Uttar Pradesh J .Zool. 27(1) : 31-38, 2007 ISSN 0256-971X

IN VITRO EFFECTS OF ALBENDAZOLE ON THE HISTOLOGY AND HISTOCHEMISTRY OF *TRICHURIS GLOBULOSA* (NEMATODA), A PARASITE OF SHEEP AND GOAT

HARPREET KAUR AND MONIKA ANEJA
DEPARTMENT OF ZOOLOGY, PUNJABI UNIVERSITY,
PATIALA-147 002, INDIA.

In the present study and attempt has been made to observe the *in vitro* effect of albendazole (ABZ), an anthelmintic drug, on the histology and histochemistry of *Trichuris globulosa*. The parasites were incubated in different working concentrations of the drug ranging from 50-200 ppm. Histologically the body wall and intestine of the treated worms were most effected by ABZ. No prominent effect of ABZ was observed on the reproductive parts, however, swelling of the polar plugs was observed in treated worms. Lipids (general lipids, neutral and acidic lipids, phospholipids and neutral fats) and carbohydrates (glycogen and acid mucopolysaccharides) were observed in lesser concentration in bodywall, intestine and reproductive parts of treated worms. Increased concentration of phospholipids in intestinal epithelium, increased concentration of glycogen and acid mucopolysaccharides in developing oogonia and oocytes was detected in treated worms.

Key words: Albendazole, *Trichuris globulosa*, histochemistry.

INTRODUCTION

Many workers such as Wang & Saz (1974), Borgers *et al.* (1975), Kaur & Sood (1982). Ahmad & Nizami (1987) and Mogambo (1998) have studied the *in vitro* effect of anthelmintics on various nematode parasites. *In vitro* effect of albendazole and fenbendazole on the histochemical localization of some enzymes of *T. globulosa* was studied by Kaur & Sood (1992). Histochemical studies on the gut of *Haemonchus contortus* were also made by Sood & Sehajpal (1978). An attempt has been made in the present study to observe the *in vitro* effect of albendazole on histology and histochemistry of *Trichuris globulosa*.

MATERIALS AND METHODS

Live nematode parasites *T. globulosa* were collected from the large intestine of sheep and goat, washed in saline solution, incubated in different working concentrations of drug stock solution. 200 ppm was found to be lethal concentration. Treated and control parasites were fixed in different fixatives *i.e.* Carnoys and Formal Calcium. Formal Calcium fixed parasites were embedded in gelatin at 37^{0}C. Blocks were stored in formal calcium in refrigerator after preparation. 10 μm thick sections were cut at -7^{0}C. Carnoys fixed parasites were embedded in paraffin wax and section were cut of 7-10 μm thickness. Sections were subjected to use for histological and histochemical studies adopted from Pearse (1968). The localization of glycogen was done by Best's Carmine test and of acid mucopolysaccharides by Alcian blue method. For lipids, in general, Sudan Black B test; for neutral and acidic lipids, Nile Blue Sulphate test; for neutral fats, Oil red O method and for phospholipids, Acid Haematin method was used.

Table I : Histochemical localization of various types of lipids in various tissues of *T. globulosa*

Histochemical Test	Treatment	Cuticle	Muscle cells			Intestine		Ovary			Uterine epithelium	Eggs			Testicular epithelium	Spermatocytes
			Hypodermis	Contractile Part	Non-contractile Part	Epithelium	Bacillary band	Epithelium	Oogonia	Oocytes		Polar Plug	Lipid layer	Cytoplasm		
Sudan Black B	Control	+	+++	+	-	++	++	+++	++	+++	++	-	+	+++	++	+
	60 ppm	+	+	++	-	+	+	++	++	++	++	-	-	++	+	-
	100 ppm	-	+	++	+	+	+	+	++	+	++	-	-	++	+	-
Nile Blue Sulphate	Control	-	+++	++	+	+	-	++	+++	+	+++	++	++	+++	++	++
	60 ppm	-	+	+	+	+	-	+	++	+	++	++	++	+	+	++
	100 ppm	-	+	-	+	+	-	+	++	+	+	+	+	+	+	+
Oil Red O	Control	-	+++	+	+	+++	++	++++	+++	++	+	-	+	+++ +	++	++
	60 ppm	-	++	+	+	++	+	+++	++	+	+	-	-	+	+	+
	100 ppm	-	+	+	+	+	-	++	+	+	++	-	-	+	+	+
Acid Haematin	Control	-	++	+	+	+	+	+++	+++ +	+	+	-	+	+++ +	++	+
	60 ppm	-	++	-	-	++	+	++	+++	+	+	+	++	+	++	+
	100 ppm	-	+++	-	-	++	+	+	++	+	+	+	++	+	+++	+

Keys to Abbreviations : ++++ Very intense, +++ intense, ++ moderate, + less, - nil.

RESULTS

Histomorphological changes : Body wall of treated worms showed single large infoldings at higher concentration of the ABZ. Cuticle showed wrinkling, peeling and inflations; also exhibited separation from hypodermis at many points at 100 ppm of ABZ. Intestine represented marked morphological changes. Narrowing and even the closure of intestinal lumen, disruption of its bacillary band and vacuolization in intestinal cells was observed in treated worms. No histomorphological change was observed in the reproductive parts due to ABZ except in the eggs present in the uterus. The polar plugs of eggs became swollen after drug incubation.

Histochemical changes

Lipids (Table I) : Concentration of various types of lipids in different parts of the nematode body was greatly effected due to albendazole. Lipids, in general; acidic and neutral lipids; phospholipids and neutral fats were present in reduced amount in hypodermis as revealed after reaction with Sudan black B, Nile blue sulphate, Acid haematin and oil Red O, respectively. Treatment with albendazole resulted in increase in concentration of general lipids and decrease in concentration of phospholipids in muscle cells but reduction in acidic lipids only in the contractile part of muscle cells (Figs. 1-4). Neutral fats were observed in reduced amount in the intestinal epithelium, bacillary band and intestinal lumen whereas general lipids and acidic lipids were detected in lower concentration in the bacillary band and intestinal cells respectively, however, increased concentration of phospholipids was observed in the intestinal epithelium. The amount of acidic lipids was decreased in uterine epithelium in treated worms as revealed by faint blue colour after reaction with Nile blue sulphate. Ovary, developing oogonia and oocytes and egg cytoplasm showed reduced concentration of general lipids, acidic and neutral lipids. Phospholipid concentration was observed to be lower than control worms in ovarian epithelium and developing oogonia (Figs.5-8). Testicular epithelium and spermatocytes exhibited decrease in the concentration of various types of lipids.

Glycogen (Table II) : No alteration in glycogen concentration in cuticle and hypodermis was observed, however, musculature exhibited decreased amount of glycogen. No effect on glycogen concentration in intestine was found in treated worms however, increased concentration of glycogen was revealed in uterine epithelium, ovarian epithelium and developing oogonia. Amount of glycogen in egg layers and egg cytoplasm remained unaltered (Figs. 9-10).

Acid Mucopolysaccharides (Table II) : Reduced amount of mucopolysaccharides in hypodermis, musculature and uterine epithelium and increased concentration in the luminal surface of intestinal cells, ovarian epithelium, developing oogonia was confirmed in treated worms after reaction with Alcian blue (Figs. 11-12)..

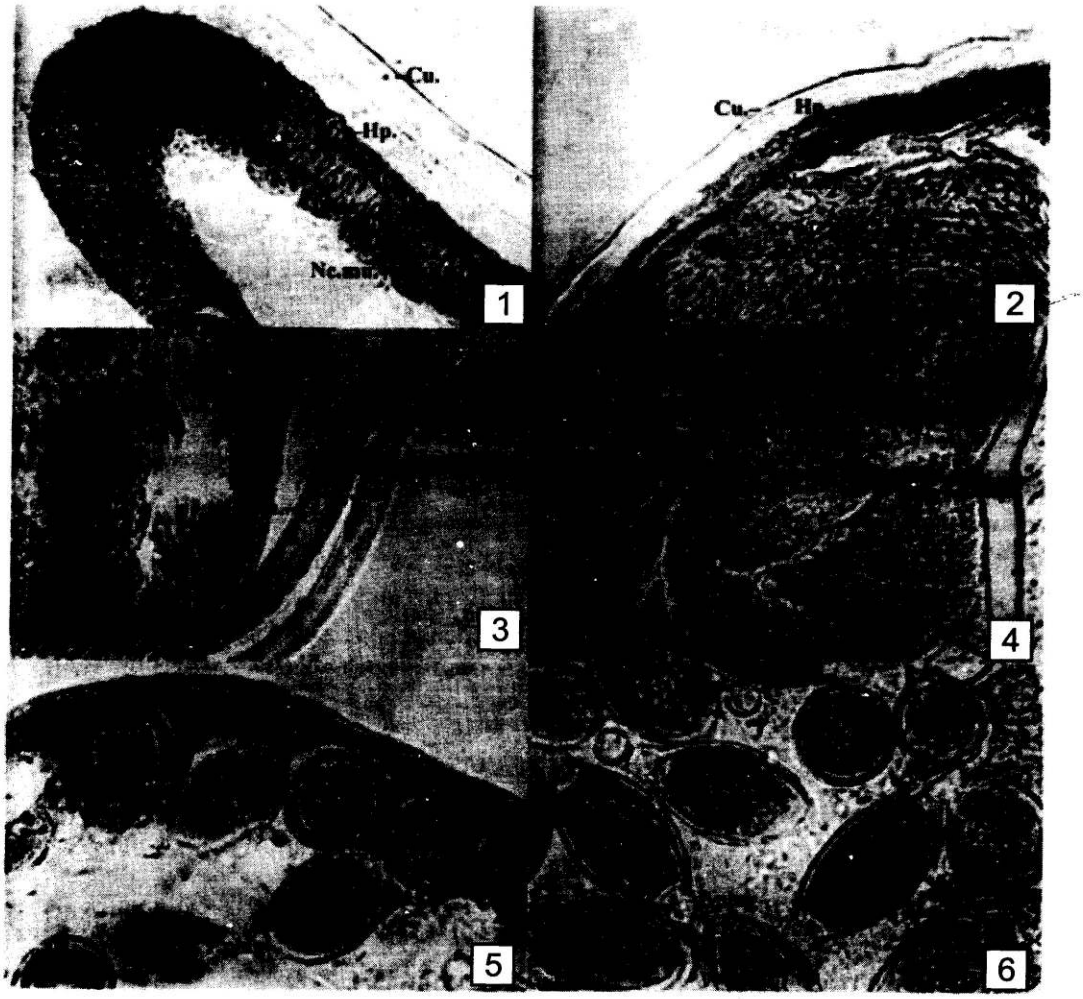
DISCUSSION

During the present study, lipids were present in reduced amount in treated worms. Lipids, in general, serve as potential energy reserves for the worm in hypodermal cells

Table II : Histochemical localization of glycogen and acid mucopolysaccharides in various tissues of *T. globulosa*

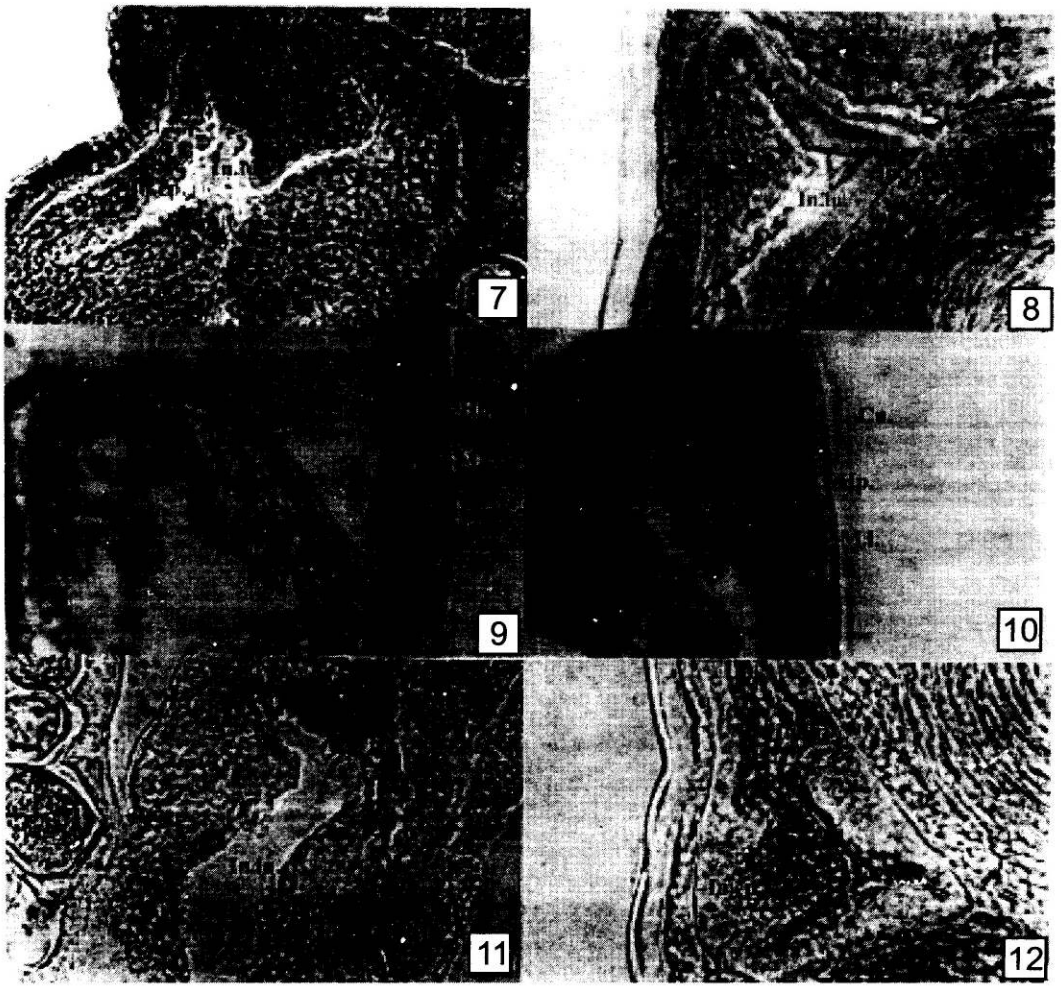
Histochemical Test	Treatment	Cuticle	Muscle cells			Intestine		Ovary			Uterine Epithelium	Eggs			Testicular Epithelium	Spermatocytes
			Hypodermis	Contractile Part	Non-contractile Part	Epithelium	Bacillary Band	Epithelium	Oogonia	Oocytes		Polar Plug	Lipid Layer	Cytoplasm		
Alcian blue	Control	+	+++	++	+	++	+	+	+	+	++	+	+	+	+	+
	60 ppm	-	+	+	+	++	+	+	++	++	+	-	++	+	-	-
	100 ppm	-	-	-	-	+	+	++	++	++	+	-	+++	+	-	-
Best Carmine	Control	+	++	+++	+++	++	+	++	+	+	+	++	-	++	x	x
	60 ppm	+	+	++	++	++	+	+++	++	+	++	++	-	++	x	x
	100 ppm	+	+	+	+	++	+	+++	+++	++	++	++	-	+	x	x

Keys to Abbreviations : ++++ Very intense, +++ intense, ++ moderate, + less , - nil, x not seen.



Figs. 1-6 : *Trichuris globulosa*. **1**. General lipids in hypodermis, contractile part of muscle cells; non contractile part of muscle cells in control worm. SBB; **2**. General lipids in body wall of ABZ treated worms. SBB: **3**. Acidic lipids in body wall and intestine in control worms, NBS: **4**. Acidic lipids in intestine of ABZ treated worms, NBS: **5**. Localization of acidic and neutral lipids in uterine epithelium, developing eggs and lipid layer of egg shell, NBS: **6**. Swelling of polar plugs and localization of acidic and neutral lipids in the eggs and treated worms, NBS. (Magnification x400).

(Lestan *et al.*, 1974). Muscle cells exhibited increase in concentration of general lipids, decrease in phospholipid concentration in both contractile and non-contractile layers whereas concentration of acidic lipids was reduced only in the contractile part. Lipids in body wall mainly forms structural elements in *Trichuris ovis* (Johal & Shivali, 1996) and also act as stored food material to be used by the muscle during starvation (von Brand, 1966). Marked reduction in the amount of general lipids was observed in intestinal epithelium and bacillary band after albendazole incubation. Lipids formed the main constituent of epithelial cell walls in the intestine (Johal & Shivali, 1996). The intestinal epithelium serves as a storage site for lipids (Sood & Sehajpal, 1978).



Figs. 7-12. *T. globulosa*. **7**. Phospholipids in intestinal epithelium, bacillary band and intestinal lumen in control worms, AH; **8**.Phospolipids in intestine of ABZ treated worms, AH; **9**. Glycogen in body wall in control worms, BC; **10**. Glycogen in body wall of ABZ treated worms, BC; **11**. Acid mucopolysccharides in the intestinal epithelium and intestinal lumen in control worms; **12**. Closure of lumen and distribution of mucopolysaccharides in ABZ treated worms AB. (Magnification x400).
(Abbreviations for Figs. 1 to 12 : Bc.b.=Bacillary band; Cu=Cuticle: Hp=Hypodermis: C.mu. =Contractile part of muscles: Ne. mu.=non contractile part of muscles; In.ep.=Intestinal epithelium; In.lu.=Intestinal lumen; M.I.=Muscle layer; Ut.e.=Uterine epithelium: Eg.cyto=Egg cytoplasm; P.plug=Polar plug)

Uterine epithelium exhibited decrease in the concentration of acidic lipids. Reduction in the concentration of general lipids, acidic and neutral lipids was observed in ovarian epithelium, developing oogonia and oocytes. The female reproductive system of nematodes contained greater amount of neutral lipids which served as substrate for carbohydrate synthesis in the developing embryos (Fairbairn, 1955; Tarr, 1972). The growth region of ovary of *Ascaridia galli* contained phospholipids which might be used in the synthesis of

cellular and subcellular membranes (Fairbairn, 1957). The significance of stored lipid which can be converted into glycogen whenever required has been discussed by Passey & Fairbairn (1957) and Barrett *et al.* (1970) in *Ascaris lumbricoides*. A rich concentration of lipids are incorporated in all the stages of developing ova both as a cytoplasmic as well as a structural element, thus contributing to egg shell formation has also been confirmed by Duggal & Harpreet (2000a). Reduced amount of glycogen concentration in muscle cells and acid mucopolysaccharide concentration in hypodermis was exhibited in ABZ treated worms. Elizabeth *et al.* (1998) also found decrease in glycogen content in ABZ treated *Paradistomum orientalis,* thus supporting the present study. The significance of the muscle cell body as the main storage site for glycogen in the large ascarids is well documented (Toryu, 1933; Fairbairn, 1957). Carbohydrates are the stored food material which may either be used by the muscle itself for its own activity or by the whole body during starvation or under unfavourable conditions and is related to availability of oxygen in its environment (von Brand, 1966). Glycogen is converted into simple sugars whenever required by glycogenolysis in muscle cells (Lee and Atkinson, 1976). Mucopolysaccharides provide selective permeability towards certain ions, gases and water but act as barrier towards the large molecules of secretions which may be harmful to the organisms (Pearse, 1966). Increased acid mucopolysaccharide concentration was observed at luminal surface of intestinal cells after ABZ treatment. Microvilli helps in the absorption of simple carbohydrates, which are conveyed to and stored in the form of glycogen in the intestinal epithelium (Johal & Shivali, 1996). Brandt & Pappas (1960) and Fawcett (1961) suggested that acid mucopolysaccharides protect the microvilli from proteolytic enzymes of host which are ingested by the parasite alongwith the food material.

High concentration of glycogen and low concentration of acid mucopolysaccharides in uterine epithelium was revealed in treated worms after performing Best Carmine test and Alcian blue test. Anya (1964) postulated that the acid mucopolysaccharides may be facilitating the extrusion of eggs by its lubricant effect as also suggested by Duggal & Harpreet (2000b) in *T. globulosa*. Both glycogen and acid mucopolysaccharide concentration was exhibited to be increased in ovarian epithelium and developing oogonia.

Johal & Joshi (1993) determined that the ovarian epithelium was rich in carbohydrates in the *T. ovis* with a considerable quantity located in the anterior portion of uterus, polar plugs of mature ova and epithelial lobes situated at the utero-vaginal junction in *T. ovis,* thus supporting the present study. Loss of acid mucopolysaccharides concentration was observed in polar plugs of eggs of ABZ treated female worms. Johal & Joshi (1993) detected good concentration of acid mucopolysaccharides in the polar plugs of eggs of *T. ovis*. Glycogen and lipids act as egg-shell precursors and may also be helpful in the survival of eggs outside in the soil (Duggal & Harpreet, 2002). Loss of acid mucopolysaccharides concentration was observed in testicular epithelium and spermatocytes also.

REFERENCES

ANYA. A.O. 1964. The distribution of lipids and glycogen in some female oxyuroids. *Parasitology,* **54** : 556-566.

BARRETT, J., WARD, C.W. & FAIRBAIRN, D. 1970. The glyoxylate cycle and the conversion of triglycerides to carbohydrates in the developing eggs of *Ascaris lumbricoides*. *Comp. Biochem. Physiol.*, **33**: 577-586.

BRANDT, P.W. & PAPPAS, G.B. 1960. An electron microscopic study of pinocytosis in *Amoeba*. I. The surface attachment phase. *J. Biophys. Biochem. Cytol.*, **18** : 675-687.

DUGGAL, C.L. & HARPREET KAUR 2000a. Egg shell formation in *Ascaridia galli*. *J. Punj. Acad. Sci.*, **2** (1) : 173-175.

DUGGAL, C.L. & HARPREET KAUR. 2000b. Comparative morphological, histological and histochemical studies on the female reproductive tracts of some parasitic nematodes. *J. Parasit. Disease*, **24** (2) : 123-134.

DUGGAL C.L. & HARPREET KAUR 2002. Comparative histochemical and SEM studies on the mature eggs from the distal uterus of some parasitic nematodes. *J. Parasit. Appl. Biol.*, **11** (1&2) : 1-8.

ELIZABETH, K.M., KISHORE, B. & HANUMANTHA RAO, K. 1998. The effect of Albendazole on *Paradistomum orientalis* (Trematoda) of *Calotes versicolor*. *J. Parasit. Appl. Anim. Biol.*, **7** (2) : 63-66.

FAIRBAIRN, D. 1955. Lipids of the female reproductive organs in *Ascaris lumbricoides*. *Can. J. Biochem. Physiol.*, **34** : 39-45.

FAIRBAIRN, D.1957. The Biochemistry of *Ascaris*. *Exp. Parasit.*, **6** : 491-554.

FAWCETT, D.W. 1961. Cilia and Flagella. In *The Cell*, Vol. II (ed. J. Brachet and A. E. Mirsky). Academic Press, New York, pp. 217-297.

JOHAL, M. & JOSHI, A. 1993. Histochemical studies on the female reproductive system of *Trichuris ovis* (Nematoda). *Current Nematology*, 4 **(2)** : 219-224.

JOHAL, M. & SHIVALI, 1996. Histochemical observations on the bodywall of *Trichuris ovis*. *J. Parasit. Appl. Anim. Biol.*, **5** (1) : 11-14.

KAUR, M. & SOOD, M.L. 1992. *In vitro* effect of albendazole and fenbendazole on the histochemical localisation of some enzymes of *Trichuris globulosa* (Nematoda : Trichuridae). *Angew. Parasitol.*, **33** :33-45.

LEE, D.L. 1960. The distribution of glycogen and fat in *Thelastoma bulhoesi* (Magalhaes, 1900) a nematode parasitic in cockroaches. *Parasitology*, **50**: 247-259.

LEE, D.L. & ATKINSON, H.J. 1976. *Physiology of Nematodes*. 2nd Ed. The Macmillan Press Ltd., London and Basingstok, 215pp.

LESTAN, P., ZMORAY, I. & DUBINSKY, P, 1974. Histochemieder gewebe von *Lumbricus terrestris* and *Ascaris Suum*. *Biologia Bratislava*, **29**: 195-205.

PASSEY, B.F. & FAIRBAIRN, D. 1957. The conversion of fat to carbohydrate during embryonation of *Ascaris lumbricoides* egg. *Can. J. Biochem. Physiol.*, **35** : 511-525.

PEARSE, D.C. 1966. Polysaccharides associated with the exterior surface of the epithelial cells, kidney, intestine, brain. *J. Ultrastruct. Res.*, **15**. 555-558.

PEARSE, A.G.E. 1968.. "Histochemistry : Theoretical and Applied" **1**. J. and A. Churchill, London.

SOOD, M.L. & SEHAJPAL, K. 1978. Morphological, histochemical and biochemical studies on the gut of *Haemonchus contortus* (Rud, 1803). *Z. Parasitenkd.* **56** : 256-273.

TARR, G.E. 1972. Ascaroside esters and other neutral lipids in the female reproductive tissues of *Ascaris lumbricoides* egg. *Dev. Biol.*, **22**: 366-387.

TORYU, Y. 1933. Contribution to the Physiology of the *Ascaris*, *Ascaris megalocephala*. Cloq. Sci. Rep. Tohoku Uni., **8**: 65-74

VON BRAND, T. 1966. *Biochemistry of Parasites*. Academic Press, New York and London.