Yonsei Medical Journal
Volume 13, 1972

Pyroninophilic Granules in Liver Cells of the Mice Treated with Alpha-Tocopherol and Thioacetamide*

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ABSTRACT

In an attempt to clarify the protective action of an antioxidant agent against acute toxicity of thioacetamide (TAA) and in order to throw some light on an satisfying concept of the mechanism of its action, a single dose of alpha-tocopherol (200 mg per kg) was given orally by stomch tube to male mice prior to the administration of thioacetamide in a dose of 200 mg per kg of body weight. Sections of liver samples, obtained from the mice which were sacrificed at intervals of 3, 6, 9, or 12 hours after TAA administration, were stained using the methyl green-pyronin technique.

At 3 hours following TAA administration, the pretreatment with alpha-tocopherol inhibited almost completely such alterations of the hepatocytes in the animals given TAA alone, as revealed by loss and clumping of cytoplasmic pyroninophilic granules in the periportal zone of the lobule.

At 6, 9, and 12 hours, the prevention of alpha-tocopherol was incomplete in degree and extent. The changes of the hepatocytes were more intense and extensive in the TAA-treated 6 to 12 hour-groups than in the 3 hour-group of TAA-treated ones.

Some discussion is given of the mechanism of TAA toxicity, with respect to the microsomal lipid peroxidation.

This Study was supported by a reaearch fund form Yonsei Medical Center and the China Medical Board.

INTRODUCTION

Thioacetamide(TAA), which has a striking effectiveness in preventing orange decay, is a well-known hepatotoxic agent, and was first reported to be hepatotoxic in albino rats by Fitzhugh and Nelson (1948). This agent has drawn the attention of many investigators ever since, and a considerable amount of later work has been undertaken on the acute toxicity of thioacetamide. It rapidly causes hepatic cell damage proceeding to central necrosis if the dose is sufficiently large(100 to 200 mg per kg) (Ambrose et al. 1949, Gupta 1956, Kleinfeld 1957, Rees et al. 1961, Hruban et al. 1966) and after prolonged administration of this compound, chronic toxicity may appear and may later develop to cirrhosis, adenomas or carcinomas of the liver(Gupta 1955, 1956 b, Miyazaki et al. 1956, Hagemann 1959, Gothosker et al. 1970).

In their studies, these investigators emphas-

ized the relatively late sequence of impairments in hepatocytes and they also provided controversial discussions on the mechanism of action of thioacetamide. Therefore, in the problem of elucidating the mechanism of TAA toxicity, the position of affairs is much complicated by the fact that the diverse suggestions cannot be easily settled and that we cannot expect a single primary action of the agent from which all subsequent derangements follow. In this connection, thioacetamide and carbon tetrachloride, both frequently used hepatotoxins in experiments, have similar light microscopic alterations.

Furthermore, the greater resolving power of the electron microscope has also shown that thioacetamide and carbon tetrachloride have in common an early dislocation of the ribosomes from the membranes of the endoplasmic reticulum of the hepatocytes (Bassi 1960, Thoëres and Bannasch 1962, Sarma et al. 1972), and they are also associated with altered ribonucleic acid synthesis and with a ribosomal disaggregation as well (Adams and Busch 1963, Smuckler et al. 1963, 1964). Lee et al. (1972) demonstrated that alphatocopherol, an antioxidant, inhibited the alteration of the cytoplasmic pyroninophilic granules of the hepatocytes in the CCl_4-intoxicated mice, on the ground of lipidperoxidation hypothesis on the mechanism of CCl_4 intoxication (Di Luzio & Costales 1965, Rao & Recknagel 1969).

It is the purpose of the present investigation to see whether the early alterations due to thioacetamide poisoning in distribution of the cytoplasmic pyroninophilic granules are also prevented by the pretreatment of alpha-tocopherol, and an attempt will be made to throw some light on a satisfying concept of the mechanism of hepatotoxicity and to find protective drugs reproduced.

MATERIALS and METHODS

Young adult male mice, weighing about 20 gm. were used in this study, and were given only casein ad libitum for 24 hours prior to the start of the experiments. They were divided into four large groups as follows:

Group 1 : normal control group, treated with liquid paraffin
Group 2 : alpha-tocopherol-treated, control group
Group 3 : thioacetamide-treated group
Group 4 : alpha-tocopherol plus thioacetamide-treated group

Alpha-tocopherol was administered orally by stomach tube, at a dosage of 200 mg. per kg of body weight, diluted with an equal volume of liquid paraffin, prior to the administration of thioacetamide (average 6 hours before).

Control animals (group 1) were given an equivalent dose of liquid paraffin under the same conditions. Animals of group 3 and 4 were given a single dose of thioacetamide in aqueous solution, 200 mg. per kg. of body weight, by a stomach tube.

Animals were sacrificed at intervals of 3, 6, 9 or 12 hours after treatment with thioacetamide. Liver samples from all the mice were fixed in Lillie's fixatives (Lillie, 1954), dehydrated, embedded in paraffin, and sectioned in appropriate thicknesses. The distribution of pyroninophilic granules in the hepatic parenchymal cells was studied, using the methyl green-pyronin technique (Rosa, 1950). Some pieces of liver tissue were frozen-sectioned and treated with oil red O stain for fat deposits, and others with hematoxylin-eosin stain.

RESULTS

A. The Control Groups; normal group(Group 1) and Tocopherol treated group(Group 2):

In all the control animals, the livers revealed normal liver histology, and showed similar microscopic pictures without respect to the different treatments and schedules, except that the hepatic parenchymal cells of the tocopherol-treated group (group 2) had a few small vacuoles in their cytoplasm which seemed to be of a lipid nature.

Moderately strong pyroninophilia was seen in every hepatocyte and the pyroninophilic granules were evenly distributed in the cytoplasm or in irregularly-sized masses.

Each of the nuclei of the hepatocytes contained one or more nucleoli which were stained clearly with pyronin and were seldom vacuolated, a small number of condensed chromatin masses, and each was bounded by a distinct nuclear membrane.

B. The Thioacetamide-Treated Group (Group 3):

1. Observations at 3 hours after thioacetamide administration.

In all the periportal areas(the outer or peripheral zones of the lobules) and some areas of the midzone adjacent to the periportal areas, the hepatocytes were swollen and more nearly rounded, and the pyroninophilic granules in them were markedly decreased in amount and were scattered in irregularly aggregated clumps throughout the cytoplasm (Figure 1 and Table 1). These severely injured cells (S-cells) contained cytoplasmic vacuoles of variable size. Oil red O revealed many lipid droplets in these cells, but some vacuoles were not stained with the dye.

In those portions of the remainder, especially in the centrilobular zone, there occurred the least-injured hepatocytes (Lt-cells) which were not involved in this severe damages, possessing a normal content and distribution of cytoplasmic pyroninophilic granules, similar to the hepatocytes in the control groups, but their cell bodies were slightly enlarged and contained many minute vacuoles (Figure 2).

No changes were shown in nuclei and nucleoli of the hepatic cells in this group. No sinusoidal dilatation was observed. However, a slight degree of cell in filtration was found in each lobule(Table 2).

2. Observations at 6, 9, and 12 hours

The changes of pyroninophilia in these groups appeared to be in the same pattern as in the preceding group (3 hour-group), but the alterations were much increased in degree and extent (Table 1).

At 6 and 9 hours after thioacetamide treatment, severely injured hepatocytes (S-cells) were located in the greater part of the lobule, extending from the outer zone to the midzone and even to the zone adjacent to the central vein. In a small remaining area of the centrilobular zone, the hepatocytes were lessdamaged (Ls-cells) and their pyroninophilia was slightly decreased, with an increased amount of vacuoles and slightly-enlarged cell bodies(Figure 4, 6). Other histologic pictures of the cells resembled those of the control groups.

At 12 hours, the severely injured cells occupied the entire lobule and some of them had aggregated granules of even more increased density in staining (Figure 8, 10). Some of their nuclei were slightly enlarged and altered in shape to be irregularly angular or indented.

In all animals of these groups, the masses of cell infiltration were increased much more in size and number than in other groups, and in some lobules small focal necrotic areas occured, which were scattered at random, and in which

the hepatocytes contained little or no pyroninophilia but showed disfigured, pyknotic nuclei. The hepatic sinusoids were markedly dilated (Table 2).

C. Thioacetamide Treatment with Pretreatment of Alpha-Tocopherol (Group 4)

As shown in table I, pretreatment with alpha-tocopherol remarkably reduced the predominent changes due to the hepatotoxicity induced by thioacetamide treatment, such as the decreased pyroninophilia, the clumping of cytoplasmic pyroninophilic granules of the hepatocytes, and focal necrosis. However, the pretreatment did not reduce the cell infiltration and sinusoidal dilatation(Table 2).

1. Observations at 3 hours after treatment of thioacetamide.

The whole lobule was occupied by the Lt-cells, the least injured hepatocytes, which had beed located in the periportal areas of the thioacetamide-treated, 3 hour-group. Serverely injured hepatocytes, of course, could not be found in any lobules(Figure 3).

A few masses of cell infiltration were still found to be scattered.

2. Observations at 6, 9, and 12 hours.

In all the specimens of these groups, the number of the severely injured hepatocytes (S-cells) was profoundly diminished but many of them still occupied the periportal area to a considerable extent. In contrast to this, the Ls-cells or the Lt-cells were correspondingly increased in number in the lobules (Figure 5, 7, 9, and 11).

Table 1. The effect of alpha-tocopherol on thioacetamide (TAA) poisoning "Pyroninophilia"

Group \ Area		Periportal area of a lobule			Midzone			Centrilobular area		
		*S Cells	*Ls Cells	*Lt Cells+	S	Ls	Lt	S	Ls	Lt
TAA	3 hrs	±	−	−	+	±	+	−	−	≠
	6 〃	≠	−	−	≠	+	−	+	+	+
	9 〃	≠	−	−	≠	+	−	±	+	−
	12 〃	≠	−	−	≠	−	−	≠	+	−
Alpha-tocopherol plus TAA	3 hrs	−	−	≠	−	−	≠	−	−	≠
	6 〃	+	+	+	−	+	+	−	+	±
	9 〃	+	+	+	−	+	+	−	+	±
	12 〃	+	+	+	−	±	±	−	+	±

*S-Cells: the severely injured cells *Ls-Cells: the less injurecells *Lt Cells: the least injured cells

Table 2. Other histological findings

Group		Cell body swelling	Cell infiltration	Sinusoidal dilatation	Necrotic area	Nuclear change
TAA	3 hrs	+	+	−	−	−
	6 〃	+	±	+	+	−
	9 〃	±	≠	+	±	−
	12 〃	±	≠	+	±	+
Alphat-ocopherol plus TAA	3 hrs	−	+	−	−	−
	6 〃	+	±	+	−	−
	9 〃	+	≠	+	−	−
	12 〃	+	≠	+	−	−

As a general rule, the extent of the damaged areas in these groups was in accordance with that of the thioacetamide-treated, 3-hour group which had not received the pretreatment of alpha-tocopherol.

Such histological changes as the cell infiltration and sinusoidal dilatation also remained in all the lobules, even with the pretreatment of alpha-tocopherol.

DISCUSSION

Gupta (1956) a reported that at 8 hours following thioacetamide poisoning, early light microscopic changes were discernible in the liver, including loss of cytoplasmic basophilia and cellular swelling. Asworth and his coworkers (1965) observed loss of the normal azurophilic aggregates and presence of minute vacuoles in the hepatocytes which did not stain for fat or gyclogen, at 5 to 7 1/2 hours after the administration of TAA in doses of 25 to 50 mg and of 100 to 400 mg per kg of body weight. These descriptions were generally supported by our observations, with the predominent changes of cytoplasmic pyroninophilia in the TAA-treated animals.

However, Rees et al. (1961) administered TAA at a dose of 200 mg per kg of body weight to rats, causing frank parenchymal cell necrosis in the liver by 12 hours, and the maximum necrosis in the centrolobular zone at 24 hours. Asworth et al. (1965) also reported that marked changes were located in the inner third of the lobule, while the cells in the outer portion of the lobule were much less severely affected. Hruban et al. (1966) noticed that continuous force-feeding off TAA resulted in centrolobular necroses in liver within 24 hours after the beginning of feeding. Meanwhile, the present investigation shows that at 6 hours after the oral administration of TAA, at a dose of 200 mg per kg, there appears a small number of focal necrotic patches scattered in the midzone of the lobule; at 9 and 12 hours, an increasing number of them are seen. Although, this discrepancy between the other investigators observations and ours cannot be properly understood, it is understandable that in the early stage of TAA poisoning, the compound attacks the hepatocytes of the periportal region at first and more intensively where the cell metabolism is believed to be at a relatively high rate(Novikoff 1959), with the additional fact that the cytoplasmic pyroninophilic granules of the hepatocytes have decreased markedly in the periportal regions of the lobule at 3 hours, and thereafter a decreasing pattern of the granules occurred extending from the periphery to the centrilobular zone in the present investigation.

Although Rees et al. (1961) roported that fatty change could not be observed in rats with TAA administration, Asworth et al. (1965) a small excess of lipid by 7 1/2 hours, and a observed greater amount of lipid at 16 hours following TAA poisoning. We also observed similar changes, but the accurate origin of the increased lipid could not yet be determined.

The pretreatment of alpha-tocopherol with a single dose of 200 mg per kg, prior to the TAA intoxicaion. almost completely inhibited such histologic changes as appeared in the 3-hour group treated with TAA alone, but it did reduce partly the alteration of decreasing granules in the midzones and centrilobular zones at 6, 9, and 12 hours. It is not clear whether this later, partial protection of alpha-tocopherol against the acute toxicity of TAA is due to the inherent action of tocopherol or may be concerned with the inadequacy of the pretreatment with alpha-tocopherol. Further work, of course, is needed to resolve this question.

It is postulated that the earlier cellular alterations of the hepatotoxins might be more indicative of the mechanism of cell injury than the later changes, and it seems highly probable that the pathogenesis of the hepatotoxins is related to basic, and yet general mechanisms and that different agents may be triggering the same mechanism in a some what different way.

Thioacetamide and CCl_4 cause similar structural alterations in both light and electron micros copy where an early dislocation of ribosomes bound to the membranes of rough-surfaced endoplasmic reticulum found(Bassi 1960, Thoë res and Bannasch 1962, Sarma et al. 1972). Significantly, they are associated with altered RNA synthesis and with ribosomal disaggregation as well (Adams and Busch 1963, Smuckler et al. 1963, 1964; Steele and Okamura 1964). In addition to this disorganization of the erga stoplasm,these substances are possibly associated with an intracellular defect in protein synthesis (Smuckler et al. 1962; Muramatsu and Busch 1962, Barkeret al. 1963). Lee and his coworkers (1972) demonstrated that pretreatment with alpha-to copherol in mice considerably reduced the progressive decomposition of pyroninophilia in the hepatocytes due to CCl_4 intoxication. The lipid peroxidation hypothesis of CCl_4 toxicity (Di Luzio and Costales 1965, Rao and Recknagel 1969) states that the free radical from CCl_4 attacks primarily the microsomal membranes causing lipid peroxidation in the lipid layer, and there is speculation that an antioxidant would inhibit the microsomal lipid peroxidation to some extent.

Since the TAA toxicity, as revealed by loss of pyroninophilia in the hepatocytes, has been considerably inhibited by the pretreatment with alpha-tocopherol, it may be suggested that TAA, like CCl_4, can primarily cause lipid-peroxidation damage to the membranes of rough-surfaced endoplasmic reticulum and then dislocation of polyribosomes attached to the membranes, resulting in suppression of protein synthesis, and it can be postulated that alpha-tocopherol will be a usefull remedy for hepatotoxins, as for as thioacetamide and CCl_4 are concerned. This awaits further attestation with more intensive and detailed experiments.

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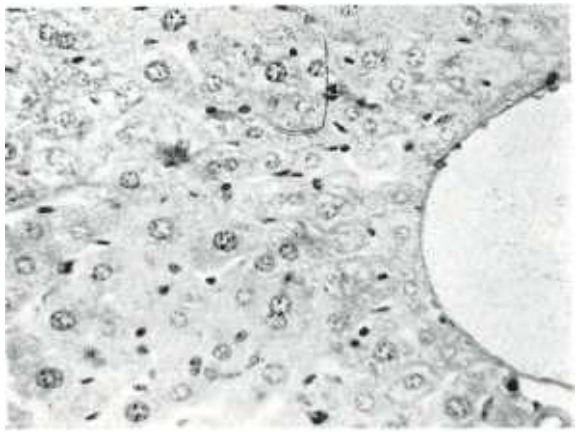

Fig. 1. The peripotal area from the group of 3 hrs after treated TAA, MGP stain, 450 ×.

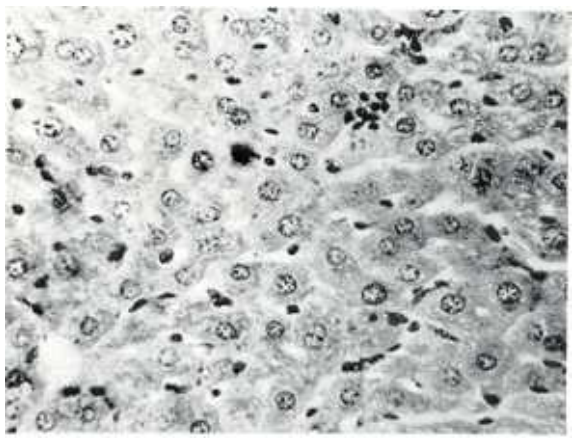

Fig. 2. Mouse liver from the group of 3 hrs after thioacetamide administration, the central vein area, stained with Methyl-green-pyronin, 450 ×.

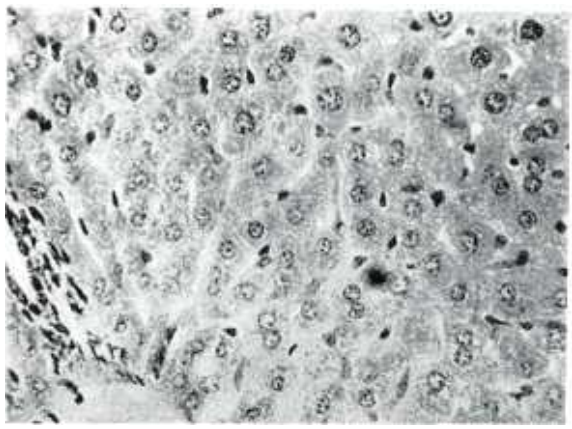

Fig. 3. Liver section obtained from the group of 3 hrs after TAA treated mouse which previously received alpha-tocopherol, MGP stain, 450 ×.

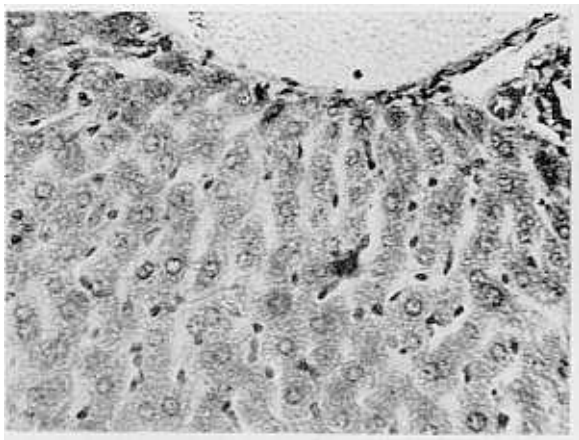

Fig. 4. The periportal area from the group of 6 hrs after treated TAA, MGP stain, 450 ×.

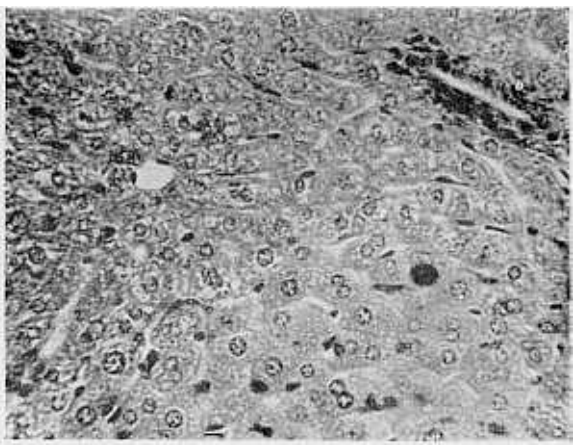

Fig. 5. The periportal area and central area from the group of 6 hrs after TAA treated mouse which previously received alpha-tocopherol, MGP stain, 450 ×.

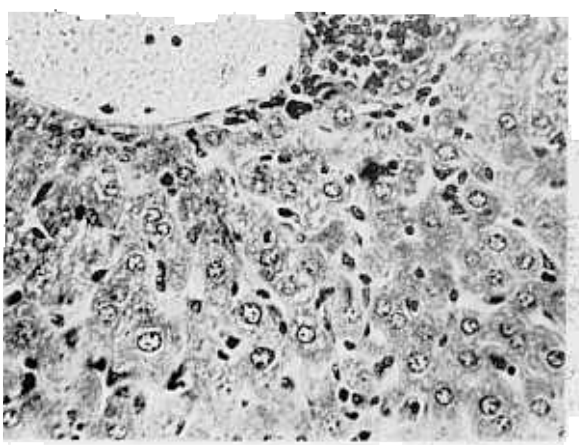

Fig. 6. The periportal area from the group of 9 hrs after treated TAA, MGP stain, 450 ×.

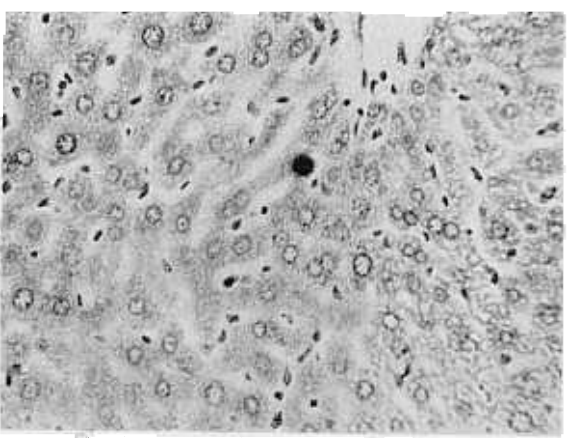

Fig. 7. The peripotal area from the group of 9 hrs after TAA treated mouse which previously received alpha-tocopherol, MGP stain, 450 ×.

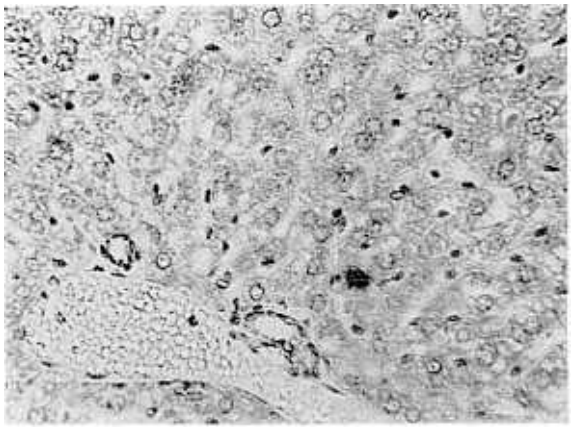

Fig. 8. The periportal area from the group of 12 hrs after treated TAA, MGP stain, 450 ×.

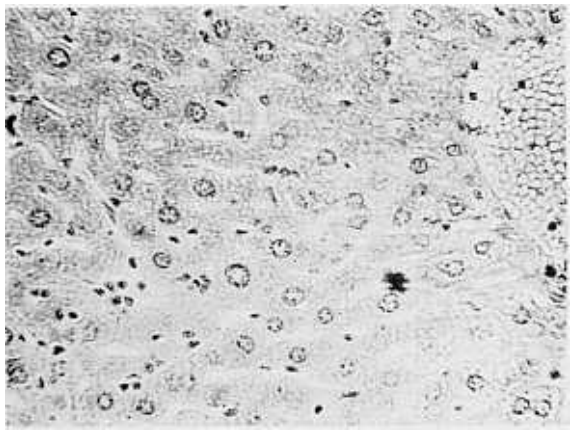

Fig. 9. The peripotal area from the group of 12 hrs after TAA treated mouse which previously received alpha-tocopherol, MGP stain, 450 ×.

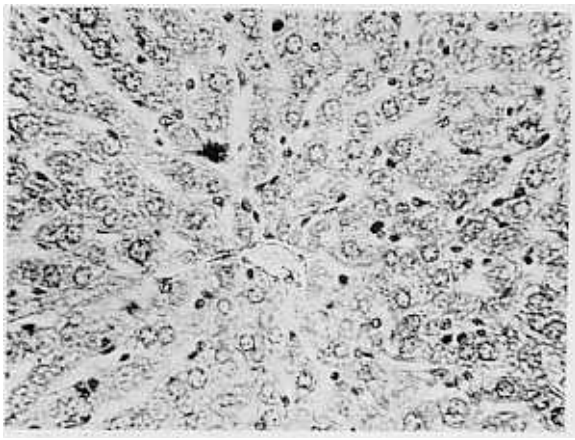

Fig. 10. The central vein area from the group of 12 hrs after treated TAA, MGP stain, 450 ×.

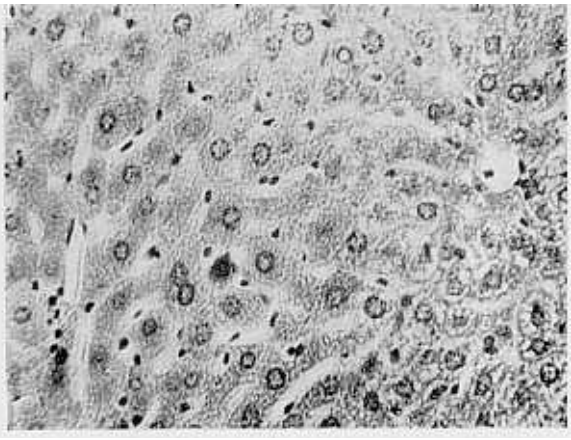

Fig. 11. The central vein area from the group of 12 hrs after TAA treated mouse which previously received alpha-tocopherol, MGP stain, 450 ×.