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EFFECT OF D-PENICILLINAMINE ON HEPATOCYTE ULTRASTRUCTURE AND ON STATE OF THE GROUND SUBSTANCE IN EXPERIMENTAL CIRRHOSIS OF THE LIVER IN RATS

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D-penicillinamine (DPA), a metabolite of benzylpenicillin hydrolysis, is nowadays used for the treatment of patients with primary biliary cirrhosis of the liver (PBC). The use of DPA in PBC rests on the broad spectrum of its action: It lowers the level of circulating immune complexes and has a copper-eliminating action [2, 6, 10], it depolymerizes pathological macroglobulins, and inhibits collagen synthesis [2, 3, 5]. However, data on the efficacy of DPA in patients with PBC are often contradictory and there are no precise opinions about side effects arising during the use of this compound. Only solitary reports of the action of DPA on hepatocyte ultrastructure could be found in the accessible literature [11].

The aim of this investigation was to study the action of DPA on hepatocyte ultrastructure and on the state of the ground substances in the liver of rats with experimental cirrhosis.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing initially 140-160 g. Cirrhosis was produced by subcutaneous injection of CCl_4 in olive oil (1:1) in a does of 01. ml/100 g body weight, twice a week for 6 months. DPA was injected perorally in a does of 200 mg/kg 5 times a week from 2 to 4 and from 2 to 6 months of the experiment. Rats with liver damage induced by CCl_4 and intact animals, kept under the same conditions as the experimental animals served as the control. For electron-microscope investigation the rat liver was prefixed by perfusion with 0.5% gluataraldehyde in 0.1 M cacodylate buffer (pH 7.2) through the portal vein for 15-20 min [1]. The liver was washed with the same buffer for 10 min by intravascular perfusion, after which tissue fragments were fixed with 1% OsO_4 for 2 h. The tissue fragments were dehydrated in a series of alcohols of increasing concentration and embedded in Araldite. Sections cut on an Ultracut F ultramicrotome ("Reichert") were stained with uranyl acetate and lead citrate and examined in the JEM-1200 EX electron microscope with accelerating voltage of 80 kV and 30-μ aperture diaphragm. Uronic acids (UA) in the liver tissue were determined by the carbazole method [7], and activity of N-acetyl-β-D-glucosaminidase (β-NAG) was studied by Weissman's method in our own modification for the liver [4].

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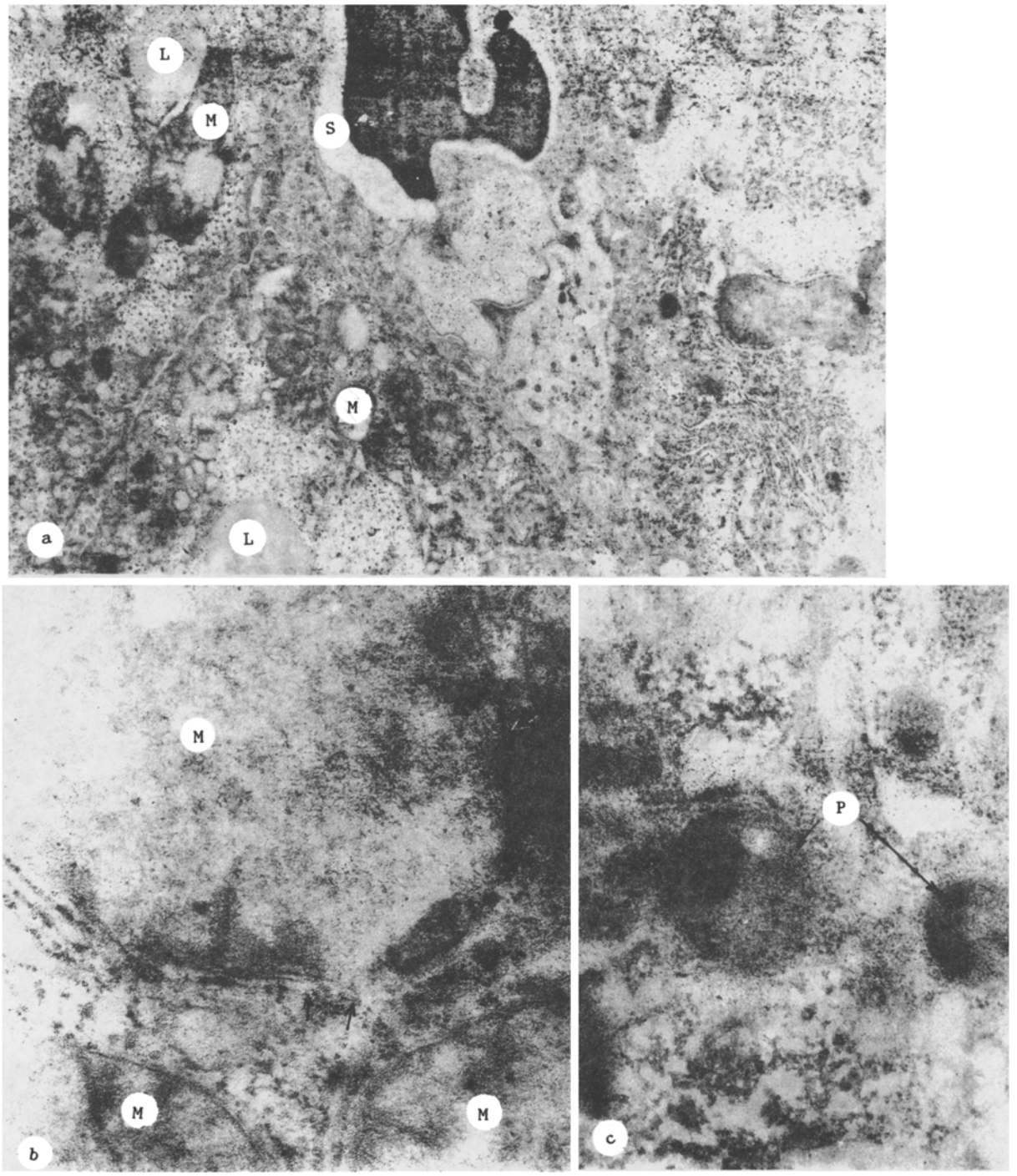

Fig. 1. Hepatocytes of rats receiving CCl_4 for 4 months and DPA for 2 months. a) Lipid inclusions (L) and altered mitochondria (M) in cytoplasm of hepatocytes. S) Sinusoid. 21,000 ×. b) Tight junction between outer membrane of mitochondria (M) and rough endoplasmic reticulum (arrow). 97,000 ×. c) Peroxisomes (P) in cytoplasm of hepatocytes. 48,000 ×.

TABLE 1. UA and β-NAG Level in Rat Liver Tissue at Different Stages of Experimental Cirrhosis (M ± m)

Group of animals	4 months		6 months	
	UA, μg%	β-NAG, μmoles/g·min	UA, μg%	β-NAG, μmoles/g·min
1. Intact control	1094,1±137,09 (n=5)	19,6±1,37 (n=12)	745,1±90,0 (n=5)	17,0±0,99 (n=11)
2. CCl_4-cirrhosis	1616,13±129,25 (n=16)	22 9±1,6 (n=8)	665,6±64,28 (n=5)	18,4±0,69 (n=19)
3. CCl_4-cirrhosis + DPA	725,7±92,24 (n=8)	19,8±1,4 (n=13)	593,9±49,4 (n=5)	21,08±0,7 (n=5)
p_1	<0,05	>0,05	>0,05	>0,05
p_2	<0,001	>0,05	<0,01	<0,01
p_3	<0,001	>0,05	>0,05	<0,05

Legend. p_1) Significance of differences between values in groups 2 and 1; p_2) groups 3 and 1; p_3) groups 3 and 2.

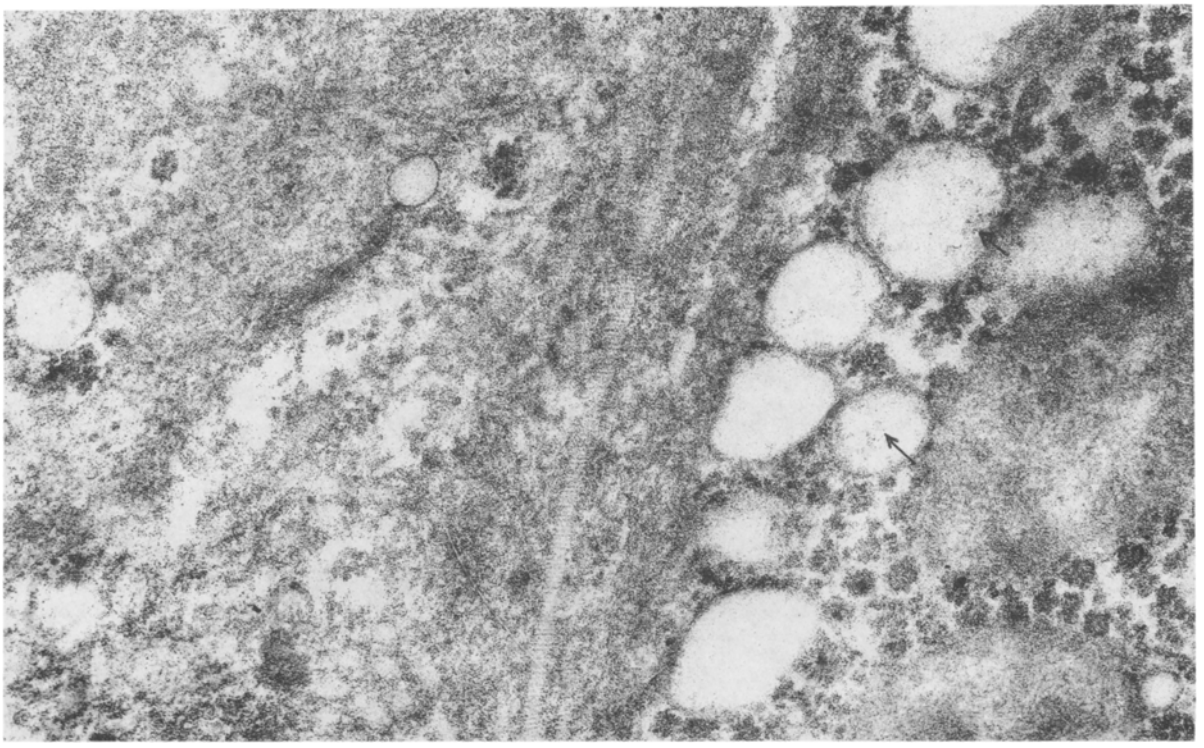

Fig. 2. Floccular protein inclusions (arrow) in swollen rough endoplasmic reticulum. 86,000 ×.

EXPERIMENTAL RESULTS

The action of DPA on the ground substance was realized by its suppressor effect on synthesis of glycosaminoglycans (GAG) [8] and inhibition of fibroblast proliferation [9], reflected in a fall in the GAG level in the media studied. In the present experiment (Table 1) a fall of the UA level was observed toward the 4th and 6th months of the experiment (DPA was given for 2 and 4 months respectively). Incidentally, the maximal UA level was observed at the 4th month of the experiment, which corresponded to the stage of compensated cirrhosis of the liver, and it fell sharply in the decompensation stage (6 months). The UA level in the group of animals receiving DPA was less than half as high as that in rats receiving CCl_4 alone. Activity of β-NAG, a GAG-hydrolase, fell toward the 4th month of the investigation and was virtually identical with the normal value, after which it greatly exceeded the level of this lysosomal enzyme in the control versions of the experiment (Table 1).

The electron-microscopic investigations showed that by the 4th month of the experiment there were definite differences in hepatocyte ultrastructure in animals with CCl_4-induced cirrhosis of the liver and receiving or not receiving DPA. Many lipid inclusions (Fig. 1a), characteristic of fatty degeneration of the liver, were observed in the cytoplasm of the

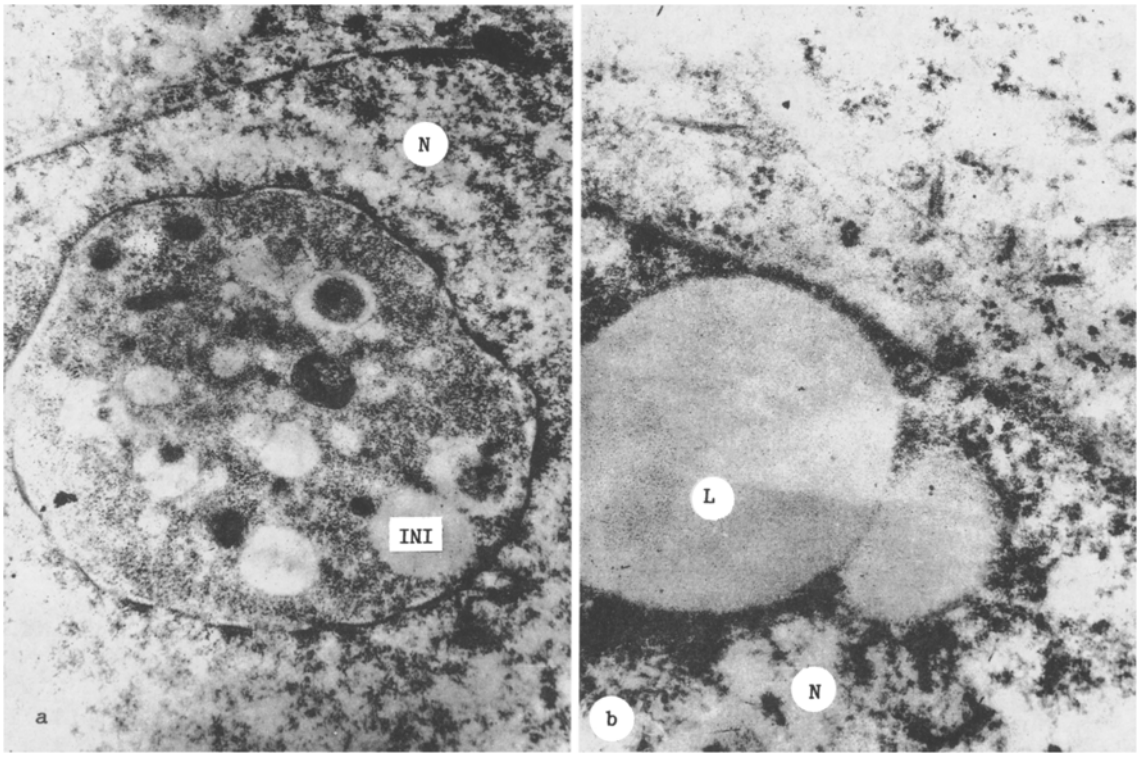

Fig. 3. Intranuclear inclusions (INI) in hepatocytes of rats receiving DPA for 6 months. a) Invaginates of cytoplasm. 22,000 ×. b) Lipid inclusions (L). 58,000 ×.

hepatocytes; the cytoplasmic matrix was reduced in density and the cytoplasma contained glycogen rosettes and granules of polysomes. The number of peroxisomes and lysosomes increased until the 4th month of the experiment (Fig. 1a, c) and fell sharply toward its end. The lysosomal population consisted of primary and secondary lysosomes, and also of the large number of coated vesicles (endosomes) with floccular contents (Fig. 2). Mitochondria had lost their normal structure, some of them were in a state of swelling, giant mitochondria occasionally appeared, and most of these organelles contained a rarefied matrix and single cristae (Fig. 1a, b). Incidentally, tight junctions and fusion of their membranes were observed between the outer membranes of the mitochondria and tubules of the rough endoplasmic reticulum (Fig. 1b). This fusion was evidently due to the need to synthesize enzymes, especially cytochrome C, and to transfer them rapidly from granules of the endoplasmic reticulum to mitochondria. The rough and smooth endoplasmic reticulum was in a state of swelling, and the tubules of the endoplasmic reticulum had "lost" their ribosomes in a part of their area (Fig. 1b). Sometimes within the lumen of the cisterns of the swollen rough endoplasmic reticulum floccules could be seen, evidently of protein origin (Fig. 2). It can be tentatively suggested that DPA, like other antibiotics, act on the protein-synthesizing function of the hepatocytes, leading to accumulation of protein synthesized on ribosomes in the cisterns of the reticulum.

After administration of DPA for 6 months two types of inclusions were found in the hepatocyte nuclei. Inclusions of the first type were evidently cytoplasmic inclusions, present in the plane of section as a result of the formation of deep invaginations of the nuclear membrane in the nuclei (Fig. 3a). Inclusions of the second type were round formations of average electron density, not bounded by a unitary membrane. In their structure and density they were indistinguishable from lipid inclusions in the heaptocyte cytoplasm and were most frequently located at the periphery of the nucleus (Fig. 3b); the nuclear membranes, moreover, could undergo lysis under these circumstances. We found no intranuclear lipid inclusions in the hepatocytes of rats with experimental CCl_4-cirrhosis, but we did find formations of this type in the liver of rats and mice treated with DPA [11].

Long-term administration of DPA during the development of experimental cirrhosis (up to 4 months) thus led to lowering of the level of ground substance in the liver and, correspond-

ingly, to a decrease in fibrosis as a whole, demonstrated by electron microscopy as a decrease in the number of collagen fibers in Disse's spaces and absence of fibrous structures in the intercellular spaces of the hepatocytes, adjoining the sinusoid. The ultrastructure of the hepatocytes in the initial stages of the experiment corresponded to the stage of fatty degeneration of the liver, and it can be tentatively suggested that treatment with DPA inhibits the formation of cirrhosis.

However, prolongation of the course of DPA in the stage of decompensated cirrhosis leads to an increase in β-NAG activity against the background of a sharp fall of the GAG level in the experimental groups, evidence of the negative effect of DPA administration in the final stage of the experiment.

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