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Formalin-induced experimental sclerosing cholangitis in the rat

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Summary. The few reported cases of sclerosing cholangitis following removal of an echinococcus cyst are thought to be a consequence of the chemical action of formalin used for sterilization of the residual cavity. The aim of this study was to assess this hypothesis.

We injected 0.15ml of 2% buffered formalin solution into the central hepatic lobe of five rats, after a midline laparotomy. At 6, 12, 18 and 24 weeks after formalin injection all rats were reoperated upon and a sample of hepatic parenchyma from both the central and the left hepatic lobe was obtained for microscopic evaluation.

Our findings, dilatation of portal tracts and bile canaliculi, thickening of the pericanalicular cytoplasm, portal and periportal inflammatory cell infiltration and fibrosis and enlargement of the perisinusoidal space of Disse, suggest that 2% formalin solution leads to the development of essential phenomena of cholestasis and sclerosing cholangitis in the rat, so thus it should be avoided in liver hydatid disease surgery.

Key words: Sclerosing cholangitis, Cholestasis, Liver hydatid disease surgery, Formalin

Introduction

Although formalin is considered to be the method of choice for residual echinococcus cavity sterilization after surgical removal of the cyst, the few reported cases (Akobianz et al., 1979; Khodadadi et al., 1981; Teres et al., 1984; Baries et al., 1985; Russo et al., 1987) of sclerosing cholangitis (SC) following surgery for that hepatic hydatid disease during recent years are thought to be a consequence of the chemical action of formalin (Hunt and Jawetz, 1979).

In an effort to test this hypothesis, we designed the following experimental model of formalin injection in the rat's liver.

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Materials and methods

Five male Wistar rats (200-250gr) were subjected to midline laparatomy, under light ether anesthesia and a total volume of 0.15ml of 2% buffered formalin solution was then injected by an hypodermic needle into the central hepatic lobe, just to the hilus.

At 6, 12, 18 and 24 weeks after formalin injection all experimental animals were reoperated upon by the same technique and a sample of tissue was obtained from both the central and the left hepatic lobe for microscopic evaluation under a light and transmission electron microscope.

At 24 weeks the animals were sacrified by cervical dislocation.

Liver specimens for light microscopy were fixed in buffered formalin and stained with hematoxylin-eosin and Van-Gieson. Specimens for transmission electron microscopy were fixed in 2% glutaraldehyde in cacodylate/sucrose 4% buffer (0.08M), postfixed in 2% osmium tetroxide and after dehydration in graded series of ethanol, were embedded in Epon resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Jeol 100cx electron microscope, operated at 80KV.

Results

All rats, although they had uneventful postoperative periods between consequent operations and good appetite, exhibited no gain in body weight throughout the six month period of the study.

The histological examination revealed dilatation of portal tracts with mild cellular infiltration. The tissue inflammatory reaction was composed of lymphocytes, eosinophils and polymorphonuclear leucocytes in association with bile ductule multiplication and fibroblastic proliferation. Intracellular bile pigment was seen in the centrolobular and periportal areas. There was a progressive portal and periportal fibrosis with

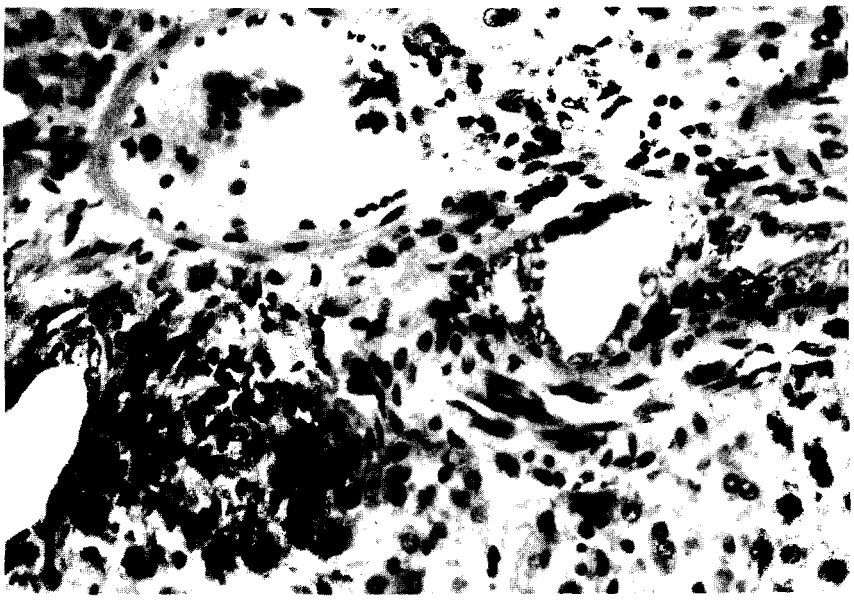

Fig. 1. Dilated portal tract with pronounced inflammatory cell infiltration and fibroblastic proliferation. H-E × 400

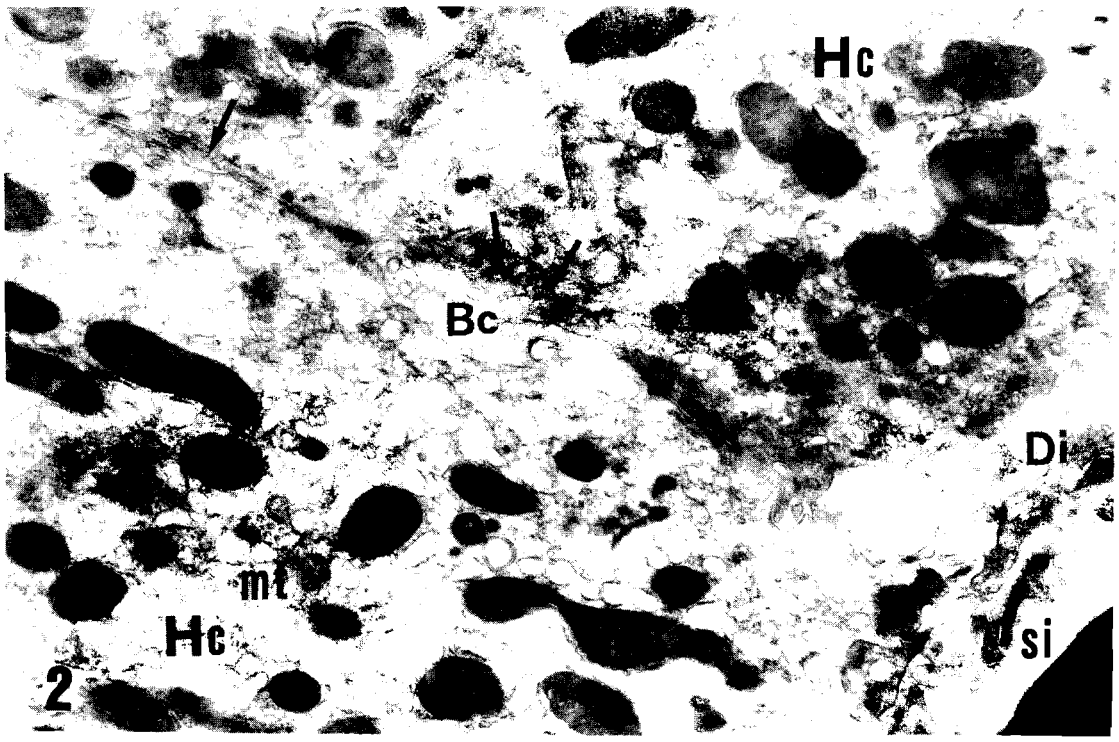

Fig. 2. Parts of two hepatic cells (Hc). The bile canaliculi (Bc) have lost their microvilli, while the pericanalicular cytoplasm is densely packed by osmiophilic material (arrows). Di = space of Disse, Si = hepatic sinusoid, D = desmosome, mt = microtubulus. × 9,000

concentric periductal lamination of collagen and marked bile duct proliferation –especially prominent with Van Gieson staining– with the time lapse from formalin injection (Fig. 1).

Our findings were common to the whole hepatic parenchyma from the 6th week after formalin injection; the severity of inflammatory reaction decreasing as fibrosis increased with the passing of time.

Transmission electron microscopy revealed dilatation of bile canaliculi with decrease or loss of microvilli (Fig. 2), some being empty or filled with a variety of materials, the most obvious being the typical «bile thrombi». While no significant alterations were noted in the organelles of the hepatocytes, only some microtubules and few microfilaments were prominent (Figs. 3, 4). However, the pericanalicular cytoplasm was densely packed by osmiophilic fine granular or filamentous material. The junctional complexes were intact. The perisinusoidal space of Disse with abundant collagen fibers and erythrocytes was enlarged. The number of microvilli

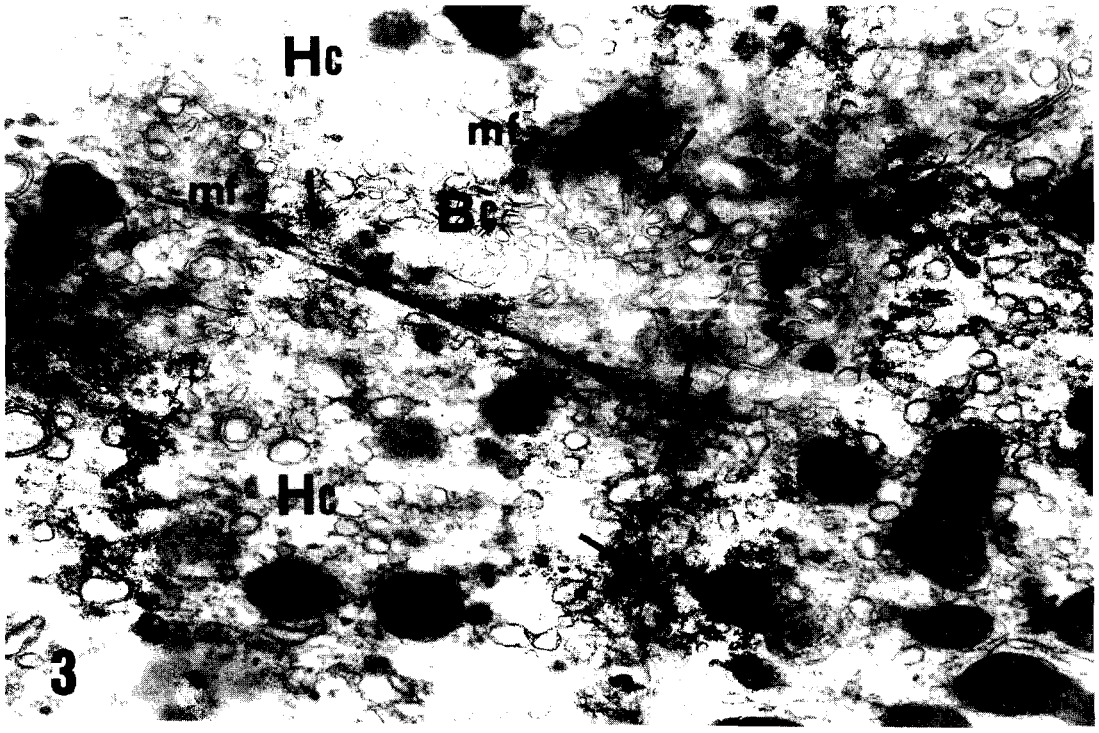

Fig. 3. Parts of two hepatic cells (Hc). Densely packed osmiophilic filamentous material (arrows) and a few microfilaments (mf) are prominent. Bc = bile canaliculus. × 12,000

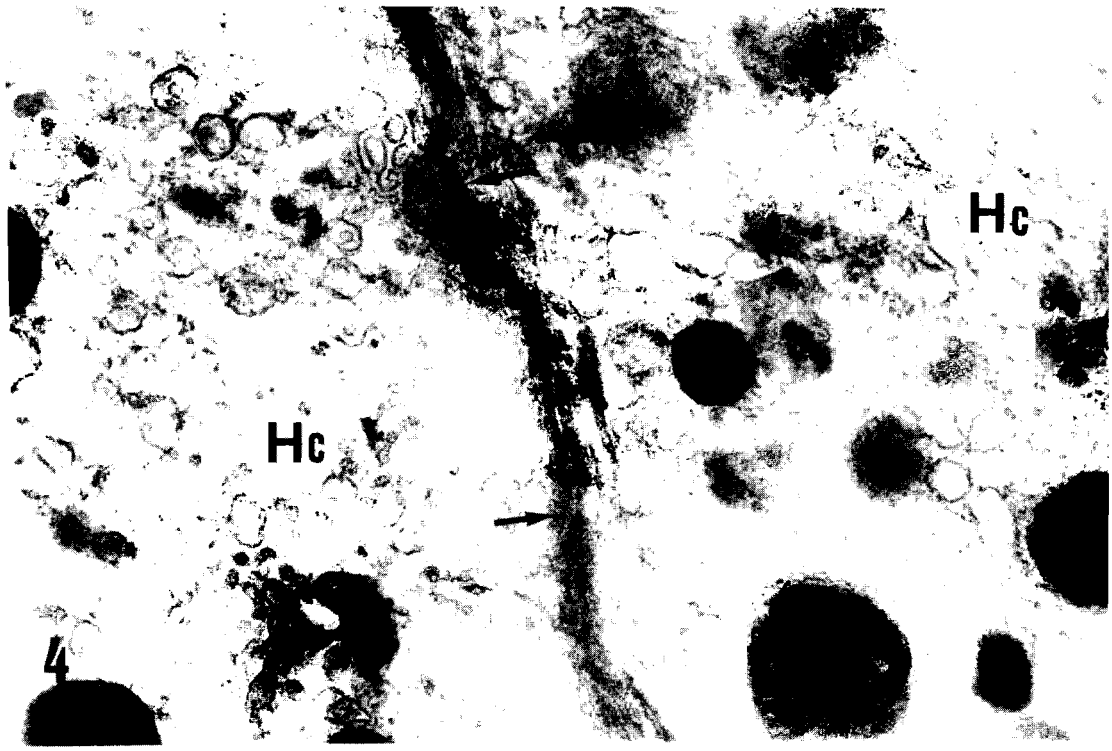

Fig. 4. Parts of two hepatic cells (Hc). Characteristic osmiophilic granulation of the pericanalicular cytoplasm (arrows). × 22,000

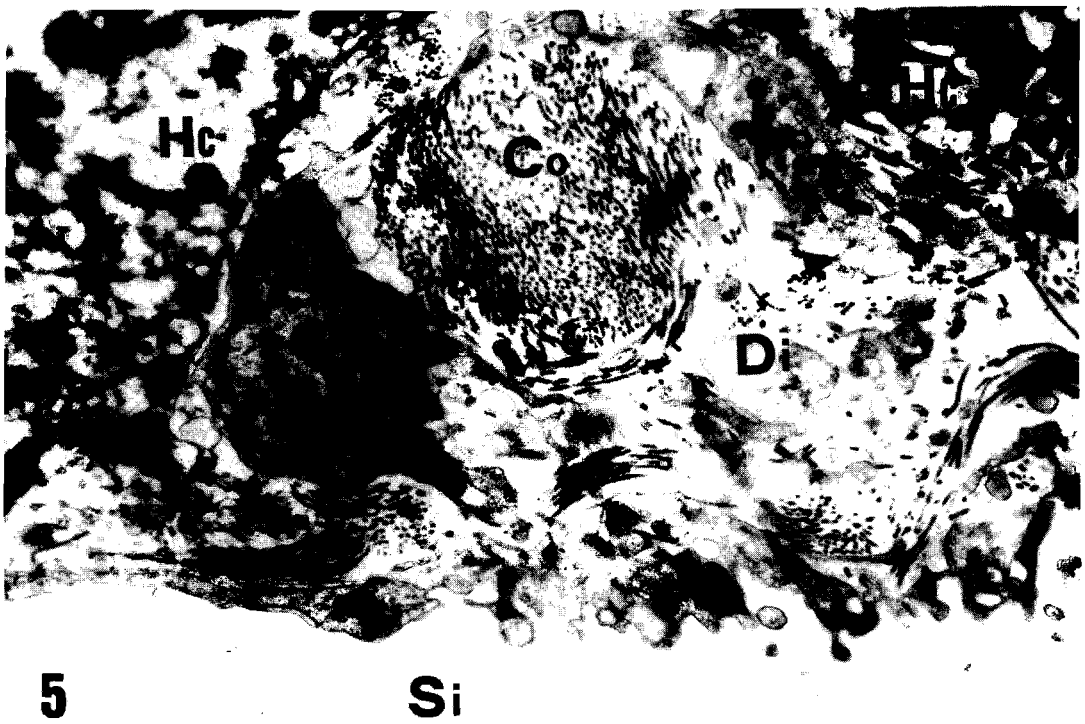

Fig. 5. Dilatation of perisinusoidal space of Disse (Di) with abundant collagen fibers (Co). Hc = hepatic cell, Si = hepatic sinusoid. × 6,000

protruding into this space were found blunted or distorted (Fig. 5).

Discussion

Primary SC was considered as a rare disease characterized by a chronic inflammatory fibrosis, thickening, round cell infiltration and, if the process persisted long enough, deposition of collagen in the wall of the intra- and extrahepatic bile ducts (Thompson et al., 1982; Panes et al., 1985).

Since the introduction of direct cholangiography (ERCP and PTC) into the routine work-up of jaundiced patients, SC has been more frequently diagnosed as secondary to common bile duct stones, congenital abnormalities of the billary tree, bile duct carcinoma, iatrogenic stenosis or continuous inflammation (Longmire, 1978; Chapman et al., 1980). Recently, it has been reported that SC has been observed following procedures for removal of an echinococcus cyst and the injection of formalin into the residual cavity (Khodadadi et al., 1981; Teres et al., 1984; Bories et al., 1985; Russo et al., 1987).

In 1982 Mirouse et al. reported in abstract form five cases of SC in man after formalin sterilization of the residual parasitic cavity and reproduced similar lesions in two dogs after injecting 20 ml of 10% and 20% formalin solution, respectively, into their gallbladders. Following this, Houry et al. (1986) and Burgeon et al. (1987) reproduced SC or pseudo-cirrhosis -like changes in rats after retrograde cannulation of the papilla of Vater through duodenotomy and 0.5% or 2% formalin injection.

Our experimental model differs in some respects from the previously described methods. We injected formalin into the hepatic parenchyma, which is more realistic than within the common bile duct, as we do not pour formalin into the residual cavity if a large opening to a bile duct exists. We used formalin 2% (i.e. the commonly used clinical solution of formaldehyde). We performed biopsies at 6 week intervals on the same animals over a period of six months, so that we were able to study any progressive changes, as well as their probable reversibility.

From our findings, there are four points that deserve discussion.

1. The presence of inflammatory cell infiltration, especially with eosinophils, represents a tissue reaction to irritant chemical or pharmaceutical stimuli (Thompson et al., 1982) apart from the common irritation from bile component leakage into the portal connective tissue.

2. Our electron microscopic observations revealed few microtubules and microfilaments, while a densely packed osmiophilic fine granular substance was prominent all around bile canaliculi. These findings are considered to be due to the disruption of pericanalicular microfilaments, thus producing canalicular dilatation with concomitant reduction in bile flow (Desmet, 1979), as it is known that the microfilaments maintain the canaliculi in a contracted state and provide tone to the canalicular system, and thus facilitate the flow of bile (French, 1976), while microtubules are involved in secretory transport towards the sinusoidal pole of

lipoproteins, albumin and fibrinogen as well as in the biliary excretion of lecithin and bile salts (Prugh, 1976; Desmet, 1977). Ichikawa et al. (1986) reported the same findings in the rat after endotoxin-induced intrahepatic cholestasis.

3. The widening of the perisinusoidal space of Disse with the blunted or distorted microvilli would be explained by the leakage of bile into this space, due to the disturbance in the secretory transport of bile through the bile canaliculi (Bergan et al., 1975; Desmet, 1979). This bilio-lymphatic reflux, however, is held responsible for the activation of fibroblasts and the collagen fiber accumulation within the space of Disse (Bergan et al., 1975; Carison et al., 1977; MacSween and Scothorne, 1979) which was observed by both light and electron microscopes. On the other hand, the presence of intact tight junctions between cells means that the hydrostatic pressure within bile canaliculi is not so high as in complete acute obstruction of the distal choledochus.

4. Another point of interest is the presence of the same morphological findings from the 6th up to the 24th week, at any site of hepatic parenchyma - adjacent or far away from the point of formalin injection. Although it is considered that diffuse and localized forms of SC reflect different stages of the disease, the diffuse involvement of liver from the 6th week means that formalin leads to diffuse hepatic damage, which seems to be irreversible, because of its persistence up to the 6th month.

We conclude that 2% formalin injection within the rat's hepatic parenchyma leads to development of essential phenomena of cholestasis and SC. The few reported clinical cases, as well as the experimental data, suggest that formalin is not a «un-dangerous» substance, so thus it must be avoided in liver hydatid disease surgery.

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