**SCANNING ELECTRON MICROSCOPY ON THE RAT LIVER WITH ALPHA-NAPHTHYLISOTHIOCYANATE-INDUCED CHOLESTASIS**

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**Summary**

Intrahepatic cholestatic rat livers were studied with the scanning electron microscope at 3, 24, 48 and 96 hours after a single dose administration of alpha-naphthylisothiocyanate (ANIT, 100 mg/kg body weight). Cholestasis reached the peak at 48 hours and decreased thereafter. On intercellular surfaces of hepatocytes, round indentations appeared at 3 hours and became larger and more numerous at 24 hours. On sinusoidal surfaces, small pits were observed at 3 and 24 hours. Bile canaliculi were dilated and their microvilli became shorter and sparse in all specimens. They became tortuous at 3 hours and after 48 hours tortuous canaliculi were numerous. Side branches of the bile canaliculi showed increase at 24 hours and were numerous at 48 hours. At the same time when changes in bile canaliculi were most remarkable, some of side branches communicated directly with the Disse space. At 96 hours canalicular changes became less observable and the communications disappeared. In conclusion, in the liver with ANIT-induced cholestasis, bile components possibly regurgitate 1) from the hepatocytes by reversed pinocytosis through intercellular surface indentations and sinusoidal surface pits in the early phase of cholestasis, and 2) from the bile canaliculus through the bile canaliculus-the Disse space communications at the peak of cholestasis.

**Key Words:** intrahepatic cholestasis, bile canaliculus, alpha-naphthylisothiocyanate (ANIT), scanning electron microscopy (SEM).

**Introduction**

The questions how and where bile components regurgitate into the bloodstream from in intrahepatic cholestasis, still remained to be unanswered. Alpha-naphthylisothiocyanate (ANIT)-induced cholestasis has been proposed as a suitable experimental model of intrahepatic cholestasis. Accordingly, many authors have studied this subject by light microscopy, histochemistry and transmission electron microscopy (TEM). In intrahepatic cholestasis, 3 possible routs of regurgitation of bile components have been proposed as follows, 1) inter hepatocellular escape from the bile canaliculus to the bloodstream, 2) transhepatocytic regurgitation to the Disse space and intercellular space by a reversed secretory polarity of the liver cell, 3) increased permeability and regurgitation through bile ductules and ducts. The regurgitation routs of ANIT-induced cholestasis have not been...
clarified. It has however been suggested that the route 2) contributes for a few hours after administration and the route 3) added after 24 hours10). The route 1) has not been confirmed in ANIT-induced cholestasis.

Scanning electron microscopy (SEM) have recently revealed new informations by demonstrating three dimensional changes of bile canaliculi in intrahepatic cholestasis induced by mono-hydroxy bile acids14-17) and cytochalasin B18). To our knowledge, ANIT-induced cholestasis has not yet been studied by SEM. In this paper, SEM observation of the hepatocyte surface morphology was attempted to elucidate regurgitation routes of bile components in rat liver with ANIT-induced cholestasis.

**Materials and Methods**

Fourteen Sprague-Dawley male rats, weighing about 150 to 250 g were used. All rats were allowed to feed ad libitum. A single dose of ANIT (Aldrich Chemical Co. Milwaukee, USA) 100 mg/kg body weight suspended in 0.2 ml olive oil was given by stomach tube to 12 rats. Two untreated rats were served as control. Three rats in each group were anesthesized with ether and sacrificed at 3, 24, 48 and 96 hours after ANIT administration. Serum bilirubin level and alkaline phosphatase activity were estimated at each time.

The liver was perfused through the thoracic aorta with Ringer solution, reperfused with 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4)19), cut into small blocks with razor blades and fixed in the latter solution for overnight. The blocks were then stained with the revised tannin-osmium method19), dehydrated through a graded series of ethanol, and dried in an HCP-1 critical point dryer using liquid carbon dioxide. The dried specimens were cracked in air by a scalpel20). Bile canaliculi and intercellular surfaces of hepatocytes were observed on both matched surfaces under the SEM (JSM-U3, HFS-2) at an accelerating voltage of 15 or 20 kV. Some specimens were observed after sputter-coating with platinum-palladium was applied in an Eiko IB-3 ion coater. Stereoscopic pairs of scanning electron micrographs were examined. Some blocks were used for light microscopy.

The bile canalicular side branches, defined as lateral extension longer than the bile canalicular diameter, were counted per 100 intercellular surfaces of hepatocytes.

**Results**

Liver cholestasis after administration of ANIT showed the peak at 48 hours and decreased thereafter (Fig. 1).

1. **Light microscopic observations**

   3 hours after ANIT administration: There were no remarkable changes in hepatocytes, bile duct epithelia and portal tracts. But a small number of hepatocytes had intracytoplasmic vacuoles and a pyknotic nucleus.

   24 hours: The hepatocytes had intracytoplasmic vacuoles mainly in the intermediate and central areas of the lobule. The vacuoles increased in number and size and located toward