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LIGHT AND ELECTRON MICROSCOPICAL OBSERVATIONS ON THE LYMPHOCYTES IN THE EPIDERMIS OF BAGRUS BAYAD (Bagrid catfish)

(With 6 Figs.)

By

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ملاحظات بالميكرسكوب الضوئي والالكتروني على الخلايا الليمفاوية في جلد سمك البياض

عبد الحكيم كيلاني ، صلاح عفيفي ، صلاح البلال

أجري هذا البحث على ستة من سمك البياض بغرض دراسة الخلايا الليمفاوية في الجلد · تم أخذ العينات من ثلاثة مناطق (الرأس ، الجذع ، الذيل) وذلك للفحص بالميكرسكوب الضوئي والالكتروني ، وقد تبين من الفحص المجهري أن الخلايا الليمفاوية تنتشر اما مفردة أو في مجموعة فوق الغشاء القاعدي للأدمة · وقد لوحظ زيادة عدد الخلايا الليمفاوية في منطقة الجذع أكثر من الرأس أو الذيل · وقد أظهر الميكرسكوب الالكتروني التركيب الفوقي للخلايا الليمفاوية مما يؤكد صورة الميكرسكوب الضوئي ·

SUMMARY

Six mature Bagarus Bayad (Bagrid catfish) of an (average weight of 150 g) were used in this study. Skin samples were taken from the head, trunk, and tail regions and processed for light and electron microscopy. Light microscopy showed small suprabasal solitary and/or aggregated distribution of lymphocytes. These cells had mature compact nuclei and were PAS-negative. Apparantely, there was a tendency of these cells to increase in the trunk rather than the head or the tail region. Electron microscopy showed the presence of intraepithelial lymphocytes with sparce cytoplasmic organells. Their nuclei had thick chromatin clumps arranged along the inner side of the nuclear membrane. The significance of these results has been discussed.

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INTRODUCTION

Bagrid catfish skin is like other vertebrates provide protection from external threats. It is composed of two layers, the epidermis and dermis. The thickness of the epidermis varies within species, part of the body, age, sex, maturation, and environmental stressors (YASUTAKE and WALES, 1983).

Fish lymphocytes have been considered as the excutive cells of the specific immune response as in mammals. Their role in rejection of transplanted tissues in fish (HILDEMANN and THOENES, 1969), presence of surface immunoglobulin (DELUCA, et al. 1978) were elaborated. Moreover, the process of homeoviscus adaptation of lymphocyte plasma membranes to environmental temperature has been investigated (ABRUZZINI, et al. 1982). The presence of lymphocytes in the epidermis of gold fish and marine fish has been demonstrated by light microscopy (PERCY, 1970; MITTAL and MUNSHI, 1971; PELETEIRO and RICHARDS, 1985).

The purpose of this study was to identify the lymphocytes in the epidermis of the River Nile species Bagrid catfish as a preliminary study. Henceforth, the dynamics of these cells in infection, wound healing, and responses to various stressors will be taken in consideration.

MATERIAL and METHODS

Fish:

Six mature Bagarus Bayad (Bagrid catfish) were obtained from Ibrahimia tributary (a branch of the River Nile) Assiut, Egypt. These fish were males and weighed about 50 g.

Light and electron microscopy:

Fish were killed by pithing the brain tissue. Skin samples representing the head, trunk, and tail regions were fixed immediately in Bouin's fixative, dehydrated in ethanol, embedded in paraffin, sectioned at 4-6 u, and stained with H. & E. stain and PAS technique. Relationship between the presence of lymphocytes and goblet cells in different regions were focused. The spleen was taken as control.

For semithin and ultrathin sections, samples were fixed in 5% glutraldehyde, post-fixed in osmic acid, dehydrated in alcohol, and embedded in epon. Semithin sections were stained with toulidine blue. While, ultrathin sections were stained with uranyl acetate and lead citrate (REYNOLDS, 1963), and examined with Jeol TEM-100c III at 80 KV-electron microscope.

RESULTS

Light microscopy:

The epidermis of Bagrid catfish presented numerous lymphocytes which are mainly concentrated in the vicinity of the basal cell layer. These lymphocytes were either solitary distributed or aggregated in small groups. They bear an unique tendancy to an increase in the neighbourhood of the mucous cells especially at the trunk region (Fig. 1). On the other hand, they were relatively fewer at the head and tail regions (Fig. 2). A feature which was also accompanied by an allied decrease of the mucous cells. The lymphocytes presented the same morphological features observed into the splenic white pulp. The lymphocytes were mostly surrounded by a hollow area. The lymphocytes showed a negative reaction for PAS technique (Fig. 3).

Electron microscopy:

The intraepithelial lymphocytes were identified as cells with wide nucleus cytoplasm ratio. Their chromatin was thick and arranged as clumps along the inner side of the nuclear membrane. Few cytoplasmic organells were observed (Fig. 4, 5). Mucous cells had an apical cytoplasm, which was completely filled with membrane bounded secretory granules of moderate electron dense material. They were attached to the neighbouring cells by a tight Junction (Fig. 6).

DISCUSSION

In the present study light and electron microscopical observations of the skin in Bagrid catfish were similar to other studies reported in different species of fishes (PELETEIRO and RICHARD, 1985). However, in our studies there were an apparent increase and association between the lymphocytes and mucous cells in the trunk region. PICKERING (1974) found that there was a tendency for an increase in mucous cells in the anterior regions of trout epidermis. The differences in the present study could be attributed to species and/or sex. It is also possible that the abundance of mucous cells in the trunk region could be controlled by the turnover of cells. In other words, the rate of turnover of mucous cells in the trunk region was lower than the head or tail.

PELETEIRO and RICHARD (1985) showed by immunoperoxidase technique and electron microscopy that the lymhocytes in trout epidermis contain immunoglobulin. Their study suggested that the immunoglobulin might be secreted and stored in the mucous cells. Despite the fact that we did not demonstrate the presence of immunoglobulin in lymphocytes, it seems that such preliminary study can be helpful for further studies on the dynamics of these cells against various infectious agents.

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REFERENCES

Abruzzini, A.F.; Ingram, L.O. and William, L. (1982): Temperature mediated processes in Teleost immunity: Homeoviscous adaptation in Teleost lymphocytes. Proceedings of the Society for Experimental Biology and Medicine, 169: 12-18.

Du Luca, D., Warr, G.W. and Marchalonis, J.J. (1978): Phylogenetic origins of immune recognition: lymphocyte surface immunoglobulins and antigen binding in genus Carassius (Teleostii). European Journal of Immunology, 67: 525-530.

Hildmann, W.H. and Thoenes, G.H. (1969): Immunological responses of pacific hagfish. Skin transplantation immunity. Transplantation, 7: 506-521.

Mittal, A.K. and Munshi, J.S.D. (1971): A comparative study of the structure of the skin of certain air-breathing freshwater teleosts. Journal of Zoology, London, 163: 515-532.

Peletero, M.C. and Richards, R.H. (1985): Identification of lymphocytes in the epidermis of the rainbow trout, Salmon gairdneri Richardson. Journal of fish Diseases, 8: 161-172.

Percy, L.R. (1970): Wandering cells in the epidermis of the gold fish (Carassius auratus). Proceedings of the University of New Castle upon Tyne Philosophical Society 1: 189-193.

Pickering, A.D. (1974): The distribution of mucous cells in the epidermis of the brown trout Salmo trutta (L.) and the char Salvelinus alpinus (L.). Journal of Fish Biology, 6: 111-118.

Reynolds, E.W. (1963): The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. Journal cell Biology, 17: 208-212.

Yasutake, W.T. and Wales, H.J. (1983): Microscopic anatomy of Salmonids: An Atlas. US Dept. of the interior. Fish and wild life Service, Washington D.C. Chapter III Skin & Scales (Hawkes W.J.) 14-23.

LEGENDS

Fig. 1: Skin of Bagrid catfish (Trunk region) showing heavy distribution of intraepithelial lymphocytes (↑) between the basal cells (B). Alarm cells (A), mucous cells (M), stratum compactum (S). H & E., X 40.

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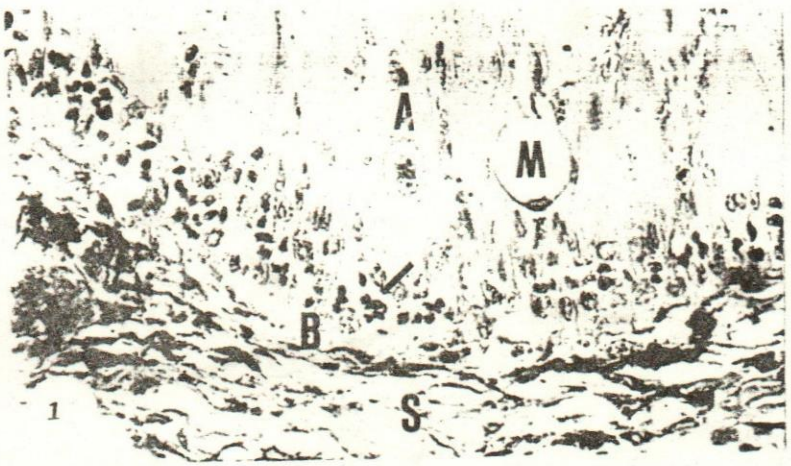
Fig. 2: Skin of Bagrid catfish (Tail region) showing few intraepithelial lymphocytes (↑) between the basal cells (B). H. & E., X 25.

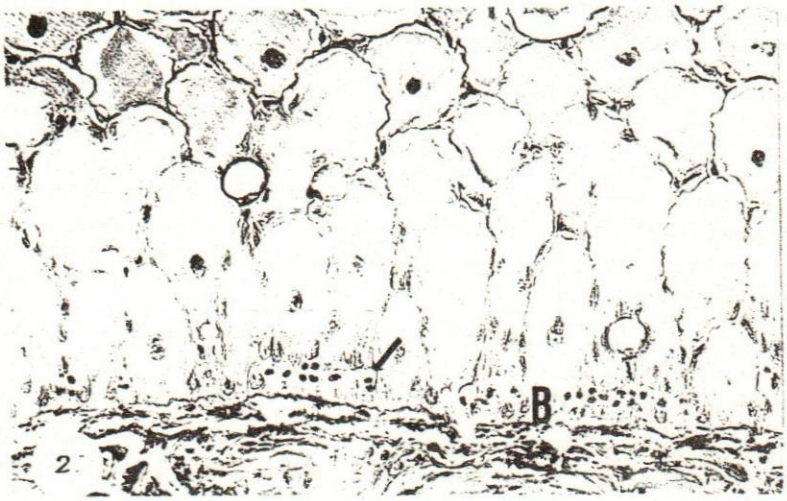
Fig. 3: Skin of Bagrid catfish showed negativity of lymphocytes for PAS technique (↑) while the mucous cells (M) are strongly PAS positive. PAS technique, X 40.

Fig. 4: The inset represented light microscopy of the intraepithelial lymphocytes between basal cells (↑). Transmission electron micrograph represent the ultrastructure of the inset. The intraepithelial lymphocytes (L) is located between two basal cells (B). (uranyl acetate and lead citrate) X 6700.

Fig. 5: Higher magnification electron microscopy demonstrating the fine structure of intraepithelial lymphocyte Nucleus (N), Chromatic (C). (uranyl acetate and lead citrate) X 10000.

Fig. 6: Electron micrograph demonstrating the ultrastructure of mucous cells (M). The apical cytoplasm is packed with mucous granules (MG). [Epidermal cells (E), tight junction (T)] (uranyl acetate and lead citrate X 2700.





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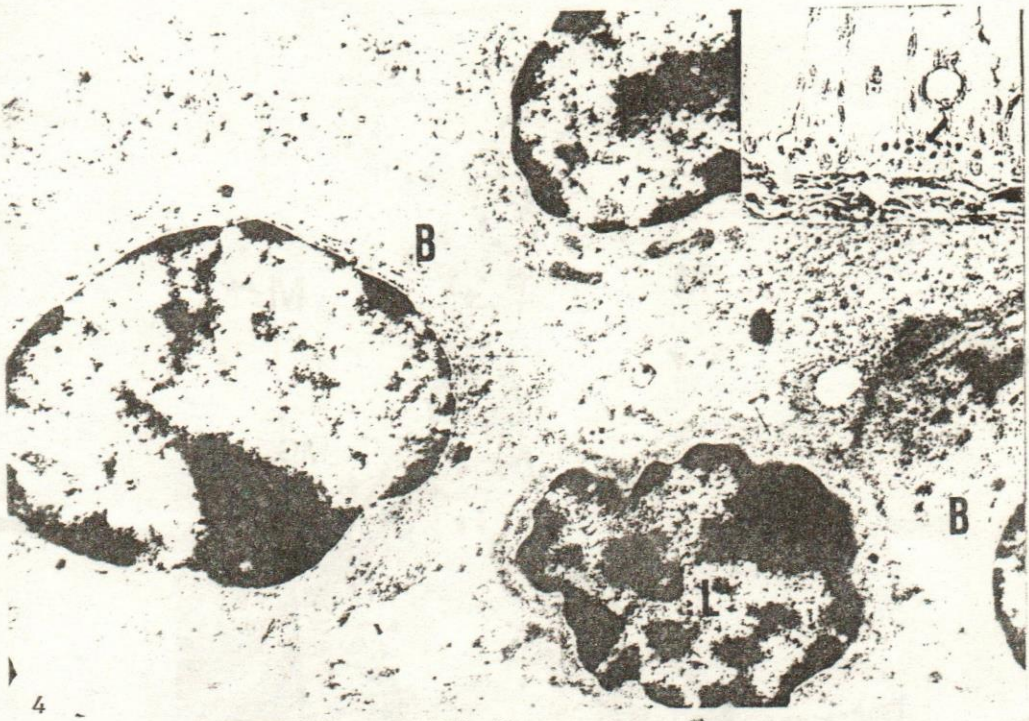

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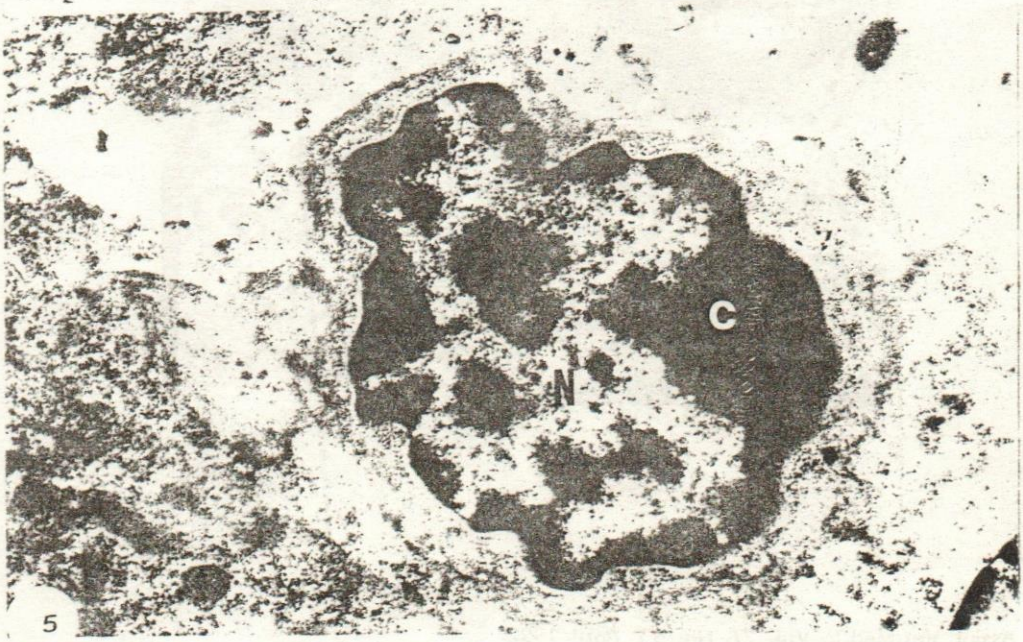

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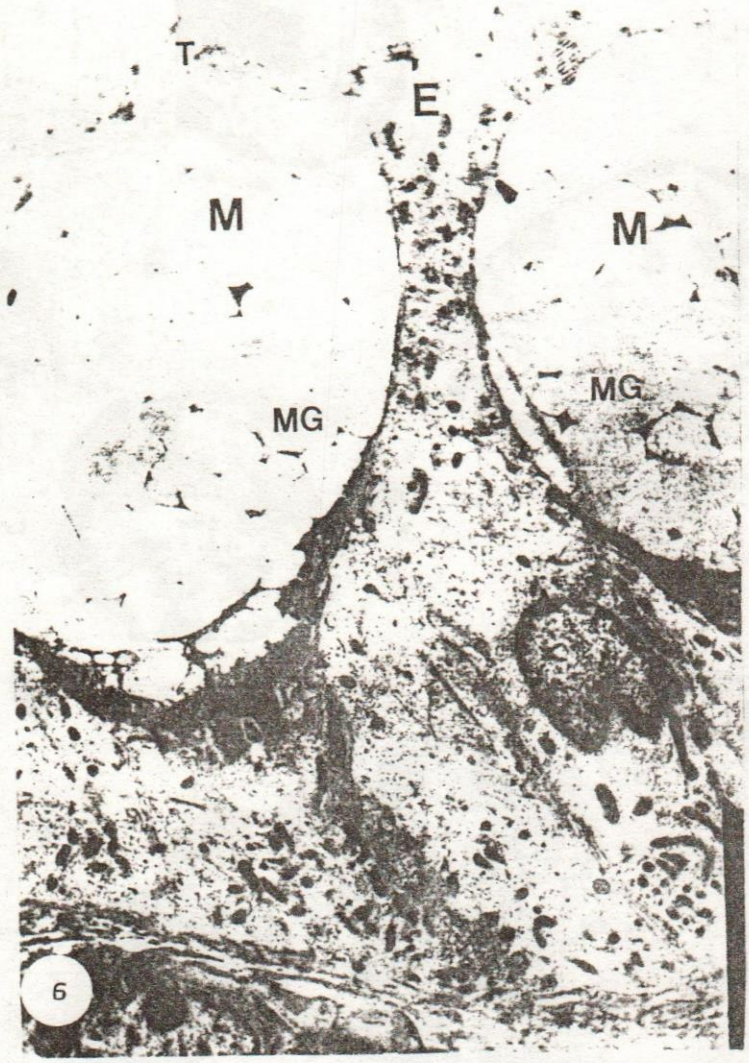
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