Origin And Classification Of The New Spermatogonia Of Ohrid Belvica (*Acantholingua Ohridana*) In The Period After The Spawning

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Abstract

This article contains the description of the origin and clasification of the new spermatogonia of Ohrid belvica (Acantholingua ohridana) in the period after the spawning. As to the new spermatogonial population we can conclude that in the latter phase of the postspawning period it is more present in the wall of the seminiferous lobules, an intesive spermatogonial proliferation, i.e. multiplication can be noticed, especialy in seminiferous lobules which contain rare sperm residues. The new spermatogonial generation in Ohrid belvica (Acantholingua ohridana) is represented by spermatogonia of A and B type, which are single or organised in cysts. The primary spermatogonia of type A are hypertrophied, they possess bright cytoplasm, clear cell borders, well seen nucleus with a bigger diameter, emphasized nuclear contours, euchromatic characteristics, peripheraly located chromatin, cenrally located nucleolus. In the contrast of these spermatogonia, in the wall of some seminiferous lobules, spermatogonia of the second generation can be seen, i. e. groups of spermatogonia of smaller dimensions, that is of smaller diameter of the nucleus and presence of more heterogenous chromatin, i. e. spermatogonia of type B. In some spermatogonia, two nucleoli can be noticed in their nucleus, which means that these cells are in the process of preparation for division (mitosis) or are in the course of division. Very often in the some spermatogonia accompanying precursor Sertoli cells with triangle shape of nucleus or in the shape of halfmoon can be noticed. They have prominent nucleolus.

Key words: Ohrid belvica (Acantholingua ohridana), spermatogonia of type A and B, postspawning period

Introduction

As to the new spermatogonial population which has been initiated in postspawning period there are many literature data. It has been studied in the scope of the investigations of the reproductive cycle with different species of Teleostei (Hyder, 1972; Ruby & Mc Millan, 1975; Saksena, 1976; Carrillo & Zanuy, 1977; Hurk et al., 1978; Baccetti et al., 1984; Patzner & Seiwald, 1987; Russel & Griswold, 1993). In previous investigations which concern the origin of the new spermatogonial population in different Teleostei, there are many explanations. Some authors think that the new spermatogonial population originate from spermatogonia which are in latent condition before the spawning ("resting spermatogonia) (Saksena, 1976; Tavciovska-Vasileva, 1992, 1994, 1999; Tavciovska-Vasileva & Dimovska, 1996; Dimovska & Tavciovska-Vasileva, 1998). According to other authors in different Teleostei in forming of the new spermatogonial population primordial (precursor) germinal cells participate on a level of the interstitium (Ruby & Mc Millan, 1975). The investigations of Hurk et al. (1978) pointed to double origin of the new spermatogonial population, i.e. the origin from the interstitium and from the latent spermatogonia. In *Salmo gairdneri* the new spermatogonia where thought to originate from the germinal cells of the seminiferous tubules during the months when spermatogenesis and spawning happen (Boddingius, 1975). Hurk et al. (1978) also, proved that in *Salmo gairdneri* the interstitial primordial germinal cells are future dormant spermatogonia. It has been concluded that a new generation of germinal cells originate from the interstitial primordial germinal cells, as well as from the dormant spermatogonia. In experimental conditions the spermatogonial proliferation in the trout was analysed by Loir (1994). All the facts previosly mentioned have pointed out that there are many differences concerning the interpretation of the origin of spermatogonia in different species of Teleostei, and this problem remains discussible. In *Oryzias latipes* a more contemporary analysis and classification concerning different generations of the germinal cells, i.e. spermatogonia was given by Hamaguchi (1979) and Kanamori et al. (1985), and in *Anguilla japonica* by Saksena et al. (1995).

Material and methods

Testes of sexually mature Ohrid belvica (*Acantholingua ohridana*) males caught in Ohrid Lake in a period of 3 years have been analysed. Analyses have been done with light microscope. For the light microscopy, parts of testes have been taken immediately after the decapitation of the alive samples, and fixed in Bouin Fixative and 4% neutral formaline. Standard paraffin technique has been used for the preparation of microscopic slides. The sections are 5 μm thin and they are stained with Hemalaun-Eozin and Floranten methods. The microphotographs for light microscopy have been taken with Leitz-Wetzlar Ortholux microscope, camera Orthomat. The histological analysis of the material has been done on the paraffin sections, as well as on semithin slides, prepared according to the following procedure: The sections with the thickness 0,5-1 μm have been cut on Reichert-Yung "Ultracut" ultramicrotome, using glass knives for electronic microscopy. The section have been stained by the method of permanent monochromatic staining with toluidin blue. The microphotographs of the semithin slides have been done using Leitz-Wetzlar Ortolux microscope, camera Orthomat. The photodocumentation of parafin and semithin slides has been done using Kodak Ektapress Multi Gold II PJM-36 slides, with blue filter.

Results

In order to have a better comprehension of the genesis of the new spermatogonial population during the postspawning period, a partial analysis of the same in the period directly before the spawning has been done.

Prespawning period

As to the new spermatogonial population we can conclude that in the prespawning period it is represented with rare, single spermatogonia, located in the wall of the seminiferous lobules. These spermatogonia are in the phase of relative rest (latent, dormant spermatogonia) which later, after the spawning, will initiate the new spermatogenesis for the next year. It has to be noted that in Ohrid belvica (*Acantholingua ohridana*) in the prespawning period single spermatogonia or small groups in subcapsular region have been observed, but still their presence is minor (Fig. 1). In individuals which are in a more progresive stage (near the spawning) except individual spermatogonia, there have been observed such spermatogonia which are organised in cysts (Fig. 2) which are attached to the wall of seminiferous lobules. These spermatogonia are characterised by clear borders and bright cytoplasm. Their nucleus is clearly seen, with equally dispersed chromatin. They possess nucleolus with central location (in some spermatogonia peripheraly) (Fig. 2). Some spermatogonia possess two nucleoli, which means that these cells are prepared for division or are in the phase of division. Among these spermatogonia, spermatogonia with homogenous cytoplasm have been noticed (Fig. 3). Very often, in some spermatogonia accompanying precursor Sertoli cells with triangle shape of nucleus or in shape of halfmoon can be noticed (Fig. 2, Fig. 3, Fig. 4).

Postspawning period

As to the new spermatogonial population we can conclude that in the later phase of the postspawning period it is more present in the wall of seminiferous lobules, i.e. an intensive spermatogonial proliferation can be noticed, a multiplication (which is one of the characteristics of this period) especialy seen in seminiferous lobules which contain rare sperm residues (Fig. 5, Fig. 6). The new spermatogonial generation in Ohrid belvica (*Acantholingua ohridana*) is represented by spermatogonia of A and B type, which are single or organised in cysts (Fig. 5, Fig. 6, Fig. 7). The primary spermatogonia of type A are hypertrophied, they possess bright cytoplasm, clear cell borders, well seen nucleus with a bigger diameter, emphasized nuclear contours, euhromatic characteristics, peripheraly located chromatin, centrally (rarely peripheraly) located nucleolus (Fig. 7). In the contact to these spermatogonia, in the wall of some seminiferous lobules, spermatogonia of the second generation can be seen, i.e. groups of spermatogonia of smaller dimensions, that is of smaller diameter of the nucleus and presence of more heterogenous chromatin, i.e. secondary spermatogonia of type B (Fig. 8, Fig.9). In some spermatogonia

two nucleoli can be noticed in their nucleus, which means that these cells are in the process of preparation for division (mitosis) or are in the course of division (Fig. 5, Fig. 9, Fig. 10). Very often on light microscope in some spermatogonia accompanying precursor Sertoli cells with triangle shape of nucleus or in the shape of halfmoon can be noticed (Fig. 6). They have prominent nucleolus.

Discusion

As to the new spermatogonial population which has been initiated in postspawning period there are many literarure data. It has been studied in the scope of the investigations of the reproductive cycle with different species of Teleostei (Hyder, 1972; Ruby & Mc Millan, 1975; Saksena, 1976; Carrillo & Zanuy, 1977; Hurk et al., 1978; Baccetti et al., 1984; Patzner & Seiwald, 1987; Russel & Griswold, 1993). In Ohrid belvica (*Acantholingua ohridana*) the new spermatogonial population which appears in the postspawning period becomes more representative. It initiates the following reproductive cycle and originates from rare single spermatogonia. We can see cysts located in the wall of the seminiferous lobules which until the spawning they are in one latent (dormant) condition. Their intensive multiplication starts immediately after the spawning. In this way their number progresively increase, especialy in the period of regeneration. In the previous investigations there is different interpretation of the new spermatogonial population, in different Teleostei. As to the family Salmonidae, concretely with *Salmo gairdneri,* Boddingius (1975) thinks that new spermatogonia originate from the germinal cells which remain in condition of resting along the wall of the seminiferous lobules during the months when the spermatogenesis and the spawning happens, which is in agreement with our findings. Some authors think that new spermatogonial population at Teleostei originates from spermatogonia which are in latent condition before the spawning ("resting spermatogonia") (Saksena, 1976; Tavciovska-Vasileva, 1992, 1994, 1999; Tavciovska-Vasileva & Dimovska, 1996; Dimovska & Tavciovska-Vasileva, 1998).The permanent liferation of germinal cells in the wall of the seminiferous lobules, called dormant, "resting", residual germinal cells or spermatogonia was described by Saksena (1976). According to other authors in forming of the new spermatogonial population in different Teleostei, primordial (precursor) germinal cells on a level of the interstitium participate as precursors of a new spermatogonial material (Ruby & Mc Millan, 1975). In thimidine-treated *Culea inconstans* the authors Ruby & Mc Millan (1975) in experimental conditions demonstrates the transfer of the thimidine from the interstitial germinal cells into the cysts with spermatogonia. This shows that the interstitium has a big role of mediator in supply, i.e. liferation with germ cells. The investigations with *Salmo salar* by Hurk et al. (1978) demonstrated that the intestitial primordial germinal cells are future dormant spermatogonia. They point to the double origin of the new spermatogonial population, i.e. from the interstitium and from the spermatogonia in latent (dormant) condition. As to the sourse of the primordial germinal cells, at Teleostei, two theories were suggested (Russell & Griswold, 1993). The first one says that there is a reserve of dormant, primordial germinal cells in the wall of the lobules during the year. During every annual cycle they are exposed to mitotic activites (in waves), but only one part enters the spermatogonial cycle. The other cells serve as a sourse of germinal cells for the folloving cycles. The second theory suggests that siccesive generations of germinal cells are obtained from gonocytes which migrate onto the seminiferous lobules from the other parts of the testis (Ruby & Mc Millan, 1970; Hurk et al., 1978). However, none of these studies with help of light microscopy as morphological evidence can not determine the authentic origin of these migratory cells. In some species of Teleostei, the spermiation is followed by presence of spermatogonia which are a dormant type of germinal cells. In other species the spermatogenetic activity happens, but it has been stopped on a level of primary or secondary spermatogonia. The primary spermatogonia are the most stable stage in which the development of germinal cells has been stoped, while the secondary spermatogonia are the most sensitive stage and they will be responsible for initiation of the spermatogenesis for the next year, initiating rapid begining of maturation of the germinal cells (Ruby & Mc Millan, 1970; Hurk et al., 1978). The spermatogonial proliferation in the trout in experimental conditions was analysed by Loir (1994). All this points out that there are significant differences in the interpretation which concern the genesis of spermatogonia in different species of Teleostei, with which this problem is still discussible. As to the new spermatogonial population in the postspawning period with Ohrid belvica (*Acantholingua ohridana*) different generations of spermatogonia have been noted. Some are hypertrophied, primary spermatogonia of type A, while others are secondary of type B, with smaller dimensions. More contemporary analysis and classification of different generations of spermatogonia with *Oryzias latipes* was made by Hamaguchi (1979) and Kanamori et al. (1985), with *Anguilla japonica* by

Saksena et al. (1995). Also, with the testes of Dojran perch (*Perca fluviatilis macedonica* K a r.) presence of different generations of spermatogonia (of A and B type) was noted by Tavciovska-Vasileva (1992, 1994). In construction of the new spermatogonial population with Ohrid belvica (*Acantholingua ohridana*) the presence of nondifferentiated Sertoli cells, i.e. folowing precursor Sertoli cells has been well noticed (Tavciovska-Vasileva, 1999).

Conclusions

From our investigations we can draw the following conclusions:
1. The multiplication of the new spermatogonial population with Ohrid belvica (*Acantholingua ohridana*) starts in the course of the postspawning period and is a representative of the new reproductive cycle for the following year.
2. Paralelly with the degenerative processes which take place in the lobules, an initial proliferation of spermatogonia, visible individuals or in cysts among Sertoli cells, happens.
3. The spermatogonial population is composed of hypertrophied primary spermatogonia (type A) and secondary (type B) with different, but with significantly smaller dimensions.
4. In contact with spermatogonia accompanying Sertoli cells with characteristic triangle or in the shape of halfmoon nucleus can be noticed.

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M I C R O P H O T O G R A P H S

Figure 1 Highly extended tunica albuginea (TA) with surface mesothelium (small arrows). In subcapsular region latent (dormant) spermatogonium (arrow). Seminiferous lobule with sperm cells (S). Hemalaun-Eozin, 40x.

Figure 2 On the surface of the testis well seen tunica albuginea (TA). Seminiferous lobule (SL) with sperm cells (S). Sertoli cells with endotheliomorphic appearance (thin arrows). Presence of single or in cysts spermatogonia (arrows) with accompanying precursor Sertoli cells (small arrows). Floranten, 40x.

Figure 3 A part of two seminiferous lobules with mature sperm cells (S). In the cytoplasm of Sertoli cells lipid vacuoles (arrows) with various dimension. Spermatogonia (arrows) with accompanying precursor Sertoli cells. Interstitium (I) with macrophages (MF). Semithin section, toluidin blue, 40x.

Figure 4 A part of 4 seminiferous lobules (SL) with mature sperm cells (S). Sertoli cells (small arrows) with endotheliomorphic appearance. Single spermatogonia with accompanying precursor Sertoli cells (arrows). Spermatogonium with two nuclei (white arrow). Hemalaun-Eozin, 40x.

Figure 5 A part of 4 seminiferous lobules (SL). Sertoli cells with pycnotic nucleus (black arrows). Destruction and delamination of the Sertoli cells, lysis of the detritus originated from Sertoli necrotic material (thin black arrows). Presence of different generations of spermatogonia with accompanying precursor Sertoli cells (small black arrows). Hemalaun-Eozin, 40x.

Figure 6 Seminiferous lobules with rare sperm residues (SR). Different generations of spermatogonia, single or organised in cysts (white arrows) with accompanying precursor Sertoli cells. Hemalaun-Eozin, 40x.

Figure 7 Semeniferous lobules (SL) with sperm residues (SR). Different generations of spermatogonia, single (small black arrows) or in cysts (thin black arrows) Sertoli cells (small white arrows). Azan, 40x.

Figure 8 Seminiferous lobules (SL) with rare sperm residues (SR). Sertoli cells with lipid vacuoles (thin black arrows) with various dimension. In the wall of the lobules presence of different generations of spermatogonia with accompanying precursor Sertoli cells (big white arrows). Hemalaun-Eozin, 40x.

Figure 9 Seminiferous lobules with rare sperm residues (SR). Sertoli cells with polymorphic nucleus (small whit arrows). Spermatogonia with “nuages” particles (small black arrows), some of them in division (black arrow). Spermatogonia in degeneration (white arrows). Macrophages (small black arrows) in the lobules and in the interstitium (I) rich with blood vessels (KS). Hemalaun-Eozin, 40x.

Figure 10 Seminiferous lobules with sperm residues (SR). Visible spermatogonia, some of them in division (black arrows). Sertoli cells (SK) with polymorphic nucleus (small black arrow). Interstitium (I) with macrophages (thick black arrows) and blood vessels (KS). Hemalaun-Eozin, 40x.

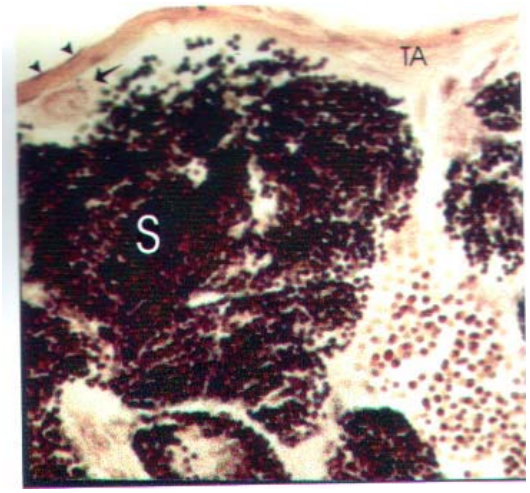


Fig. 1

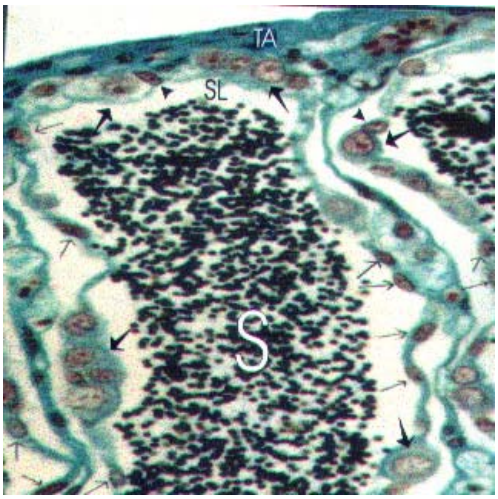
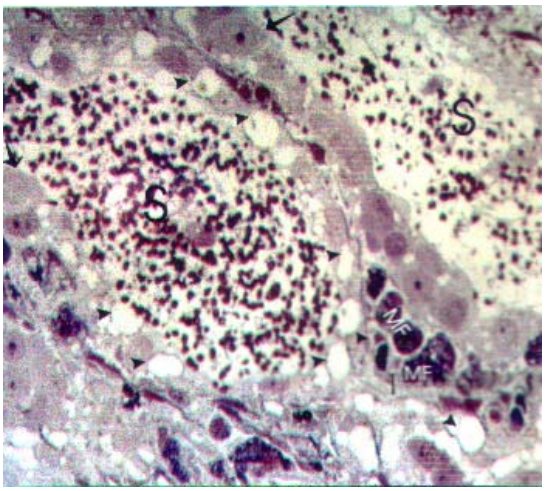


Fig. 2



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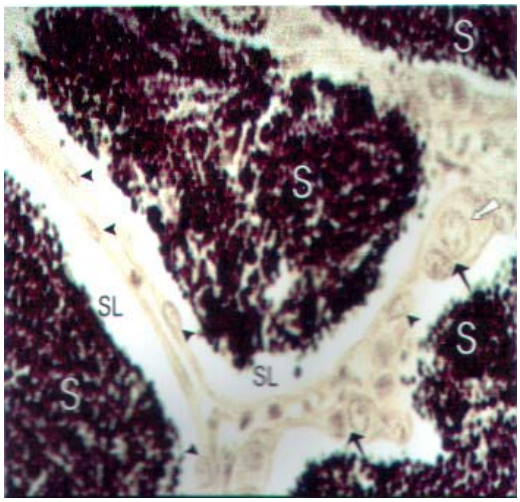

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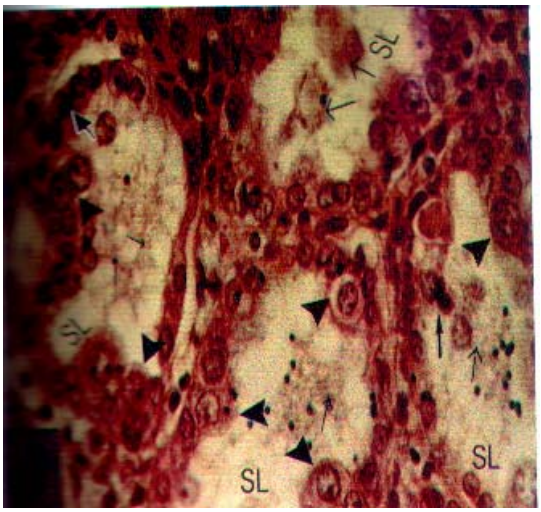

Fig. 5

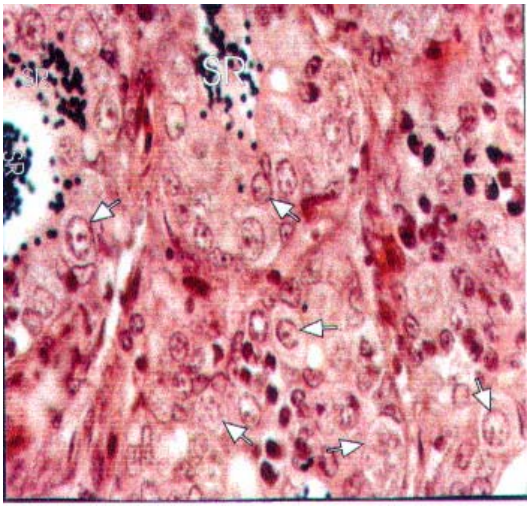

Fig. 6

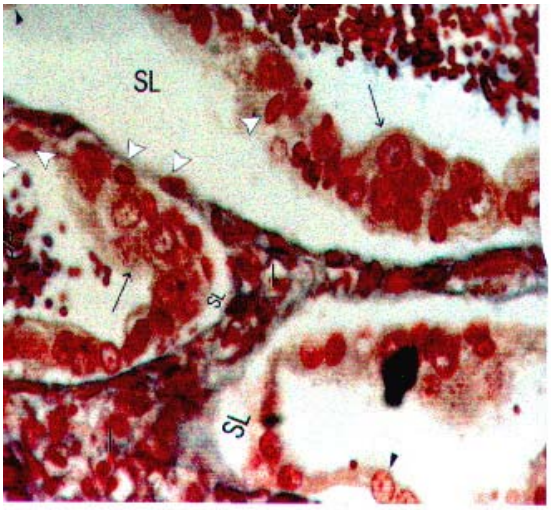

Fig. 7

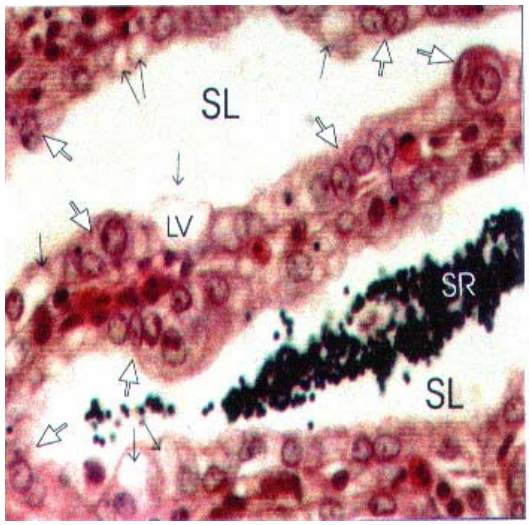

Fig. 8

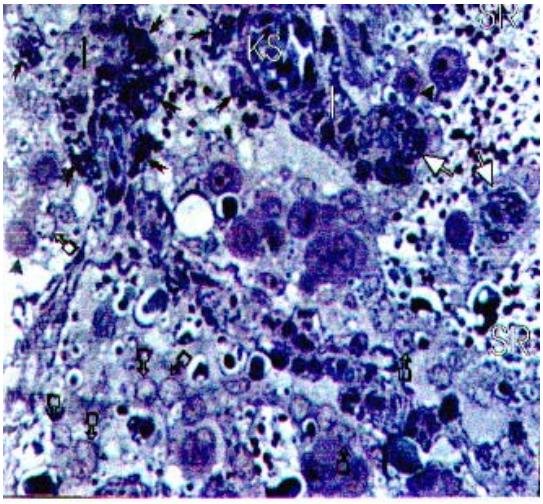

Fig. 9

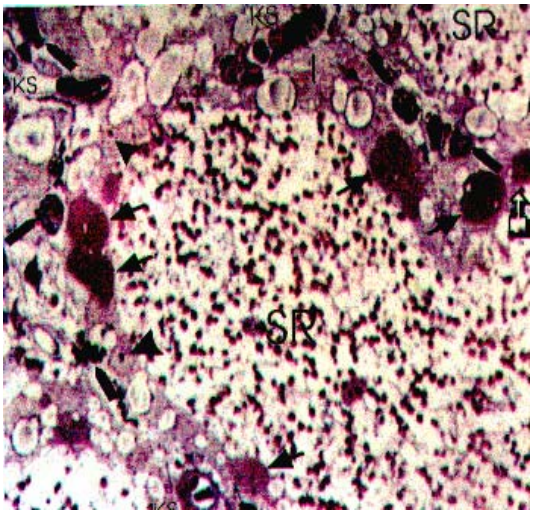

Fig. 10