57 CHARACTERIZATION OF THE TAUROCHOLATE TRANSPORT SYSTEM IN NORMAL AND TRANSFORMED HEPATOCYTES. Daniel Levy, Peter Drain and Patricia von Dippe. Department of Biochemistry, University of Southern California, School of Medicine, Los Angeles CA 90033.

The taurocholate transport system in normal and transformed hepatocytes has been characterized using transport kinetics and photoaffinity labeling techniques. In normal hepatocytes the sodium dependent uptake process had a Km of 26 μM and a Vmax of 0.73 nmol/mg protein/min. The uptake of taurocholate by hepatoma tissue culture (HTC) cells and by H4 II EC3 hepatoma cells derived from the Reuber hepatoma, however, was less than 2% of the value observed in normal hepatocytes. A membrane associated bile acid carrier was identified utilizing a photoreactive 7-diazirine derivative of taurocholate which was shown to be a substrate for this transport system with a Km of 25 μM and a Vmax of 0.88 nmol/mg protein/min. A membrane protein with a molecular weight of 54,000 was specifically modified when affinity labeling was carried out on either intact cells or purified plasma membranes. The incorporation of the photoprobe could be inhibited when photolysis was carried out in the presence of taurocholate. Membrane subfractionation indicated that the carrier was located on the blood sinusoidal surface domain. Photolabeling of HTC cells, in contrast, did not result in the incorporation of the photoprobe into any membrane protein, a result consistent with the loss of transport activity in this cell system. The labeled carrier was shown to be an intrinsic membrane protein based on its solubility characteristics and its labeling from within the lipid bilayer with the photoreactive glycolipid, 12-(4-azido-2-nitrophenoxy)-stearoylglucosamine. This carrier protein has been substantially purified using affinity and ion exchange chromatography. The transport and labeling results suggest that the 54,000 dalton protein is a component of the hepatocyte bile acid transport system whose capacity is greatly reduced in several hepatoma cell lines as a result of possible alterations in the synthesis, processing, membrane content or structure of this carrier protein. (Supported by NIH Grant AM 25836.)

58 NON-A, NON-B POST TRANSFUSION HEPATITIS: DISASTER AFTER DECADES? RL Koretz, O Stone and GL Gitnick. UCLA School of Medicine, Los Angeles, California.

Between 1973 and 1978, 66 patients in a prospective study were identified who developed non-A, non-B (NANB) post transfusion hepatitis. These patients have been followed until the alanine aminotransferase (ALT) abnormalities had resolved (on at least 3 consecutive determinations at least 1 month apart) or until the present time, unless death supervened or the patient refused further followup. This report details the outcome to date in these 66 patients.

Results: 21 patients had liver biopsies (obtained 6 or more months after the onset of illness) demonstrating chronic hepatitis and/or cirrhosis, and 2 others had ALT abnormalities for more than 2 years without liver biopsy. An additional 12 patients either died or were lost to followup with abnormal ALT levels. Thus the overall incidence of chronic liver disease was between 35% (23/66) and 53% (35/66). Although the majority of patients had no significant long term symptoms, histologic confirmation of cirrhosis has been established in 4 of them (at months 6, 61, 76, and 80 respectively). One of these had a hepatic coagulopathy, and a second may have had hypersplenism, as clinical manifestations of cirrhosis. All 4 patients with cirrhosis had abnormal ALT values.

Conclusions: 1) NANB post transfusion hepatitis commonly results in chronic liver disease (35%-53%). 2) Cirrhosis has occurred in at least 6% of those developing NANB post transfusion hepatitis (after 4-9 years of followup) and exclusively in those with persistently abnormal ALT values. 3) Patients with NANB post transfusion hepatitis should be followed for many years after the onset of disease if biochemical resolution fails to take place. 4) Cirrhosis develops in a clinically silent fashion and usually only after years of activity.

59 GRISEOFULVIN INDUCED MALLORY BODIES AND GAMMA GLUTAMYL TRANSPEPTIDASE ACTIVITY. J. Tazawa, T. Irie, S. Akeda, T. Ihrig, H. Benson, and S. W. French., Dept. of Pathology, VA Medical Center, Martinez, and University of California, Davis, CA.

To evaluate whether mallory bodies (MBs) are linked to the induction of the oncofetal enzyme gamma glutamyl transpeptidase (GGT) mice were fed 2.5% griseofulvin (GF). The mice livers were biopsied three times, i.e. after 4 months' continuous GF feeding, 1 month after GF withdrawal and 13 days after GF refeeding. Livers from 12 experimental and 6 control adult male C3H mice were examined histologically, histochemically and immunocytochemically. The number of GGT positive spots and the total number of spots/mm^2 area were assessed each time by morphometry. To localize MBs inside GGT positive cells a double staining method was used where GGT positive cells were identified histochemically followed by anti-MB antibody staining using the unlabeled immunoperoxidase technique. MBs (number/mm^2 area) were found in all but one GF liver in H and E sections at first operation (0.72 ± 0.20, Mean ± SEM) and decreased after withdrawal of GF (0.11 ± 0.059, P<0.025) and increased again after refeeding of GF (3.33 ± 1.38, P<0.05). No MBs were found in control livers. GGT spots were found in all GF livers. The number of spots/mm^2 area was 3.55 ± 0.56 at the first operation and decreased after withdrawal (0.49 ± 0.04, P<0.001). The percent GGT spot area/total area analyzed was 6.23 ± 1.28 at the first operation and decreased after withdrawal (0.39 ± 0.16, P<0.005) and increased again after refeeding (4.68 ± 1.19, P<0.005). No GGT spots were detected in the control livers The percent GGT spot area/total area and the number of MBs/total area in the GF livers at the third operation were closely correlated (r=0.8362, P<0.01). In double staining of GGT positive cells and MBs in the GF livers from the third operation, the number of MBs/GGT spot area (146.5 ± 42.4) was significantly larger than the number of MBs/GGT negative liver area (0.68 ± 0.22, P<0.005). The results support the conclusion that MBs are associated with GGT positive cells in GF fed mice livers. This indicates that MBs, like GGT, are a result of a phenotypic change induced by GF.

60 BILIARY BICARBONATE SECRETION: INFLUENCE OF CHLORIDE AND DIISOTHIOCYANO DISULFONIC ACID STILBENE (DIDS). MS Anwer and WGM Hardison. Department of Medicine, Division of Gastroenterology, University of California at San Diego and VA Medical Center, San Diego, California.

Biliary bicarbonate secretion is a major determinant of bile acid independent bile formation. The present study was designed to find out if chloride plays a role in biliary bicarbonate secretion, as in other epithelia, and if this information can help define the mechanism of bicarbonate secretion. Isolated rat livers, perfused with 100 ml recirculating bicarbonate buffer (pH 7.4) containing balanced electrolytes, were used in all studies. Replacement of chloride by relatively impermeable isethionate decreased both bile flow and bicarbonate secretion by 50% without affecting bicarbonate concentration in bile. These decreases were not seen when chloride was replaced by permeable nitrate. Neither ion substitution altered perfusion flow, oxygen consumption rate, or endogenous bile acid secretion rate. These results suggest that part of biliary bicarbonate secretion is dependent on the presence of a permeable anion. To determine if this dependency is through a carrier-mediated chloride/bicarbonate exchange, the effect of DIDS, a potent inhibitor of anion exchange, was studied. At 90 nmol/min, DIDS did not change bile flow but decreased both concentration and secretion of bicarbonate by 25% without affecting chloride secretion. The same change in bicarbonate secretion with DIDS occurred when isethionate was substituted for chloride. DIDS did not affect the liver viability and was concentrated in bile. This indicates that chloride/bicarbonate exchange is not a significant mechanism in bicarbonate secretion and that the effect of DIDS is independent of its known action on anion exchange. We conclude that about 50% of biliary bicarbonate secretion is dependent on the presence of a permeable anion and may involve a passive exchange of bicarbonate for permeable anions (chloride under physiological conditions) to preserve electroneutrality. The persistence of bicarbonate secretion and the effect of DIDS in the absence of permeable anions indicate that at least part of biliary bicarbonate secretion is independent of chloride.