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Effect of sodium selenite on the hepatotoxicity induced with carbon tetrachloride

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ABSTRACT

The authors have demonstrated the effect of sodium selenite on the hepatotoxicity due to carbon tetrachloride, by observing the distribution and disaggregation of the pyroninophilic granules in the hepatic cells of the mature male albino mice.

Each experimental mouse of the selenite and the selenite plus carbon tetrachloride groups was given a single dose of 4 ug. of sodium selenite per kilogram of body weight and that of the control and the carbon tetrachloride groups was given 0.1 ml. of distilled water alone.

Six hours after the first administration of distilled water or sodium selenite, the experimental mice of the carbon tetrachloride and the selenite plus carbon tetrachloride groups were given a single dose of 1.0 ml. of carbon tetrachloride per kilogram of body weight and those of the selenite groups were given 0.1 ml. of paraffin oil alone.

Following the last administration of carbon tetrachloride or paraffin oil, the mice were sacrificed by bleeding (cutting the common carotid artery) at the intervals of 2, 3, 4, 6, 8, and 12 hours respectively. Histochemical preparations were stained by the methyl-green and pyronin method and oil red 0 method.

The hepatotoxicity due to the administration of carbon tetrachloride was evident in the hepatic cells; the pyroninophilic granules were partly reduced in volume in the hepatic cells of the centrilobular and the intermediate zones as early as the 3 hour-period, and markedly reduced or disappeared in the centrilobular and some part of the intermediate zones associated with hydropic degeneration as well as in the 6 hour-period. Thereafter marked reduction or dissolution of the pyroninophilic granules was found and extended as the periportal zone at the 12 hour-period.

However, the pyroninophilic granules in the hepatic cells of selenite plus carbon tetrachloride group showed no significant changes in the hepatic cells of these zones, compared to the histochemical feature of the granules in the hepatic cells of the control and the selenite groups.

Consequently it is suggested that the lipid peroxidative decomposition of the microsomal membranes, which is induced with carbon tetrachloride, would be prevented by a previous administration of sodium selenite.

INTRODUCTION

Recently a new hypothesis linking together lipoperoxidation to the toxic action of carbon

tetrachloride, which was associated with destructive peroxidation of lipids in the endoplasmic reticulum of the hepatic parenchymal cells, was reported by Di Luzio and Costales (1965), Recknagel and Ghoshal (1966). Recknagel (1967), and Rao and Recknagel (1968).

It is well known that the lipid peroxidation of the microsomal phosphlipid membranes caused by free radicals was inhibited by lipid antioxidants. Teppel (1952) described that the Linoleic acid, linolenic acid and arachidonic acid of the biological essential fatty acids are unstable for oxidation in general, and unsaturated poly-fatty acids release free radical chains.

Additionally it was reported that the free radical chain reaction of unsaturated poly fatty acids was inhibited by antioxidants. Zalkin et al. (1960), and Hamilton and Tappel (1963) suggested that the antioxidants terminated the free radical which could initiate lipid peroxidation, and also inhibited propagation of free radical chain reaction, thus protecting against peroxidative decomposition of the microsomal phospholipid caused by carbon tetrachloride hepatotoxicity.

The authors have demonstrated the effect of sodium selenite on the hepatotoxicity due to carbon tetrachloride which could induce peroxidative decomposition of the microsomal phospholipid and alteration of the microsomal ribosomes by observing distribution and disaggregation of the pyroninophilic granules in the hepatic cells histochemically.

MATERIALS AND METHODS

87 healthy mature male albino mice were divided into 4 groups: 1) the control, 2) the selenite, 3) the carbon tetrachloride, and 4) the selenite plus carbon tetrachloride groups. The experimental animals have been deprived of food except distilled drinking water up to their sacrifice.

Each mouse of the selenite and the selenite plue carbon tetrachloride groups was given a single dose of 0.1 ml. of sodium selenite solution in which 4 ug. of sodium selenite (Kishida Chemical & Co.) per kilogram of body weight was contained. The control and the carbon tetrachride groups were given 0.1 ml. of distilled water alone.

6 hours after the previous administration of sodium selenite solution or distilled water, each mouse of the carbon tetrachloride and the selenite plus carbon tetrachloride groups was given a single dose of 0.1 ml. of carbon tetrachloride solution in which 1.0 ml. of carbon tetrachloride (Merk Co.) per kilogram of body weight was diluted in 4.0 ml. of paraffin oil. The control and the selenite groups were given 0.1 ml. of paraffin oil alone.

The sodium selenite solution or distilled water and the carbon tetrachloride solution or paraffin oil were given by means of gastric tube.

Following the last administration of carbon tetrachloride or paraffin oil, the animals of each group were sacrificed by bleeding (severance of the common carotid artery) at intervals of 2, 3, 4, 5, 6, 8, and 12 hours, respectively. The posterior lobe of the liver was excised after laparotomy, fixing in Lillie's solution and histochemical preparation was made by paraffin section cut 6 u. in thickness and stained by the methyl-green and pyronin method (Rosa, 1950) for RNA granules in the hepatic cells. A piece of the liver tissue was fixed in 10% formalin solu-

tion and histochemical preparation was made by cutting scetions 6 u. thick with cryostat and then the lipid of the hepatic cells, which was stained in oil red 0, was observed by the light microscope.

RESULTS

A. The control group:

In this control group the histological feature of the hepatic cell was not significantly different from that of the intact hepatic cell except that there were enlarged vesicles in the cytoplasm. The pyroninophilic granules were evenly distributed in granular form in the hepatic cells. The vesicles, which were enlarged slightly in the hepatic cells, were considered to be lipid deposits and this was conformed by lipid stain.

B. The selenite group:

As in the control group the general histological feature of the hepatic cell was not altered to any marked degree except for slight increment of the cytoplasmic vesicles. The pyroninophilic granules in the hepatic cell and the vesicles increased in the hepatic cell slightly and showed a similar picture to those of the control group.

C. The carbon tetrachloride group:

About 2 hours after administration of carbon tetrachloride the general histological feature of the hepatic cell was not significantly different from the cells of the former group.

3 hours after the administration of carbon tetrachloride there were narrow hepatic sinusoids, light hydropic degeneration of the cytoplasm of the hepatic cell associated with the karyorrhaxis or pyknosis of the hepatic cell nuclei, and leucocyte infiltration around the involved area. The pyroninophilic granules in the hepatic cell of the centrilobular zone were slightly reduced. In the intermediate zone of the hepatic lobule the vesicles of the hepatic cell were fairly enlarged.

About 6 hours after administration of carbon tetrachloride the pyroninophilic granules of the hepatic cells in the centrilobular zone and in a part of the intermediate zone were markedly decreased or disappeared and the involved zones were associated with marked hydropic degeneration, karyorrhaxis or pyknosis, and leucocyte infiltration. In this stage of the 6 hour-period many vacuoles in the hepatic cells, which were positively localiz ed in the intermediate and the periportal zones, were added to the characteristic histopathological picture of hepatotoxiciy due to carbon tetrachloride. Those vacuoles in the hepatic cell were confirmed as lipid droplets.

In the 12 hour-period after administration of carbon tetrachloride the degenerative changes of the centrilobular hepatic cells were markedly increased in association with the progressive development of degenerative changes of the hepatic cells in the intermediate area and partly in the periportal area. However, the degenerative changes of the hepatic cells in the intermediate and periportal zones were relatively less than those changes of the centrilobular hepatic cells.

D. The selenite plus carbon tetrachloride group:

In this group the histological and histochemical structures of the hepatic cells were fairly similar to those of the hepatic cells in the control and the selenite groups, sh-

owing moderately positive pyronophilic granules without disaggregation in the hepatic cell.

Consequently it was postulated that the prior administration of sodium selenite prevented the above mentioned degenerative changes of the hepatic cells caused by the administration of carbon tetrachloride.

DISCUSSION

Stowell and Lee (1950) demonstrated that the pyroninophilic granules in the cytoplasm of the central zone of the liver became absent or reduced by the administration of carbon tetrachloride. Chopra et al. (1972) observed that a single intragastric dose of carbon tetrachloride induced in the control animals a reproducible loss of glucose-6-phosphatase, loss of cytoplasmic pyroninophilia, fatty change, and necrosis occuring in an orderly sequence.

Zalkin et al. (1960) demonstrated that dieteary selenium compounds lead to the in vivo formation of antioxidants; dietary selenium could inhibit in vivo lipid peroxidation in the chick and in vitro lipid peroxidation in chick liver.

Gallagher (1962) demonstrated that the prior administation of alpha-tocopherol acetate, sodium selenite or NN^1-diphenyl-p-phenylenediamine afforded protection to rats given lethal doses of carbon tetrachloride. In biochemical observation the loss of reduced pyridine nucleotides was completely prevented by DPPD, and the loss of oxidized pyridine nucleotides was largely prevented by DPPD, or alpha-tocopherol acetate and to a lesser degree by sodium selenite. In the histological observation a single dose of DP PD injected intraperitoneally before an administration of carbon tetrachloride also largely prevented hepatic necrosis, and decreased the fatty change.

Lee et al. (1972) demonstrated that the hepatotoxicity caused by carbon tetrachloride was evident by the clumping or aggregation of the pyroninophilic granules in the cytoplasm of the hepatic cells of the centrilobular area occuring as early as the 3 hour-period. Thereafter a decrease or disappearance of the pyroninophilic granules was observed as late as the 12 hour-period. However, a considerable number of the pyroninophilic granules in the hepatic cells of the alpha-tocopherol plus carbon tetrachloride group persisted in the hepatic cells of all zones of the hepatic lobules. Consequently it is suggested that decomposition of the microsomal membranes induced by carbon tetrachloride, would be reduced by a previous administration of alpha-tocopherol.

In this study the authors have demonstrated the effect of sodium selenite to prevent the hepatotoxicity of carbon tetrachloride by observation of the histological and histochemical, picture of the hepatic cells in albino mice, compared to those of the control which were quite similar to the normal histological and histochemical picture of the liver. The pyroninophilic granules showed even distribution in granular form without disaggregation in the hepatic cells by the prior administration of sodium selenite, protecting against the hepatotoxicity induced by carbon tetrachloride.

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LEGENDS OF FIGURES

Fig. 1. Mouse liver, the pyroninophilic granules were moderately distributed in the hepatic cells of the 3 hours control group, methyl-green pyronin stain, 100×

Fig. 2. Mouse liver, the pyroninophilic granules were moderately distributed in the contrilobular hepatic cells of the 3 hours control group, methyl-green pyronin stain, 450×

Fig. 3. Mouse liver, the pyroninophilic granules were moderately distributed in the hepatic cells of the 3 hours selenite group, methyl-green pyronin stain, 100×

Fig. 4. Mouse liver, the pyroninophilic granules were moderately occurred in the centrilobular hepatic cells of the 3 hours selenite group, methyl-green pyronin stain, 450×

Fig. 5. Mouse liver, the pyroninophilic granules were partly disappeared in association with a karyorrhaxis or pyknosis in the centrilobular hepatic cells and the intermediate hepatic cells of the 3 hours CCl_4 group. methyl-green pyronin stain, 100×

Fig. 6. Mouse liver, the same picture of Fig. 5 in the centrilobular area, methyl-green pyronin stain, 450×

Fig. 7. Mouse liver, the pyroninophilic granules were moderately present in the hepatic cells of the 3 hours selenite plus CCl_4 group, methyl-green pyronin stain, 100×

Fig. 8. Mouse liver, the pyroninophilic granules were moderately occurred in the centrilobular hepatic cells of the 3 hours selenite plus CCl_4 group, methyl-green pyronin stain, 450×

Fig. 9. Mouse liver, the pyroninophilic granules were markedly decreased or disappeared in association with a marked hydropic degeneration, karyorrhaxis or pyknosis and leucocyte infiltration in the centrilobular and intermediate zones of the 6 hours CCl_4 group, methyl-green pyronin stain, 100×

Fig. 10. Mouse liver, the same picture of Fig. 9, in the centrilobular zone, methyl-green pyronin stain, 450×

Fig. 11. Mouse liver, the pyroninophilic granules were moderately observed in the hepatic cells of the 6 hours selenite plus CCl_4 group, methyl-green pyronin stain, 100×

Fig. 12. Mouse liver, the same picture of Fig. 11 in the centrilobular hepatic cells, methyl-green pyronin stain, 450×

Fig. 13. Mouse liver, the pyroninophilic granules were markedly reduced or diappeared in association with a hydropic degeneration of the centrilobular, intermediate zones and partly the periportal zone of the 12 hours CCl_4 group, methyl-green pyronin stain, 100×

Fig. 14. Mouse liver, the same picture of Fig. 13 in the centrilobular hepatic cells, methyl-green pyronin stain, 450×

Fig. 15. Mouse liver, the pyroninophilic granules were moderately distributed in the hepatic cells of the 12 hours selenite plus CCl_4 group, methyl-green pyronin stain, 100×

Fig. 16. Mouse liver, the same picture of Fig. 15 in the centrilobular hepatic cells, methyl-green pyronin stain, 450×

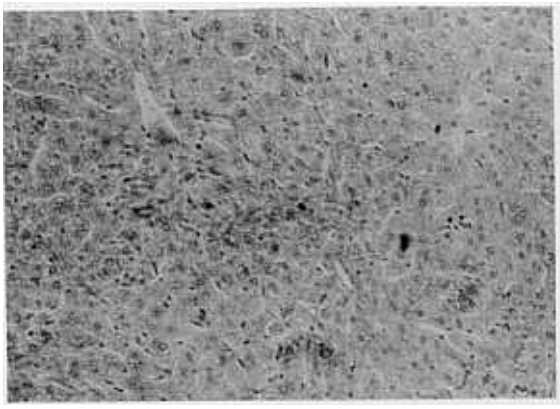


Fig. 1

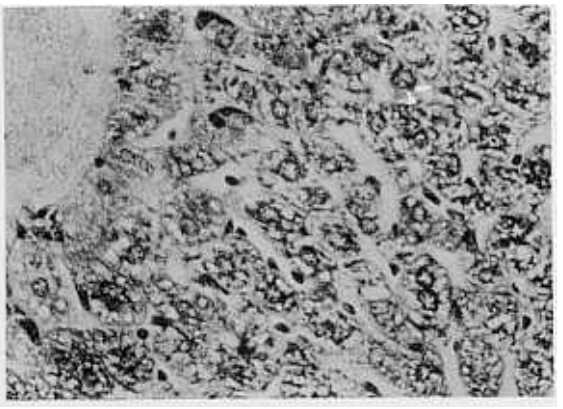
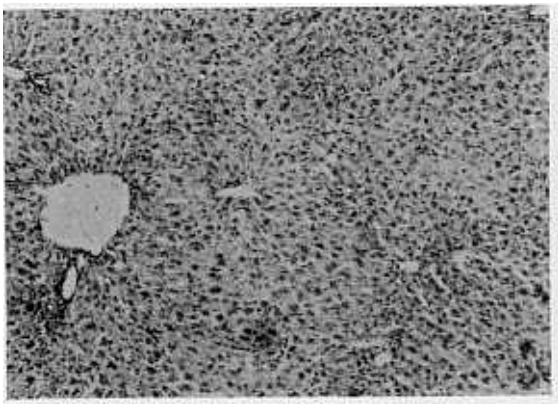


Fig. 2



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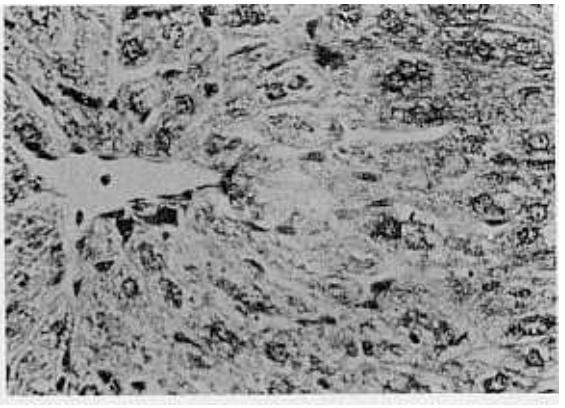

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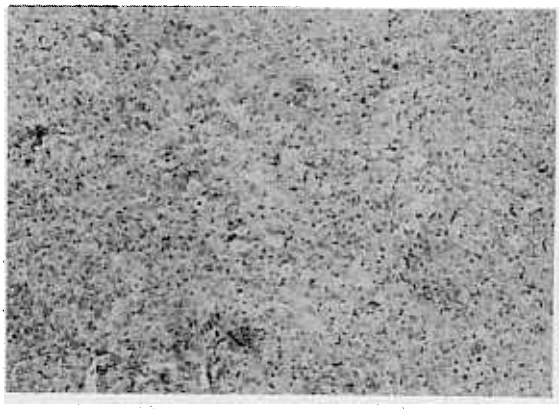


Fig. 5

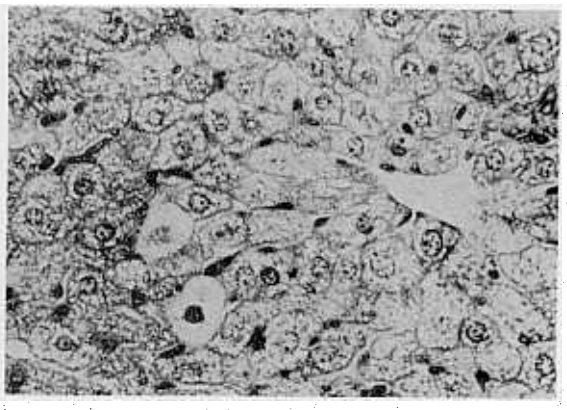


Fig. 6

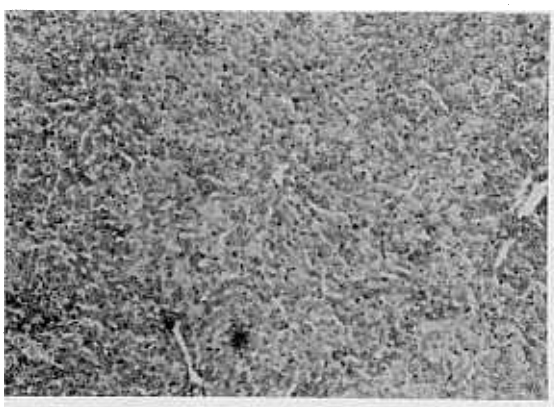


Fig. 7

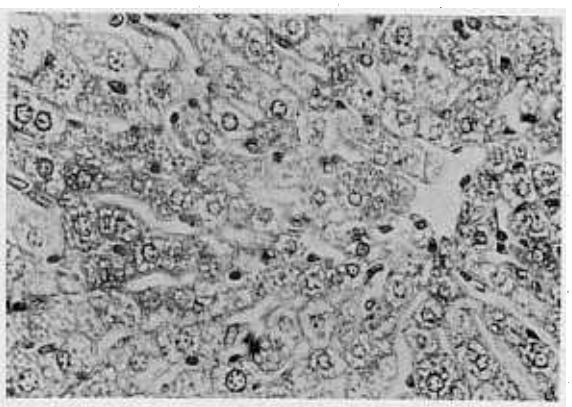


Fig. 8

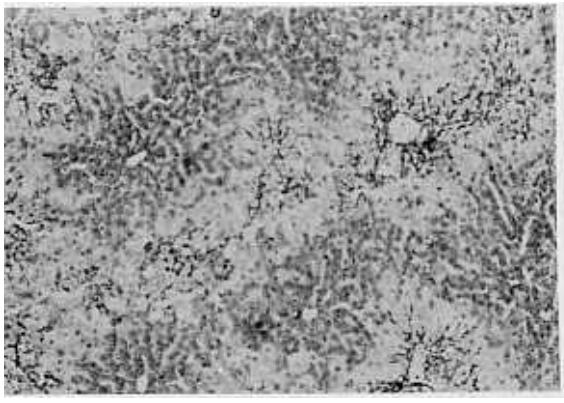


Fig. 9

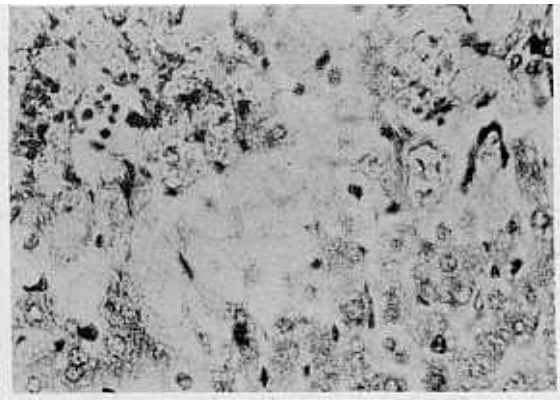


Fig. 10

Fig. 11

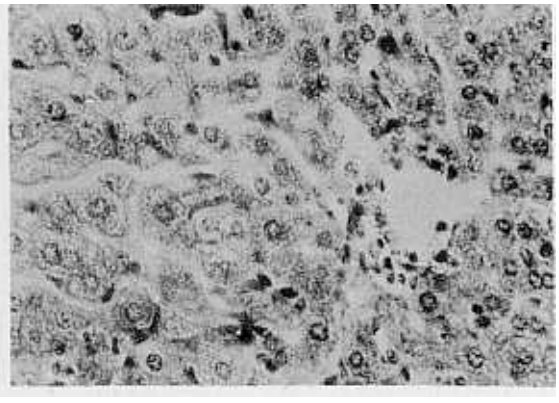


Fig. 12

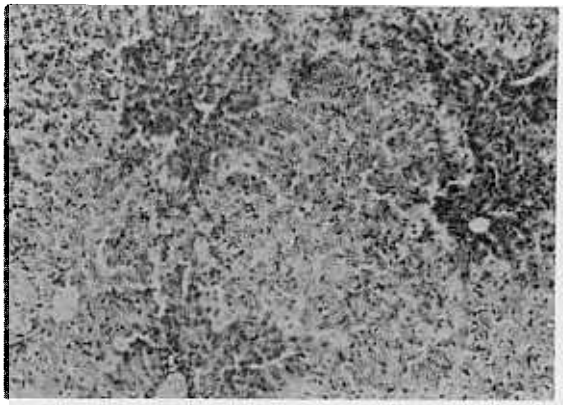


Fig. 13

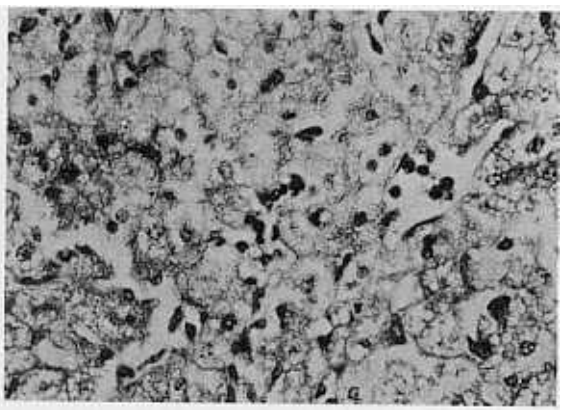
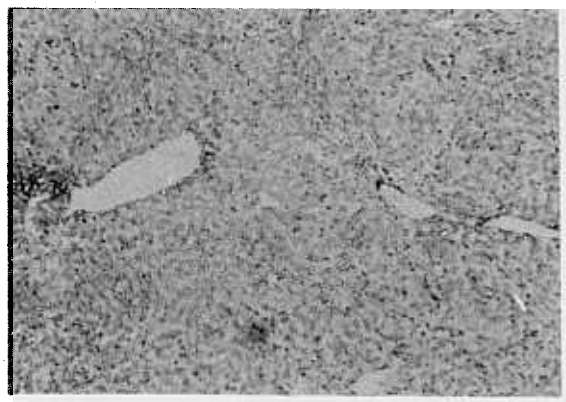


Fig. 14



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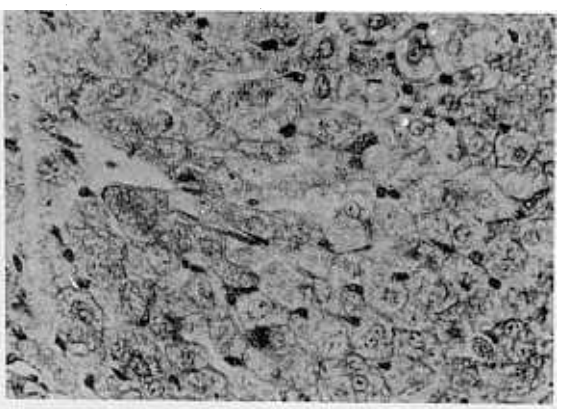

Fig. 16