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Cytological Studies in Albino Rats after Oral Administration of Manganese Chloride

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Manganese is used in the manufacture of steel, dry battery cells, ceramics, calico-printing and in various chemical industries. Recently it has been used in gasoline and other fuels as an antiknock agent, which has posed the problem of environmental pollution (Tolonen, 1972). Cases of suspected manganese poisoning have been reported after drinking well water with high content of manganese (Report on Manganese, 1973). Thus, the industrial workers as well as population at large are exposed to the hazards of manganese toxicity. The deleterious effects of this metal on the central nervous system and testis are well documented (Mella, 1924, Pentschew et al. 1963, Rodier, 1955, Chandra et al. 1974, Imam and Chandra 1975). While many chemicals are known to produce cytogenetic changes in cell systems of mammals, there are no such reports on the effects of manganese. The present communication reports the cytological changes in bone marrow and testis of rats after chronic exposure to manganese chloride.

Materials and Methods

Male albino rats of ITRC colony (initial body weight 60 ± 10 g) were maintained on ad libitum pellet diet from Hindustan Levers Ltd., India and in an air conditioned room throughout the experiment. Group I comprising of 10 rats were given orally $MnCl_2.4H_2O$ (50 µg/kg) daily for a period of 180 days. Another group II with same number of animals were given orally 1 ml. of distilled water daily for the same period and served as controls.

Chromosome analysis

Colchicine was administered to each rat (4 mg/kg) by i.p. injection 4 hrs before killing by decapitation. Bone marrow from femur bones and seminiferous tubules were quickly collected in separate tubes in Hank's balanced salt solution (HBSS, pH 7.2). Cell suspensions were made by careful maceration and subjected for centrifugation (800 rpm). The cells were

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washed in HBSS, and exposed to hypotonic treatment for 10 minutes at 37°C. After recentrifugation, the cell pellet was fixed in methanolacetic acid (3:1) mixture. Air dried and flame fixed cells were stained with Giemsa.

The slides were numbered at random. Scoring was made to determine mitotic index and chromosome damage in bone marrow and spermatogonial cells. Mitotic index was calculated as % cells at metaphase after scoring a total of 100 cells per slide. Fifty metaphases were analysed from each treatment. Selection of metaphase plates for analysis was carried out with low power objective (X10). Chromosome damage was analysed for chromatid breaks, chromosome breaks and exchange figures, under X 1000 magnification. Cells having wide spread chromatids with minimum overlapping were selected for scoring.

Results and Discussion

Mortality - None of the animals died during the course of the experiment.

Cytogenetic study - Microscopic observations and scoring of metaphase plates revealed that manganese did not produce any significant chromosomal damage either in bone marrow or spermatogonial cells of male rats. Occurrence of chromatid gaps and a few chromatid breaks were the only type of observations in the bone marrow cells (Fig. 1, 2). The spermatogonial cells were devoid of these mild changes. Also the

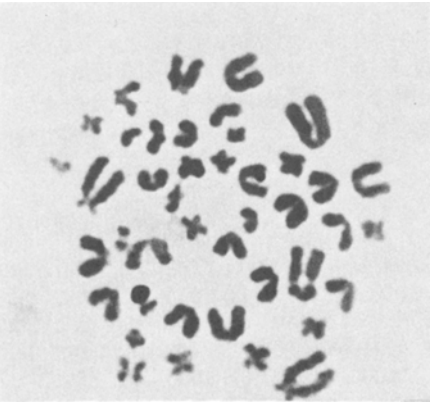

Fig. 1: Metaphase chromosomes from the bone marrow cells of normal rat (control) Giemsa stain; Ca x 1920.

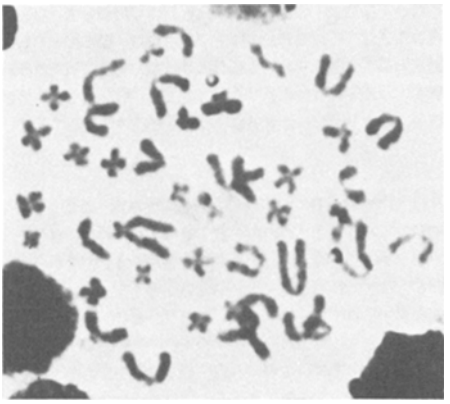

Fig. 2: Metaphase chromosomes from the bone marrow cells of rat treated with $MnCl_2 \cdot 4H_2O$ (50 µg/kg) for 180 days.
Note: Normal structure of chromosomes. Giemsa stain; Ca. x 1920.

exchange figures, chromosome breaks or the ring chromosomes were totally absent in both the cell systems (Table 1).

TABLE 1

FREQUENCY OF CHROMOSOMAL ABERRATIONS & MITOTIC INDEX IN THE BONE MARROW OF MANGANESE EXPOSED RATS(50 µg/kg).

Animals	Metaphases scored	Type of chromosomal aberrations				Mitotic index	Per cent aberrations
		Chromatid break	Chromosome break	Chromatid exchanges	Multiple breaks		
Exptl.							
1	50	2	-	-	-	3.1	4.0
2	68	5	-	-	-	3.8	2.9
3	60	-	-	-	-	2.7	-
4	55	3	-	-	-	3.0	5.4
Control							
1	50	-	-	-	-	4.0	-
2	50	-	-	-	-	3.4	-
3	50	2	-	-	-	3.2	4.0

Mitotic Index - Scoring of large number of cells showed that the mitotic index of bone marrow as well as spermatogonial cells of rats treated with manganese did not differ from that of control. In other words the metal did not induce any appreciable mitotic index in male rats *in vivo*.

The possible mutagenic action of several chemicals present in our environment has caused serious concern all over the world. The identification of mutagenic properties of heavy metals is all the more very important in view of their varied utility in the ever growing industries. *In vivo* cytogenetic assay in systems of mammals constitute an addition or alternative to the mutagenecity tests. Our earlier studies and also of other workers have indicated that bone marrow is a good media for screening chromosomal damage since it offers large number of mitotic cells (Legator *et al*. 1969, Kato, 1969, Schmid 1971, Dikshith and Datta, 1977 and Dikshith *et al*. 1977).

Manganese chloride administered for a prolonged period of 180 days did not induce any discernible chromosomal damage either in the bone marrow or spermatogonial cells of rats. However, earlier observations have shown that manganese does cause histopathological and enzymatic alterations in different tissues of laboratory animals (Chandra 1972, Chandra and Imam, 1973, Imam and Chandra, 1974). In this context it is significant to note that cadmium which is known for its acute and specific effects on mammalian testis and also for the production of temporary sterility in animals did not cause any chromosomal damage at a dosage as high as 3.0 mg/kg (Gilliavod and Leonard, 1975). Similarly it did not cause any noticeable changes in leukocytes (Pattin and Allison, 1972). Mitomycin C showed no cytogenetic effects in male germ cells while it did produce toxic effects on the spermatogonial cells (Gilliavod and Leonard, 1971, Leonard and Gilliavod, 1973).

It is known that action of manganese is strongly dependent upon the physiological conditions within the cell. Lack of mutagenic effects of this metal in fungi has been accounted to these physiological factors (Aurbach, 1973). Manganese chloride though caused a systemic damage in experimental animals, yet failed to induce demonstrable cytogenetic changes in the male rats.

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