ACTION OF COLCHICINE ON HEPATIC SCHISTOSOMAL GRANULOMA

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Amorphous material and altered collagen fragments within dilated secretory vesicles and cisternae of fibroblast cytoplasm were the main ultrastructural changes seen in hepatic periovular granulomas formed in mice infected with Schistosoma mansoni *and treated with colchicine. Despite promoting ultrastructural changes in the fibroblasts found in hepatic periovular granulomas, colchicine administration to infected mice did not significantly change the light microscopic appearance of the hepatic schistosomal lesions, did not diminish the amount of total hepatic collagen, and did not change the collagen isotypes in the granulomas, as observed after a comparative study with non-colchicine treated infected control mice. When administered to mice two weeks after curative treatment of schistosomiasis with praziquantel, colchicine did not seem to increase extracellular collagen degradation or to induce a more rapid resorption of hepatic periovular granulomas, although still promoting ultrastructural alterations in fibroblasts.*

Key words: *Schistosoma mansoni* – fibrosis – colchicine – periovular granuloma

Colchicine an alkaloid obtained from the plant *Colchicum automnalis* has anti-inflammatory and anti-fibrosing properties (Malawista, 1968). Its physiological activities are rather complex, since among other things colchicine inhibits microtubule assembly and interferes with collagen secretion (Scherft & Heersche; 1975, Cho & Garant, 1981, 1985), increases the production of collagenase (Harris et al., 1971), decreases the production by circulating monocytes of factors that stimulate fibroblast proliferation *in vitro* (Kersenobitch et al., 1984) and increases hepatic alkaline phosphatase activity (Wilfred, 1977).

Although its therapeutical use in humans may be accompanied by neuromuscular toxicity (Kuncl et al., 1987), beneficial results have been observed in patients with active liver cirrhosis (Kershenobich et al., 1979), chronic active hepatitis (Silva et al., 1978), sclerosing cholangitis (Leiser & Kadish, 1986), primary biliary cirrhosis (Kaplan et al., 1986), besides its century old analgesic effect in gout.

When colchicine was administered to rats with carbon tetrachloride-induced cirrhosis it diminished fibrosis and improved liver function (Bolarin et al., 1987). Recently it has been claimed that colchicine given to mice with *Schistosoma mansoni* infection causes marked reduction of fibrogenesis in hepatic periovular granulomas (Nigro et al., 1988).

Fibrosis induced by periovular schistosomal granuloma is a major factor in the production of hepatic disease in schistosomiasis and the possibility of therapeutical interference in fibrosis production also appears as extremely important. These aspects stimulated further studies on this subject.

Present investigation is a parasitological, histological and ultra-structural study, combined with methods for the evaluation of collagen concentration and collagen immunotyping in mice infected with *S. mansoni* and treated since the onset of oviposition with colchicine. The possibility that the drug could accelerate the process of collagen degradation after the cure of schistosomiasis was also investigated in infected mice previously submited to treatment with praziquantel.

MATERIALS AND METHODS

First experiment – Young adult outbred Swiss mice of both sexes, maintained on a

commercial balanced diet and water *ad libitum*, were infected each with 50 *S. mansoni* recently eliminated cercariae, by the transcutaneous route. Forty days after exposure, the animals were treated daily with 1mg/kg b.w. of colchicine (Enila — SK & F) administered by gavage. Treatment lasted for 5 weeks. Each week 5 colchicine treated animals and 5 controls (a group of mice infected at the same time, but left untreated) were killed and their livers submitted to the techniques to be described below.

Before the administration of colchicine, all the animals included in the study were previously proved to be eliminating viable schistosome eggs in the stools.

Second experiment — Swiss mice were similary infected with 30 *S. mansoni* cercariae and 8 weeks later, when they were eliminating viable eggs in the stools, they were submited to treatment with 400 mg/kg b.w. praziquantel (Bayer), given in four doses of 100 mg each, all on the same day, by gastric intubation. Two weeks after treatment, the animals were divided into two groups: one was treated daily with 1 mg/kg b.w. of colchicine, administered by gavage; the other was left untreated and served as controls. Five days after the beginning of colchicine treatment 5 animals from each group were killed. This procedure was repeated 2 and 5 weeks afterwards.

Parasitology — At the time of sacrifice the animals were anesthetized with ether and exsanguinated after opening of the abdomen and severing of the abdominal aorta. A needle was introduced into the left ventricule and a perfusion of the liver with saline was done according to Duval & DeWitt (1967) for the recovering of adult worms. The number of eggs in the liver was determined by the Cheever's method (1968).

Histology — Fragments of the liver were fixed in Bouin's fluid, embedded in paraffin and the 5 μm thick sections stained with hematoxylin and eosin, Picrosirius-red, Schiff-PAS and Gomori's method for reticulum.

Electron microscopy — Small pieces of the liver were quickly fixed in cold 2% glutaraldehyde in cacodylate buffer and post fixed in 1% osmium tetroxide. After embedding in Epon resine, semi-thin and ultra-thin sections

were obtained in an automatic Reichert ultramicrotome. The grids with the ultra-thin sections were contrasted with lead citrate and uranyl acetate and examined with a Zeiss EM-109 electron microscope at 60 kv.

Immunotyping of collagens — Fragments of the liver were snap frozen in liquid nitrogen and kept at $-70\,^{\circ}C$ until the moment when they were cut in a cryotome at $-20\,^{\circ}C$. The sections were prepared according to standard immunofluorescence technique for the identification of either Types I, Pro-III, III and IV collagens. The specific fluoresceinated antibodies used, as well as the process for the obtaining of their respective antigens have been described elsewhere (Andrade & Grimaud, 1988).

Dosage of total collagen — Representative floating paraffin sections of the liver were submitted to a colorimetric method for the quantitative estimation of total collagen and protein (Lopez de Leon & Rojkind, 1985; Guerret et al., 1988). The method is based on the selective binding of Pricrosirius-red and Fast-green to collagen and non-collagenous proteins respectively. After elution of these dyes from the sections, lectures were made in a Beckman spectrophotometer at 540 and 605 nm.

RESULTS

Infection with 50 cercariae yielded 10-12 adult worms, with a slight predominance of males. The mean number of eggs per gram of liver tissue was 3,453.302.

The histological sections were coded and analyzed. The periovular granulomas appeared in several stages of evolution with different degrees of inflammation and fibrosis, the observer being unable to discriminate between colchicine-treated and untreated cases, just by looking at the microscopic slides. Even when the slides were decoded, no clear cut differences could be ascribed to either group, when the amount of collagen tissue and the aspect of collagen fiber arrangement in Picrosirius-red stained slides, the degree of periovular necrosis, the numbers of macrophages and eosinophils were critically evaluated.

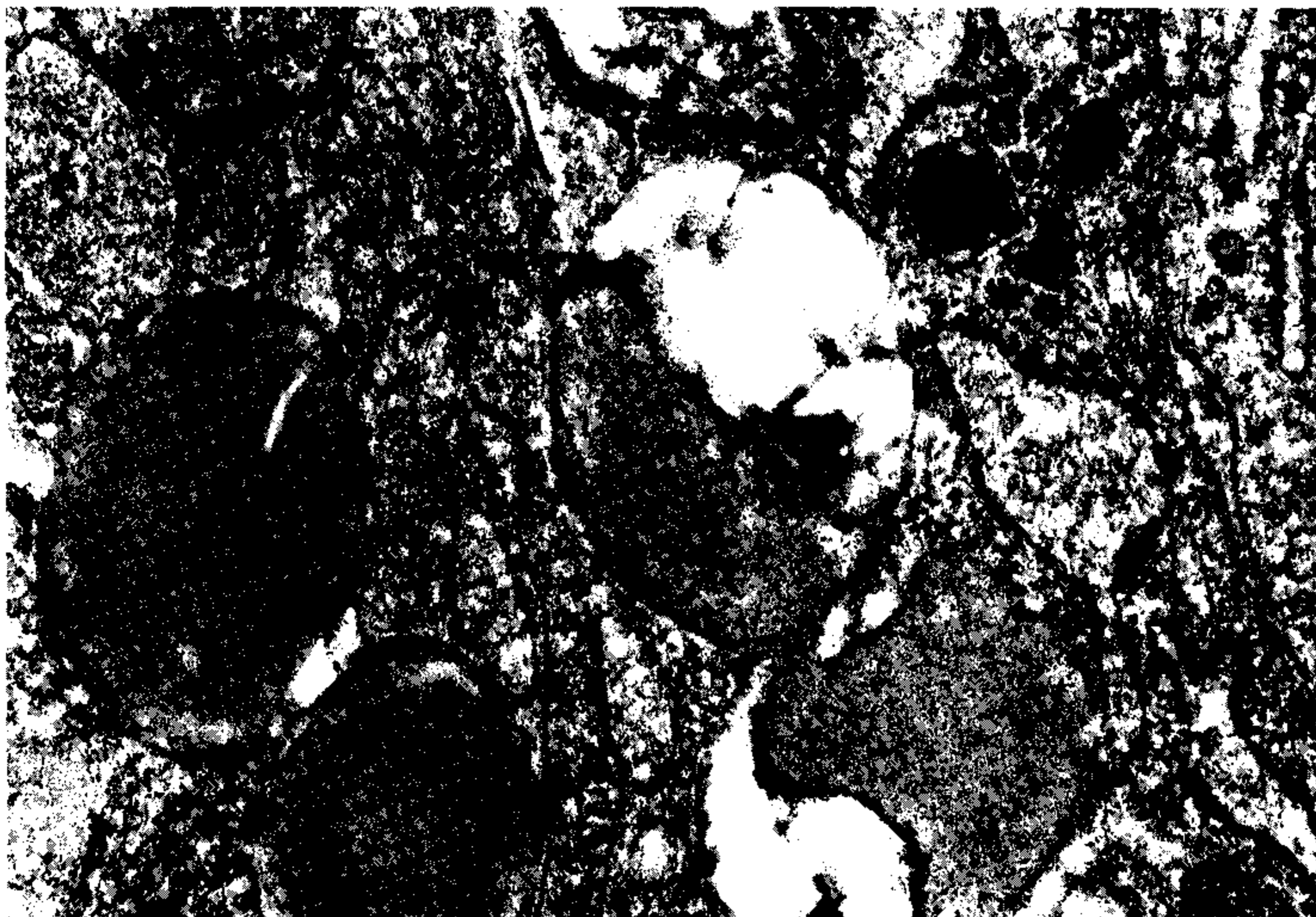Estimation of collagen content in the sections of livers with comparable number of

Fig. 1: dense amorphous material present within dilated vesicles in the cytoplasm of a fibroblast in a periovular schistosomal granuloma of a mouse treated with colchicine during 2 weeks. Electron microscopy, x 20,000.

Fig. 2: cell presenting hyperplastic endoplasmic reticulum and some dilated vesicles containing electron dense homogeneous material, probably fat droplets. Electron microscopy, x 12,000.
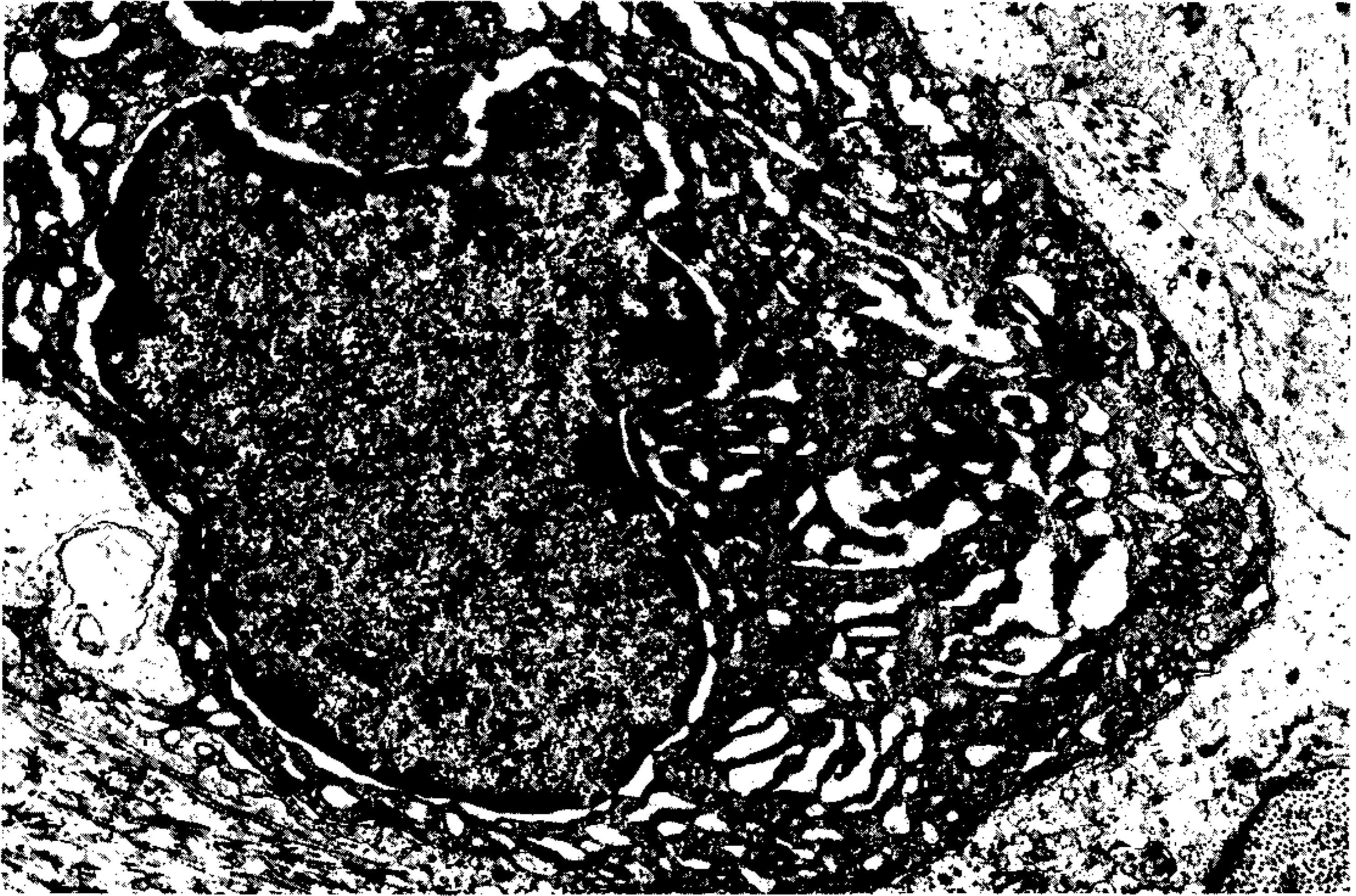
Fig. 3: round cell with eccentric, rather homogeneous nucleus and a well developed and dilated endoplasmic reticulum plus several lysosomes. From a periovular granuloma in a mouse which had been treated with colchicine for 2 weeks. Electron microscopy, x 7,000.

periovular granulomas performed by means of a colorimetric method is shown in the Table. The numbers obtained for micrograms of collagen per gram of proteins did not reach statistical significance when the two groups were compared.

Immunofluorescent study revealed that Types I and III collagens were present in the periovular granulomas. The staining appeared brighter and disclosed more fibers for Type III collagen (and especially for Pro-III collagen) than for Type I collagen. However, no evident differences were registered when the two groups were considered. Type IV collagen was not represented within the hepatic granulomas from either group.

Electron microscopic examination showed the fusiform cells in the granulomas of colchicine-treated animals to present dilated cisternae and vacuoles in the rough endoplasmic reticulum which contained a homogeneous material with variable electron densities (Figs. 1, 2). Other cells, especially plasmocytes (Fig. 3), also presented cytoplasmic clear vacuoles. In the animals treated during 4 or 5 weeks with colchicine some vacuoles were quite large and presented dense and heterogeneous material, sometimes arranged in alternating clear and dark parallel lines ("zebra bodies", Fig. 4). Fragments of collagen fibrils and fibers with its characteristic cross striation and with variable degree of alterations were frequently observed within the vacuoles, as well as concentric electron dense membranous material ("myelin bodies", Fig. 5). Such findings were not observed in non-treated animals, although their granulomas usually contained fibroblat-like cells with considerable hypertrophy and hyperplasia of the rough endoplasmic reticulum and Golgi complexes. Preparations examined did not allow a critical differential evaluation of the microtubules within the several cell-types from the different experimental groups. Signs of extra-cellular collagen deposition and collagen breakdown, as well as the presence of activated macrophages and many partially degranulated eosinophils were similarly observed in granulomas taken from either group.
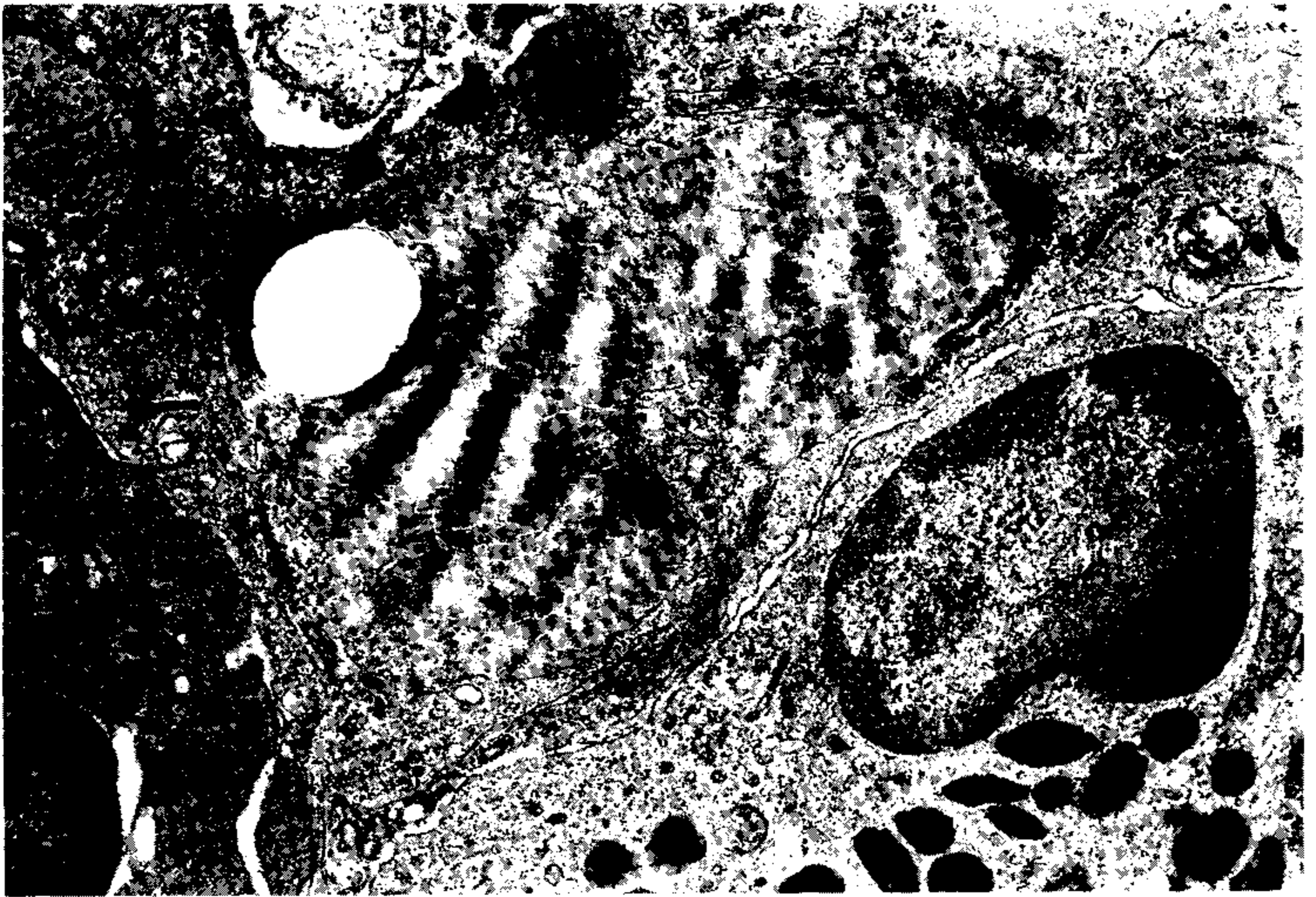
Fig. 4: a dilated cisternae containing dark striated material identified as intracellular collagen fibrils, separated one from the other by clear zones ("zebra-body"). The spherical empty space is probably an artifact. At one side of the cisternae one can see a neutrophil and an eosinophil leukocytes. Five-week colchicine treated mouse. Electron microscopy, x 20,000.
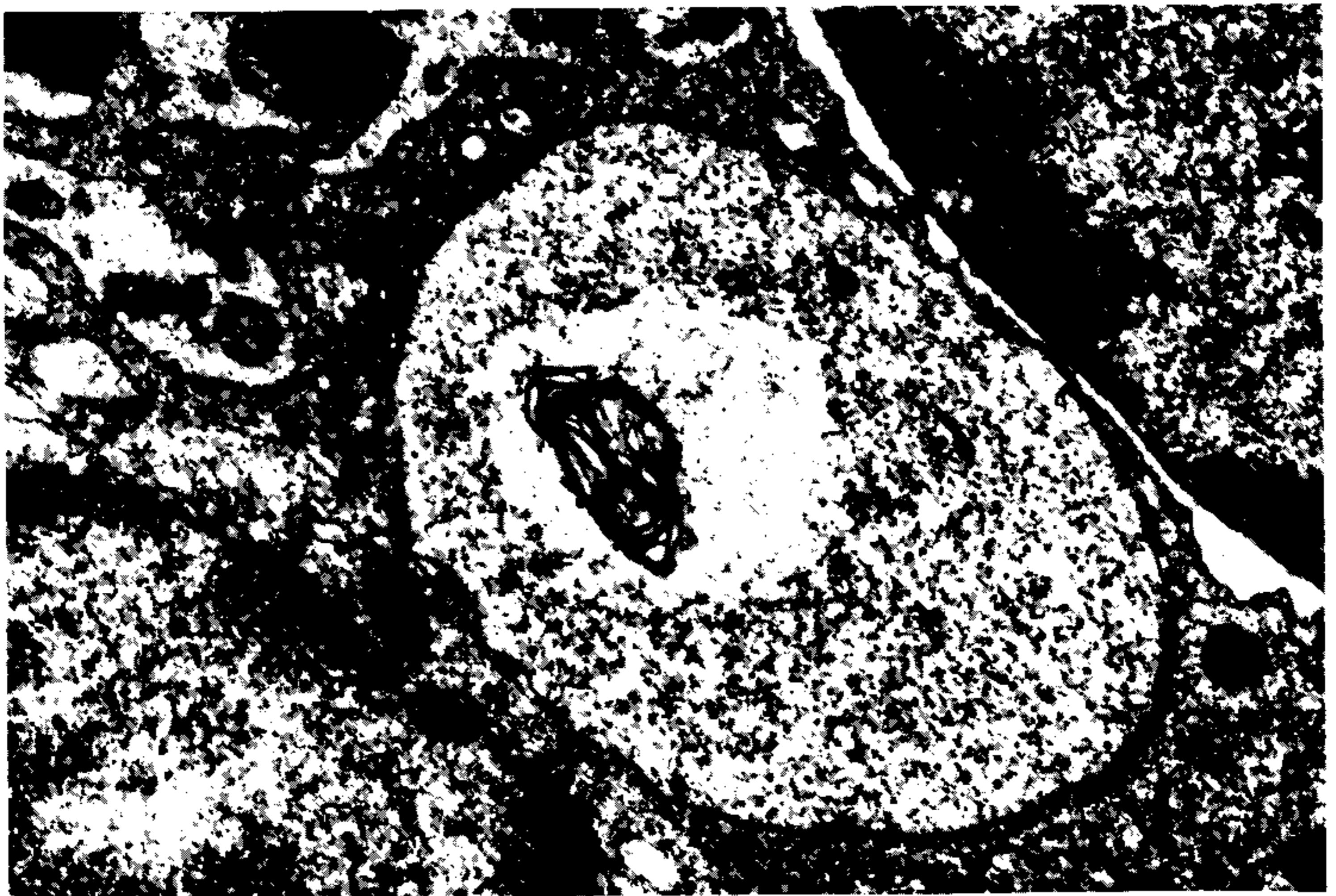
Fig. 5: dilated vesicle containing a "myeline figure" in its center. Mouse infected with *Schistosoma mansoni* and treated for 5 weeks with colchicine. Electron microscopy, x 20,000.

TABLE

Collagen estimation by colorimetric method, made
in the livers of mice infected with *Schistosoma
mansoni*, treated and non-treated with colchicine
(data from the first experiment)

Groups	No. of animals	μ g collagen / mg protein	SD
Treated	9[a]	42,1221	6,0651
Non-treated	14	38,5572	5,2417

[a]Animals treated for 2, 3, 4 and 5 weeks with colchicine.

In the material from the second experiment,
the livers from animals treated with praziquantel
showed the well known signs of involution
of the schistosomal lesions (see Andrade & Grimaud,
1986, 1988). The administration of
colchicine resulted in the ultrastructural changes
in fibroblasts similar to those described above,
but otherwise no other differential change was
observed. The method for total collagen estimation
was not applied to the material belonging
to this experiment.

During the first two weeks after administration
of praziquantel the presence of cells
(especially fibroblasts) with internalization of
collagen fragments was a frequent finding.
After that period such internalization was no
more seen. When colchicine begun to be
administered, two weeks after praziquantel, no
reappearance of collagen internalization
occurred.

DISCUSSION

The administration of colchicine to mice
infected with *S. mansoni* caused ultrastructural
changes in active fibroblasts in hepatic periovular
granulomas, but this was apparently not
sufficient to promote a crucial change in total
collagen deposition when a comparison was
made with granulomas in control animals,
non-colchicine-treated. Even when an estimation
of total collagen was made by a quantitative
method no differences between colchicine-treated
and controls could be disclosed. Also,
the ratios and general appearance of the genetic
types of collagen deposited around schistosome
eggs in the liver did not differ in the two
groups of animals.

Since the colchicine treatment of the schistosome-infected
animals was started before
maturation of the eggs in the liver, it can also
be said that such treatment did not prevent the
accumulation of collagen during granuloma
formation. These findings differ from those
of Nigro et al. (1988), who used colchicine
(a daily drop of a solution of 0.5 g of colchicine
in 71 ml saline) to treat mice after the 40th day
of a 35-cercaria infection and found that
hepatic fibrogenesis was marked decreased by
a qualitative light microscope examination.

It is well known that colchicine like others
microtubule-disrupting drugs increase the numbers
of secretory vacuoles in several cell-types,
impairing the secretion of collagenous and non-collagenous
proteins as well, although not their
synthesis (Scherft & Heersche, 1975). Microtubes
are considered as part of a mechanism for
collagen granule translocation from the Golgi
complex to the cell periphery (Cho & Garant,
1980). Collagen secretion being microtube-dependent
is inhibited by colchicine and the
collagen precurssors retained within the cells
may undergo partial polymerization to form
striated fibrils (Cho & Garant, 1985).

Present findings are in keeping with the
above observations, since colchicine-treated
animals showed fibroblasts and other cells
with dilated vacuoles, and even the presence
of collagen fragments were seen in some cytoplasmic
cisternae, sometimes forming the so-called
"zebra-bodies" (Cho & Garant, 1985).
Why these changes did not seem to have a major
impact on collagen deposition is schistosome
granuloma formation is not clear. May be the
duration of treatment was not sufficient or
probably not all collagen-synthesizing cells
are equally sensitive to colchicine. It is also
possible that colchicine-affected cells may go
on producing a fair amount of collagen, despite
the ultrastructural alterations. Only further
studies can clarify these matters. However, the
present findings are not suggestive that colchicine
can be a tool to either prevent or remove
fibrosis in hepatic schistosomiasis.

Concerning colchicine role in accelerating
the collagen breakdown that follows the cure
of schistosomiasis, the present results were
essentially negative. Expectations for the
effectiveness of the drug on this regard rested
on the findings of others which indicated that
colchicine treatment increases collagenase

production (Harris et al., 1971) and also the number of T-suppressor cells while decreases the basal production of interleukin 1 and the production by circulating monocytes of factors that stimulate fibroblast proliferation *in vitro* (Kerschenobich et al., 1984).

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