Rec. zool. Surv. India : **104** (Part 1–2) : 91-100, 2005

HISTOLOGICAL STUDIES OF TISSUE DAMAGES ASSOCIATED WITH *CYATHOCEPHALUS TRUNCATUS* (CESTODA : SPATHEBOTRIDEA) INFECTION IN THE RAINBOW TROUT *SALMO GIARDNERI*

C. E. OKAKA*

Department of Pure and Applied Biology, University of Leeds, Leeds LS2 9JT, England

INTRODUCTION

Previous studies have shown that *Cyathocephalus truncatus* infection in salmonid fishes do result in host tissue destruction at the site of the tapeworm's attachment (Wisniewski, 1932; Vik, 1955; Awachie, 1966; Catalini *et al.*, 1978; Okaka 1990). These studies were based only on already established infections in the pyloric caeca of fish collected from the wild and in some cases lacked detailed histological descriptions of damages on the infected tissues.

In the present report, the histopathological changes seen in the pyloric caeca and other tissues infected by the tapeworm were studied in laboratory infected rainbow trout, *Salmo giardneri* over a 2-years period.

KEY WORDS : Tapeworm, *Cyathocephalus truncatus*, rainbow trout *Salmo giardneri*, histopathology.

MATERIALS AND METHODS

Sixteen hatchery-bred *Salmo giardneri* varying between 10 cm and 14 cm in length were put two per tank into eight tanks and maintained in the laboratory by being fed with uninfected amphipods, *Gammarus pulex*. Sixty infected *G. pulex* were later fed in each tank to establish infections in the fish after which they were continuously fed with uninfected gammarids. The infected *G. pulex* were earlier raised and infected in the laboratory as described by Okaka (1989).

**Present Address for Correspondence : Department of Zoology, University of Benin, P.M.B. 1154, Benin City*

Field infected fish and amphipods from which laboratory infections of amphipods were established, were originally collected at the Southburn Beck tributary of the River Hull at Driffield, England. Two control experiment tanks were set up in which the fish were only fed with non-infected gammarids over the period of study.

The experimental fish were examined 4 times (*i.e.* 4 fishes in 2 tanks at a time) for the state of establishment of infection. The infected tissues studied histologically were those of infected fish examined after one week, one month, one year and two years of infections respectively. 10 mm thick longitudinal and transverse sections of the infected tissues were made using paraffin wax routine histological techniques and employing Ehrlich's Haemotoxylin / Eosin and Masson's trichome staining techniques as stated by Humason (1962). Histological studies were also conducted on the tissues of fish in the control experiment tanks and on transverse sections of the entire infected fish at the pyloric region.

RESULTS

The prevalence of infection was highest (90% and 88.3%) for the fish examined one month after infections and lowest (60% and 58.3%) for the fish examined 2 years after infection as shown in Table 1.

The histological section of the uninfected pyloric caecum (Fig. 1) shows clearly the body wall layers of the *Serosa, Muscularia, Stratum compactum* of the submucosa and the mucosa as previously described by Weinreb and Bilstad (1955), Kimura (1973) and Ezeasor and Stockoe (1981). In the one-week-old infection, the tapeworms were recovered from the pyloric caeca where they were attached to the mucosa at the distal blind end. The histological section of the one week old infection (Fig. 2a) shows that the villi was already being eroded and inflammation set up at the body wall of the distal end. (Fig 2b). Collagenous fibres which gave green colour stain with Massons technique were being deposited in the *Tunica propria*, *Stratum compactum* and *Stratum granulosum* layers. Detailed studies also showed increase eosinophils and macrophage cells in the sections when compared to similar tissue of the uninfected pyloric caeca.

The histological section of the one-month-old infection (Fig. 3) shows that the tapeworm's attachment at the distal end of the caecum advanced into the *Muscularis* layer of the body wall. The scolex was seen to be surrounded by inflammed tissue reaction. Inflammation of tissues and blood vessels and collageneous fibres deposition were also seen in the *Serosa* layer.

The histological section of the one-year-old infection (Fig. 4) shows the attachment deep into the heavily inflammed tissues at the distal end of the caecum. The original layers of *Muscularis* and *Serosa* have been lost with the entire tissue being filled with collageneous fibres, which were

also noted to be deposited in tissues of other organs in the body cavity that are attached to the mesothelium of the *Serosa*. By tissue reaction, effects of the infection thus spreads to other organs of the body that have any bodily contact with the infected caeca.

The histological sections through the pyloric caeca region of the two year old infection (Fig. 5, 6 and 7) shows that in some cases the scolex was at the *Serosa* end while in others, the worms having completely eroded through the caecal body wall, were now attached to the abdominal musculature, submucosa and skin of the fish. Infection was also seen to have spread to the wall of the duodenum and small intestine, and to tissues of the liver and gonads lying in the body cavity of the fish.

Table 1. : Experimental infection of *Salmo giardneri* with *Cyathocephalus truncatus* using laboratory infected *Gammarus pulex* harbouring the tapeworm procercoid.

Track Number	**No. of fish**	**No. of infected *G. pulex* fed to fish**	**Time of examination**	**No. of tapeworm recovered from fish**	**Percentage of tapeworm established (%)**	**Percentage of tapeworm lost (%)**
1	2	60	After 1 week	21, 19 } 40	66.7	33.3
2	2	60	„	17, 22 } 39	65.0	35.0
3	2	60	After 1 month	28, 26 } 54	90.0	10.0
4	2	60	„	27, 26 } 53	88.3	11.7
5	2	60	After 1 year	25, 23 } 48	80.0	20.0
6	2	60	„	22, 24 } 46	76.7	23.3
7	2	60	After 2 years	20, 16 } 36	60.0	40.0
8	2	60	„	21, 14 } 35	58.3	41.7
9	2	Control	A fish each after 1 week and 1 month	–	–	–
10	2	Control	A fish each after 1 year and 2 years	–	–	–

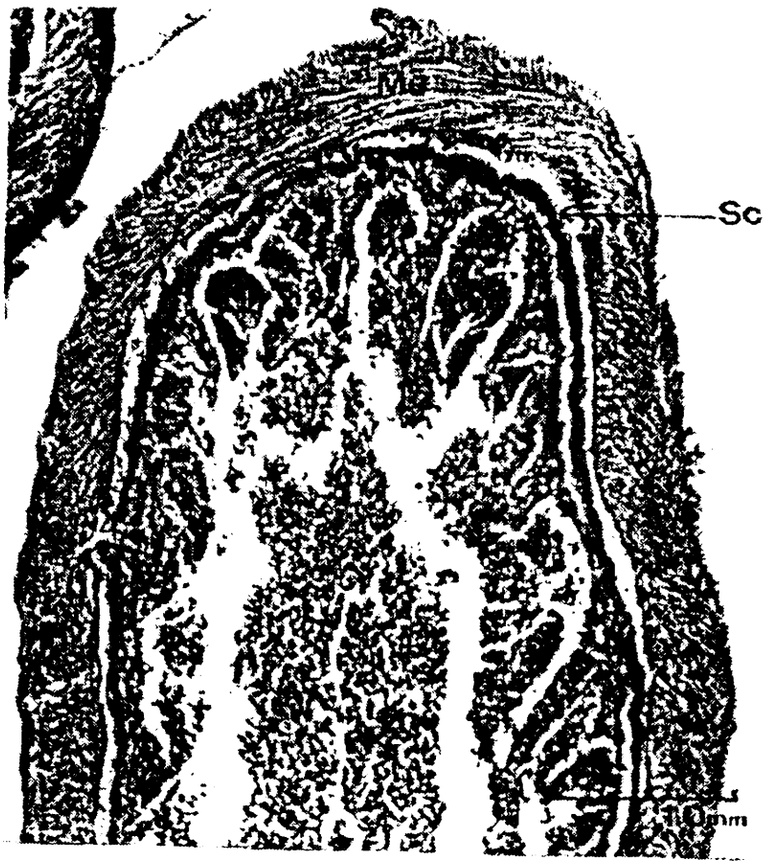

Fig. 1. Longitudinal section of the uninfected pyloric caecum of rainbow trout *Salmo giardneri*.

Fig. 2(a). Longitudinal section of a one-week old infected pyloric caecum with the tapeworm *C. truncatus* attached to the epithelium of the mucosa.

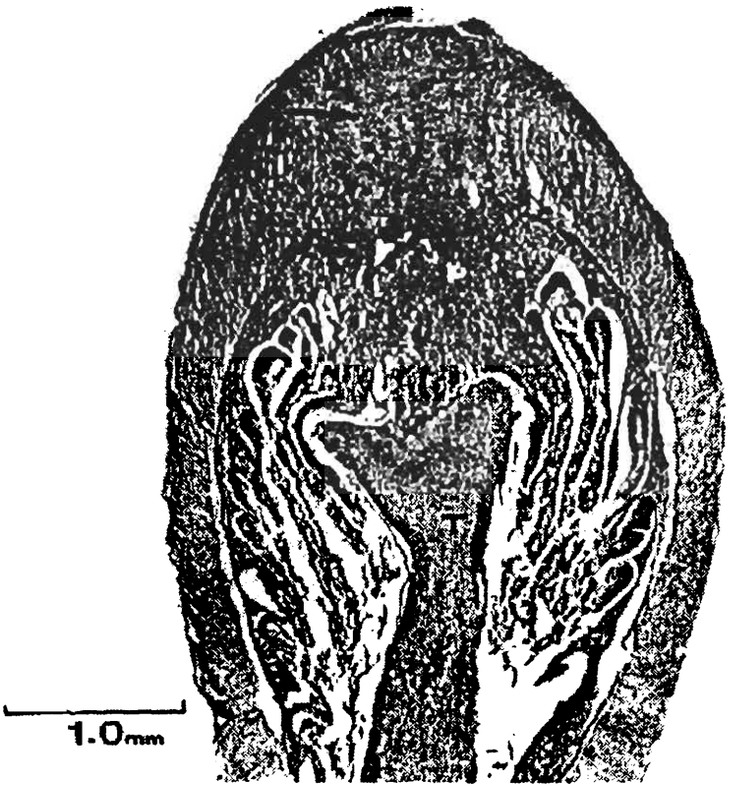

Fig. 2(b). Longitudinal section of a one-week old infected pyloric caecum with the tapeworm *C. truncatus* attached to the epithelium of the mucosa.

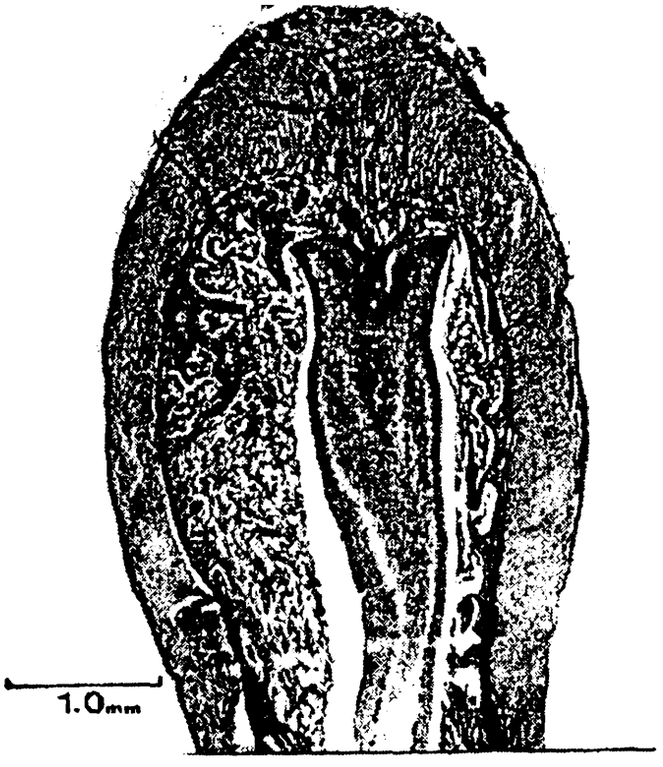

Fig. 3. Longitudinal section of a one-month old infected caecum with *C. truncatus* attached to the muscularis layer of the body wall.

Fig. 4. Longitudinal section of a one-year old infected caecum with *C. truncatus* attached to the inflammed and heavily swollen tissue at the distal tip. Note that the *muscularis* and *serosa* layers are no longer distinguishable at the site of attachment.

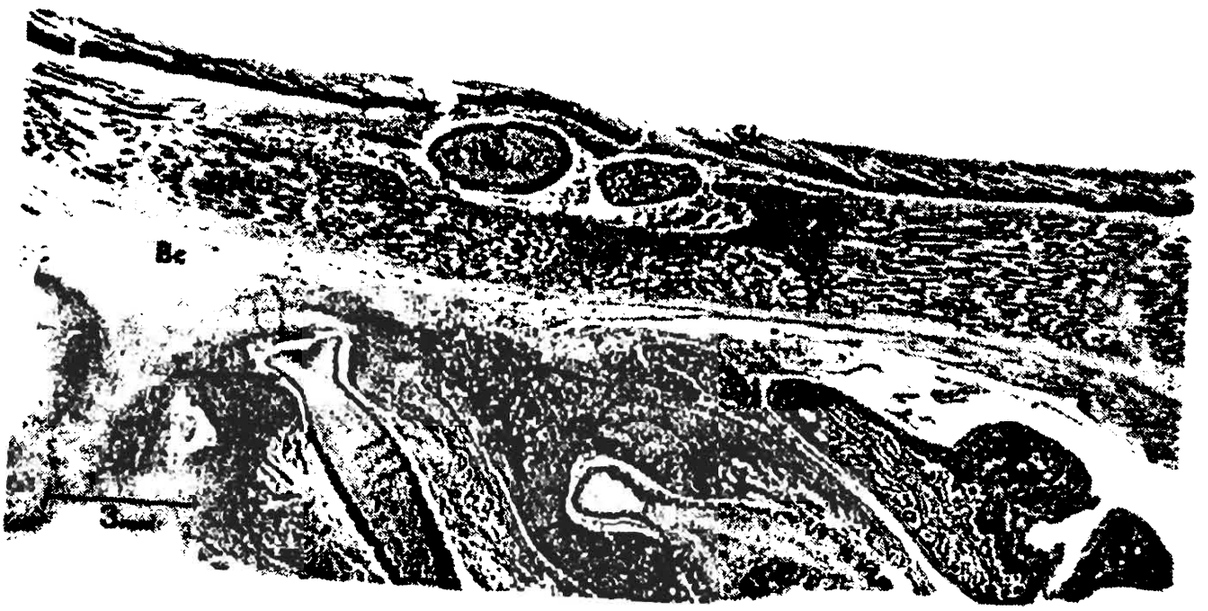

Fig. 5. Transverse section of an infected *S. giardneri* at the pyloric region showing the tapeworm *C. truncatus* at the serosa layer and advancing towards the body cavity of the fish. Note another worm is cross section (arrowed) already lodged in the skin of the fish.

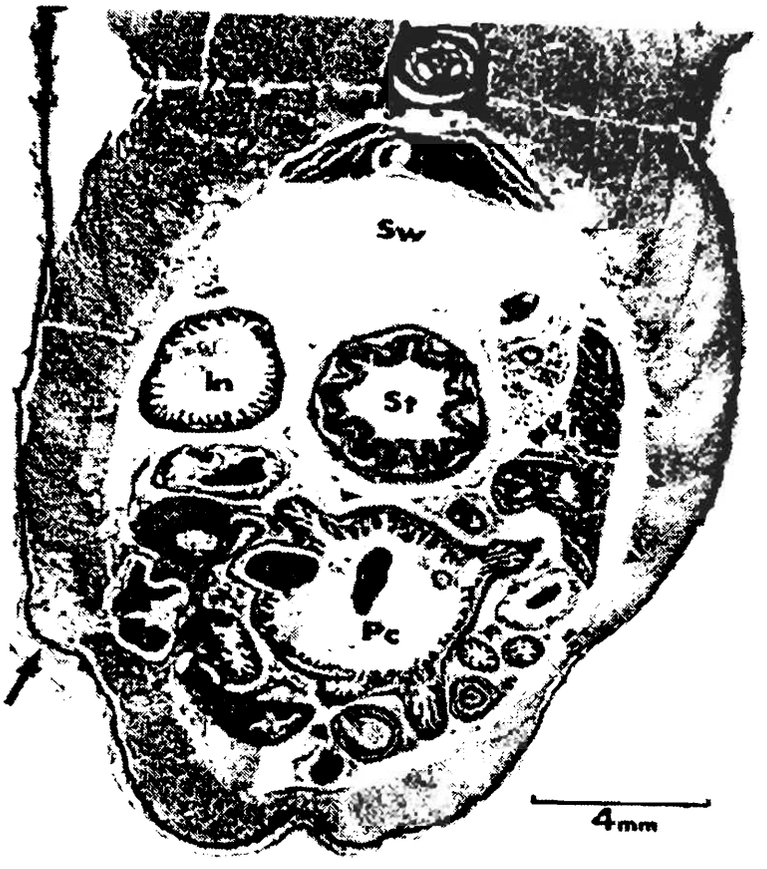

Fig. 6. Transverse section of an infected *S. giardneri* at the pyloric region showing the tapeworm at the body cavity of fish. Note the destruction (arrowed) of the body wall musculature of the fish.

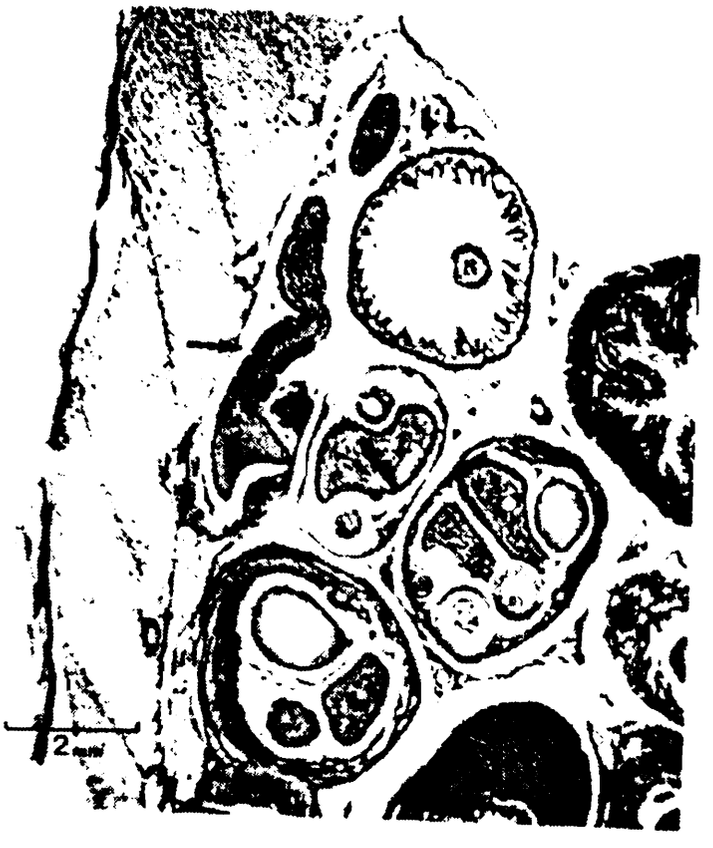

Fig. 7. Transverse section of an infected *S. giardneri* showing the tapeworm (arrowed) in the body cavity of the fish.

DISCUSSION

The results of experimental infection show that a high percentage of worms establish on infection and only few worms are lost. Loosing of tapeworms by this method of infection may be due to inability of the worms to quickly form an attachment to the intestinal body wall but swept out of the gut by peristalsis. Inability to attach at a specific site may also be due to competition for space among the tapeworms (Chappell, 1969), but this may be unlikely for *C. truncatus* because Okaka (1984) reported the occurrence of more than one worm in a single pyloric caecum.

The present work has also shown that once the tapeworm infection is established a strong attachment is already formed one week after infection. The firm attachment elicited tissue reactions, which resulted in infiltation of fibres and inflammation or swellings of the site of attachment in fish. This is similar to the observations of Bullock (1963). McDonough and Gleason (1981) on histopathology of the intestine of fish infected with acanthocephalan parasites. The tapeworm *C. truncatus* in the present study was seen attached to this inflammed tissue for over one year of infection but by the second year the tapeworm was seen to have eroded through this tissue to reach other organs like the liver, gonads, abdominal body wall and even the skin of the fish. This ability of the infections to spread to other organs in long standing infections resulting in impairment of the organs and possibly can result in the death of the fish. Wisnewski (1932) and Vik (1954) have reported high rate of mortality of fish infected with *C. truncatus*.

The tendency of *C. truncatus* to penetrate the host tissue of the pyloric caeca to get into other organs at the abdominal region could support the views of Wisniewski (1932) and Okaka (2000) that the tapeworm in fish possesses neotenic features but attained maturity in the fish at a stage when it should have been a plerocercoid encapsulated in the muscular tissues.

SUMMARY

Histological studies of tissues and organs of rainbow trout (*Salmo giardneri*) infected with the tapeworm *Cyathocephalus truncatus* revealed tissue damages in the pyloric caeca, body wall of small intestine, liver, gonads, lower abdominal body wall and skin. Typical sections of the damaged tissues characteristically showed the followings : a longitudinal or cross-section of the tapeworm, heavy presence of collagen fibres, eosinophils, macrophage cells and general loss of natural texture. Tissue damages were seen to be more severe as infection ages. Two years old infection can lead to impairment of the infected organs and possibly death in heavily infected fish.

LABELS

Amu–Abdominal musculature of fish; In–Intestine of fish; Li–Liver; Lu–Lumen of intestine; Mu–Muscle layers; Muc–Mucosa layer of pyloric caecum; O–Gonads; Pc–Pyloric caeca region;

Sc–*Stratum compactum* layer of submucosa; Sk–Skin of fish; St–Stomach; Sw–Swim bladder; T–Tapeworm *Cyathocephalus truncatus*.

ACKNOWLEDGEMENT

Thanks are due to Peter Broughton for maintenance of fish in the aquarium, Mike Ayers, Geoff Standley and Peter Evennette for technical assistance and Arthur Holliday for photography.

REFERENCES

Awachie, J. B. E. 1966. Observations on *Cyathocephalus truncatus* Pallas, 1781 (Cestoda : Spathebothriidae) in its intermediate and definitive hosts in a trout stream, North Wales. *J. Helminth.*, **40** : 1-10.

Bullock, W. L. 1963. Intestinal histology of some Salmond fishes with particular reference to the histopathology of acanthocephalan infection. *J. Morphol.*, **112** : 23-24.

Catalini, N., Orecchia, P., Paggi, L. and Todaro, P. 1978. Parassitofauna di *Salmo trutta* L. del Fiume Tiriono. Nota 111, Osservazioni istologiche sui parassiti reperti a Livello intestinale. *Parassitologia*, **20** : 169-170.

Chappell, L. H. 1969. Competitive exclusion between two intestinal parasites of the three spined stickle back, *Gasterosteus aculeatus* Linnaeus *J. Parasit.*, **55** : 775-778.

Ezeasor, D. N. and Stockoe, W. M. 1981. Light and electron microscope studies on the absorptive cells of the intestine, caeca and rectum of the adult rainbow trout, *Salmo gairdneri* Rich. *J. Fish. Biol.*, **18** : 527-544.

Humanson, G. L. 1962. *Animal Tissue Techniques*. W.H. Freeman and Co., Publishers, London.

Kimura, N. 1973. Fine structure of the epithelial cells in the pyloric caecum of the rainbow trout, *Salmo gairdneri. Jap. J. Ichthyol.*, **20** : 13-24.

McDonough, J. M. and Gleason, L. N. 1981. Histopathology in the rainbow darter. *Etheostoma caeruleum* resulting from infections with the acanthocephalans *Pomphorhynchus bulbocolli* and *Acanthocephalus dirus. J. Parasit.*, **67**(3) : 403-409.

Okaka, C. E. 1984. Studies on the biology of *Cyathocephalus truncatus* (Pallas, 1781). (Cestoda : Spathebothridea) in its fish and crustacean hosts. Ph.D Thesis, University of Leeds, England.

Okaka, C. E. 1989. Studies on the development of the oncosphere and procercoid of *Cyathocephalus truncatus* (Cestoda) in the intermediate host *Gammarus pulex. Zoologica scripta*, **18** : 205-209.

Okaka, C. E. 1990. Histological demonstration of occurrence of *Cyathocephalus truncatus* in the body cavity and body wall of *Salmo gairdneri. Bioscience Research Communications*, **2** : 61-66.

Okaka, C. E. 2000. Maturity of the procercoid of *Cyathocephalus truncatus* (Eucestoda : Spathebothriidae) in *Gammarus pulex* (Crustacea : Amphipoda) and the tapeworm's life cycle using the amphipod as the sole host. *Helminthologia*, **37** : 153-157.

Weinreb, E. L. and Bilstad, N. M. 1955. Histology of the digestive tract and adjacent structures of rainbow trout *Salmo gairdneri irideus* Copeia, **3** : 194-204.