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THE STUDY OF CELL AND TISSUE MECHANISMS OF THE HOMEOSTASIS OF „HELMINTH-HOST" SYSTEM AT MUSCLE TRICHINELLOSIS BEFORE AND AFTER THE ADMINISTRATION OF A HERB BIOSTIMULATOR AND ANTHELMINTHIC

SERGEI MOVSESSIAN[1], GALINA GONCHAROVA[1], ASHOT ASATRIAN[1], MANIA NIKOGHOSSIAN[1] AND ANDRZEJ MALCZEWSKI[2]

[1]Institute of Parasitology RAS, Lenin' av. 33, 119071 Moscow, Russia, E-mail: movses@iparan.msk.ru; [2]W. Stefański Institute of Parasitology, PAS, Twarda 51/55, 00-818 Warszawa, Poland; E-mail amalczip@twarda.pan.pl

ABSTRACT. Results of micromorphological and histological studies of larvae of *Trichinella spiralis* and *T. pseudospiralis*, as well as, muscles, liver and small intestine of the rat-host before and after biostimulator administration of phytohemagglutinin and phytoanthelminthic were presented.
It has been established that rats with *Trichinella* larvae of both species developed unspecific allergic angiomyositis, hepatitis, cholangitis, and erosio-haemorrhagic enterocolitis in the host's organism on the 35[th] day after infection. Furthermore, processes of compensatory hypertrophy, that support the host's (rats) homeostasis, on cell and tissue levels, were observed at histodestructional and morphofunctional deficiency.
It has been revealed that phytohemagglutinin, biostimulator injected into the host's organism before infection, is of immunostimulating nature and partially destroys the larvae of *Trichinella.*
The phytoanthelminthic produces a significant trichinellocide effect: RNA synthesis and glycogen is intensified in the organs of the treated animals, their pathomicromorphogenesis weakened, and their compensatory and regenerative processes were observed. The combined use of the phytohemagglutinin and phytoanthelminthic fails to intensify the mentioned effect.

Keys words: disfunction, mixed trichinellosis, pathomicromorphology, phytohemagglutinin, phytoanthelminthic, therapy.

INTRODUCTION

Currently, the study of parasite-host relation problems are still highly actual in parasitology. They are so many-sided and complex that interpretation of some questions on how to solve them differs a great deal. To date, studies based on various species of homeostasis of adaptational mechanisms at antagonistic relations of partners in „helminth-host" system in the norm and after the anthelminthic and biostimulator effect, were incomplete.

The study of versatile effect of anthelminthics and biologically active combinations on helminth tissue and organs and their hosts enables to determine the lability of some homeostatic mechanisms – structural and histofunctional. The biological sense of lability includes development, deepening or elimination of pathostructural and pathomorphofunctional processes, as well as divergence compensations from microstructural and histofunctional indicators.

A number of works dealing with the study of trichinellocide effect of some anthelminthics with partial restoration of the structure and function of skeletal muscles of the infected host have been published in recent years. As a result, data on the results of their usage in the experiment and clinical practice of akrichin, chlorophos, monomicin, NRV, thiabendazolum (Froltsova et al. 1965, 1966; Łapszewicz et al. 1969; Gerwel et al. 1974; Kocięcka et al. 1974); mebendazolum (De Nollin et al. 1974; Thienpont et al. 1974, Mittermayer and Spaldonova 1981), chlophozolum, benzimidazolum, fenbendazolum, karbendazolum, imidazolum, and benzimidazolum (Ozeretskovskaya et al. 1969a, 1976, 1978 a, b; Pereverzeva et al. 1976, 1981; Pereverzeva and Ozeretskovskaya 1979) have been obtained. The therapy of experimental trichinellosis with liposomes and liposomal anthelminthics was considered to have perspective (Novik et al. 1997; Skvortsova et al. 1996a, b). The patient therapy with steroide hormones and combined usage of steroides, adrenocorticotrophic hormone, and anthelminthics was of considerable clinical effect (Ozeretskovskaya et al. 1958, 1966a, b; Ozeretskovskaya 1965; Kocięcka and Budzyńska 1985). Analogical results were obtained from experimental treatment of trichinelliosis (Cocker 1956, Pereverzeva 1975).

Anthelmintics with immunomodulating activities e.g. levamisolum gave unsatisfactory results both in experimental intestinal and muscle invasion and clinical study (Borzuchowska and Kuroczycka-Seniutycz 1988). Data in literature on efficacy of Thymus Factor X (TFX Polfa) preparation in treatment of intestine and muscle trichinellosis are of great interest. Detailed descriptions of its composition, pharmacological features and immune action mechanism were presented by Giełdanowski et al. (1980), Jaszcz et al. (1980), Ślopek et al. (1980) and Kocięcka et al. (1989).

The authors mentioned showed that TFX Polfa acted selectively and specifically on blood picture, lymphatic and immune systems. It influenced T-lymphocyte maturing and function. Through T-suppressor and T-helper, not directly, it (TFX Polfa) regulated B-lymphocyte activities and antibody production. According to Kocięcka et al. (1989, 1992) the preparation administered to *Trichinella*-infected mice at different periods post infection had a trichinellocide effect, e.e. it induced mortality of adult intestine forms, retarded larvae encapsulation in muscles or provoked their destruction and influenced capsule wall thickening. The authors mentioned above observed the prolonged action of TFX Polfa. Reliable results were obtained in clinical trials of the preparation against human trichinellosis (Kocięcka et al. 1989).

Literature lacks information on the therapy of trichinellosis with biologically active combinations and anthelminthics of plant origin, apart from a brief statement on NRV. The latter was used in animals infected with the larvae of *Trichinella spiralis* for three days beginning from the second day of the infection (Froltsova et al., 1965).

The aim of this work was to study the biostimulator effect of phytohemagglutinin and phytoanthelminthic on the structure and functional activity of tissues of *Trichinella* larvae, as well as on the structure and microfunctional state of muscles, liver, and intestine of their hosts at mixed infection with the larvae of *Trichinella spiralis* and *T. pseudospiralis*.

MATERIALS AND METHODS

Larvae of *T. spiralis* and *T. pseudospiralis* and rats of the Vistar line as their hosts were used in modelling of experimental trichinellosis. Rats, males (7 months old) weighing 245-250 g were infected per os with *T. spiralis* and *T. pseudospiralis* larvae. The infection dose was 10 larvae from each species per 1 g of rat body mass. The *Trichinella* larvae were obtained from digested in pepsine muscle tissue of Vistar rats infected separately with *T. spiralis* and *T. pseudospiralis*.

25 experimental animals were divided into 5 groups of 5 rats.

Group 1 – intact animals;
Group 2 – infected control animals;
Group 3 – animals that receive a biostimulator daily for 5 days before the infection with *Trichinella*;
Group 4 – infected animals receiving phytoanthelminthics daily for 5 days from the 16th day after the infection;
Group 5 – infected animals receiving a biostimulator daily for 5 days before the infection, and phytoanthelminthics daily for 5 days from the 16th day after the infection.

A collection of herbs such as: *Plantago major* L., *Satureja montana* L., and *Ranunculus acris* L. were used in 2:2:1 ratio, accordingly.

The composition of the anthelminthic was elaborated and its theurapeutic dose tested at the Institute of Zoology, NAS of Armenia. The biostimulator phytohemagglutinin is successfully used in poultry industries as an immunostimulator. Phytohemagglutinin (70 mg/kg per animal) and phytoanthelminthic (50 mg/kg per animal) were mixed with fodder.

Animals of different groups were slaughtered on the 35th day after infection. Tissue samples of a volume of 0,5-0,7 cm^3 (musculus rectus, liver, and small intestine) were fixed in Zenker liquid with an addition of iced vinegar acid, in 10% formalin, in Schabadasch and Carnoy fixators. After the treatments specific to each method, the animal pieces were dipped into parafin with subsequent preparation of parafin cuts of 7-8 μm thickness. For the purpose of morphological studies, the deparafin cuts were stained with hematoxylin Carazzi-eosin and by the method of Mallory. Also, histochemical reactions were conducted on deparafin cuts: RNA was found by the Brachet method, and polysacharides – with the use of the SCHIK-reaction by the Schabadasch method using the Schiff reagent – iodine acid.

The reliability of histological tests for the identification of RNA and polysacharides was confirmed by appropriate control reactions.

RESULTS

Studies indicated that the pathomicromorphological changes in organs of infected animals that received phytohemagglutinin for five days before infection with *Trichinella* larvae did not differ from those of infected control animals.

A desintegration of myofibrils and local loss of longitudinal and diametrical lines was observed in muscle tissue. Foci of granulare breakdown, karyopycnosis and karyorhexis were found in the sarcoplasma. Majority of the nuclei which survived were hypertrophied. Considerable hyperplasia of the endomysium and perimysium was observed. Tissue cells (histocytes, fibroblasts, not numerous plasma and mast cells) were hypertrophied.

Moreover mast cell cytoplasma was filled with closely lying and metachromatically dyed granules.

Changes in the blood vessel wall texture were observed; here the vessels were full-blooded. Haemorrhage islets occurred in the perivascular area. Cellular-inflammatory infiltration was seen around larvae of *T. pseudospiralis* and capsules with

Fig. 1. Larva of *T. pseudospiralis* in destructed muscle fibers of musculus rectus in controlly infected rats at mixed infection with the larvae of *T. spiralis* and *T. pseudospiralis*. Longitudinal section, 8μm. 10% formalin, hematoxylin Carazzi-eosin. Mag. 500x.

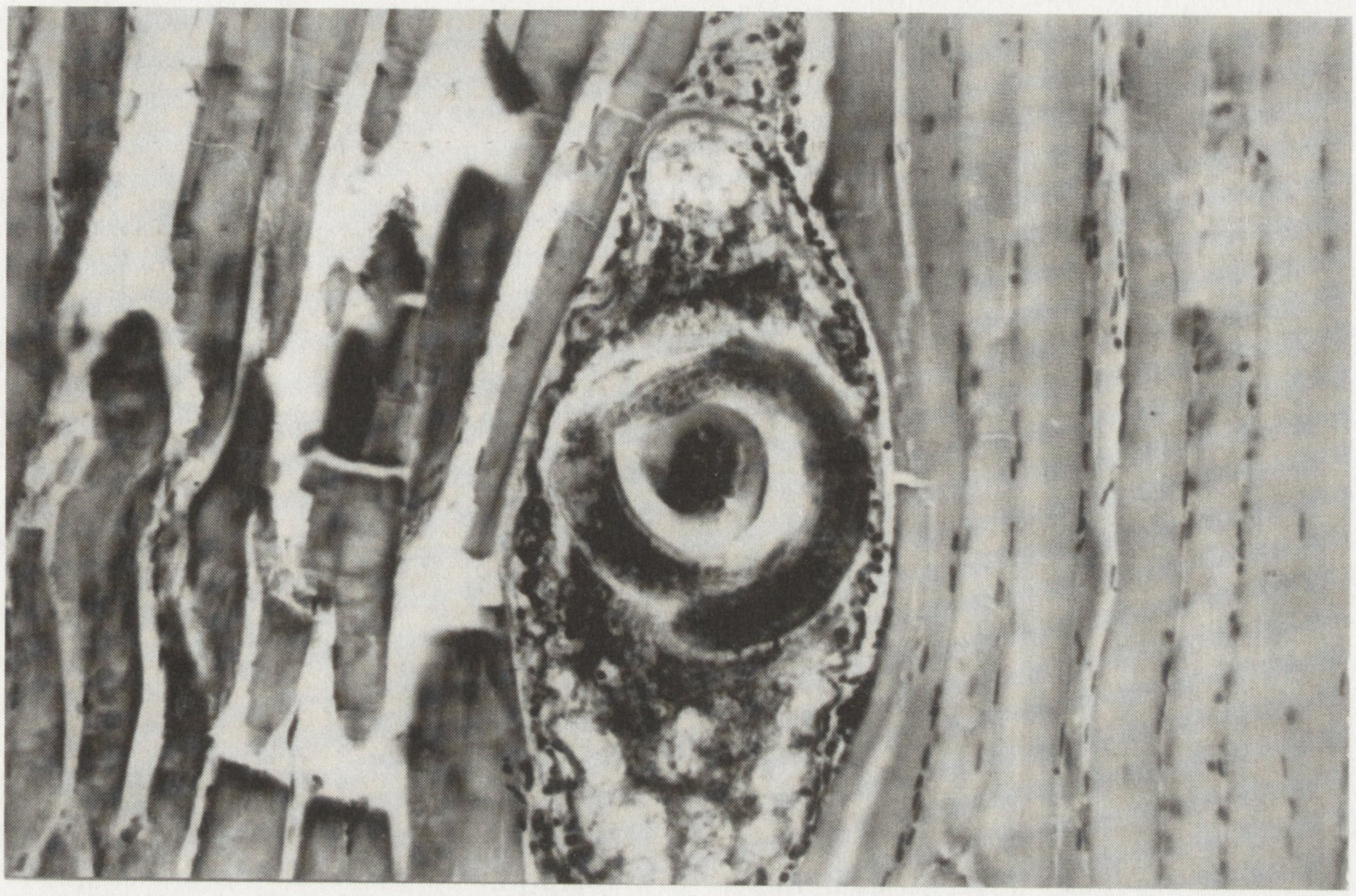

Fig. 2. Encapsulated larva of *T. spiralis* in destructed muscle fibers of musculus rectus in controlly infected rats at mixed infection with larvae of *T. spiralis* and *T. pseudospiralis*. Longitudinal section, 8 μm. 10% formalin, hematoxylin Carazzi-eosin. Mag. 500x.

larvae of *T. spiralis* inside, as well as in some areas of the endo – and perimysia. Lymphocytes predominated in the infiltration; there was a significant number of plasmatic cells, histiocytes, and fibroblasts, whereas there was a small number of eosynofils surrounding the capsules (Figs. 1, 2).

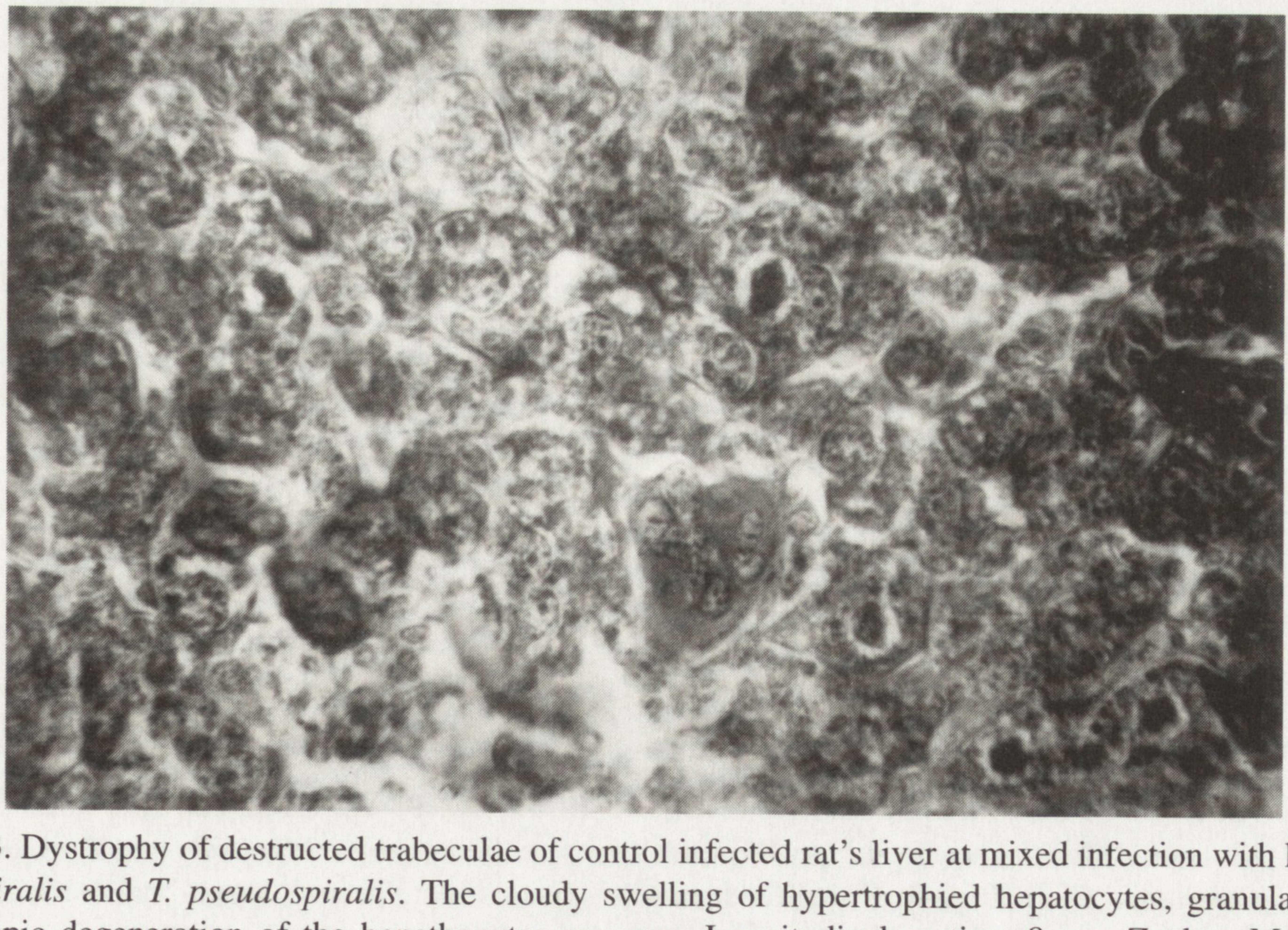

Fig. 3. Dystrophy of destructed trabeculae of control infected rat's liver at mixed infection with larvae *T. spiralis* and *T. pseudospiralis*. The cloudy swelling of hypertrophied hepatocytes, granular and hydropic degeneration of the hepathocytes are seen. Longitudinal section, 8 μm. Zenker, Mallory. Mag. 900x.

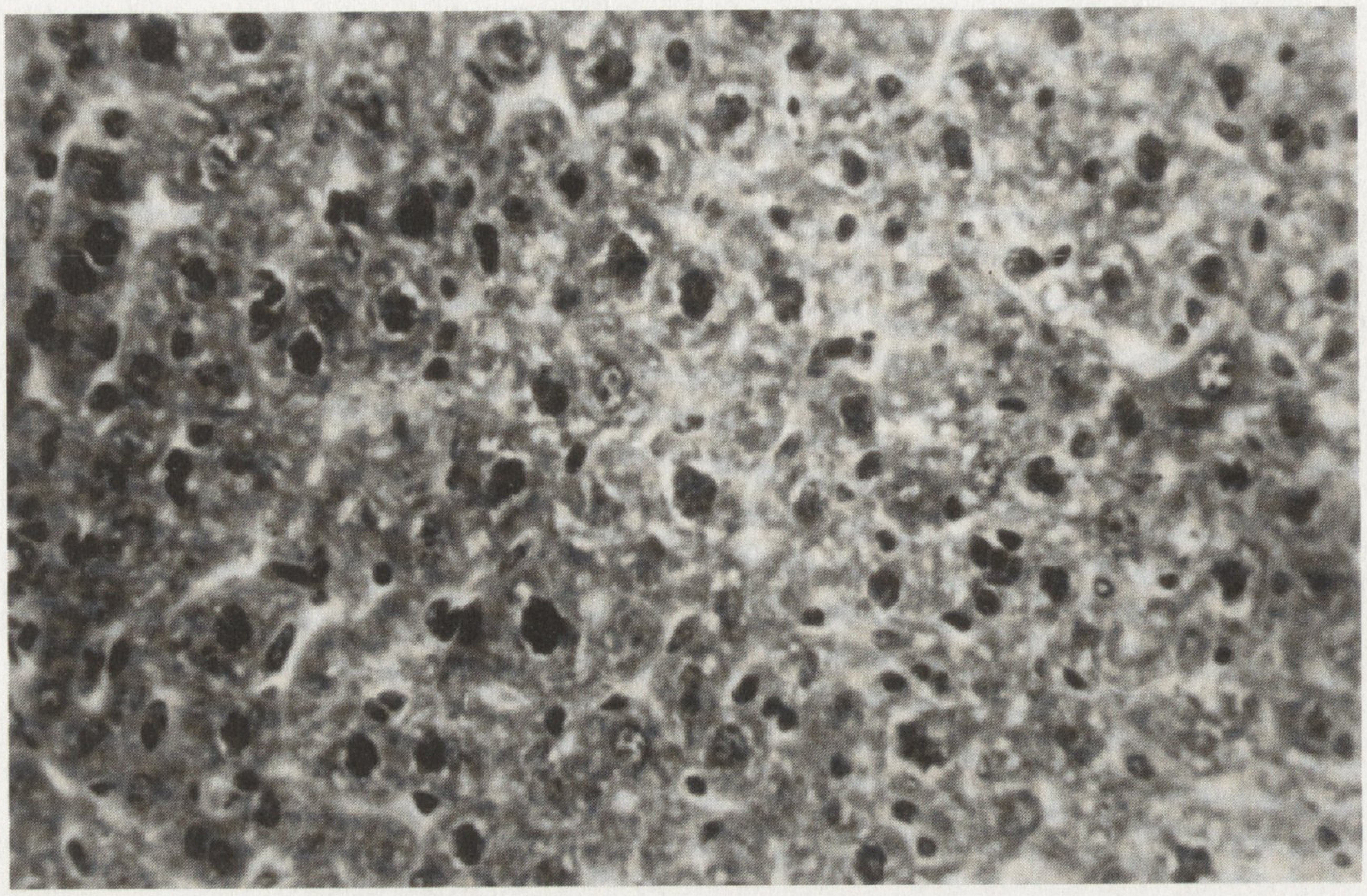

Fig. 4. Liver of a control infected rat at mixed infection with the larvae of *T. spiralis* and *T. pseudospiralis*. Blocked break-up of hepatocyte cytoplasm, karyopycnosis and karyorhexis are seen. Longitudinal section, 8 μm. Zenker, Mallory. Mag. 500x.

Local breaches of the trabecula structure, severe distrophic changes in hepatocytes and foci of parenchymatouse necrosis were observed in the liver. A hepatocyte distrophy was found to develop, as a result of granular and hydropic degeneration of the cytoplasm. A cloudy swelling of the cytoplasm was seen in some hepatocytes (Fig. 3). The nuclei were in the state of karyopycnosis and karyorhexis in

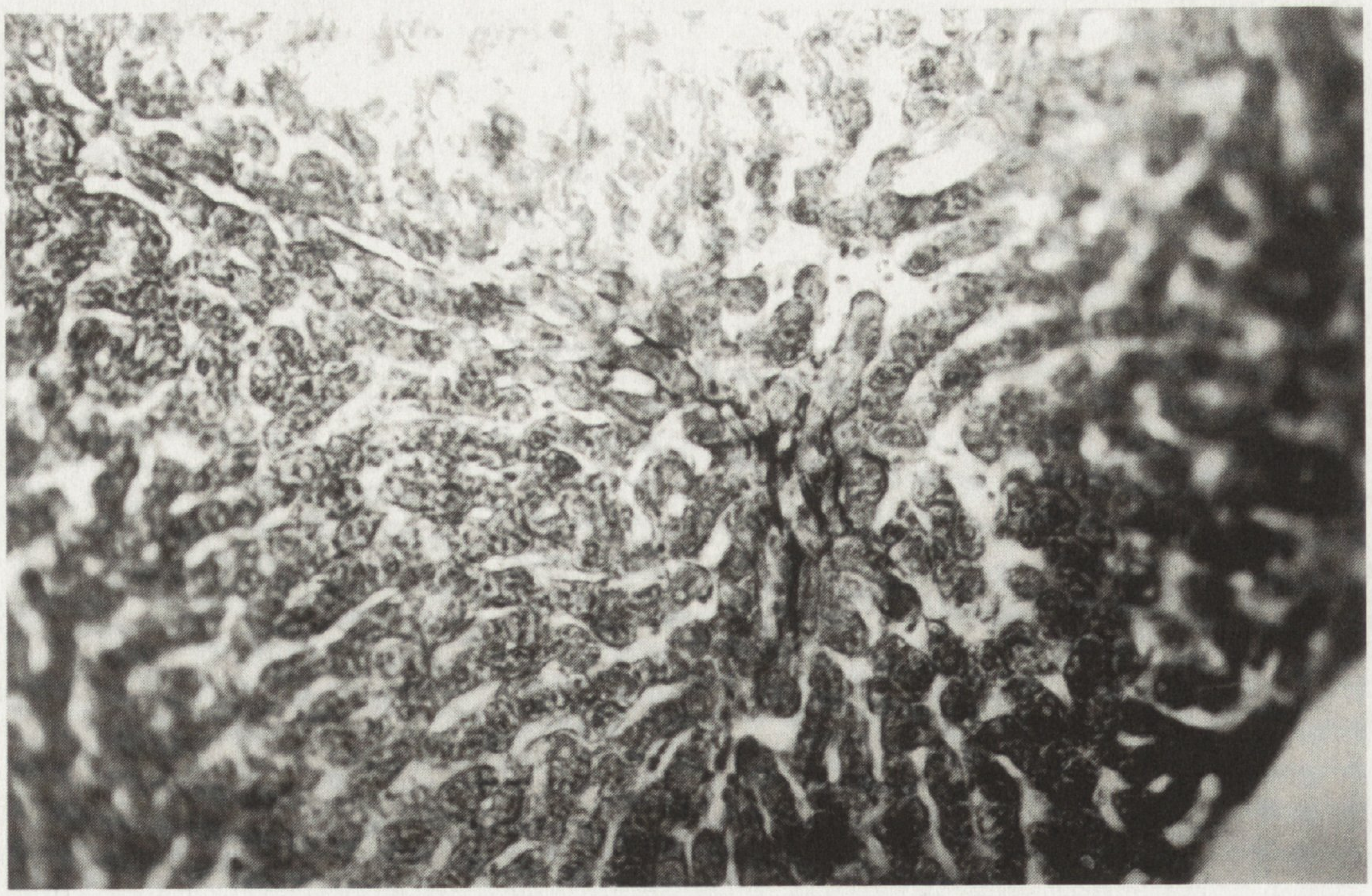

Fig. 5. Liver of control infected rat at mixed infection with the larvae of *T. spiralis* and *T. pseudospiralis*. Thickening of reticuline fibers and widening of sinusoides are seen. Longitudinal section, 8 μm Zenker, Mallory. Mag. 250x.

certain areas of the hepatocytes, while the cytoplasm was degenerated according to the type of the break-up (Fig. 4). Growing of interlobular binding tissue occurred in the parenchyma of the liver. Particularly, reticular fibers developed a vast net and migrated along the trabecula on a large area (Fig. 5). This parenchyma was moderately infiltrated by lymphocytes and mononuclears. The biliary ducts were enlarged. Hyperplasy and hypertrophy of the epithelium wall of the large biliary ducts were observed. Interlobular arteria and veins, as well as central veins were full-blooded. Hyperplasia of the tunica adventitia of the interlobular vesseles was clearly shown. The enlarged sinusoidea were filled with erythrocytes. Cavities filled with an indefinite mass were developed at the expense of a section of the most enlarged sinusoids (Fig. 6).

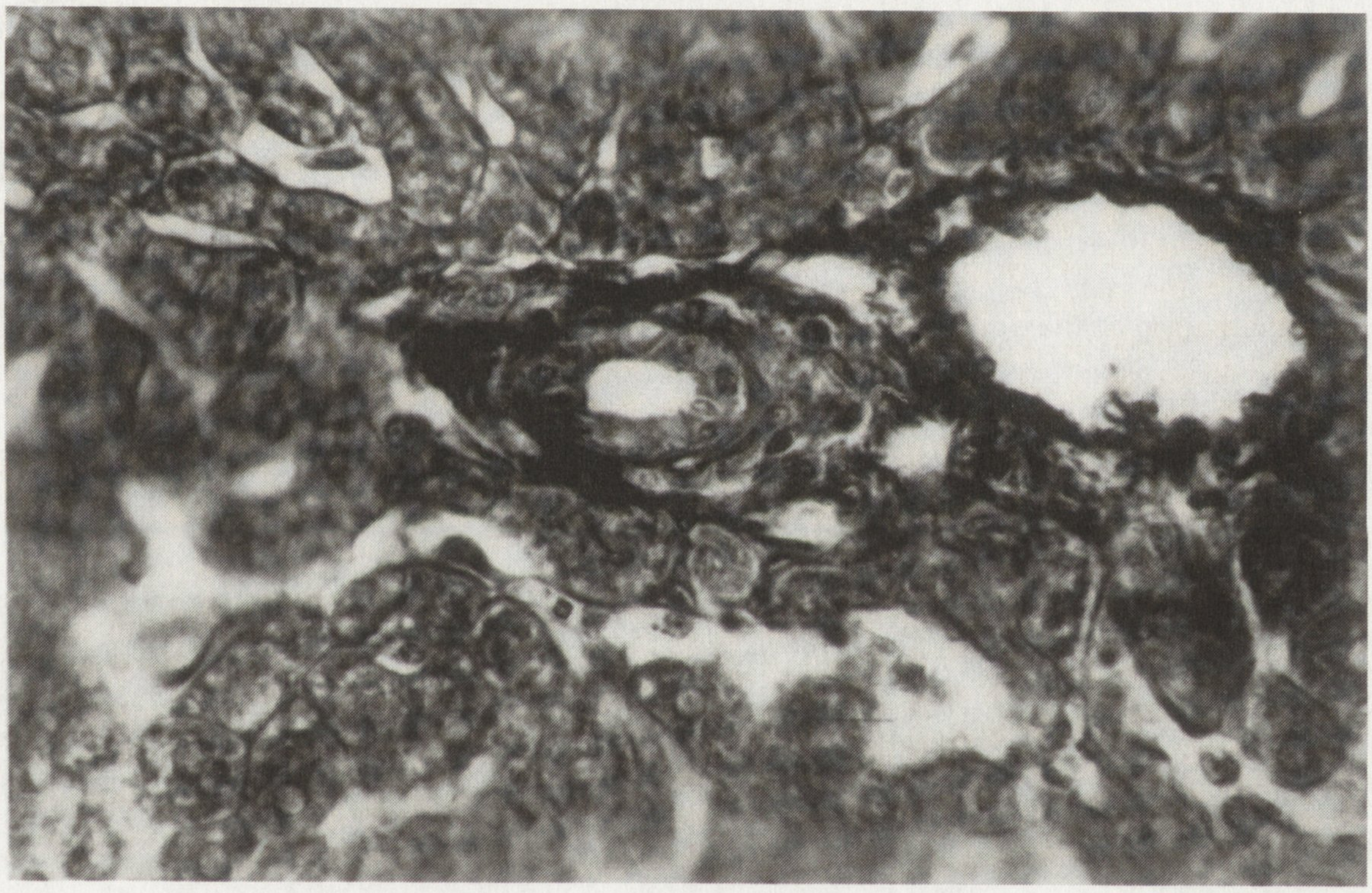

Fig. 6. Rat liver at mixed infection with larvae of *T. spiralis* and *T. pseudospiralis* after phytohemagglutinin effect. Sinusoides of the parenchyma cavity, hyperplasia of the adventitial membrane of interlobular vein, hypertrophy and hyperplasia of the gall duct epithelium. Longitudinal section, 8 μm. Zenker, Mallory. Mag. 500x.

The wall of the small intestine was oedematous and full-blooded. Punctate haemorrhage and tissue infiltration by leucocytic elements were revealed in the submucous layer. Villi of the intestinal mucosa were destructured and their apexes were fused. As a result, desquamative masses were developed and shedded in the intestine cavity (Fig. 7). The basal sections of enterocytes of intact fragments of villi were infiltrated intensively mainly by lymphocytes, and, partially, by plasmacyte cells. Some characteristic indicators of hydropic degeneration were found in a number of enterocytes. The hypertrophied gobletlike cells in the state of hypersecretion were seen. The swollen layer of the mucuous membrane of villi contained lymphocytes and a small number of eosinophils, while lymphatic and blood vessels were

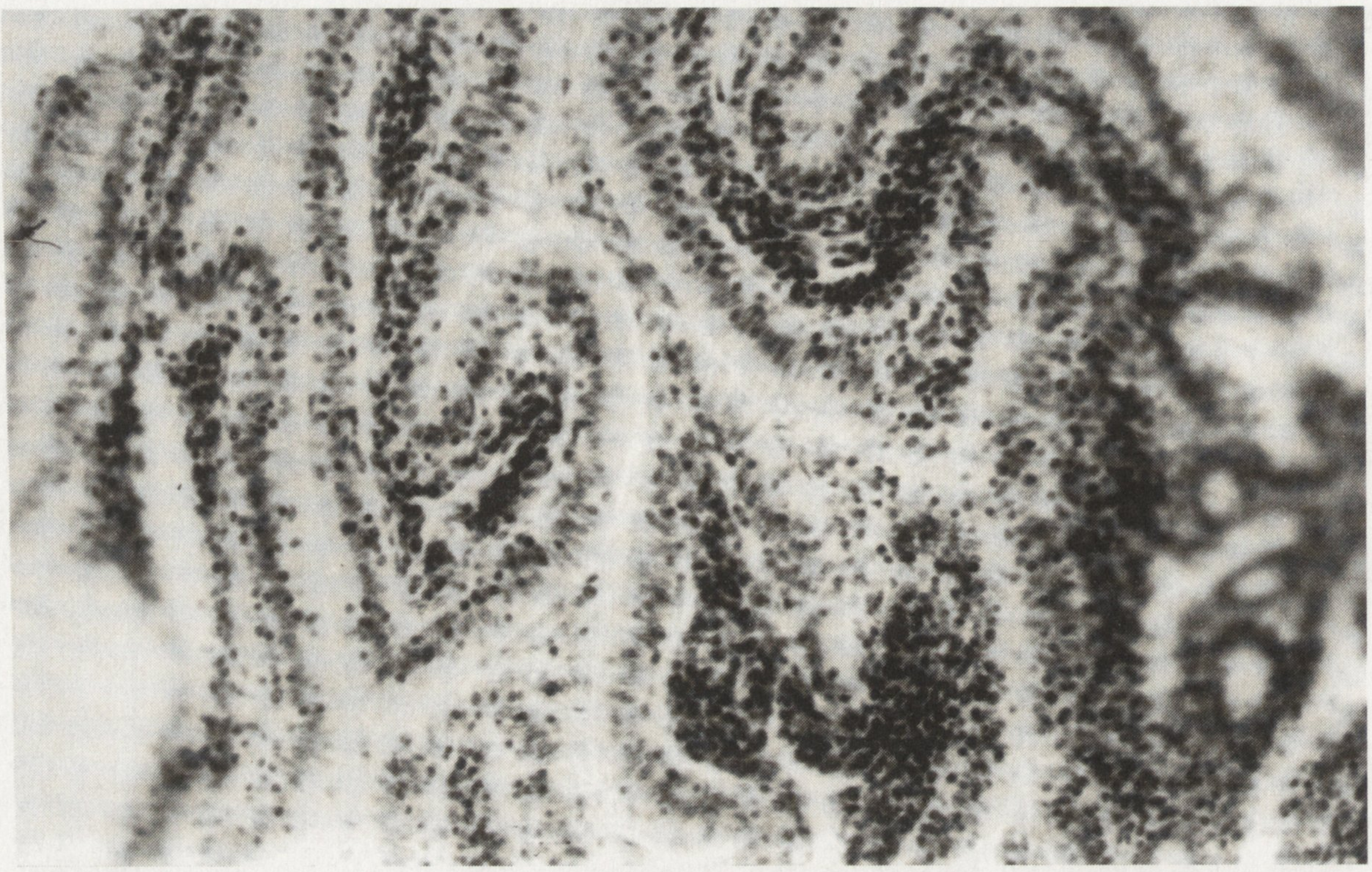

Fig. 7. Desquamation of the epithelium of the large intestine microvilli at mixed infection with larvae of *T. spiralis* and *T. pseudospiralis*. Longitudinal section, 8 μm. 10% formalin, hematoxylin Carazzi-eosin. Mag. 250x.

enlarged (Fig. 8). Cell proliferation and increase in lymphocyte number were observed in intervillous lymphatic follicules.

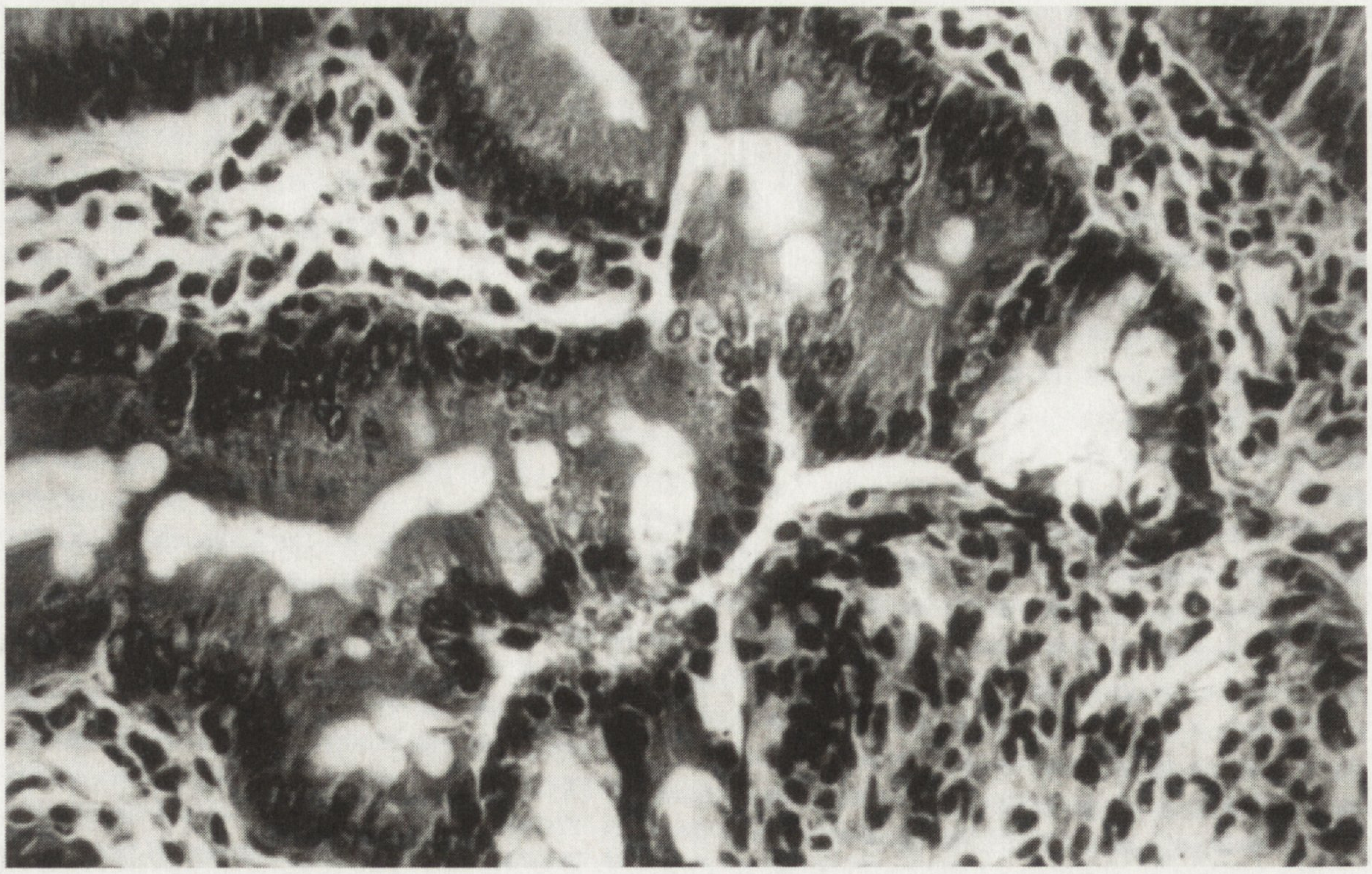

Fig. 8. The large intestine of the rat at mixed infection with larvae of *T. spiralis* and *T. pseudospiralis* after phytohemagglutinin effect. Infiltration of the enterocytes and swollen lamina propria of microvilli of the mucous membrane. Hypersecretion of the gobletlike cells. Longitudinal section, 8 μm. 10% formalin, hematoxylin Carazzi-eosin. Mag. 500x.

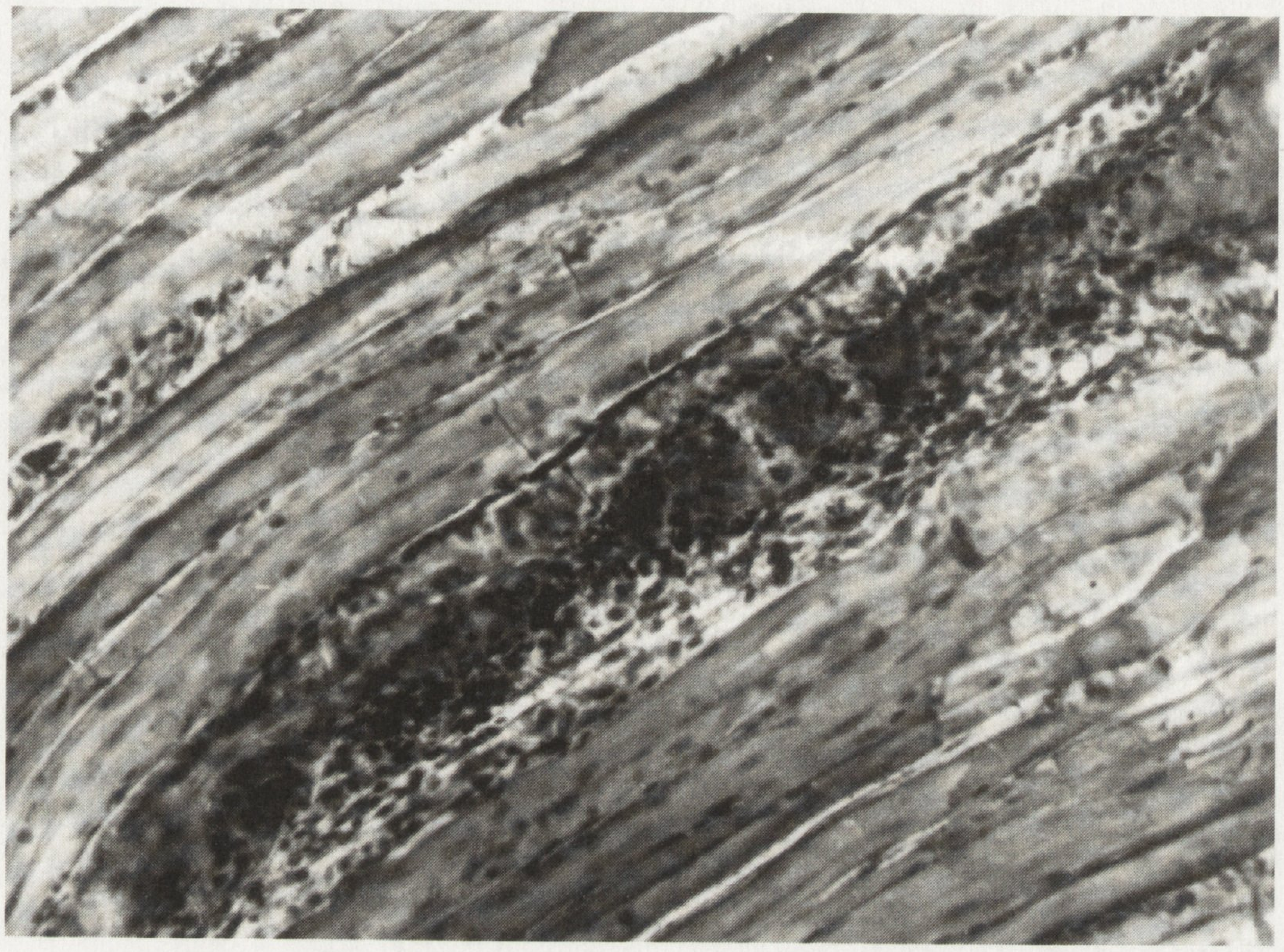

Fig. 9. Cell infiltration around the larvae of *T. pseudospiralis* after using phytohemagglutinin. Longitudinal section, 7 μm. 10% formalin, hematoxylin Carazzi-eosin. Mag. 900x.

However, a 5-day phytohemagglutinin administration before the infection of rats with *Trichinella* produced a positive effect. Particularly, the pathomorphological changes in muscle tissue were smaller than those in infected control animals. Hypertrophy of the nuclei and their redistribution in the sarcoplasma was stimulated. Some of the *T. spiralis* and *T. pseudospiralis* larvae were delayed and died in the endo- and perimysium of capillaries. The larvae of *T. spiralis* were delayed with the encapsulation. The cell-inflammatory reaction was intensified particularly in the perivascular area, around the larvae of *T. pseudospiralis* and at the poles of capsules containing larvae of *T. spiralis.* Lymphatic elements prevailed in the infiltrates, however, histiocytes and plasmatic cells also occurred there (Fig. 9). There was a greater number of Kupffer cells in the liver; they were hypertrophic. Dark granules emerged in the cytoplasma. The number of binuclear hepatocytes increased, and the number of cells in the state of turbid swelling decreased. Foci of round-cell infiltration were found in the parenchyma, particularly in the area of decomposition of the trabecules (Fig. 10). The mucous forming function of goblet cells and desquamatose reaction were decreased in the intestine. The reactive centers of the lymphatic follicules were abruptly activated in relation to producing lymphoblasts and lymphocytes. The infiltration rate of the mucuous membrane layer of villi by lymphocytes, mononuclears, and eosinofils were increased (Fig. 11).

Histochemical studies indicate that the biostimulator which was added to the fodder before the infection failed to effect the composition and cytotopography of RNA and the glycogen in *T. spiralis* and *T. pseudospiralis*. The number and distri-

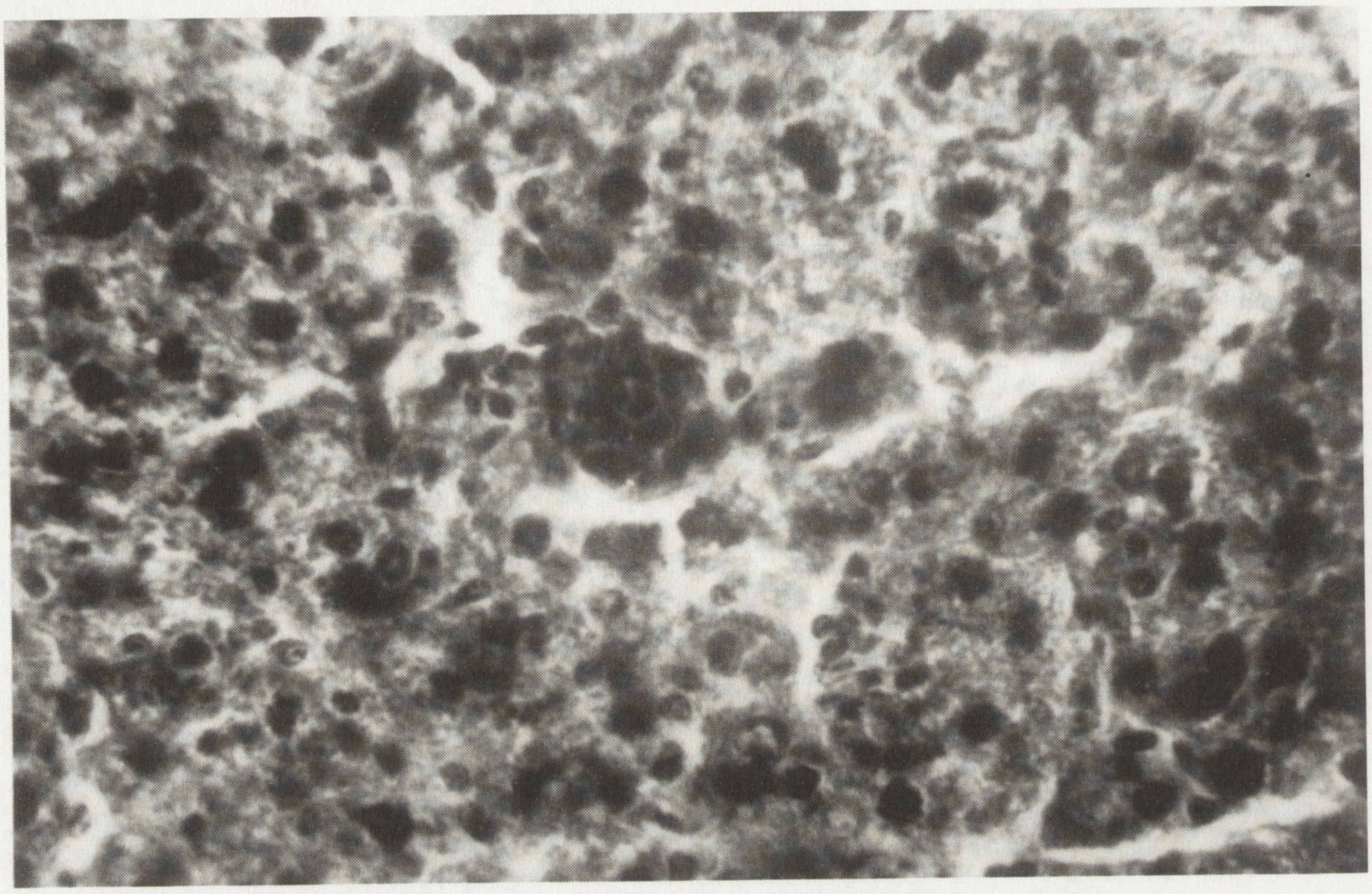

Fig. 10. A focus of round-cell infiltration in rat's liver at mixed infection with larvae of *T. spiralis* and *T. pseudospiralis* after phytohemagglutinin effect. Cross-section, 8 μm, Zenker, Mallory. Mag. 250x.

bution of these vitally important indicators were similar to those of the larvae of infected control animals. The histichocytes demonstrated a maximum positive reaction for the presence of RNA and glycogen. Hard pyroninophilia and SCHIK-reac-

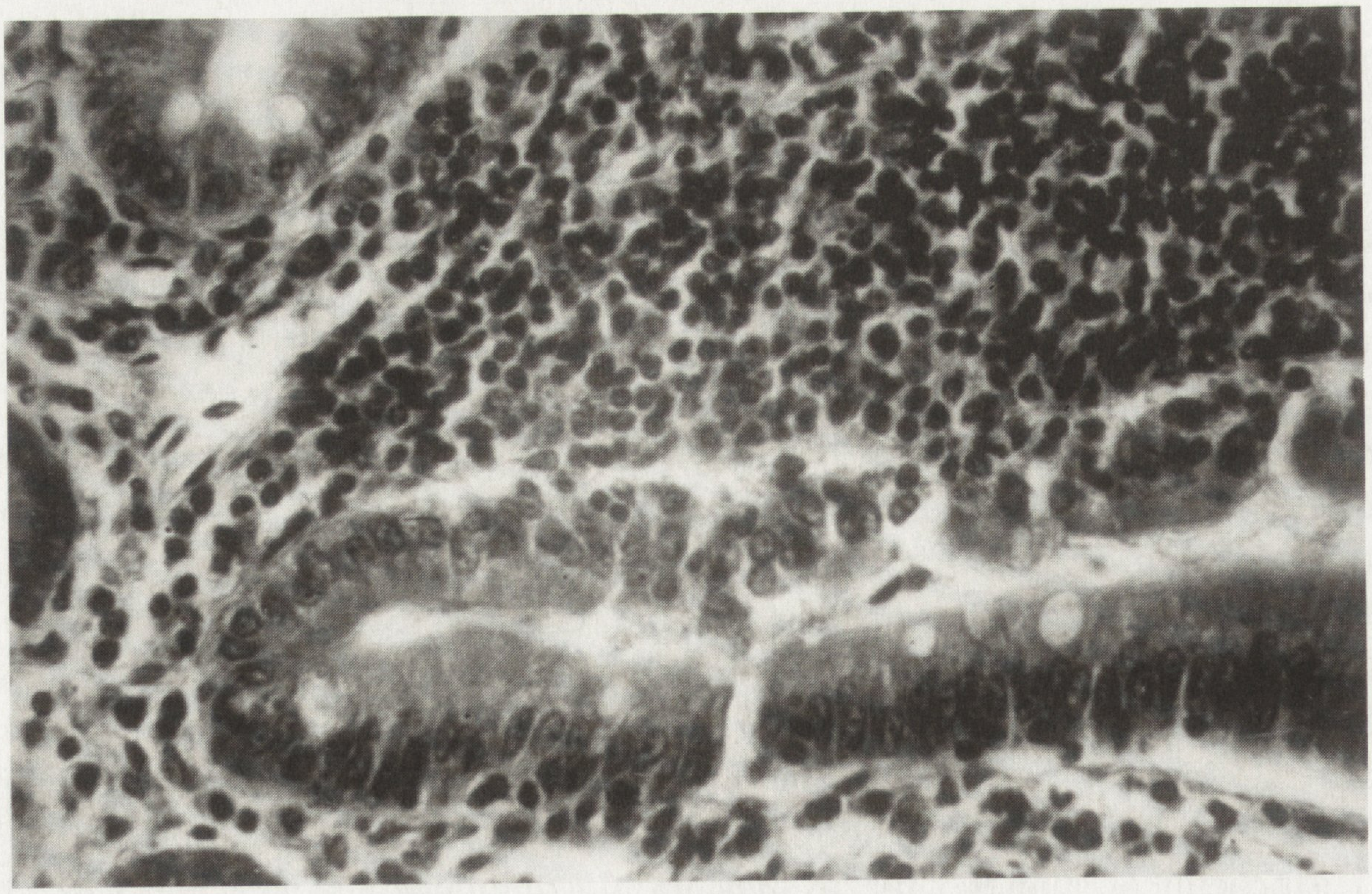

Fig. 11. Hyperinfiltration of the mucous membrane lamina propria of a rat's large intestine microvilli at mixed infection with larvae *T. spiralis* and *T. pseudospiralis* after phytohemagglutinin effect. Longitudinal section, 8 μm. 10% formalin, hematoxilin Carazzi-eosin. Mag. 500x.

tion were typical to the hypodermium and plasmatic sacks of the somatic muscle cells: RNA was dyed diffusively while the glycogen was concentrated in hard blocks. Moderate reactions identifying RNA and glycogen were typical for the middle intestine and genital glands of larvae. As for the capsules with larvae of *T. spiralis*, a moderate reaction with acid polysacharides was shown only in their external layers; glycogen and RNA were absent in the intracapsular sarcoplasma.

The composition of RNA and glycogen in muscles, liver, and intestine increased slightly compared with that in analogical organs of infected control animals. However, the nature of cyto and histotopography of these molecules did not change. Thus, the reaction to RNA and glycogen was negative in destroyed muscle tissues. Pyroninophilia and SCHIK-reaction were rather weak in destroyed tissues; and RNA and polysacharides were distributed in the sarcoplasma zonally.

In livers of both the rats which received biostimulator before the infection and infected control rats, the intensity of reactions on glycogen and RNA varied from moderate to weak compared with intact animals, their livers having highly intensive pyroninophilia and SCHIK-reaction. These changes were distributed zonally (Fig. 12).

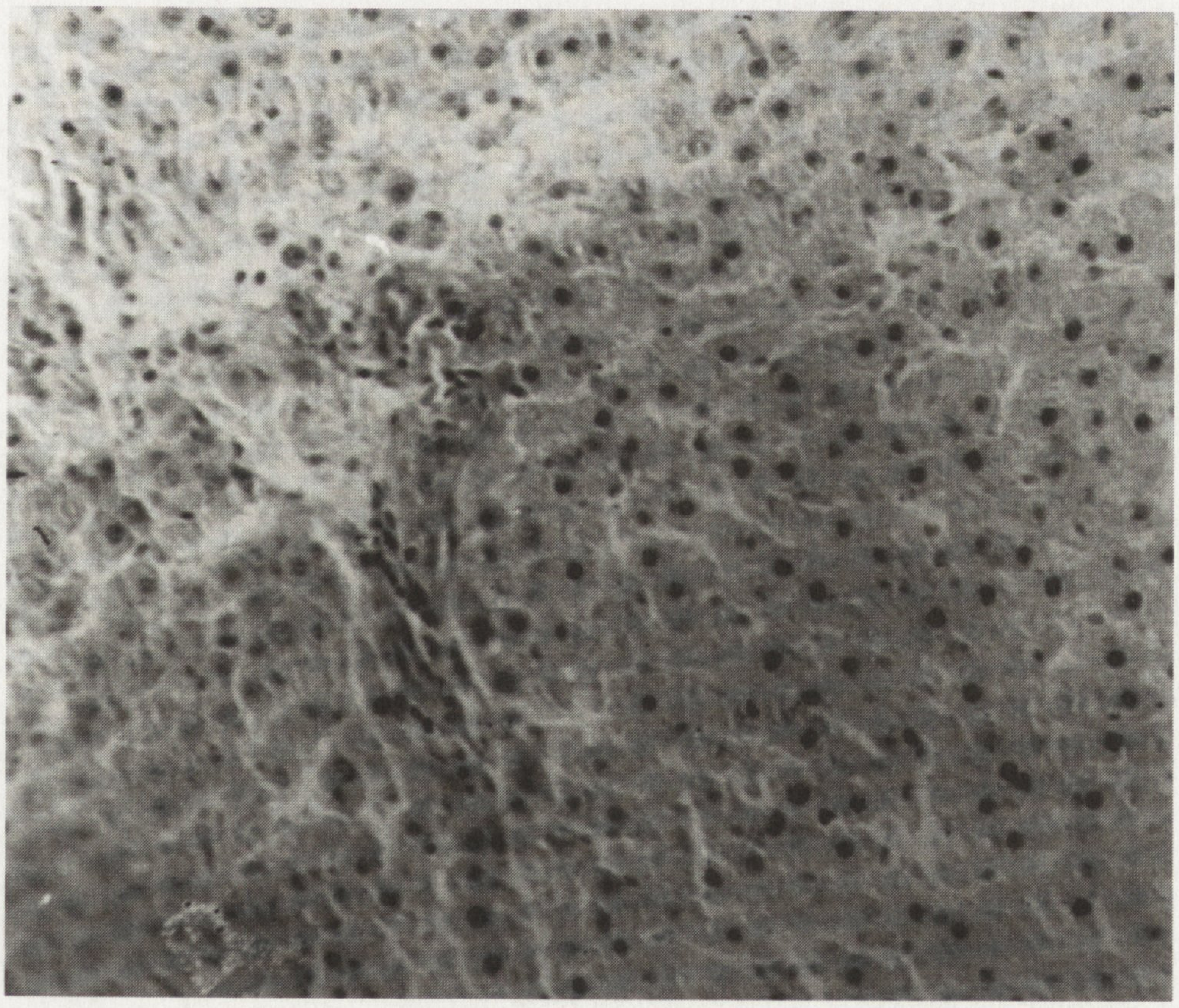

Fig. 12. Inlaid distribution of RNA in rat's liver at mixed infection with larvae of *T. spiralis* and *T. pseudosliralis* after the usage of phytoanthelminthics. Cross-section, 7 μm, Carnoy, reaction of Brachet. Mag. 250x

Glycogen was concentrated only in hepatocytes in such areas of the trabecula which were located only near to the central vessel of the lobulae; the SCHIK-reac-

Fig. 13. Glycogen in a rat's liver at mixed infection with larvae of *T. spiralis* and *T. pseudospiralis* after phytoanthelminthic effect. Cross-section, 7 μm. Schabadasch, SCHIK-reaction. Mag. 500x.

tion was negative in the periportal area and in the periphery of the lobulae (Fig. 13). The reaction intensity in various tissues of the intestine wall varied.

Weak pyroninophilia and a moderate positive SCHIK-reaction were especially characteristic for the muscle plate, and RNA and the polysacharides were dyed diffusely. The intensity of both reactions increased at a sub-mucous layer; the dyeing

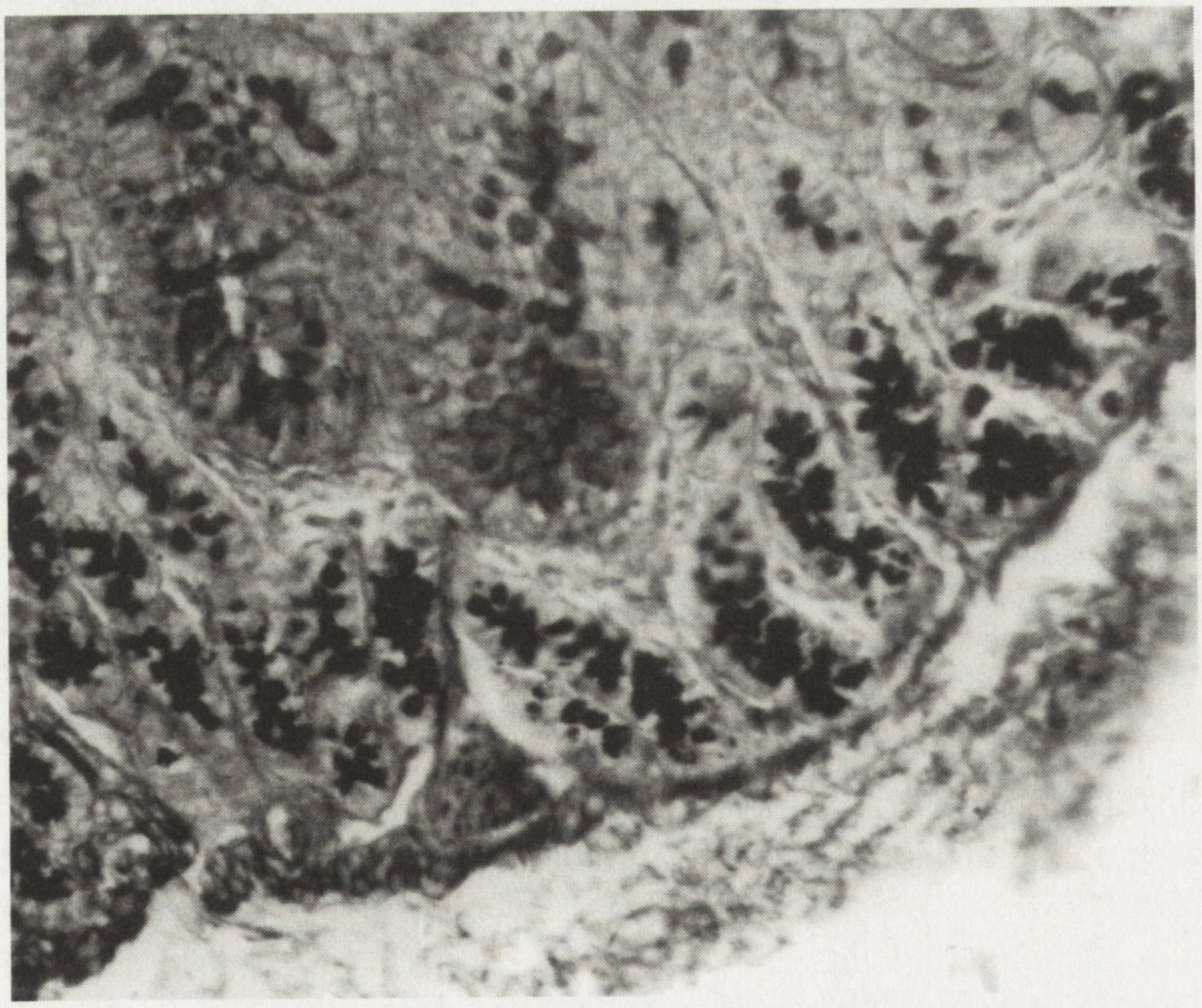

Fig. 14. Polysacharides in structural elements of a rat's large intestine microvilli at mixed infection with larvae of *T. spiralis* and *T. pseudospiralis*. Squaint section, 7 μm. Schabadasch, SCHIK-reaction. Mag. 250x.

of the tissue was also diffused. Villi of entherocytes of the mucous membrane were pyroninophiled, whereas dyeing of the cytoplasm – bright and diffused. However, the intensity of SCHIK-reaction oscillated from weak to considerably moderate. In the first case, the enterocyte cytoplasma was dyed diffusingly, in the second – tiny glycogen granules ocurred in the weakly dyed background.

The secretion of the majority of goblet-like cells produced a very positive SCHIK-reaction in the presence of neutral mucopolysacharides with diffusional dyeing (Fig. 14).

Destruction of *Trichinella* larvae of both species and the capsules around *T. spiralis* was observed after a 5-day phytoanthelminthic therapy of animals began at the 16th day after the infection. The shape and the outline of the majority of capsules were changed, the gyaline layer was festoon-like as a result of irregular thickening. The intracapsular sarcoplasma was heavily vacuolized or turned into a rough and granular mass, but was separated from the larvae of *T. spiralis* (Fig. 15). Some of the capsules underwent necrosis and lysis of the intracapsular sarcoplasma. Abrupt activation of cellular-inflamatory reaction was seen on each pole of the capsules: the infiltrate contained lymphocytes, neutrophils, eosinophils, histocytes and fibroblasts.

Tinctorial features of the cuticulae were destroyed in a number of larvae of *T. spiralis* and *T. pseudospiralis*, and the cuticulae became very knobby and was torn away from the hypodermis in some areas. The hypodermis and the plasmatic areas of the muscle cells were heavily vacuolized. Locus exfoliation was revealed in all

Fig. 15. Destruction of the intracapsular sarcoplasma and larvae of *T. spiralis*. Cross-section, 8 μm. Zenker, Mallory. Mag. 900x.

components of skin-muscle sack. Metachromasia of the genital glands and gastrointestinal tract was observed. An abrupt rate of destruction of frontal and intermediate areas of the stichosome was noted; the shape and sizes of the back area of the stichosome in the intact stichocytes were changed. The dead larvae of *T. pseudospiralis* were revealed in the preparations. The larvae which underwent necrosis were replaced by a large tight inflammatory cell infiltrate composed of lymphocytes, neutrophiles, monocytes, plasmatic and giant multi-nuclei cells (Fig. 16). Fragments of the dead larvae of *T. spiralis* were locked in the capsules.

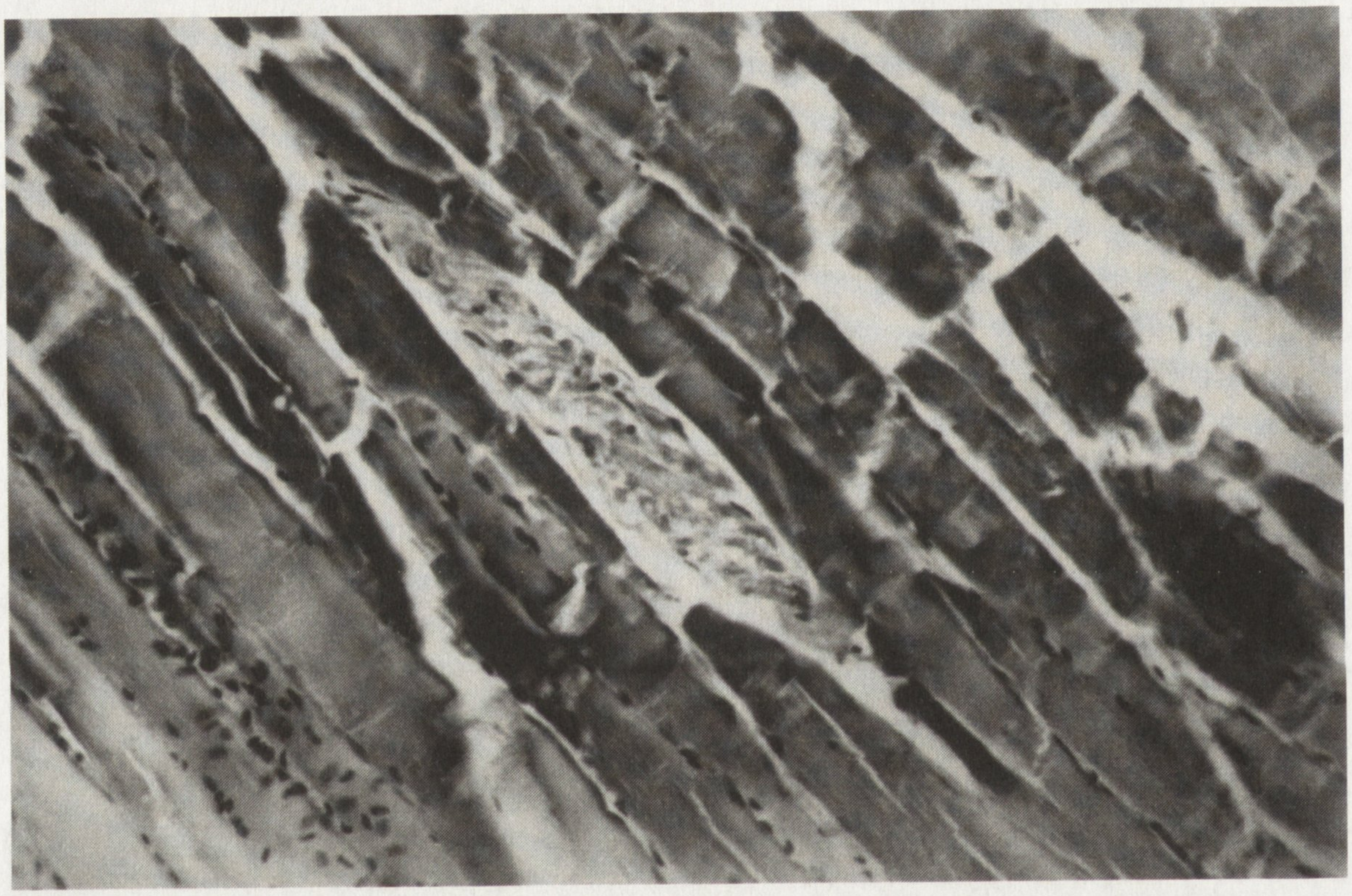

Fig. 16. Musculus rectus of a rat at mixed infection with larvae of *T. spiralis* and *T. pseudospiralis* after phytotherapy. Cellular-inflammatory infiltration around necrotized *T. pseudospiralis* larva. Squaint section, 7 μm. Zenker, Mallory. Mag. 500x.

The pathogenesis indicators weakened in muscles, liver, and intestine, and characteristic features of compensatory and regeneratory processes were noted.

Tissue-binding hems, that fill the destroyed muscle fibres and their fragments, were particularly developed in the muscle tissue. Partial regeneration of the muscle tissue at the expense of the mioblastic elements – observed. The shape of the muscles' tissue and longitudinal and diametrical lines in their sarcoplasma were also observed. Furthermore, compensatory hypertrophy was characteristic to the miosinplast's nuclei. Redestribution of nuclei was found to occur: the majority of them were located in peripheric areas of the sarcoplasma and made a chain under the sarcolemma. This phenomenon gave evidence about the tendency to restore the normal structure of muscle tissues. Now and then, amitotic division of the nuclei occurred; as a result, the number of nuclei lost in karyopycnosis and karyorexis increased.

Partial restoration of the structure and architectonics of the tuberculae, in marginal areas of hepatic lobulae in particular, were seen in the liver. In some areas, the liver parenchyma was lowered by a strip that was formed by slightly differentiated

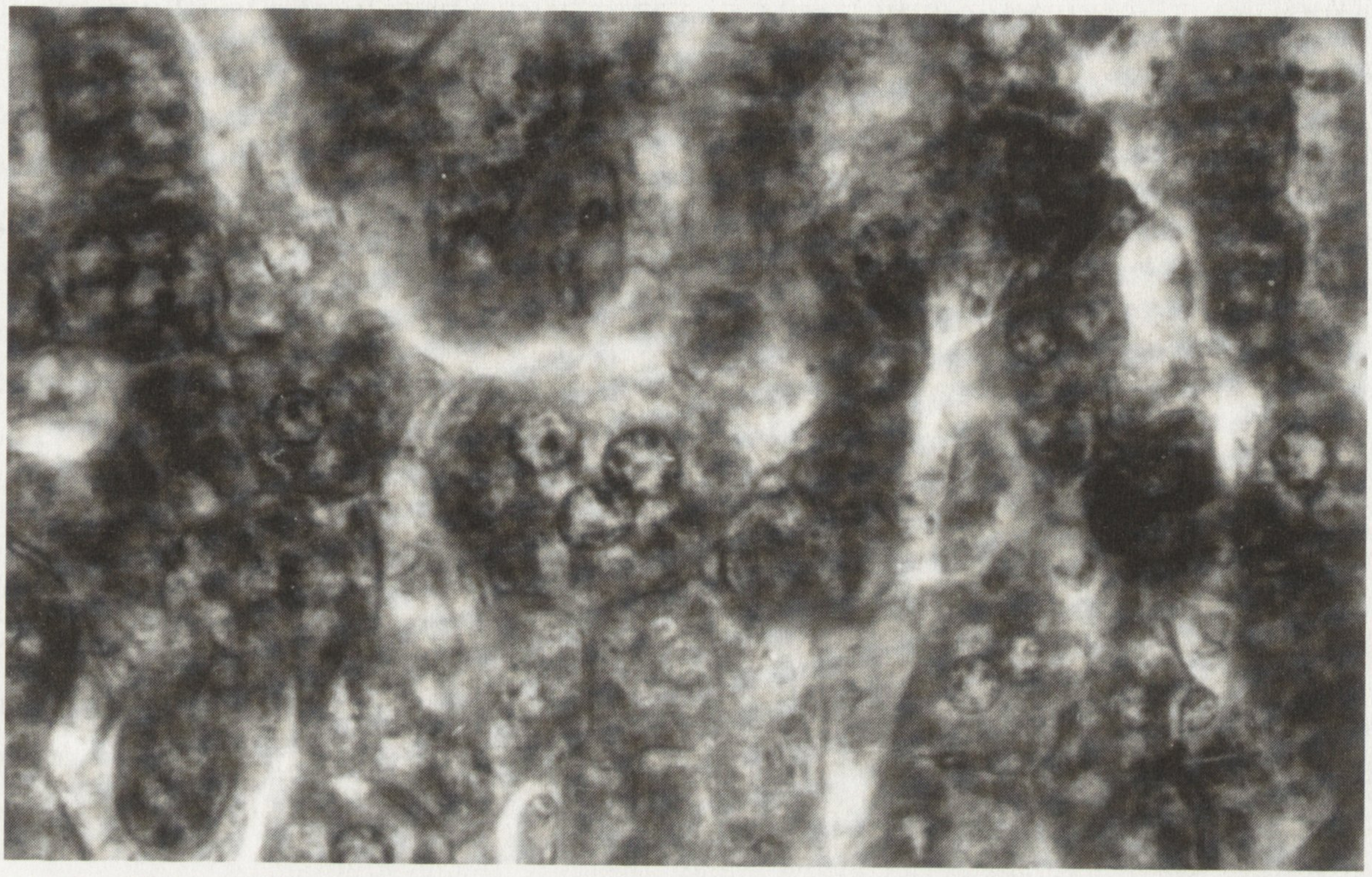

Fig. 17. Rat liver at mixed infection with larvae of *T. spiralis* and *T. pseudospiralis* after phytoanthelminthic effect. False trabeculae and two-nuclei hepatocytes. Longitudinal section, 8 μm. Zenker, Mallory. Mag. 750x.

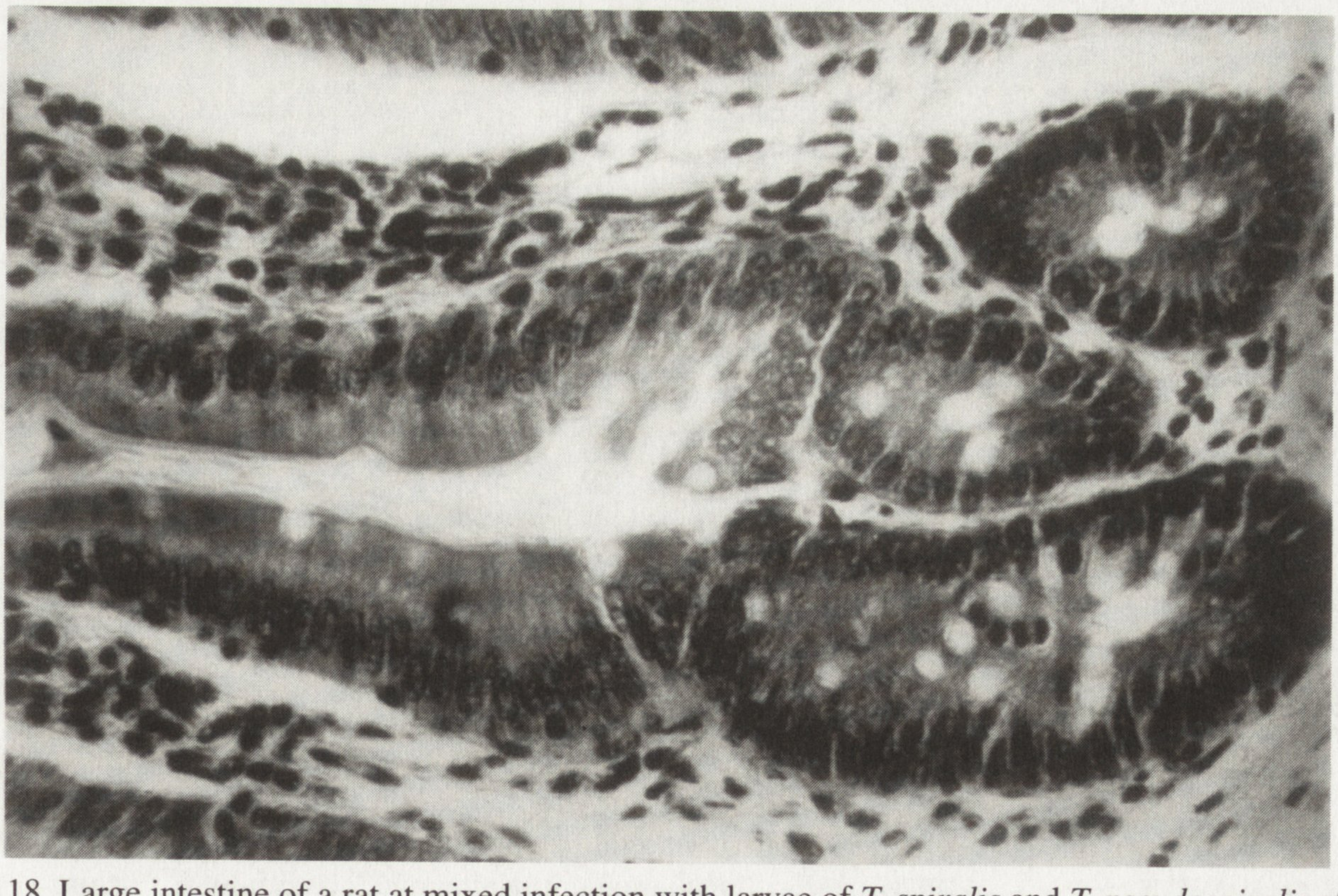

Fig. 18. Large intestine of a rat at mixed infection with larvae of *T. spiralis* and *T. pseudospiralis* after phytotherapy. Longitudinal section, 8 μm. Zenker, Mallory. Mag. 500x.

epithelioid cells. The strip developed false trabeculae (Fig. 17). In treated rats the dystrophic changes of a number of hepatocytes underwent reverse development. As a result of the phytotherapy the infiltration of the liver parenchymae by leucocytae elements was decreased. Some traces of the infiltration remained around the gall ducts, full-blooded varicose central veins and portal venose vesseles. The width of the sinusoid gaps and the number and sizes of the Kupffer cells reached the norm.

Weakening of desquamatose reaction and restoration of villous structure were observed in the intestine. The mucous-forming function of the goblet-like cells and their sizes also reached the norm. The number of intraepithelium lymphocytes decreased in enterocytes. The oedema of the mucous, sub-mucuous, and tissue tunics of the intestine wall, as well as of villi of the mucous membrane's lamina propria and its infiltration by leucocytae elements decreased ubruptly (Fig. 18). There was also a decrease in gaps of lymphatic and blood capillaries of the villi mucous; however, the blood vessels of the sub-mucous base and muscle plate of the intestine wall remained varicose.

Histochemical studies indicate that after the treatment of rats with the phytoanthelminthic the reaction on glycogen and pyroninofiles was rather weak in tissues of alive *Trichinella* larvae of both species. The stichocytes of middle and back areas of the stichosome indicating moderate pyroninofilia and SCHIK-reaction were exceptions.

Fig. 19. Inlaid distribution of glycogen in the periportal parenchyma of a rat liver at mixed infection with larvae of *T. spiralis* and *T. pseudospiralis* after phytotherapy. Longitudinal section, 7 µm. Schabadasch, SCHIK-reaction. Mag. 250x.

Acid mucopolysacharides were noted in the capsule wall with larvae of *T. spiralis*. RNA synthesis and glycogen formation were intensified in rat muscles, liver, and intestine. The RNA dyeing was diffused as usual; the glycogen occurred in a variety of granule forms and sizes while neutral mucopolysacharides occurred in the secretion of goblet-like cells — bright and diffused. Cyto- and histotopography of RNA and glycogen were changed, too. Thus, a considerable number of RNA and glycogen granules were seen in the cytoplasm of periportal parenchyma hepatocytes (Fig. 19). Also, there was a simultaneous increase in the intensity of histochemical reactions that reveal these granules in the cytoplasma of the trabecula hepatocytes by the central veins of liver lobulae, as well as in the parenchyma, in the area of gall ducts. The enterocytes of intestine villi demonstrated moderate pyroninophilia and produced a positive reaction to glycogen: RNA was dyed diffusely, while the glycogen emerged in small round granules. The secretion of the gobletlike cells contained intensively stained neutral mucopolysacharides (Fig. 20).

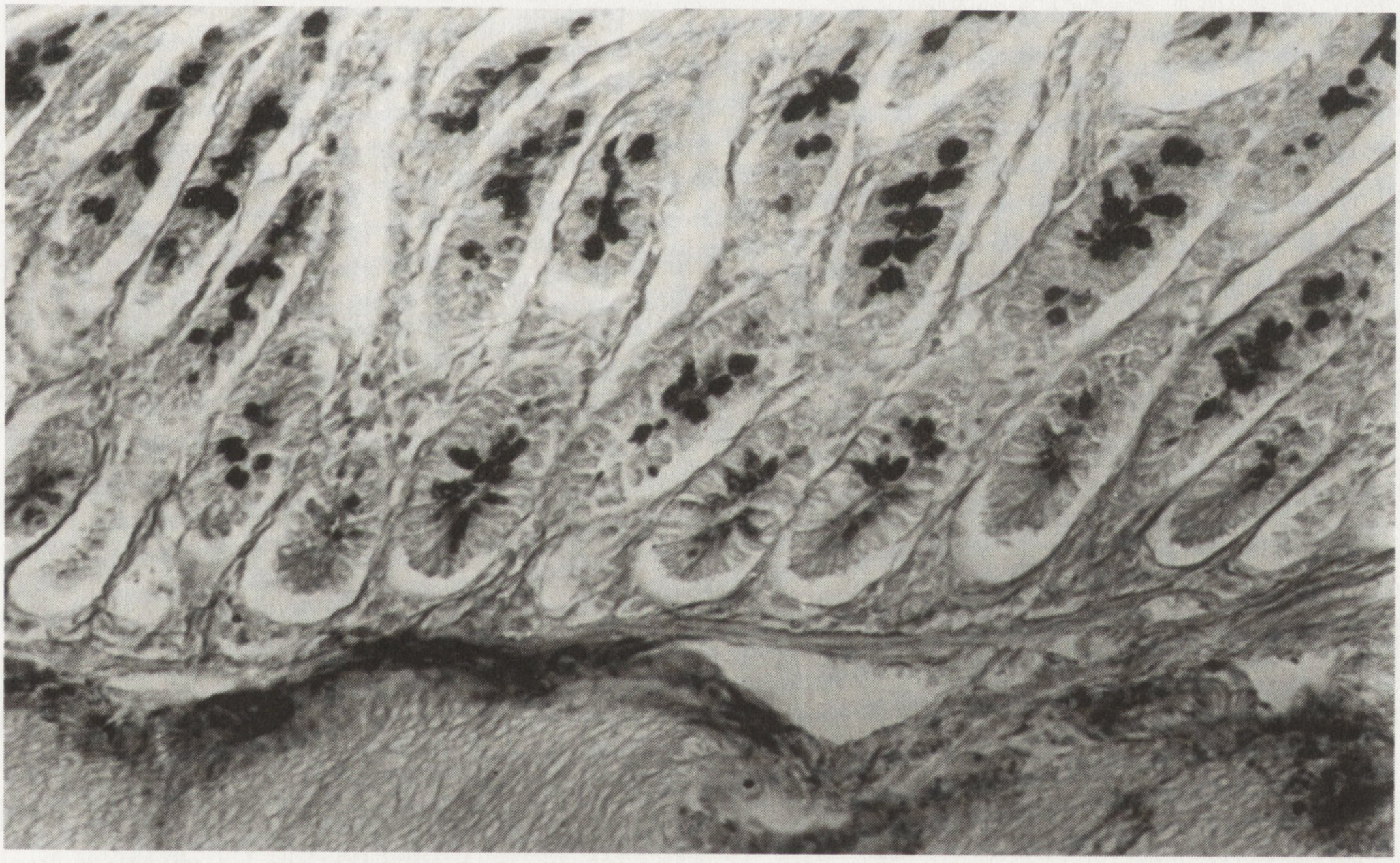

Fig. 20. Large intestine of a rat at mixed infection with larvae of *T. spiralis* and *T. pseudospiralis* after phytoanthelminthic effect. Glycogen in enterocytes and neutral mucopolysacharides in the secretion of gobletlike cells. Cross-section. 7 μm. Schabadasch, SCHIK-reaction. Mag. 250x.

After the combined usage of two phytopreparations (biostimulator – for 5 days before the infection and phytoanthelminthic – for 5 days, from the 16th day after infection) the pathomicromorphological changes in the larvae and capsules were the same as after the therapy with phytoanthelminthic. The microstructure and the nature of compensatory and restoration processes in rat muscles, liver, and intestine did not change at all. However, some definite intensification in regeneration – of which one of the characteristic features was an increase in two-nuclei hepathocytes – was noted in the liver.

The RNA and glycogen composition and topography in tissues of alive larvae of *T. spiralis* and *T. pseudospiralis*, and in rat muscles, liver, and intestine remained on the same level and within the same limits as in the case of applicating one phytoanthelminthic.

DISCUSSION

It was established that the pathomicromorphological and histofunctional changes in rat muscles at mixed infection with the larvae of *T. spiralis* and *T. pseudospiralis* were analogical to those of animals infected only with one of the *Trichinella* species (Goncharova et al. 1997 a, b). The data obtained by us on the pathogenesis and micromorphofunctional reconstruction of the muscle tissue of hosts with trichinellosis are compatible with those in literature (Stoyanov and Nenov 1965; Błotna 1967; Pereverzeva 1967, 1976; Gabryel et al. 1969).

Both our and other authors' studies on destructive and dystrophic changes in muscle tissue determine a clear picture of non-specific angiomiositis solely typical to trichinellose infection. However, trichinellosis provokes pathogenesis and disfunction of other organs of the infected organism, for instance, the heart, brain, spinal cord, lungs, liver, intestines, and adrenal glands. Bekish and Nikulin (1996), Ozeretskovskaya (1970), Ozeretskovskaya et al. (1966a, b, 1969b), Januszkiewicz et al. (1969) studied the dynamics of the pathogenesis of organ and systemic involvement in trichinellosis. The above authors have concluded that all the organ and systemic effects are non-specific and are of immunomorphological and immunochemical nature.

Results of our experimental studies on the barrier organs' (liver and intestine) pathogenesis and comparison with literary data (Tsvetaeva 1970, Bogoyavlensky et al. 1992) gave rise to two hypotheses. Firstly, the type of pathogenesis mechanism and reaction of barrier organs is likely to exist in different helminthoses (ascaridiosis, heterakidosis, tominxosis, opistorchosis, fasciolosis, echinococcosis etc.), and secondly, organ damages are likely to appear regardless of whether helminthes had contact with the organ.

The published data on the effect of various chemotherapeutic preparations on *Trichinella* larvae indicated that their anthelminthic effect develops as a result of oppression of *Trichinella* tissue metabolism. This gives birth to various forms of dystrophy, destruction, and necrobiotic processes (Froltsova et al. 1965, 1966; Ozeretskovskaya et al. 1969b, 1976, 1978a, b; Pereverzeva et al. 1976; Thienpont et al. 1974; Skvortsova et al. 1996a, b). According to some authors, all the used anthelmithics had side-effects.

For the first time in our studies, we used a biostimulator and anthelminthic of plant origin for the therapy of experimental trichinellosis. It was revealed that the biostimulator phytohemagglutinin, which was added to fodder in a dosage of 70 mg/kg of rat body weight during 5 days before infection, stimulated the function of lymphoide tissue and activated cell-inflammatory reactions in the host's tissues; this gave evidence about the strengthening of cell immunity of animals with trichinellosis. Destruction of *T. spiralis* and *T. pseudospiralis* larvae in the endomysium and perimisium of vessels was noted. It is characteristic that a similar phenomenon was described by Kocięcka et al. (1989) in treatment of mice trichinellosis with an immunomodulating preparation TFX Polfa which, according to the authors, had a prolonged action. They observed a delay in *T. spiralis* larvae encapsulation as well as changes in capsule structures right up to their full destruction in muscles of infected animals. Encapsulated larvae were found to be destructed to a different extent, necrosis being the furthest. There was cumulation of cellular infiltrates around capsules which was an indication of cell-immune response. The same pattern could be seen in experimental animals and humans treated with thiabendazolum and mebendazolum in later stages of trichinellosis (Łapszewicz et al. 1969, De Nollin et al. 1974, Kocięcka et al. 1974). Our results showed that phytohemagglutinin stimulated compensatory hypertrophy in practically all structures of the muscle tissue, liver, and intestine. In addition, synthesis was strengthened in single structural elements of the tissues of these organs.

The phytoanthelminthic, added to the fodder in a dosage of 50 mg/kg body weight for 5 days from the 16^{th} day after infection, stimulated cell immunity. Simultaneously, the phytoanthelminthic not only delayed the encapsulation of *T. spiralis* larvae but, most likely, having a prolonged effect, also caused the deformation and destruction of developing capsules. Furthermore, it caused deep irreversible structural and micromorphofunctional changes in *Trichinella* larvae, as a result of which they perished. Both strengthening of compensatory hypertrophy indicators and restoration processes took place in muscle tissue, the liver and intestine after the phytotherapy of the host.

Compensatory hypertrophy developed on an organelle and cellular level which caused the restoration of intracellular homeostasis. The structural and functional bases of intracellular homeostasis are qualitative and quantitative changes and redistribution of nuclear and cytoplasmatic structures ensuring adequate oscillation of the cells' functional activity. Especially, the hypertrophy of the nuclei and the presence of the chromatin in a diffused state (i.e., the presence of euchromatin) in the karyoplasma gave evidence about the activation of the synthetic processes in the cell. The RNA synthesis intensification in cells is usually associated with the expansion of loosened, decondensed chromatin zones in the nuclei. The cloudy swelling of the cytoplasm of marginal area hepatocytes of the liver lobulae was caused by cell reconstruction in hyperfunctional conditions. As a result, specific functions of the hepatocytes strengthened and energetic losses increased as glycogenolysis and glycolisis in the cytoplasm matrix strengthened.

The reversion of cell dystrophy as a result of the normalization of a single metabolic process in the cytoplasm matrix was a proof of the reversibility of morphological and morphofunctional changes occurring after phytotherapy. The level of glycogen and RNA synthesis reached normal indications in the muscle fibers, in liver and intestine tissues. The wide scale rate of the functional activity does not contradict the knowledge on the normalization of tissue metabolic processes since it is explained by the regulations of structural and functional liability of the organs. Any organ – a polystructural system: cells and tissues with their internal correlations forms structural-functional complexes. Variant structural elements can be in various functional states, and some of their parts cannot function and make up the organ's reserve temporarily.

According to our studies regenerative processes were realized mainly at the expense of dividing of non-differencial cells and amitosis of the nuclei. Such cells were the myoblasts in the muscle tissue, and cells in the intestine and liver, which did not function during the differentiation shortly after the previous myotic division. Restoration of the intestinal villi and liver parenchyma took place at the expense of cells. In the liver, false trabeculae were developed from strip non-differentiated epithelium cells. Nuclei amiotosis was observed in the muscle fibers and in hepatocytes. Thus, some of the liver trabeculae were nearly wholely composed of two-nuclei hepatocytes.

The combined usage of the phytohemagglutinin during 5 days before infection and the phytoanthelminthic during 5 days from the 16th day after the infection with larvae of *Trichinella* of both species did not strengthen the effectiveness of the phytoanthelminthics in the muscle phase of mixed trichinelliosis.

CONCLUSION

It was established that at a mixed infection of rats with larvae of *T. spiralis* and *T. pseudospiralis*, on the 35th day after infection there are angiomyositis, hepatitis and cholangitis and erosive-haemorrhagic enterocolitis. All the diseases were of non-specific allergic nature. Simultaneously to histo- and cytodestructions in muscles, liver, and the intestine, processes of compensatory hypertrophy ensuring host's homeostasis on cell and tissue levels of organization were observed.

The biostimulator phytohemagglutinin used with the fodder in a dosage of 70 mg/kg body weight for 5 days before infecting rats with larvae of *Trichinella* of both species, demonstrated features of an immunostimulator. Cell immunity was intensified in such infected animals. Loss and resorption of *Trichinella* larvae were observed as well.

The phytoanthelminthic, mixed with the fodder in a dosage of 50 mg/kg body weight and used for 5 days from the 16[th] day after infecting rats with mixed *Trichinella* larvae, delayed the *T. spiralis* larvae encapsulation and caused loss of a definite number of *Trichinella* larvae of both species. Structural lesions and decrease of synthetic functions were noted in tissues of live muscle larvae. An increase in RNA and glycogen synthesis in muscles, the liver and intestine of treated animals and the weakening of the pathomicromorphogenesis and stable indicators of compensatory and regenerative processes were noted. The effectiveness of the phytoanthelminthic was not strengthened when the usage of the phytohemagglutinin and phytoanthelminthic was combined.

The results of our studies call for elaboration and usage of the anthelminthics of plant origin in helminthoses, particularly, for therapy of trichinellosis.

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