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Proteins, Lipids and Carbohydrates in the Liver and Kidney of Rats after Molybdenum and Copper Treatment

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Amongst the trace elements, molybdenum and copper are important being essential with a significant role in growth and maintenance of the body (RICHERT and WESTERFELD 1953, UNDERWOOD 1971, MERTZ and CORNATZER 1971). However, their intake in excess have been found toxic. Molybdenotic syndrome is characterized by alopecia, dermatosis and severe anemia with a deformity in the front legs (DAVIES et al. 1960). Copper like several other heavy metals can be cumulative in animal/human body causing chronic poisoning (TODD 1962, 1969). Several studies have been conducted on various aspects of their toxicity by JETER and DAVIS (1954), DUTT and MILLS (1960), EVANS and ABRAHAM (1973), RANA and KUMAR (1979b, 1980). Though the physiological antagonism between molybdenum and copper is well known (ARRINGTON and DAVIS 1953), however, their combined effects have not been studied in details. Present study reports on the individual and combined effects of molybdenum and copper on total proteins, lipids and carbohydrates in the liver and kidney of adult rat, Rattus rattus albino. The results are expected to be important from environmental health point of view since these elements from mines, factories, through urbanization and motorization of large sections of population, together with contamination of water and soil pose possible long term dangers to human health.

MATERIALS AND METHODS

Forty male albino rats, Rattus rattus albino, 90 days old and weighing 100 ± 10 gm were selected from the laboratory stock. They were divided at random into four groups, each containing ten rats. Each rat was housed separately in a suitable cage, fed on standard lab. diet (Hindustan Lever Ltd., Bombay) and tap water ad libitum and maintained under laboratory conditions. Rats of group A received orally ammonium molybdate at the dosage of 1.0 gm/kg body weight in addition to lab. diet on each day for a total of twenty days. Animals of group B were fed on 0.1 gm/kg body weight copper sulfate and group C were fed on a mixture of ammonium molybdate and copper sulfate in the ratio of 1:1 at the total dosage of 2.0 gm/kg body weight in addition to lab. diet for twenty days. Animals of control group D were provided lab. diet only and tap water ad libitum.

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Total weight of the diet was kept constant throughout the experiment.

After 20 days, all the animals were starved for 24 hr and then killed by decapitation. Liver and kidney were removed from each animal and their wet weight was recorded. They were kept in separate containers and dried at 60°C for 15 days to a constant weight. An estimation of total proteins using bovine serum albumin (BSA) as standard was performed (LOWRY et al. 1951). Total lipids were extracted from the dried samples by soxhlet extractor using chloroform and ether as solvents (COLOWICK and KAPLAN 1963). Carbohydrates were determined using orcinol reagent (FRANK-CONSOLAZIO et al. 1963). The student 't' test described by FISHER (1950) was employed to calculate the statistical significance between control and experimental values.

RESULTS AND DISCUSSION

Observations on moisture contents of the liver did not show any marked effect of these elements whereas in kidney a significant elevation was noted after molybdenum treatment. However, copper had reverse effects. Data on proteins in these tissues and its comparison with controls showed that protein contents decreased after molybdenum and copper treatments. However, elevated values were obtained in the liver of rats fed on both the metals. A comparative data on total lipids showed that molybdenum stimulated hepato-renal lipids. After copper and Mo + Cu treatments, the lipid contents decreased in the liver but increased in kidney. Carbohydrate contents too were inhibited by molybdenum and copper treatments, however, their simultaneous feeding stimulated carbohydrates in the kidney only. The average values for moisture, proteins, lipids and carbohydrates in the liver and kidney of rats thus obtained are shown by Tables 1 and 2 respectively.

Since most of the naturally occurring metal ions are bound by proteins, they have been found useful probes for studying protein induction processes. Available examples of metal induced protein synthesis (DRYSDALE 1968, BRYAN and MORGAN 1970, WISNIEWSKA-KNYPL and JABLONSKA 1970) indicate that specific responses are elicited by these elements. However, protein induction defined as an adaptive increase in the number of molecules of a specific protein or decreased rate of its degradation is a possible selective means for regulating levels of specific proteins. Present results suggest that protein systems undergo a regulation as a response to changes in environmental parameters and

TABLE 1

Percentage of moisture, total proteins, lipids and carbohydrates in the liver of control and metal(s) fed rats.

Components	Control	Treatment					
		Molybdenum	% Alter.	Copper	% Alter.	Mo + Cu	% Alter.
% moisture	70.49±2.05[a]	71.87±2.62	1.95(+)	71.92±1.65	2.02(+)	71.21±0.99	1.02(+)
% total proteins	8.50±1.02	7.63±1.20	10.23(-)	5.80±0.53*	31.76(-)	11.32±1.21	33.17(+)
% total lipids	13.81±1.06	17.50±0.92*	26.71(+)	11.52±1.20	16.58(-)	10.89±0.85*	21.14(-)
% total carbohydrates	5.36±0.98	1.49±0.02**	72.20(-)	4.91±0.91	8.39(-)	4.60±0.89	14.17(-)

Values are significant at - *P< 0.05; **P< 0.01 (Fisher's 't' test).
a, Values expressed as mean ± mean standard error (5 observations).
+, Indicate % stimulation.
-, Indicate % inhibition.

TABLE 2

Percentage of moisture, total proteins, lipids and carbohydrates in the kidney of control and metal(s) fed rats.

Components	Control	Treatment					
		Molybdenum	% Alter.	Copper	% Alter.	Mo + Cu	% Alter.
% moisture	74.43±1.16[a]	80.16±1.04**	7.69(+)	74.31±0.92	0.16(-)	75.34±0.89	1.22(+)
% total proteins	12.50±1.05	6.41±0.98**	48.72(-)	9.60±0.89	23.20(-)	10.07±1.10	19.84(-)
% total lipids	8.09±0.92	11.04±1.00*	36.46(+)	12.60±1.08*	55.74(+)	8.38±0.56	2.96(+)
% total carbohydrates	3.63±0.51	1.36±0.10**	62.53(-)	2.93±0.62	19.28(-)	4.46±0.98	22.86(+)

Values are significant at - *P< 0.05; **P< 0.01 (Fisher's 't' test).
a, Values expressed as mean ± mean standard error (5 observations).
+, Indicate % stimulation.
-, Indicate % inhibition.

support the observation of JACOB and MONOD (1961) and FILNER et al. (1969).

Though copper is an essential constituent of many enzymes, it is directly bound to the blood protein ceruloplasmin. In view of significant accumulation of copper in the liver of Cu fed rats, total proteins were estimated in liver and kidney both. Contrary to general belief (FIREDEN 1968) that Cu ions react with proteins more strongly and function in biological system as a component of metalloproteins, it was found that it inhibited proteins in both the tissues. Though the earlier observations of DOWDY (1969) seems interesting that liver has a functional compartment for storing absorbed copper, however, when the dietary intakes of copper were excessive and the storage capacity of the liver exceeded, the absorbed Cu might have found its way out of the compartment effecting the kidney also. In both the tissues unexpectedly, it did not promote the biosynthesis of proteins. Similarly absorbed molybdenum inhibited the synthesis of proteins in liver and kidney both as shown in Tables 1 and 2. In biological system molybdenum is bound through carboxyl groups or hydroxyl groups of tyrosine and serine residues. Cysteine is also a likely binding site of the metal ion. SMITH and WRIGHT (1975) suggested that high Mo intakes induce the formation of stable Cu-Mo-protein compounds in plasma. Whether the protein -Cu-Mo complex are formed in the plasma from normally existing plasma proteins or whether different plasma proteins which can enter in to combination with copper and molybdenum are formed in response to molybdenum is not yet known. Nevertheless, present results show a loss of proteins from these two tissues which might have been the means of adaptive behaviour of their parenchyma to exceeding limits of copper and molybdenum in their fluid.

Present observations on total lipids show that Mo manipulates their elevation in liver and kidney both, however, Cu inhibited their synthesis only in liver. In all, there seems to be four explanations for the accumulation of fat in the liver and kidney (i) there is increased synthesis of fat by the parenchymal cells, (ii) there is decreased oxidation of fat by the parenchymal cells, (iii) there is increased mobilization of depot fat and (iv) there is decreased release of fat from the liver to the general circulation. At present nothing more is available on the fact that Mo increases L-α-glycerophosphate generation that diverts the fatty acids away from the β-oxidation pathway of fatty acids catabolism to reactions forming triglycerides and phospholipids. Enzymological studies reported elsewhere (RANA and KUMAR 1979a) further support the above conclusion. Differential action of Cu on the lipids of liver

and kidney suggests two different mechanisms. Copper might have decreased the input of free fatty acids (FFA) averting the formation of triglycerides while the reverse mechanism explains their accumulation in kidney. In view of the significant accumulation of Cu in the liver of Cu fed rats (RANA and KUMAR 1978) it can be speculated that excess of copper might have activated pre-existing enzyme(s) resulting in an increased capacity of the liver microsomes to desaturate fatty acids as observed in pigs by ELLIOT and AMER (1973).

Present data on carbohydrates of liver and kidney both show inhibitory effect of molybdenum and copper. Since, endoplasmic reticulum participates in the metabolism of carbohydrates, an injury to this organelle seems to be caused by these elements.

The phenomenon of physiological antagonism exhibited by Mo and Cu could be evidenced through these parameters. Their simultaneous feeding to rats helped in bringing the values approximate to controls. Different mechanisms have been reported in the past (DICK 1954, 1956, MILLS 1961) for the antagonistic behaviour of Mo and Cu. The most widely accepted view is that of HUISINGH et al. (1973) who showed that Mo and Cu form a biologically unavailable complex called cupric molybdate which appears to be absorbed, transported and excreted as a unit and makes both Cu and Mo less available. Though the basic mechanism for these changes remains unknown, an insight in to suggested mechanisms could come from further studies on metal induced biochemical responses.

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