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The element manganese in parasitic helminths - A preliminary note

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Abstract

The distribution of manganese in parasitic helminths belonging to the trematodes, cestodes or nematodes was analysed by the use of an atomic absorption spectrophotometer. The results of these analyses have demonstrated that growing trematodes (smaller forms) with active oogenesis and spermatogenesis contained more manganese than older forms (large or very old adults) with empty uteri and large lobulated testes. In cestodes, the neck region of cysticerci and immature proglottids showed more manganese concentration than the mature and gravid proglottids of worms or cyst proper. Similarly, the youngest endogenous daughter cysts of *Echinococcus granulosus* showed more manganese in their walls than those of larger forms. The element was found more concentrated in nematode eggs than in adult females. The significance of this element in parasitic helminths—both adult and larval stages is briefly discussed.

Keywords : Helminth, *Echinococcus granulosus,* Biochemical analysis, Manganese profile.

Introduction

Since long it is known that small quantities of manganese is essential for growth and reproduction. It is required by man, animals, plants as well as microorganisms. However, data on its occurrence in parasitic helminths are scanty (Von Brand, 1973).

Earlier, there have been few studies to identify and quantify this element in individual helminths (Singh *et al.*, 1978; Sood and Kapur, 1980; Goldsmid, 1986; Pandey and Chowdhry, 1989) or individual elements in different groups of helminths (Chowdhury and Singh, 1989; 1995). In the present paper, we have made an attempt to analyse the distribution of manganese in different groups of helminths in order to understand the probable function(s) of this trace element.

Materials and Methods

The different groups of helminths (Table 1) analysed in this study were obtained from freshly-slaughtered or autopsied animals, rinsed quickly 3-4 times (total time 1 min) in normal saline, and dried in moist blotting paper before taking measurements and wet weight. Cysticerci and endogenous daughter (hydatid) cysts of *Echinococcus granulosus* were deflated to remove the fluid and the cyst walls, after inversion, were rinsed. Uteri of gravid female nematodes were removed after longitudinal incision. Adult helminths or larval stages were digested with triple acid in the ratio 10:3:1 (concentrated nitric acid +70% perchloric acid + concentrated sulphuric acid). Manganese contents of the digests were determined with the help of an atomic absorption spectrophotometer (Model AA6 Varian Techtran) with aqueous calibration standards as in the earlier studies (Chowdhury and Singh, 1989; 1995). The observations were supplemented with histological studies of haematoxylin and eosin-stained 5-6 μm sections.

Results and Discussion

It is imperative from the Table-1 that younger forms of trematodes had comparatively higher concentrations of manganese than medium and larger forms. In cestodes, the concentration of manganese was higher in the neck or in immature regions than in mature and gravid proglottids or in the cyst proper. The smallest endogenous daughter cysts of *Echinococcus granulosus* showed the higher concentration of the element in the walls and its concentration decreased as the cyst increased in size. In nematode, *Ascaris lumbricoides* no demonstrable manganese was found (Von

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Table 1: Manganese concentrations in various helminths

Helminth	Host	Predilection site	Size L x B or Diam (x) (mm)	Wet wt (mg)	Mg %
TREMATODES					
Gigantocotyle explanatum	Buffalo *(Bubalus bubalis)*	Bile duct	Immature 11 x 5	300 (10)	0.52
			Mature 13 x 5.5	673 (10)	0.38
			Gravid 14 x 5.5	712 (10)	0.28
Cotylophoran cotylophorum	Sheep	Rumen	Immature 5 x 1.5	56 (12)	4.16
			Mature 7 x 2.5	323 (12)	0.72
			Gravid 9 x 4.5	887 (12)	0.26
Gastrothylax crumenifer	Goat	Rumen	Immature 6 x 1.5	32 (7)	14.58
			Mature 10 x 3	296 (7)	0.39
			Gravid 14 x 5	304 (7)	0.76
CESTODES					
Taenia hydatigena	Dog	Small intestine	Neck 10 x 2.5	8 (7)	35.00
			Mature 2 x 4.5	18 (5)	15.55
			Gravid 8.7	35 (7)	8.00
Cysticercus *(tenuicollis)* of *T. hydatigena*	Goat	Peritoneal cavity	Neck 10 x 2.5	67 (2)	29.15
			Cyst proper 35 x 30	303 (2)	8.58
Hydatid cyst (endogenous daughter cyst) of *Echinococcus granulosus*	Buffalo *(B.bubalis)*	Lung	5-6	30 (2)	38.50
			7-10	95 (2)	8.55
			11-12	90 (2)	8.10
			14-15	245 (2)	3.14
			16-18	448 (2)	1.71
NEMATODES					
Ascaris suum	Pig *(Susscrofa domestica)*	Small intestine	Female 245 x 5	275 (1)	0.50
			Uterus 50 x 1	7	7.00
Toxocara vitulorum	Buffalo calf *(B. bubalis)*	Small intestine	Female 195 x 5	214 (1)	1.30
			Uterus 60 x 1	13	32.30
Ascaridia galli	Poultry	Small intestine	Female 78 x 1	315 (5)	3.66
			Uterus* (60 x 0.3)	10	5.83

Figures within bracket indicate number of worms or proglottids; *Approximate measurement.

Brand, 1973). However, manganese concentrations (along with other elements) have been reported from nematodes *Haemonchus contortus* and *Ternidens diminutus* (Sood and Kapur, 1980; Goldsmid, 1986). In the present study, the concentration of manganese was higher in eggs than in all the three species of adult nematodes. In general, the distribution of manganese among three groups of helminths appears to be the more in cestodes than in nematodes and low in trematodes.

Earlier studies have discussed that zinc and cobalt are important trace elements. In small forms of trematodes there is a rapid oogenesis and spermatogenesis along with body growth. Our previous studies have shown that immature form of trematodes have comparatively higher concentration of zinc and cobalt than the medium and large forms. Evidently, manganese might be playing a definite role along with the above trace elements in the reproductive processes and body growth (Table-1). The reduction in quantity of the element in the gravid worms where degenerative processes might have been already started also support this hypothesis. In cestodes, stem cells in the unsegmented neck behind the scolex is the germinative region from where proglottids continuously regenerate and is the active site of DNA and RNA synthesis. The two important trace elements—zinc and cobalt in elevated concentrations required by helminths for these processes in the cell multiplication and growth (Chowdhury and Singh, 1989; 1995). The results of the present analyses of manganese along with the anterio-posterior axis of the adult tape worms beginning with the "neck" region, cyst walls of different sizes have indicated that this element most likely play a role on the survival, utilization of the element for ongoing developmental processes both in the adult tapeworms and larval forms of the cestodes particularly younger form of the hydatid cyst when compared with the larger forms.

In nematodes most intra-uterine eggs examined were found in various stages of multiplication. Sood and Kapur (1980) and Goldsmid (1986), respectively reported occurrence of manganese in *Haemonchus contortus* and *Ternidens diminutus* but they did not interpret their findings. Interestingly, *in vitro* studies have also shown that addition of several trace elements including zinc, iron, manganese, cobalt at 0.0002-10 mg/l^{-1} resulted in a 16-40% increase in the biomass of yeast *Saccharomyces cerevisiae* (Khrycheva, 1970). Vulfs and Wilks (1968) found that manganese was less toxic and addition of 10 mg% of $MnSO_4$ in the medium stimulated multiplication of *S. cerevisiae*; its presence in small quantities with cobalt in the nutrient medium enhances the production of riboflavin, uptake of iron, biosynthesis and immunological activity of zymonsan produced by the yeast cells, and these are essential components for both growth and development of microorganisms (Enari, 1958; Bass-Shadkhan and Galina, 1961; Bass-Shadkhan, 1965).

Further, Leach (1971) and Doisy (1972) observed that manganese is essential in the biosynthesis of glycoproteins, mucopolysaccharides and sterols in higher mammals. In helminths, it was demonstrated that both nematode *Caenorrhabditis briggsae* and cestode *Hymenolepis microstoma* needed sterols and haem (lecithin-haemin particles) during their growth *in vitro* (Khan, 1974; Vanfleteren, 1974). Since data on this element in helminths, particularly on physiological and/or biochemical aspects are scanty to compare with our results, it is suggested that along with spectrophotometer, more biochemical analyses of different groups of helminths are necessary to gather more information of its occurrence for a meaningful interpretation of the involvement and to elucidate its possible function(s).

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References

Bass-Shadkhan, K.F., 1965. The influence of Mn, Cu and Co in the formation of biologically active materials synthesized by *Saccharomyces cerebrisiae*. Mikroelementry V Sel'. Khos. i. Med. Ukr. Nauch-Issled. Inst. Fiziol. Rast. Akad. Nauk Ukr. SSR; Materially 40 go (Chetvertogo). Vses. Soveshch., Kiev., 640-3.

Bass-Shadkhan, K.F. and Galina, I., 1961. Influence of certain trace elements on quantity and quality of zymosan formation in yeast. I. Certain chemical indexes of zymosan prepared from yeast in nutrient medium supplemented with cobalt, copper and manganese. Latvijas PSR Zinatnu Akademijas Vestis. No. 12: 69-74.

Chowdhury, N. and Singh, R., 1989. Distribution of zinc in parasitic helminths. J. Helminthol., 63: 149-152.

Chowdhury, N. and Singh, R., 1995. Distribution of cobalt in parasitic helminths. J. Helminthol., 69: 259-261.

Doisy, E.A. Jr., 1972. Micronutrient controls on biosynthesis of clotting proteins and cholesterol. In: D. Hemphill (Ed). Trace substances in environmental health. VI. Proc. Univ. Missouri 6th Ann. Conf. Trace Substances Environ, Hlth. University of Missouri, Columbia, MO. p. 193.

Enari, T.M., 1958. Uptake of cobalt and iron and their effect on the production of riboflavin by *Candida guilliermondii*. Ann. Acad. Scientific. Fen. Sec AII No. 90: 8-42.

Goldsmid, J.M., 1986. Inorganic elements in adult *Ternidens diminutus* (Nematoda : Strongylidae : Oesophagostominae) from humans and baboons. J. Helminthol., 60: 147-148.

Khan, Z.I., 1974. Some physiological and biochemical studies on *Hymenolepis microstoma* grown *in vitro*. Ph.D. Dissertation. State University of Gent, Belgium.

Khrycheva, A.I., 1970. Effects of trace elements on the growth of *Sacharomyces cerevisiae* biomass. Prikle. Biochem. Mikrobiol., 6: 307-312.

Leach, R.M. Jr., 1974. Biochemical role of manganese. In: Hockstra, W.G., Buttie, J.W., Ganther, H.E., Mertz, W. (Eds). Trace element metabolism in animals. Baltimore, University Park Press. pp. 51-59.

Lal, S.A. and Kumar, S., 1985. Total ash and organic substances of five species of nematodes from ruminants. Indian J. Parasitol., 9: 153-158.

Pandey, K.C., Chowdhry, S., 1989. Inorganic elements in adults of *Ascaridia galli* (Shrank, 1768). J. Helminthol., 63: 75-76.

Singh, B.B., Singh, S., Ghosal, A.K. and Dwarkanath, P.K., 1978. Inorganic calcium, magnesium and phosphorus in *Thysaniezia giardi*. Indian J. Parasitol., 2: 37-38.

Sood, M.L. and Kapur, J., 1980. Inorganic elements in the adults of *Haemonchus contortus* (Nematoda : Trichostrongylidae). J. Helminthol., 54: 253-254.

Van Fleteren, J.R., 1974. Nematode growth factors. Nature, 284: 255-257.

Von Brand, T., 1973. Inorganic substances biochemistry of parasites. Academic Press Inc. pp. 1-47.

Vulfs, L. and Wilks, S., 1968. Effect of some trace elements on the growth of *Sacharomyces cerevisiae* and biosynthesis of B vitamins. Latiujas PSR Zinatnu Akademijas Vestis No. 8: 98-104.