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COBALT AND MANGANESE INTERACTION IN THE LIVER OF RAT

S. V. S. Rana, R. Prakash and V. P. Agrawal

School of Environmental Contamination and Toxicology, Department of Zoology, D. A. V. (P. G.) College, Muzaffarnagar, India

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The effects of cobalt and manganese on some enzymes in the rat's liver were studied. Manganese invariably inhibited the activity of alkaline phosphatase, acid phosphatase, glucose-6-phosphatase, cholinesterase and lipase, whereas cobalt affected only the activities of acid phosphatase, glucose-6-phosphatase and lipase. After a combined treatment with both cobalt and manganese (a mixture containing one half of LD_{50} each) only the activity of acid phosphatase decreased, while the activities of other enzymes remained within the normal range, indicating an antagonistic behaviour of these essential trace elements.

Today, significant quantities of trace elements are generated through atmospheric pollution specially in the areas adjacent to mines and factories. A manifold increase in the number of sources of xenobiotics as a result of increasing urbanization and motorization constitutes a long-term danger to human health. Although a few trace elements, like manganese, cobalt, zinc, molybdenum etc, are of low toxicity to mammals, health impairment may be due to their excessive intake through food, drinks, occupational exposure or industrial contamination.

Manganese poisoning is characterized by a severe psychiatric disorder known as *Locura manganica*. Cobalt, although less toxic, causes true polycythaemia, hyperplasia of the bone marrow, reticulocytosis and increased blood volume. Frequent use of cobalt and manganese both in steel and glass industries (1, 2) has increased the risk of their combined effects. Reports on their individual effects (3—8) show that excessive accumulation occurs in the liver, kidney and bones. However, their physiological relationship is not known. Since enzymological changes determine the nature, the extent of metabolism and thus the action and fate of the chemicals in the animal, the present work on alkaline phosphatase

(EC 3.1.3.1), acid phosphatase (EC 3.1.3.2), glucose-6-phosphatase (EC 3.1.3.9), cholinesterase (EC 3.1.1.7), and lipase (EC 3.1.1.3) was undertaken in the liver of rats fed on cobalt and manganese supplemented diets.

MATERIALS AND METHODS

Forty adult albino rats of both sexes and of the same age and weight (100 ± 10 g) were selected from the laboratory stock. After having adapted to laboratory conditions they were randomly classified into four groups each containing ten rats. Each rat was housed in a separate galvanized cage, fed on a standard diet (Hindustan Lever Ltd., Bombay), provided tap water *ad libitum* and maintained under standard laboratory conditions. The rats from group A received a sublethal dose of cobalt as cobalt acetate (50 mg/kg body wt/day/rat), those in group B received a sublethal dose of manganese as manganese chloride (250 mg/kg body wt/day/rat) and rats in group C a sublethal dose of the equimolar mixture of cobalt and manganese (150 mg/kg body wt/day/rat). The rats from group D fed on a standard laboratory diet only (without cobalt or manganese) were taken as controls. Metals were administered to the rats by gavage daily for thirty days.

The rats were starved for twenty-four hours and then killed by decapitation. Slices of liver were quickly excised and frozen. Tissue homogenates were prepared in 0.25 M ice cold sucrose solution (10 % w/v). During homogenization the temperature was maintained near 0 °C. The homogenates were centrifuged for 20 minutes at 500 g and respective clear supernatant fluids were used as enzyme source. The activities of alkaline and acid phosphatase (9), glucose-6-phosphatase (10), cholinesterase (11) and lipase (12) were determined spectrophotometrically. For each enzyme triplicate samples were analysed and the incubations were repeated three times. Student's t-test (13) was applied to calculate statistical significance. Total protein content was determined following the method of *Lowry and co-workers* (14).

RESULTS

Manganese invariably inhibited the activity of all the enzymes selected for the present study. However, cobalt influenced acid phosphatase, glucose-6-phosphatase and lipase only. The results on their reciprocal effects as shown in Table 1 approximated control values favouring a physiological antagonistic mechanism between the two metals.

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Table 1.

Enzyme activity in the liver of control and experimental rats

Enzyme	Control	Treatments		
		Cobalt	Manganese	Cobalt + Manganese
Alkaline phosphatase[a]	0.40(0.020)	0.44(0.011)	0.29(0.013)***	0.37(0.016)
Acid phosphatase[a]	0.44(0.016)	0.27(0.006)****	0.20(0.008)****	0.38(0.004)***
Glucose-6-phosphatase[a]	0.70(0.028)	0.57(0.019)***	0.61(0.027)*	0.75(0.025)
Cholinesterase units	42.0(3.25)	45.0(2.69)	27.0(2.64)**	38.0(3.58)
Lipase units	22.0(2.89)	12.0(2.35)*	8.0 (1.43)***	18.0(1.57)

Values are significant at *$P < 0.05$; **$P < 0.02$; ***$P < 0.01$; ****$P < 0.001$.

a: Activity is expressed in mg of inorganic phosphate liberated/mg protein/h at 37 °C.

DISCUSSION

Earlier studies have shown that manganese is involved in a wide range of enzyme activities in a variety of tissues and that mitochondrial structure and function are particulary affected in manganese deficiency (15). Furthermore, a dietary deficiency of manganese in mice was found to cause alterations in the integrity of cell membranes. Endoplasmic reticulum was swollen and irregular mitochondria were found with elongated stacked cristae in the liver, heart and kidney cells and there was an overabundance of lipid in liver parenchymal cells (16). However, several reports on the effects of manganese have, nevertheless, been limited to brain only (17). Enzymological studies in the brain by *Chandra and Shukla* (18) have shown that manganese inhibits the activity of succinate dehydrogenase (SDH) and alkaline phosphatase, whereas it stimulates the activity of monoamine oxidase (MAO). Although no comparative data on liver enzymes are available, present results are primarily the manifestations of manganese accumulation in the liver. Impairment of oxidative respiration ond mitochondrial alterations (19, 20) seem to be the events preceding enzymological disorders.

Enzymological effects of cobalt have not been studied in detail. However, cobalt salts are known to decrease cytochrome P_{450} (21) and nicotinamide adenine dinucleotide phosphate hydratase (NADPH) in the liver. An impairment of the drug metabolizing enzyme system (DMES) (22) has also been reported. In addition, a phenomenon such as inhibition of lipid peroxidation via a direct chain termination activity is also provoked by cobalt (23). A significant inhibition by cobalt of acid phosphatase, glucose-6-phosphatase and lipase activities in the liver can undoubtedly be related to lysosomal injury and disorders in carbohydrate and lipid metabolism.

The interaction between cobalt and manganese, which may be at the absorption or post absorption level, clearly shows antagonism. The inhibition of cation competition for absorption may be speculated upon, but nothing is certain until the biological significance of the »gut compartment« for cobalt and manganese is established. Also, cobalt may modify manganese toxicity through vitamin B_{12}. It is important to note that the complex interrelationships among essential minerals are as significant as those among heavy metals.

The amount of enzyme (protein) may be controlled by factors affecting their rate of synthesis and/or their rate of breakdown. Although a large number of compounds of different molecular types may affect the same enzyme system, there may be a number of enzymes which are affected by one class of compound and not by another. Variations in enzyme activity are further known to be the result of change caused by allosteric interaction of the toxicophore with the enzyme or of differences in the content of enzyme protein induced by a change in the synthesis or de-

gradation of the protein. The physical and chemical nature of membranes, the specific interaction with other proteins, and lipids may further contribute to the process of enzyme regulation.

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Sažetak

INTERAKCIJA KOBALTA I MANGANA U JETRI ŠTAKORA

Opisani su učinci kobalta i mangana na neke enzime u jetri štakora. Utvrđeno je da mangan inhibira aktivnost alkalne fosfataze, kisele fosfataze, glukoza-6-fosfataze, kolinesteraze i lipaze dok kobalt remeti aktivnost samo kisele fosfataze, glukoza-6-fosfataze i lipaze. Aplikacijom po 1/2 LD_{50} vrijednosti jednog i drugog metala bila je smanjena aktivnost samo kisele fosfataze, dok je aktivnost ostalih enzima ostala nepromijenjena u odnosu na kontrolne životinje. Ovi rezultati upućuju na to da vjerojatno postoji antagonistički učinak ovih dvaju esencijalnih metala.

Škola za onečišćenje okoliša i toksikologiju, Odjel za zoologiju, Muzaffarnagar, Indija

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