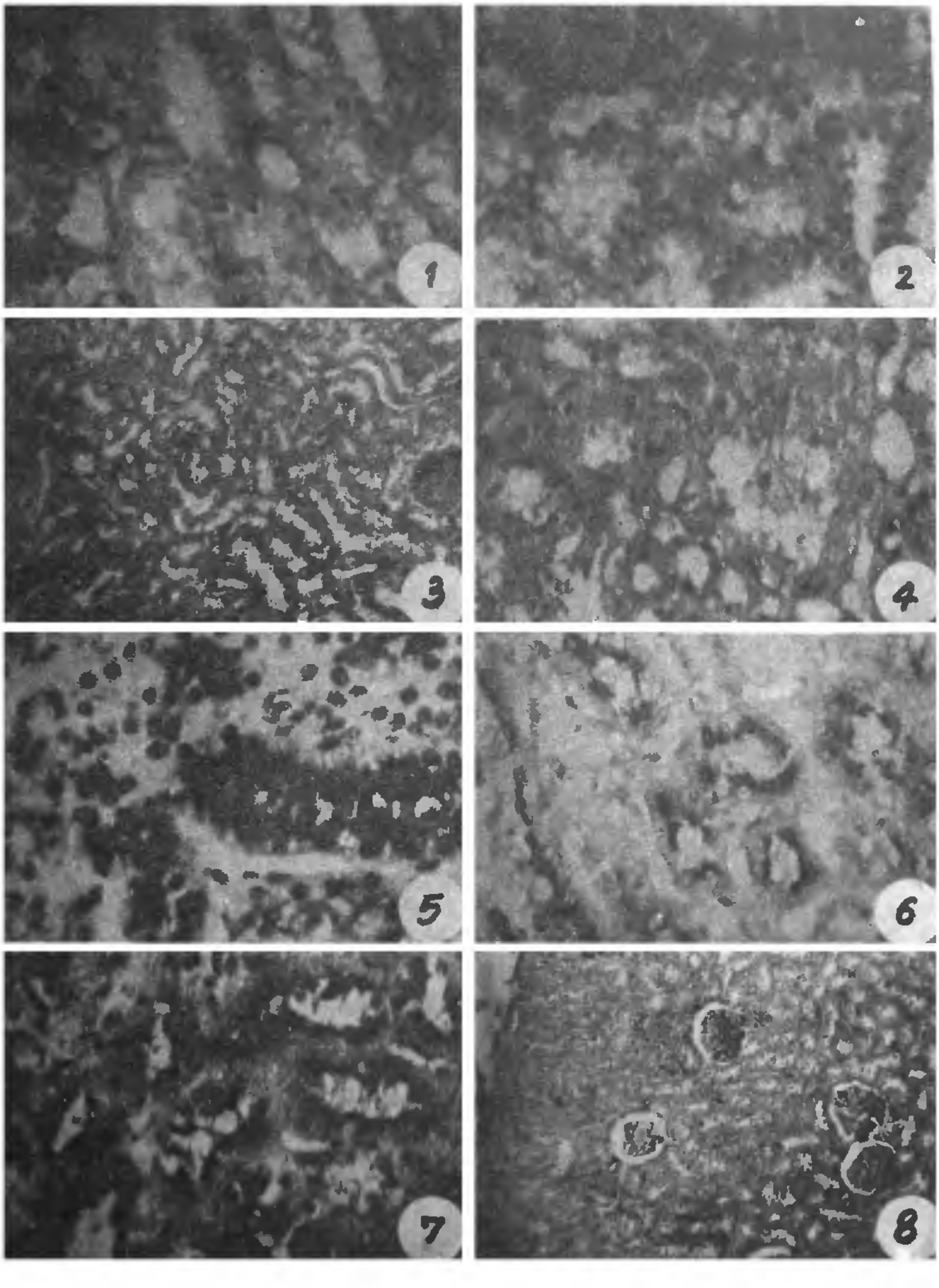
Prolonged treatments with cobalt induced renal atrophy and extensive tubular necrosis (figures 1,2). It inhibited all the key enzymes in rat kidney after 30 days treatment (figures 3,4), whereas activities were found elevated in the kidney of rats fed on cobalt for 60 days (figures 5–8). However, no differences in the activities of these enzymes could be observed in the kidney of normal rats after 30 or 60 days. Changes in their distribution thus noted are presented by table 1.

TABLE 1

Enzyme activity in the kidney of cobalt fed rats.

Enzyme	Control				Treatment							
					30 days				60 days			
	A	B	C	D	A	B	C	D	A	B	C	D
Alkaline phosphatase	+	+	±	−	±	−	−	−	++	+	−	−
Acid phosphatase	+	++	−	−	−	±	−	−	+	+	−	−
5-Nucleotidase	++	+	−	−	$+^n$	$+^n$	−	−	++	+	±	−
Cholinesterase	−	−	+	−	−	−	−	−	±	±	+	−

++, strong activity; +, moderate; ±, dull; −, no acitivity; $+^n$, moderate activity only in nucleus. A = Proximal convoluted tubules, B = Distal convoluted tubules, C = Glomeruli; D = Medulla.



Figures 1–8. 1. A transverse section of kidney shows extensive tubular necrosis after 30 days treatment of rats with cobalt. ×320. **2.** Almost similar were the observations in the kidney of 60 days treated rats. ×320. **3.** No reaction for alkaline phosphatase was noticed in the kidney of rats fed on cobalt for 30 days. ×80. **4.** Only the nuclei exhibited a positive reaction for 5-nucleotidase in renal cortex after 30 days treatment of rats with cobalt. ×320. **5.** Enhanced reaction for acid phosphatase was observed in the kidney of rats fed on cobalt for 60 days. ×320. **6.** A moderate reaction for alkaline phosphatase could be noticed in the kidney of rats fed on cobalt for 60 days. ×320. **7.** A 60 days treatment with cobalt activated 5-nucleotidase in renal cortex. ×320. **8.** After 60 days treatment with cobalt cholinesterase could be localized in the glomeruli. ×80.

Cobalt is one of the essential and least toxic elements. However, it develops true polycythemia, hyperplasia of the bone marrow, reticulocytosis and increases blood volume[9]. Excessive accumulation of cobalt does not occur in any particular tissue or organs but the liver, kidney and bones usually carry highest concentrations of this element. It induces renal atrophy, tubular necrosis and fibroproliferation. The processes responsible for these lesions could account for disappearance of these enzymes from renal cortex through mechanisms, *i.e.* feed back regulation or competitive inhibition. However, enzyme reversal thus recorded after longer treatment does not reflect reversal of renal injury. Although exact mechanism is to be explored, certain generalizations could be made. Firstly cobalt plays an important role in biological redox processes. As free radical (during excess feeding) it might change microenvironment of the cell and its organelle. Finally depression in enzyme activities takes place through chemical reactions. *viz.* phosphorylation, adenylylation, ADP ribosylation, oxidation of thiol groups and also through the respective reverse reactions. The reversibility of modifying reactions can also be achieved by separate enzymes catalyzing the irreversible attachment and removal of the modifying group. However, after prolonged treatment, complete adaptation takes place at membrane level and their stabilization results in enzyme elevation. Although there may exist a separate mechanism of inhibition of each of these enzymes, common metabolic pathways presumably help in enzyme reversal. Universal existence of this concept even in metal toxicity, appears far from being acceptable, however, results from liver also support present observations[10]. The idea of this phenomenon being specific to essential elements only, is opposed by observations already made on mercury in the liver of a fresh water fish[11]. Nevertheless, degree of adaptation may depend on a number of factors *viz*, element, tissue, animal age and nutritional status etc.

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1. Reed, L. J. and Muench, H , *Am. J. Hyg.*, 1938, **27**, 375.
2. Defrawy, E. I., Masry, E. I., Mannering, G. I., *Drug metabol. Disp.*, 1974, **2**, 279.
3. Higgins, M., Kawachi, T. and Rudney, H., *Biochem. Biophys. Res. Commun.*, 1971, **45**, 138.
4. Deimling V. O., *Prog. Histochem. Cytochem.*, 1970, **1**, 1.
5. Burstone, M . S., *Enzyme histochemistry and its applications to the study of Neoplasms*, Acad. Press, New York, London, 1962.
6. Gomori, G., *Microscopic histochemistry principle and practice*, Univ. Chicago Press. Chicago. Illinois, 1952.
7. Pearse, A. G. E. and Reis, J. L., *Biochem. J.*, 1952, **50**, 526.
8. Koelle, G. B. and Periendenwald, J. S., *Proc. Soc. Exp. Biol. Med.*, 1949, **70**, 617.
9. Grant, W. C. and Root, W. S., *Physiol Rev.*, 1952, **32**, 449.
10. Rana, S. V. S., Agrawal, V. P. and Prakash, R., *Ind. Hlth.*, 1983, (In press).
11. Rana, S. V. S., Rekha Sharma, *Toxi. Lett.*, 1983, **128**, 1.

NEW SPECIES OF THE GENUS *SHINDEOBOTHRIUM*, SHINDE AND CHINCHOIKAR 1975 FROM *TRYGON* SP. AT RATNAGIRI.

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THE Genus *Shindeobothrium* was erected by Shinde and Chincholikar in 1975 with *S. indica* as the type species from *Trygon* sp. The present communication describes a new species of the genus.

Four specimens were collected from the *Carcharias acutus*, fixed in 4% formalin and stained with Harri's haematoxylin. Drawings were made with the help of a Camera lucida and the measurements are in millimeters.

Description: S. carchariasi n.sp.

Worms measure 7 cm. in length, scolex with four bothridia, measuring 0.004–0.006 × 0.002–0.004 in diameter. Neck present, measuring 0.05 in length and 0.016 in its maximum width.

Mature segments longer than broad (0.54 × 0.12). Testes oval, in two rows, 10 in number, pre ovarian (0.022–0.025 × 0.012–0 013). Cirrus pouch oval, submarginal, at 1/4 from the anterior margin of the segment (0 038 × 0.024). Cirrus thin, straight, unarmed (0.038 × 0.001). Vas deferens runs slightly posterior (0 034 × 0.001). Genital pores irregularly alternate, submarginal and oval.

Ovary 'H' shaped, situtated at 1/6 of the segment from anterior margin and measure 0.07–0.009 × 0.003. Vagina anterior to cirrus pouch, elongated, thin tube, runs trasversely, and measures 0.42 × 0.003. Genital pores oval, submarginal, irregularly alternate and measures (0.019 × 0.01). Ootype oval, small (0 038 × 0.012). Uterus elongated thin tube, up to ante-