month indicated that this might not be feasible since PBV appeared to be absorbed and transported by other vessels as well(2). In studies of local spread of dye(4) and cannulation of the lymphatics(3), the dyes appeared in blood from venous capillaries and urinary excretion of PBV was not indicative of lymphatic transport alone but of total vascular uptake from an area. There was an inverse relationship between rate and area of spread of the dye at site of injection and rate of urinary excretion. The rates and areas of local spread were greatest in those subjects with edema while urinary excretion was slowest except when they were rapidly losing fluid under treatment. This relationship suggests that decrease in urinary output of dye by these subjects was due, in large measure, to disturbed uptake by either lymphatics or capillaries. A lower PBV:PSP ratio further demonstrated that decreased renal function was not a prime factor in differences observed between subjects with edema and controls, and that diminished uptake by local vessels and/or slower transport to the general circulation were the most reasonable explanations. No correlation between sluggish uptake of PBV and other substances in edema fluid has been attempted, but the implications are apparent.

Summary. Patent Blue V, used to visualize lymphatics is excreted in urine in measurable amounts after intradermal injection of 0.1 ml 11% solution. Differences in rates of excretion between edematous and nonedematous subjects suggested that this may be useful in studying rates of uptake of interstitial substances. Control subjects excreted approximately 10% of injected dose in 24 hours, ½ of which was excreted in the first 2½ hours. Except for patients who were rapidly losing edema under treatment, rates of excretion were less for subjects with edema of various causes. Correlation of urinary excretion of PBV with that of PSP administered simultaneously but intravenously, and correlation of urinary excretion with rates and areas of local spread of intradermally injected dye, indicate that the principal factor in differences in urinary excretion is decreased uptake and transport by vessels at site of injection.

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Effect of Carbon Tetrachloride Upon Glucuronide Formation by Guinea Pig Liver.* (25682)

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Glucuronide conjugation is a detoxification mechanism important in metabolism of many endogenous and exogenous compounds possessing alcoholic, phenolic or carboxyl radicals (1). This conjugation occurs primarily in liver and involves a microsomal enzyme (glucuronyl transferase) and uridine diphosphate glucuronic acid (UDPGA)(2,3). Although it seems likely that derangements in glucuronide formation might occur in hepatocellular disease, clinical and laboratory investigations have been contradictory. Both Englert *et al.*(4), and Peterson and associates(5) found no delay in clearance of tetrahydrocortisone (THE) from plasma of patients with liver disease and concluded THE-glucuronide formation was not impaired. In contrast, Cohn and Bondy(6) measured plasma levels of THE-glucuronide in patients with hepatic disease and found only trace quantities of these conjugates, while free THE was present in plasma in significant amounts. Barniville and Misk(7) found a lower urinary glucuronic acid excretion after oral salicylamide adminis-

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TABLE I. Hepatic Glucuronyl Transferase Activity in Normal and CCl_4 Injected Guinea Pigs.

	Liver wt (g)	Glucuronyl transferase activity* Per g liver	Per total liver wt	Per 100 mg microsomal protein
Controls (9)	8.12 ± 1.52	.66 ± .11	5.25 ± .29	2.88 ± .83
Exp. (9)†	9.7 ± 2.0 ($p < .1$)	.34 ± .08 ($p < .01$)	3.14 ± .79 ($p < .01$)	2.75 ± .83
% change from control	+19	−48	−39	

* Activity is expressed in μmoles p-nitrophenol conjugated in 30 min. Results are mean values ± S.D.

† 24 hr after inj. with CCl_4, 0.1 ml/kg.

tration in patients with liver disease. Their study, however, was based solely on urinary excretions so that any derangement in renal function of their patients could have significantly influenced their results. Kunin *et al.* (8) found a decrease in rate of disappearance of chloramphenicol from plasma of patients with hepatocellular disease and suggested that this represented impairment in formation of chloramphenicol glucuronide. Streeten(9) concluded that glucuronide conjugation is not impaired in liver disease. In view of these conflicting reports we studied the glucuronide conjugating capacity and microsomal glucuronyl transferase activity in animals subjected to acute liver injury with carbon tetrachloride. Guinea pigs were selected because of their significant levels of hepatic glucuronyl transferase(3).

Materials and methods. Guinea pigs weighing 200 to 300 g were injected intraperitoneally with carbon tetrachloride (0.1 ml/kg). The animals were then fasted and sacrificed at varying intervals. Normal guinea pigs, fasted for similar periods served as controls. Animals were killed by cervical dislocations and livers perfused with 20 ml of 0.1 M KCl. A 20% liver homogenate (0.1 M KCl) was then prepared with a Potter-Elvehjem homogenizer. The microsomes were isolated by ultracentrifugation(10), and then homogenized in 3 to 5 ml of 0.1 M KCl to yield a suspension with a protein concentration of 30 to 80 mg/ml. Protein measurements were made by turbidimetric technic using Klett colorimeter (11). For studies of glucuronyl transferase activity, p-nitrophenol (Sigma Chemical Co., St. Louis, Mo.) was used as acceptor and its conversion to p-nitrophenol glucuronide measured by decrease in optical density at 400 mμ (3). UDPGA was prepared from UDPG according to Strominger *et al.*(12). The incubation medium consisted of 0.5 μM p-nitrophenol, 0.8 μM UDPGA, 10 μM Mg Cl, 50 μM Tris buffer† pH 7.4 and 0.1 ml microsomal suspension (3 to 8 mg protein) in total volume of 0.75 ml. All incubations were carried out in duplicate for 30 min at 37°C. Three ml of ethanol were then added and aliquots of 0.5 ml removed. After addition of 3 ml of 0.1 N NaOH, optical density at 400 mμ was determined in a Coleman Jr. colorimeter. The micromoles of p-nitrophenol conjugated were calculated on the basis of molecular extinction coefficient of 18 for free p-nitrophenol(3).

Results. Injection of carbon tetrachloride produced changes in gross appearance of livers, apparent as early as 2 hours. Livers instead of their normal brown color had a mottled (brown-gray) appearance, with occasional hemorrhagic areas. Microscopically there was evidence of varying degrees of necrosis and fat infiltration in the centrilobular and midzonal areas, similar to findings described by Myron(13). Cells in the periphery of liver lobule appeared normal both at 2 hours and 24 hours. Activity of glucuronyl transferase 24 hours after carbon tetrachloride injection is shown in Table I. Liver glucuronyl transferase activity was significantly reduced in experimental animals when expressed on weight basis. Thus there was a 48% decrease in activity/g of liver and 39% decrease on the basis of total liver weight. The difference between these 2 values is due to slight increase in liver weight of CCl_4 treated animals. Diminution in hepatic activity of the enzyme could be demonstrated earlier than 24

† Tris = tris (hydroxymethyl) aminomethane.

hours. A reduction in enzyme activity of 19% was observed within 2 hours and as much as 25% within 6 hours after injection of CCl_4. It should be noted in Table I (column 6) that glucuronyl transferase activity of isolated microsomes themselves was not diminished after CCl_4 treatment. The observed reduction in hepatic glucuronide conjugation capacity was therefore due to decrease in amount of microsomes that could be isolated by ultracentrifugation from liver homogenates of CCl_4 treated animals.

Discussion. These results indicate that acute liver damage by CCl_4 produced a significant reduction in total hepatic glucuronide conjugation as measured *in vitro*. Of interest is the observation that glucuronyl transferase activity in microsomes isolated from damaged liver tissue was not diminished. The histochemical experiments of Myron are pertinent in this connection(13). He observed that activity of succinic dehydrogenase, another particulate enzyme, was not diminished in cells peripheral to midzonal area of liver lobule. The present experiments also suggest that reduction in enzyme activity was on a quantitative basis and did not represent a qualitative alteration of all liver cell microsomes. Electron microscopic data will be needed to resolve this matter definitively. It is likely that structural and functional microsomal alterations may occur in clinical forms of liver disease and thus contribute further to a reduction in hepatic conjugation capacity.

Use of a phenolic substrate such as p-nitrophenol may not reflect ability of microsomes to form glucuronides of other acceptors with alcoholic, phenolic or carboxyl groups. However, to date, no conclusive evidence for more than one glucuronyl transferase has been obtained. Thus, Gunn rats with congenital deficiency of glucuronyl transferase had impairments in formation of glucuronides of such different substrates as o-aminophenol, o-aminobenzoate, menthol, bilirubin(14) and 4-methyl umbelliferone(15). Chojecki and Kern(16) also reported *in vitro* reduction of hepatic glucuronide conjugation in rats with dietary cirrhosis (protein deficiency plus alcohol) and CCl_4 poisoning. However their studies were carried out with whole liver homogenates and no comment was made on activity of isolated microsomes.

That patients with liver disease usually have elevated levels of conjugated bilirubin in serum has often been considered as evidence that hepatic glucuronide conjugation is not impaired, even in severe hepato-cellular failure. It should be recalled however, that non-glucuronide conjugates of bilirubin also occur (17) and contribute to the elevated bilirubin levels under these conditions. Furthermore, in presence of liver disease, hepatic excretory mechanisms are usually significantly reduced and impairment in hepatic excretion of conjugated bilirubin will tend to obscure a lesser interference in glucuronide formation. Under these conditions the bilirubin glucuronide formed, although this be at a reduced rate, would not be effectively excreted into the bile and allow entry of the pigment into the blood stream. Finally, it may be of significance that organs such as kidney, stomach and intestine also have glucuronyl transferase and it is possible, as suggested by Bollman(18), that these tissues contribute to total glucuronide conjugating capacity of the organism in presence of liver disease.

Summary. Intraperitoneal injection of CCl_4 causes reduction in hepatic glucuronide conjugating capacity of the guinea pig as measured by p-nitrophenol glucuronide formation *in vitro*. This reduction is apparent in 24 hours but is demonstrable as early as 2 hours after injection. Glucuronyl transferase activity of isolated microsomes however is not diminished. Therefore, reduction in hepatic glucuronide conjugation induced by CCl_4 is due to a quantitative change in microsomal transferase activity rather than to a qualitative one.

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Placenta and Salivary Gland Fluoride Transfer in the Rat.* (25683)

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Concentration of fluoride in drinking water of a pregnant rat must be approximately 10 ppm before an appreciable placenta transfer of fluoride occurs(1). In these studies fluoride transfer occurred by comparing fluoride concentration in pups whose mothers received varying levels of dietary fluoride with comparable controls receiving no fluoride. Such a technic provides for no biological estimation of effect of fluoride. It is possible that even micro-quantities of fluoride transferred through the placenta could result in some anticariogenic effects. For example, Jenkins(2) demonstrated that concentrations of fluoride as low as 1 ppm have a detectable inhibitory effect on salivary acid production. Therefore, to estimate one effect of fluoride, namely, its antisolubility, pregnant rats were given fluoride at different gestation and lactation periods and solubility of pups' molar teeth evaluated for acid solubility.

Materials and methods. Eight pregnant Sprague-Dawley strain rats divided into 4 groups were housed in pairs in raised screen cages in air-conditioned room. Fluoride-free filter paper was placed in each cage for the mother to make nests for her pups. Group I was composed of 2 pregnant rats which served as controls and received fluoride-free drinking water. Pregnant rats in Group II received 50 ppm F (as NaF) in drinking water during pregnancy. One day before delivery she was placed in separate cage, and upon delivery she and her pups were placed on fluoride-free drinking water. Group III was composed of pregnant rats which received the same concentration of fluoridated drinking water as animals in Group II, except that they received fluoride both during pregnancy and lactation, while rats in Group IV received same concentration of fluoride during lactation. All animals received the same stock non-cariogenic diet (F = 0.01 ppm). All pups were sacrificed at 30 days of age and their mandibular teeth prepared for estimation of acid solubility by methods previously described(3). Due to high concentration of fluoride used, it was possible that some fluoride could pass into the oral cavity from salivary tissues and cause a reduction in acid solubility to molar teeth. Since this effect could not be divorced from the antisolubility effect resulting from conversion by blood fluoride of a portion of the hydroxyapatite in developing rat molars to fluorapatite, a further study was planned to investigate the effect of fluoride availability in

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