The Mechanism of Bile Regurgitation Experimental Approach with Radioisotope.

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The route of bile regurgitation in biliary obstruction and histological site of blood-bile exchange have long been the subject of extensive investigations[1,~7], but agreement has not as yet been reached. Even among electron microscopic pathologists[7,~10], argument continues as to whether or not continuous separation of liver cells occurs in biliary obstruction.

The present study aims at elucidating the kinetic mechanism of biliary regurgitation. The bile secretion pressures was measured under various conditions. Radioactive iodine (inorganic and PVP bound) and India ink particles were infused into the common bile duct in the rabbits under various biliary pressures, and the rate of transfer into the blood stream of these materials was studied with simultaneously carried out light and electron microscopy.

Materials and Methods

A total of 38 male and female adult rabbits weighting from 2.2 to 2.4 Kg. were used. The rabbits were fasted for 24 hours before any observation was made. A glass cannula was inserted into the common bile duct and connected by a polyethylene tube with a diameter of 4 mm. and the abdomen was closed. After the flow of bile was established, the polyethylene tube leading from common bile duct was set in vertical position and the bile secretion pressure was determined. The level at which the column of bile stopped was interpreted as indicating the bile secretion pressure. After measurement of the bile secretion pressure, the tube was raised slowly and the level at which the column of bile began to fall was represented as the bile back flow pressure. All these pressures were referred to the hilum of liver.

The rabbits were divided into four groups for determination of bile secretion pressures. Group 1, normal rabbits. Group 2, rabbits in which cholestasis was relieved after 5 days. Group 3, rabbits given intrabiliary injection of 4 ml of 4 per cent EDTA at the bile back flow pressure. Group 4, rabbits given intrabiliary infusion of 100 ml of Ringer's solution at a high pressure (50 cm of water). After determination of the bile secretion pressure, each of the animals

received intrabiliary injection of ^{131}I-bound PVP[11] solution containing 200–250μc or India ink* solution mixed with Na ^{131}I at high pressure (Group 5,) or back flow pressure. In each case the time required for injection was about one-half hour. Two ml of blood were taken from the femoral vein 15 and 30 minutes after the end of injection. The radioactivity of all samples was then measured using a scintillation counter (Toschiba Co.).

Results

1. Bile secretion pressure

Fig. 1 shows mean values for bile secretion pressure and back flow pressure

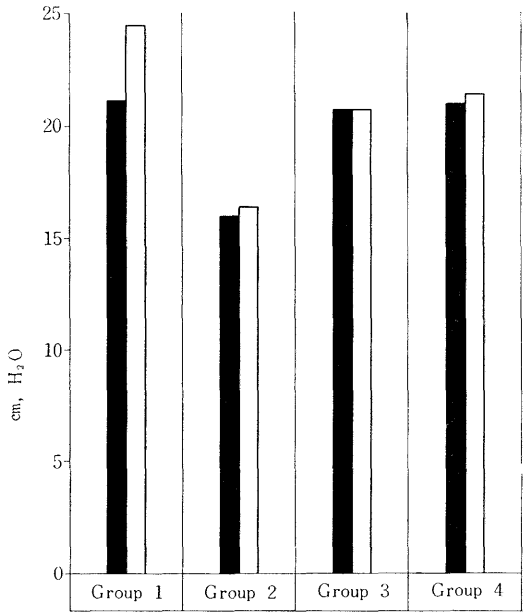

Fig 1. Mean Pressure in Common Bile Duct. (cm of H_2O)

■ Bile Secretion Pressure
□ Bile Back Flow Pressure

Group 1, normal rabbits
Group 2, rabbits in which cholestasis was relieved after 5 days
Group 3, rabbits given intrabiliary injection of EDTA at the bile back flow pressure
Group 4, rabbits given intrabiliary injection of Ringer's solution at a high pressure.

* Pelican. No. $^{11}/_{1431}$ a. Particle size 200Å.

in four groups. Bile secretion pressure in normal group ranged from 18.5 cm of water as the lowest to 24.4 cm of water for the highest. After intrabiliary injection of EDTA or experimental cholestasis, mean bile secretion pressure was decreased from 22.4 cm of water to 17.8 cm (group 3) and 16.5 cm of water (group 2), but these effects on bile secretion pressure was transient. Between the 2 and 3 groups, there was no significant difference. The time required for intrabiliary injection of 100 ml of Ringer's solution was about 5 minutes; Bile back flow was extremely rapid. After such a maneuver, bile secretion pressure fell to 17.2 cm of water (group 4). However, it was gradually restored to normal within 20–30 minutes after back flow ceased, and bile secreted thereafter was nearly normal.

2. Distribution of particles following retrograde intrabiliary injection

In Fig. 2 PVP concentration in the blood following retrograde intrabiliary injection was expressed as per cent of the dose administered. Upon retrograde injection at high pressures (group 5) of 100 ml of Ringer's solution containing

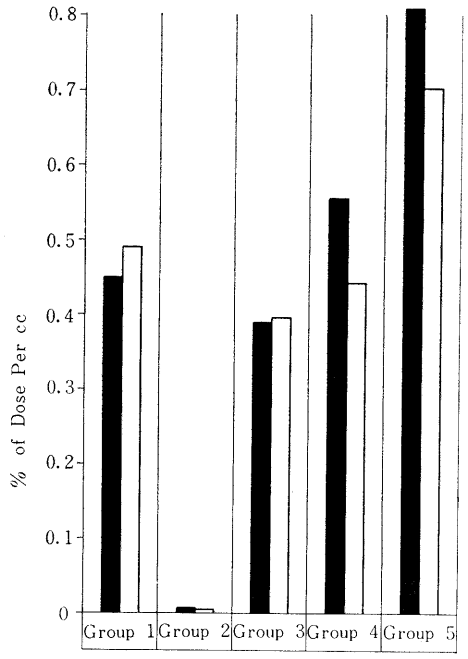

Fig 2. PVP concentration in the Blood after Intrabiliary Injection.

■ after 15min. □ after 30min.

Group 5, rabbits given intrabiliary injedtion of ^{131}I-PVP solution at 50 cm of water.

PVP, the PVP concentration in the blood was enhanced rapidly to a level which is about twice the normal. Among the groups 2, 3 and 4, no statistically significant difference in the PVP concentration was found. After cholestasis of 5 days (group 2), transter of PVP into the blood was markedly decreased; This fact may be considered to be a result of accumulation of PVP both in the dilated biliary system and the hepatic cells.

When India ink mixed with Na ^{131}I was injected into the common bile duct at back flow pressure, the radioactivity appeared in the blood within 5 to 10 minutes, but no India ink particle was transfered in the blood stream. In specimens fixed 30 minutes after the intrabiliary injection, no uptake of the India ink particles by Kupffer cells was observed. Small aggregates of the particles were found between the hepatic cells in the periphery of the lobules, and occasionally in the bile canaliculi (Fig. 3),

When India ink was injected at high pressures, the liver and conjunctiva bulbi immediately became black, and microscopically the particles were taken up by the Kupffer cells (Fig 4). These findings indicate that the India ink particles when infused at high pressures are transfered into the blood stream.

3. Electron microscopic observations

Fig. 5 and 6 demonstrate the electron micrographs of rabbit liver 3 days after complete extrahepatic biliary obstrution. The bile canaliculi were markedly dilated in all cases, the number of microvilli being decreased and many of them distorted. No evidence of rupture of bile canaliculi was found and chainlike separations between the bile canaliculi and the spaces of Disse were demonstrated. These findings suggest some interrupted communications between the

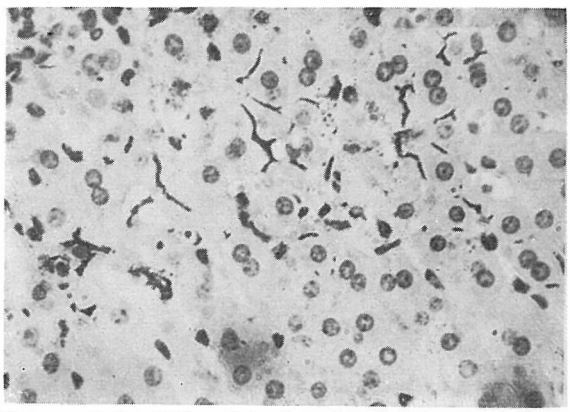

Fig. 3 Retrograde intrabiliary injection of India ink at back flow pressure (HE×400).
Small aggregates of particles are found between the hepatic cells in the periphery of the lobules and bile canaliculi.

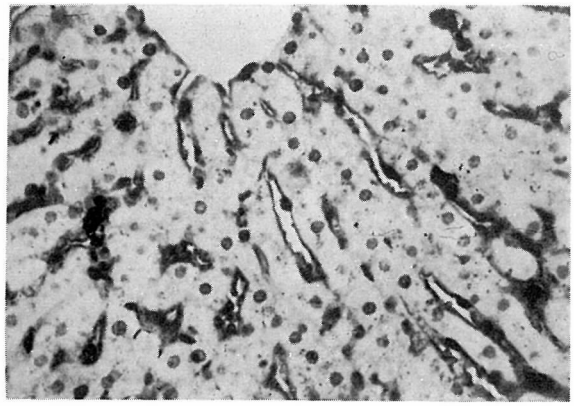

Fig. 4 Retrograde intrabiliary injection of India ink at high pressure (HE×200).
The particles are taken up by Kupffer cells.

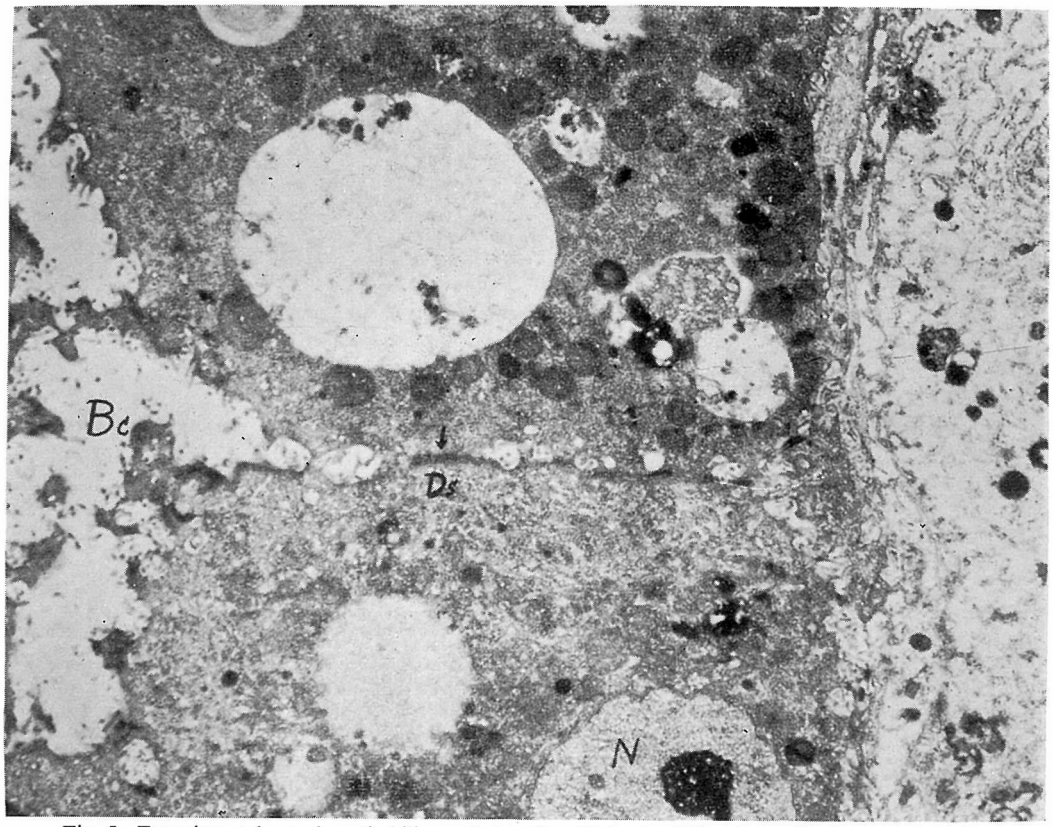

Fig. 5 Experimental extrahepatic biliary obstruction (3 days). Dilated canaliculus and chainlike separation between the bile canaliculus and the space of Disse are demonstrated (×6000).
V. vacuole N. nucleus Ds. desmosome M. mitochondria Bc. bile canaliculi

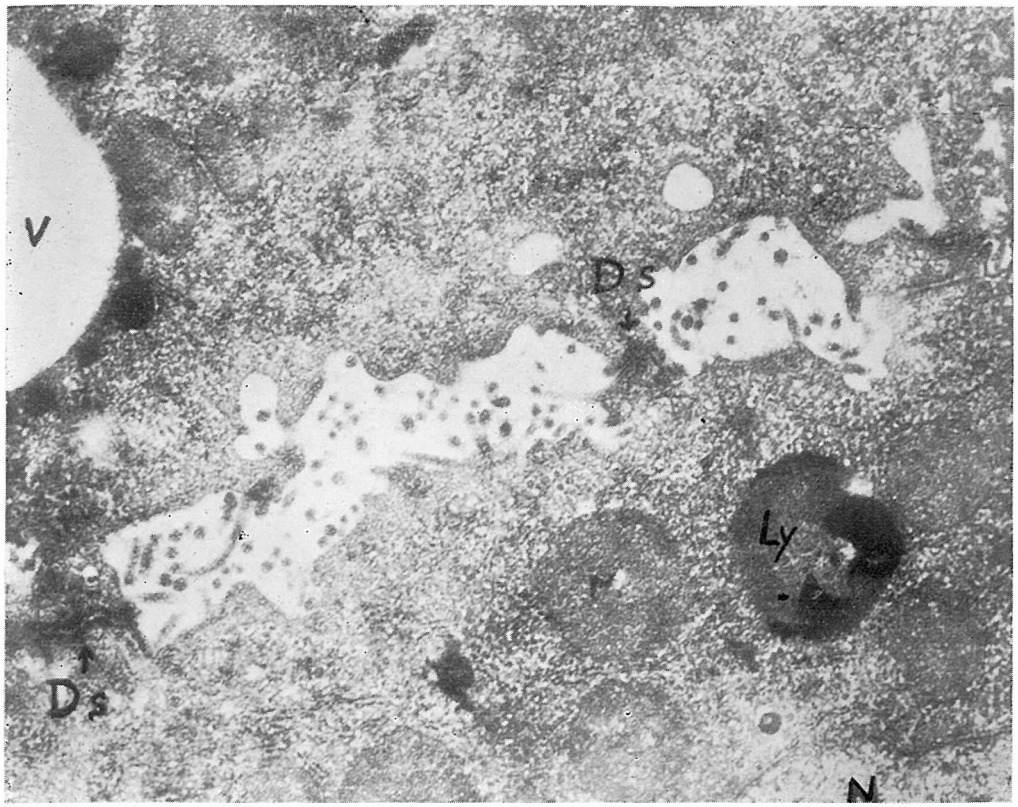

Fig. 6 Experimental extrahepatic billary obstruction (3 days). A vacuolar degeneration and increased density of mitochondrial matrix are evident in hepatic cells (×11500).

bile canaliculus and the space of Disse. Desmosomes remained intact even when the canaliculus was widely dilated. The main changes of hepatic cells were a vacuolar degeneration, increase of lysosomes and increased density of mitochondrial matrix.

Discussion

At a pressure a few cm of water higher than the bile secretion pressure, a communication was established between the biliary tree and the blood stream. Under these conditions, this communication allowed passage of PVP of molecular weight of 35,000 but not India ink particles. However, if India ink was infused into the common bile duct at 50 cm of water, approximately twice the bile secretion pressure, it was transferred into the blood stream. The wide communication between the biliary tree and the blood stream seems to be a result of high

pressure. However, when the bile pressure was reduced, even after infusion at high pressure, normal bile was secreted and no communication was demonstrable. It suggests this communication is transient and reversible.

After cholestasis of 5 days duration, bile secretion pressure dropped. Normal bile secretion pressure may not develop in biliary tree because of reflux of newly formed bile into the lymphatic or vascular stream.

A number of reports have detailed the route of biliary regurgitation and histological site of blood-bile exchange in the biliary obtruction. The juxtasinusoidal extention of bile canaliculi[12] as well as ductular region[13] and peribiliary vascular plexus[14] seem to be the site for bulk back flow. Hampton[15] demonstrated that the particles of colloidal mercuric sulfide (70 Å) administered to rats by intrabiliary injection were taken up at the biliary surface, transported through the cytoplasm, and discharged at the plasma surface of hepatic cells. On the other hand, Schaffner and Popper[7] reported that bile canaliculi rupture and permit bile to enter plasm in the space of Disse through the intercellular spaces. The results of the present study seem to contradict Schaffner and Popper's report; Electron micrographs have revealed chainlike separation (not continuous, or interrupted communication) between the bile canaliculi and the space of Disse with no rupture of bile canaliculi.

Recently, various schemas to illustrate the mechanism of bile regurgitation have been proposed by electron microscopic pathologists, but they have no three dimensional consideration. The hepatic cells are joined mainly the desmosomes. Electron microscopic picture of desmosome merely represents a cross section of desmosome which is actually a bandlike structure surrounding the surface of hepatic cells. Desmosomes are found at the bile canaliculus and near the space of Disse, and such an arrangement would provide a means whereby bile could be prevented from entering intercellular spaces[15]. Some of the desmosomes may be separated for a moment permitting bile to enter intercellular spaces, but they must be closed again by the influence of neighboring desmosomes. Thus, it seems that in the early stages of biliary obstruction, desmosomes are separated noncontinually, and bile is pressed out from the bile canaliculi into the space of Disse across the intercellular Spaces.

SUMMARY

In attempt to elucidate the kinetic mechanism of biliary regurgitation, the rabbit livers were subjected to measurement of bile secretion pressure under various conditions, and retrograde intrabiliary injection of radioactive iodine and India ink particles were carried out at various pressures to study their transfer into the blood stream. Electron and light microscopic studies were performed

simultaneously.

1. At a pressure a few cm of water higher than the bile secretion pressure, a communication was establisched between the biliary tree and blood stream. This communication allowed passage of PVP of molecular weight of 35,000, but not India ink of the size of 200 Å.

2. Under high infusion pressures (twice the secretion pressure) a wide communication was established whereby India ink was transferred in the blood stream. However, this communication was transient and no communication was demonstrable when the infusion pressure was reduced.

3. It is suggested that in the early stages of biliary obstruction, desmosomes are separated noncontinually, and bile is pressed out from the bile canaliculi into the space of Disse across intercellular spaces.

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