

HISTOPATHOLOGY OF NUCHAL LIGAMENT ONCHOCERCIASIS AND MICROFILARIAL DERMATITIS IN DONKEYS IN KAFR EL-SHEIKH GOVERNORATE

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SUMMARY

During the period from January to July 1999 a total of 26 donkeys slaughtered for feeding Zoo carnivora were carefully examined for adult worm induced lesions in ligamentum nuchae, flexor and extensor tendon, testes, penis and subcutaneous tissue. Nodules contained deeply enmeshed Filarial nematodes identified as Onchocerca cervicalis were present in the nuchal ligament. Ligament and skin specimens from ventral midline were collected. Histopathological examination of ligamentum nuchae revealed chronic active inflammatory reaction around adult worms represented by cellular infiltration (neutrophils, eosinophils, histiocytes, lymphocytes, and basophils) and fibroplasia. The eosinophilic infiltration was commonly accompanying the microfilariae bearing adult females skin specimens revealed prominent dermatitis, characterized by epidermal hyperplasia (acanthosis), hyperkeratosis, and dermal infiltration with eosinophils, histiocytes, lymphocytes and mast cells at perivascular and periadnexial locations. The reaction was more severe in deeper dermis (reticular dermis) than in the upper papillary dermis .

INTRODUCTION

Onchocerciasis in equidae caused by filarial nematodes has a worldwide distribution but its prevalence is poorly documented in Egypt. Very recently (Abd El-Wahab and Ashour, 1999; AbdEl-Wahab and Raef, 1999) recorded incidence of 65.38% of *Onchocerca cervicalis* among donkeys in Kafr El-Sheikh province. However there is controversy over the speciation and pathogenicity of this parasites (Cello, 1971; McMullan, 1972). Although the predilection site of adult *Onchocerca cervicalis* is being the ligamentum nuchae of equidae (Mellor, 1973), *Onchocerca* spp could be present in flexor and extensor tendon, testes, penis and subcutaneous tissue (Muller, 1979). The viviparous adult female produce large number of microfilariae which migrate to the dermis and localize along the ventral

abdominal midline and thorax (Mellor, 1973) inguinal and perineal area (Rabalais et al. 1974). The adult worm is involved as a major contributory factor in supraspinous bursitis (fistulous withers) (Jacobs 1986) while the microfilariae is associated with dermatitis and ocular lesions as keratitis and periodic ophthalmia (Lloyd and Soulsby 1978, Attenburrow et al 1983, Schmidt et al. 1985) The disease is transmitted through biting arthropod vector (*midge of genus Culicoides and mosquitos of genus Anopheles*) (Soulsby, 1982) .

Only small percentage of microfilaria associated dermatitis was reported in horses (McMullan, 1972) which led to the concept that presence of microfilaria in the dermis is only coincidental with no aetiological importance.

The present study is aimed to study the pathological aspect of naturally occurring onchocerciasis in donkeys in Kafr El-Sheikh governorate, Egypt

MATERIAL AND METHODS

During the period from January to July 1999 a total of 26 donkeys slaughtered for feeding Zoo carnivora were subjected to careful postmortem examination for adult worm induced lesions in its predilection sites (ligamentum nuchae, flexor and extensor tendon, testes, penis and subcutaneous tissue.) The Parasitological examination were performed as previously described for light and scanning electron microscopy (Abd El-Wahab and Ashour, 1999; Abd El-Wahab and Raef, 1999). For Pathological examination , specimens were collected from the nuchal ligament and from the skin of ventral abdominal wall and withers then were immediately and properly fixed in neutral buffered formalin 10 %, processed, paraffin embedded, sectioned at 4µ thickness, stained with H&E stain and microscopically examined after being covered and dried

RESULTS

Prevalence and Gross examination:

seventeenth out of twenty six animals examined had *O.cervicalis* in their ligamentum nuchae (65.38%). Postmortem examination revealed presence of small nodules containing superficially located easily extracted adult worm (Identified as Male *Onchocerca cervicalis*) and other larger nodules contained deeply enmeshed worm (identified as female *Onchocerca cervicalis*). The nodules were present in the two lateral sides and inner sides of lamellar part of the ligament and at its insertion in thoracic vertebrae and atlanto-occipital articulation. The affected part of skin along the ventral midline showed somewhat dry scaly skin with loose hair.

Parasitological Examination :

Parasitological examination of adult male and female worm and the microfilariae recovered from the skin showed morphologic and ultrastructural criteria of *onchocerca cervicalis* (Full details were described previously by Abd El-Wahab and Ashour, (1999) and Abd El-Wahab and Raef, (1999)).

Histopathology:

The ligamentum nuchae showed chronic active inflammatory reaction around the adult worms represented by cellular infiltration and fibroplasia. The cellular infiltration varied from mild to massive leukocytic infiltration (Fig 1, 2) They were consisted of neutrophils, eosinophils, histiocytes, lymphocytes, and basophils. (Fig. 3). The eosinophilic infiltration was commonly accompanying the microfilariae bearing adult females (Fig. 4).

Microscopical examination of the skin specimens revealed prominent dermatitis, characterized by epidermal hyperplasia (acanthosis), hyperkeratosis, (Fig. 5) and dermal infiltration with eosinophils, histiocytes, lymphocytes and mast cells at perivascular and periadnexial locations. The reaction was more severe in deeper dermis (reticular dermis) than in the upper papillary dermis and it was sometimes focal in distribution (Fig. 6). The inflammatory reactions were sometimes solely eosinophilic in nature (Fig. 7). Variable numbers of microfilariae were frequently detected in the dermis, often large numbers were detected in upper (papillary) dermis as compared to small numbers in reticular dermis (Fig. 8). They commonly showed perivascular location accompanied with perivascular oedema (Fig. 9). The reticular dermis showed prominent vascular reaction in addition to the cellular infiltration. The severity of vascular reaction and cellular infiltration were apparently not related to presence of microfilariae. There was a mild reaction in upper papillary dermis where microfilariae were heavily present and the reverse was true in reticular dermis.

DISCUSSION

The prevalence recorded herein indicate high percentage of *O. cervicalis* in donkey. This data does not differ greatly from that recorded by Klei et al. (1984.) They recorded incidence of 82.4% in horses, and 76% in ponies in USA. Concerning the gross lesion recorded in nuchal ligament. Very similar nodular lesion were reported in horses donkeys and cattle as a results of *Oncchocerca* spp. infestation (Helmy et al 1967 ; El-Sammani and Hussein, 1983; Jacobs, 1986).

Histopathological examination of the nuchal ligament revealed chronic inflammatory reaction. Similar reaction was previously described in horses (Herd and Donham 1983) donkeys (Hussein and El-Sammani 1985) and cattle (el Sennary et al, 1994). The presence of neutrophils in the reaction site could be attributed to a chemotactic effect of either secretory and / or excretory products of the adult worm. This concept was augmented by detection of neutrophils chemotactic factor in extract of female *O. volvulus*. (Rubio de Kromer et al 1998) The authors also reported that neutrophils were accumulated near and attached to the cuticle of immature females, microfilariae producing females and males, and they suggested that migration of neutrophils was elicited by excretory - secretory product of vital females

Concerning presence of eosinophils and mast cells Wildenburg et al (1996 and 1997) reported consistent presence of eosinophils associated with

microfilariae producing females, while mast cells were present in the center of the infiltrate or in around the non nodular filariae. They were not detected in direct contact with the worm cuticle or among the other cells (macrophages neutophils and eosinophils)

Histopathological examination clearly indicated that microfilariae were the cause of dermatitis. Similar finding was previously reported in horse (Schmidt, et al 1985) The presence of large numbers of microfilariae in area with mild inflammatory reaction and its absence in areas with severe reaction apparently supports the view of pathogenesis, that life microfilariae are well adapted to their host but become pathogenic after death. These inflammatory reaction are most probably initiated by inflammatory mediators released after death and disintegration of microfilariae (Herd and Donham 1983). However the pathogenesis could be explained as a sum of factors including mechanical injuries or irritation in the dermis induced by periodically appearing microfilariae and as a results of inflammatory mediators produced from life as well as dead microfilariae.

This view could be supported by the reports indicate day or night time periodicity of dermal microfilariae of onchocerca spp. as Hussein and El Sammani (1990) recorded an evening periodicity of *O. raillietti* microfilariae while Anderson et al (1975) and Davis et al. (1994) recorded a day time periodicity of *Onchocerca* spp. Moreover microfilarial dermatitis may be as a results of contribution of parasitic protease through direct enzymatic destruction of connective tissue proteins or indirectly through triggering autoimmune response to self determinants (Petralanda and Piessensn, 1994) Absence of correlation between presence of microfilariae and severity of inflammatory reaction was previously reported in horse (Schmidt et al. 1985). This could be explained as a results of death and lysis of microfilariae within such severely inflamed areas, or as a results of continuous movement of the motile microfilariae from site to another. Presence of microfilariae within areas of mild or no inflammatory reaction my in part due to periodicity of microfilariae within the dermis as well as due to suggested immune evasion property of microfilaria as a result of periodical changing of microfilarial surface antigen (Edwards et al 1990)

From this study it has been documented that onchocerca cervicalis was the cause of the described lesions in nuchal ligament and their microfilariae were the cause of dermatitis of the donkeys examined .

REFERENCES

- Abd El-Wahab, T. M. and Ashour, A., A (1999):** Scanning Electron microscopy of the two flariid nematodes *Setaria equina* and *Onchocerca cervicalis* from Kafr El-Sheikh area , Egypt. .Alex J. Vet Science Vol 15 (3) 541-549.
- Abd El-Wahab, T. M. and Raef, A. M. (1999):** Flariid nematodes in Equine and donkeys at Kafr El-Sheikh province .Alex J. Vet Science Vol 15 (3) 549-564.
- Anderson, R.I.; Fazen, L.E. and Buck, A.A. (1975):** Onchocerciasis in Guatemala . III. Daytime periodicity of microfilariae in skin., Am. J. Trop. Med. Hyg. 24(1): 62 - 65.
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- Attenburrow, D.P.; Donnelly, J.J. and Soulsby, E.J.L.(1983):** Periodic ophthalmia (recurrent uveitis) of horses: As evaluation of the aetiological role of microfilaria of *Onchocerca cervicalis* and the clinical management of the condition. *Equine Vet. J.* 2: 48-56.
- Cello, R.M. (1971):** Ocular onchocerciasis in the horse . *Equine vet. J.* 3:148-154.
- Davis, J.R.; Wasserman,S.S. and Trpis, M (1994) :** Diurnal biting activity and transmission of *Onchocerca volvulus* (Filariata Onchocercidae) by *Simulium yahense* (Diptera: Simuliidae) in Liberia *Med. Entomol*, 31(2): 217-224.
- Edwards, M.K.; Busto. P.;James, E.R. ;Carlow, C.K. and Philipp, M. (1990)** :Antigenic and dynamic properties of the surface of *Onchocerca microfilariae*. *Trop. Med. Parasitol.* 41(2): 174-180.
- El-Sammani, S.E. and Hussein, H.S.(1983):** *Onchocerca raillietti*: Adult location and skin distribution of the microfilaria in Sudanese donkeys. *J. Helmithology*, 57: 355 - 360.
- El Sinnary, K. A.; Hussein, M. F. and Hussein, S. H. (1994):** *Onchocerca gutturosa* infection of the ligamentum nuchae in two cows in the Sudan *Rev. Elev. Med. Vet. Pays Trop.* 47(2) : 183-184.
- Helmy, N ; Khamis, M.Y. and Selim, M.K. (1967):** The role of *Onchocerca reticulata* as the cause of fistulus withers and ulcerative wounds of back in solipeds and its treatment.*Vet. Med. J.* 14: 149- 164.
- Herd, R.P. and Donham. J.C. (1983):** Efficacy of Ivermectine against *Onchocerca cervicalis* microfilarial dermatitis in horses. *Am. J. Vet. Res.* 44 (6): 1102 - 1105.
- Hussein, H.S. and El-Sammani, S.E. (1985):** *Onchocerca raillietti* : Prevalence and pathology in Sudanese donkeys. *J. Helmith.* 95(3) 345-351.
- Hussein,H.S. and El-Sammani, S.E.(1990) :** *Onchocerca raillietti* : release from skin snips, maintenance in vitro and periodicity of microfilariae. *Vet. Res. Commun.* 14(1)31-39.
- Jacobs, D.R. (1986):** A colour Atlas of equine parasites. London Bailliere and Tindall pp 220
- Klei, T. R.; Torbert, B. and Chapman, M. R. (1984) :** Prevalence of *Onchocerca cervicalis* in equines in the Gulf Coast region. *Am. J. Vet. Res.* 45(8): 1644- 1647.
- Lloyd, S. and Soulsby, E.J.L (1978):** Survey for infection with *Onchocerca cervicalis* in horses in eastern United States. *Am. J. Vet. Res.* 39(12) 1962-1963 .
- McMullan W.C.(1972):** Onchocercal filariasis. *Southwest Vet.* 25:179-191.
- Mellor, P.S.(1973) :** Studies on *Onchocerca cervicalis* in (Railliet and Henerly 1910) *Onchocerca cervicalis* in British horses. *J Helminthology* 47: 97 -110.
- Muller,R. (1979):** Identification of *Onchocerca* . 17th symposium of the British society for parasitology.(Edited by Taylor,A.E.R. & Muller,R;) pp. 175-206 , Oxford; Blackwell.
- Petralanda, I and Piessens, W. F.(1994):** Pathogenesis of Onchocercal dermatitis : possible role of parasite proteases and autoantibodies to extracellular matrix proteins. *Exp. Parasitol.* 79(2): 177-186.
- Rabalais, F.C.; Eberhard, M.L. and Ashly, D.C. (1974) :** Survey for equine Onchoceciasis in the midwestern United States. *American J.Vet. Res.* 35: 125-126.

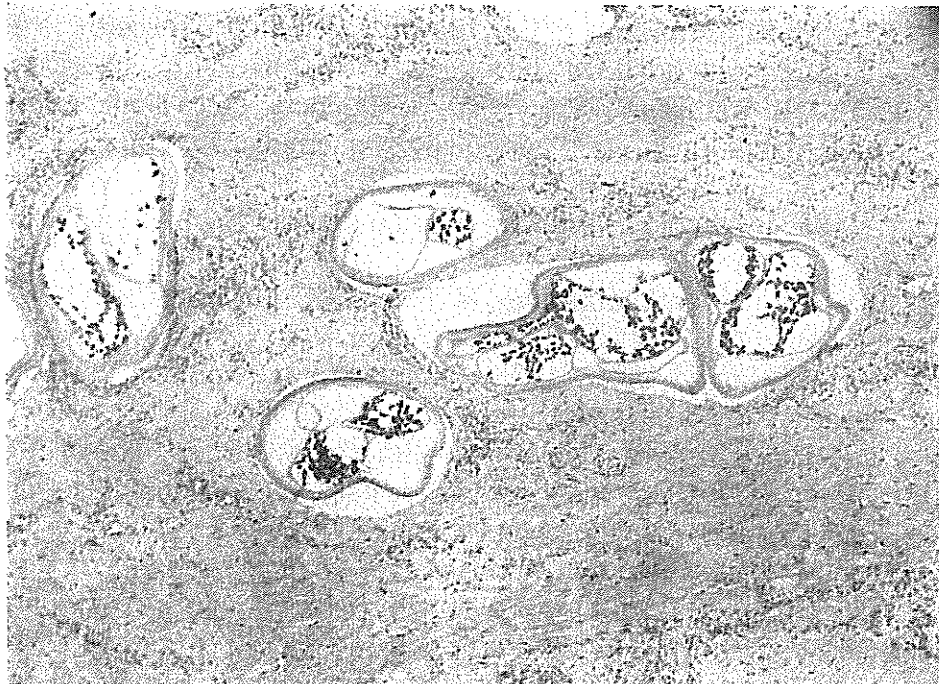
Rubio de Kromer, M.T.; Kromer, M.; Luesen, K. and Brattig, N.M.; (1998): Detection of A Chemotactic factor for neutrophils in extracts of female onchocerca volvulus. Acta Trop. 15; 71(1): 45 - 56.

Schmidt, G.M.; Coley, Sc and Leid, R.W.(1985) : Onchocerca cervicalis in horses: dermal histopathology .Acta Tropica 42(1): 55-61 .

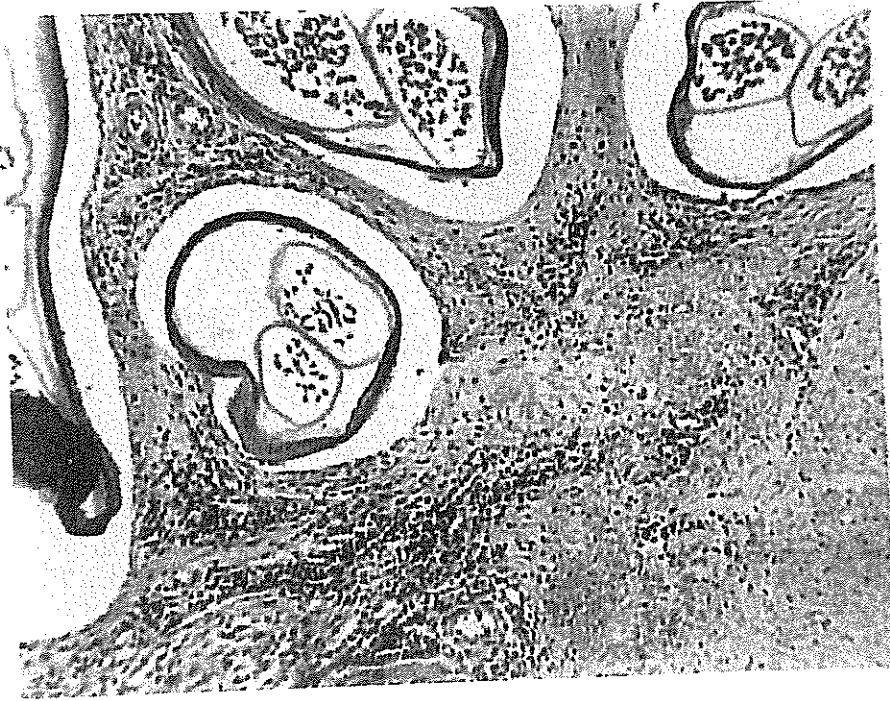
Soulsby, E.J.L (1982): Helminths Arthropods and Protozoa of domestic Animals. 17th Ed. The English Book Soc & Bailliere Tindall. London.

Wildenburg, G.; Kromer, M. and Buttner, D.W.(1996): Dependence of Eosinophil granulocyte infiltration into nodules on the presence of microfilariae producing onchocerca volvulus . Parasitol. Res. 82,(2): 117 - 124.

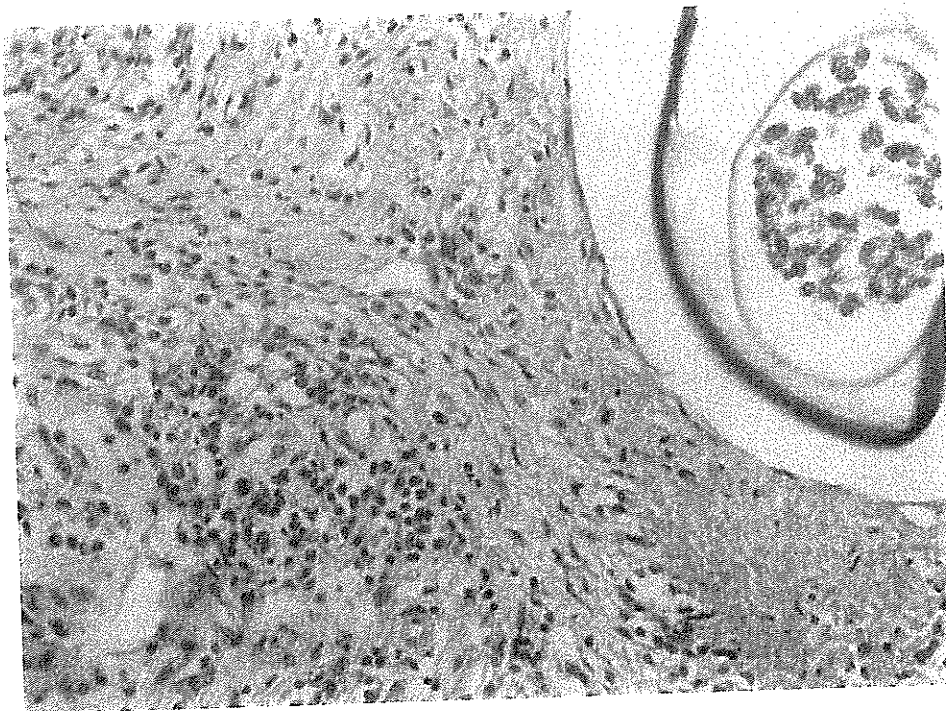
Wildenburg, G.; Plenge-Bonig, A.; Reza, A; Fischer, P; and Buttner D.W.(1997): Distribution of Mast Cells and Their correlation with inflammatory cells around Onchocerca gutturosa, O. tarsicola, O. ochengi and O. flexuosa. Parasitol. Res. 83,(2): 109 - 120.



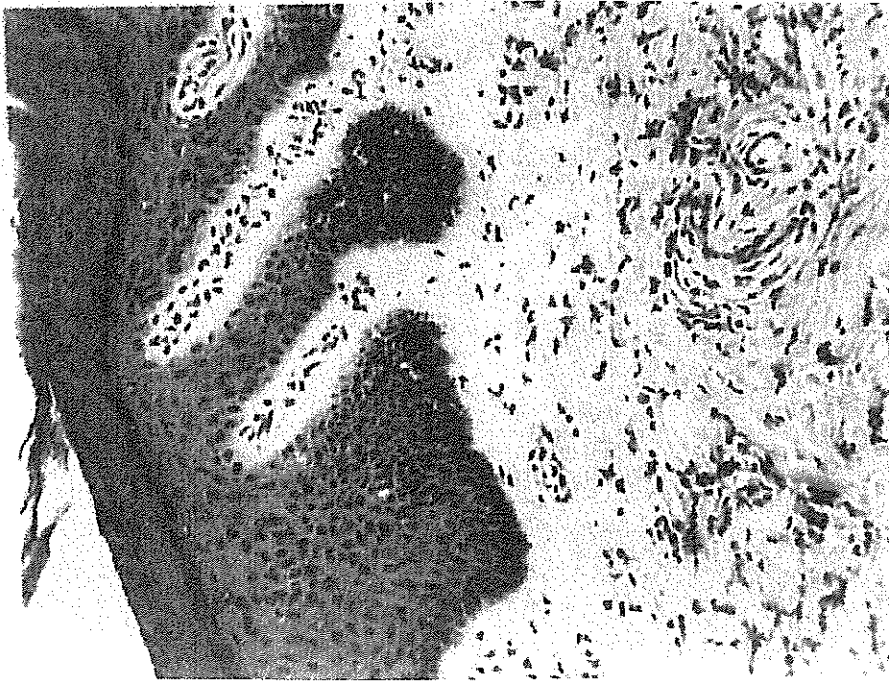
(Fig. 1): The ligamentum nuchae showing chronic active inflammatory reaction around the adult worms. Notice light density of inflammatory cells around the worm. H & E X 100



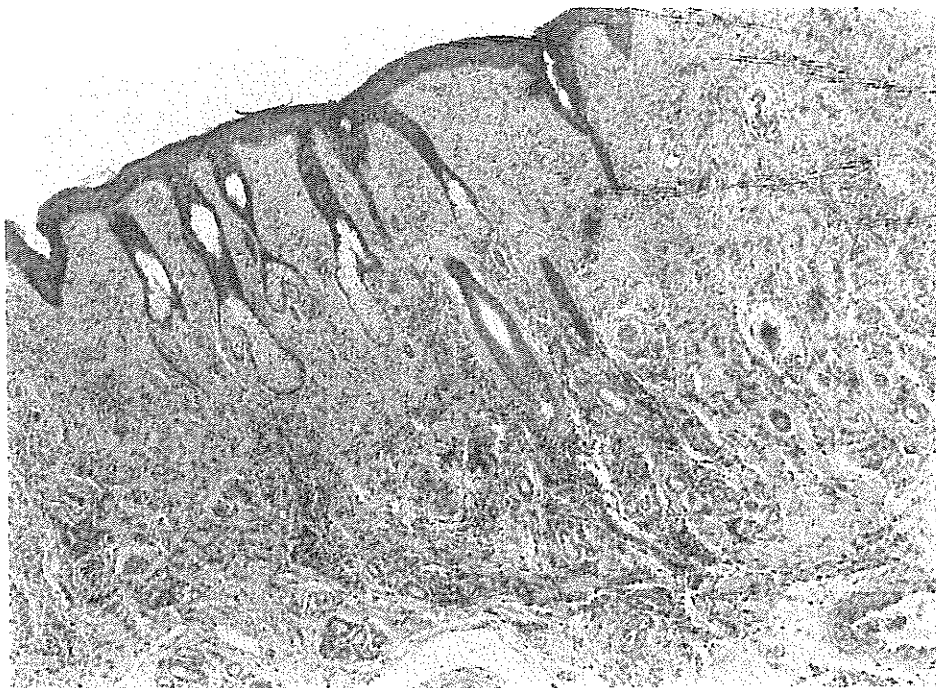
(Fig. 2): The ligamentum nuchae showing chronic active inflammatory reaction around the adult worms. Notice fibroplasia and heavy density of inflammatory cells around the worm. H & E X 200



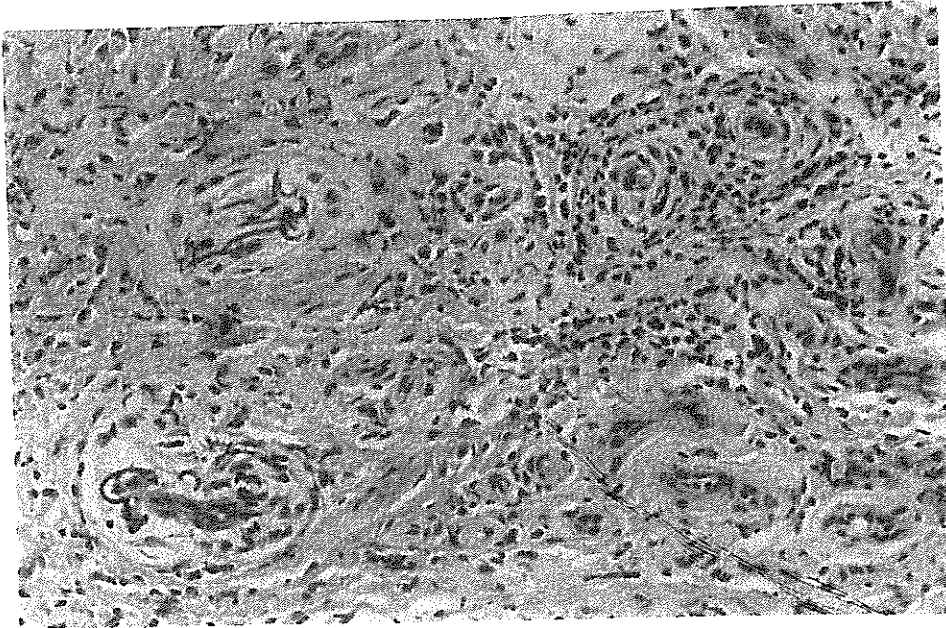
(Fig. 3):- The ligamentum nuchae showing chronic active inflammatory reaction around the adult worms. Notice the prevalence of eosinophils. H & E X 200



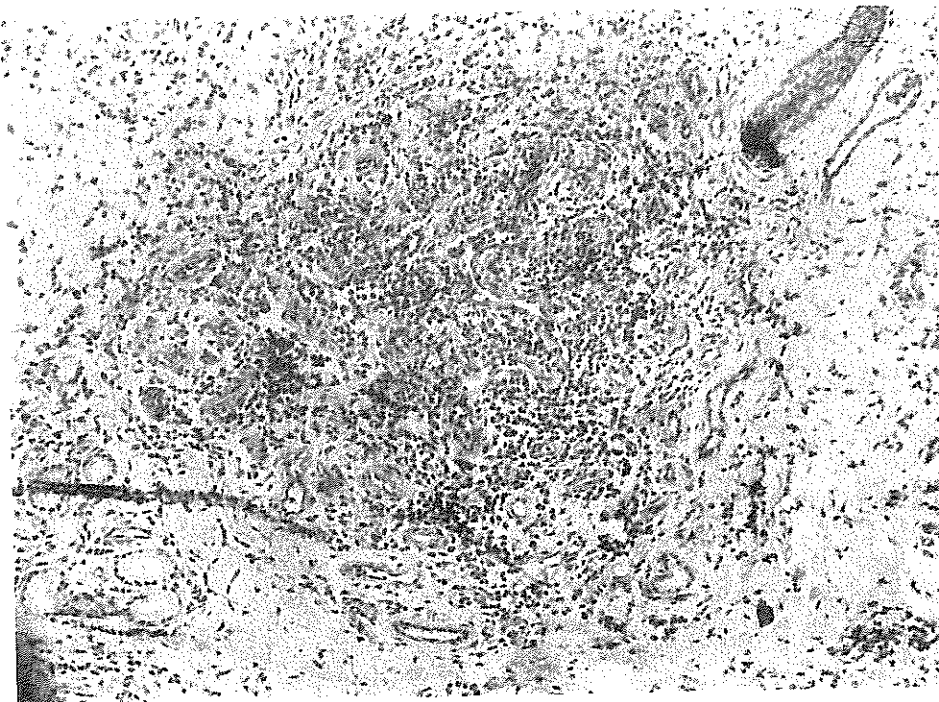
(Fig 4) :- Skin showing epidermal hyperplasia (acanthosis), and hyperkeratosis H & E X 400



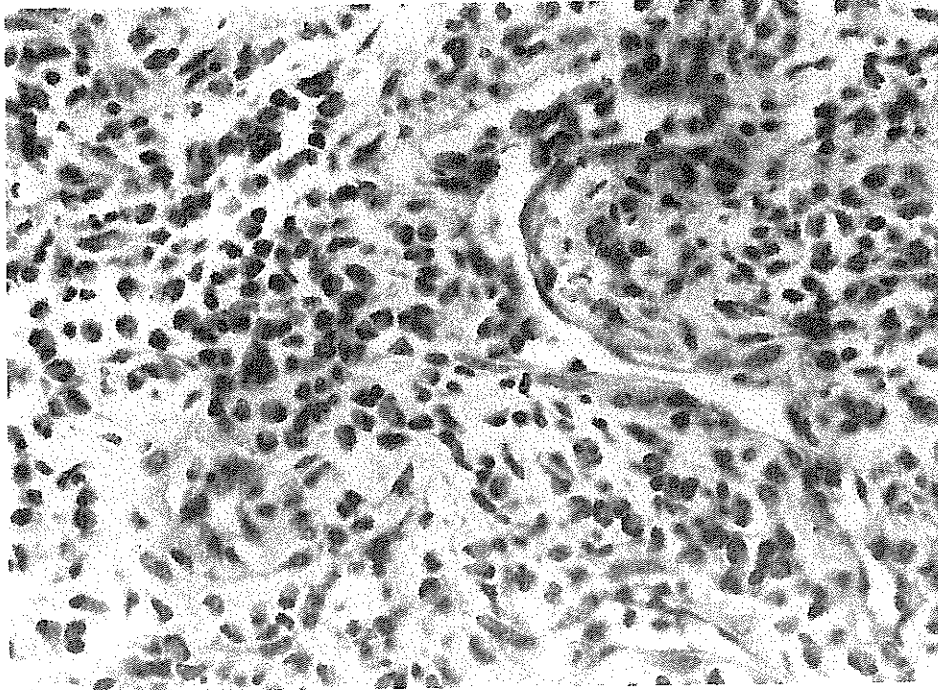
(Fig. 5):- Skin showing diffuse dermatitis Notice intense reaction in deeper reticular dermis H & E X 50



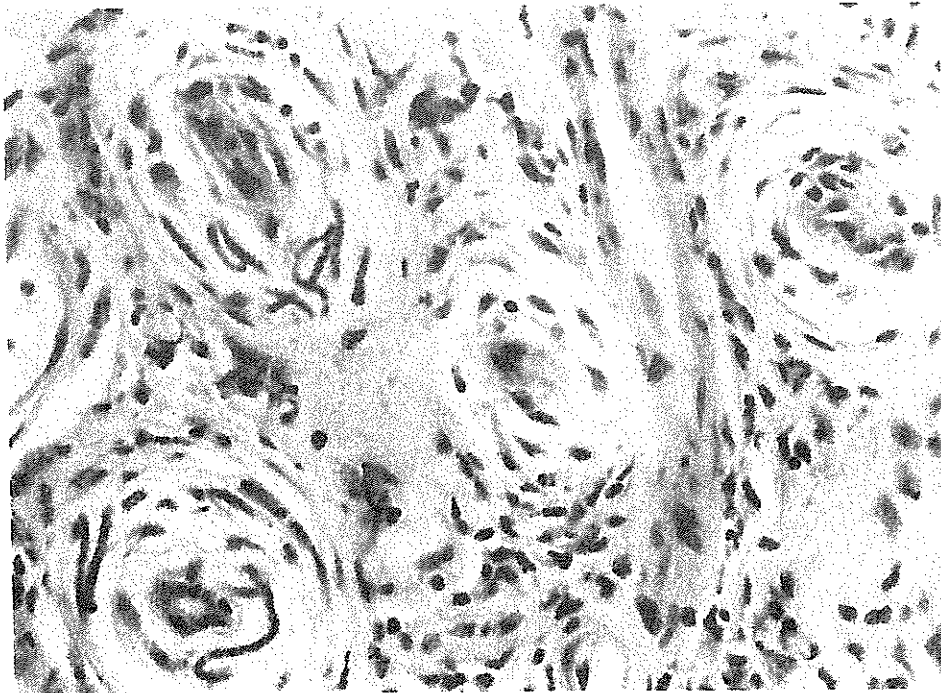
(Fig. 6):- Showing diffuse dermatitis. Notice the types of inflammatory cells and perivascular microfilariae associated with oedema. H & E X 200



(Fig.) 7:- Dermis showing focal areas of deeply sited dermatitis. Notice heavy cellular infiltration. H & E X 100



(Fig. 8):- Dermis showing predominance of eosinophils in microfilarial dermatitis. H & E X 400



(Fig. 9):- Dermis showing association of microfilariae with perivascular oedema H & E X 400