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Bilirubin liver
hepatic activity UDP glucuronyl transferase

Attempts to Induce Hepatic Uridine Diphosphate Glucuronyl Transferase in Genetically Deficient Gunn Rats by Grafting of Normal Liver Tissue*

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Extract

Liver from normal Wistar rats was grafted into the liver of homozygous Gunn rats which are deficient in UDP glucuronyl transferase (UDPGT) (bilirubin) activity. After 3 months, UDPGT activity (bilirubin) remained absent in microsomal suspensions of liver from recipient rats and no bilirubin glucuronide was detected in their bile.

Speculation

We were unable to confirm previous reports suggesting that transplantation of segments of normal rat liver into Gunn rat liver resulted in amelioration of jaundice because of UDPGT activity in the recipient liver.

Homozygous Gunn rats lack UDPGT activity with bilirubin as a substrate resulting in chronic nonhemolytic unconjugated hyperbilirubinemia (1). UDPGT activity is not enhanced in Gunn rats after treatment with phenobarbital (3).

Mukherjee and Krasner (7) transplanted small pieces of normal Wistar rat liver into the liver of Gunn rats and observed reduction in serum bilirubin concentations and virtually normal hepatic UDPGT activity (bilirubin) 3 months after the grafting procedure. Because these results have important implications for treatment of several human deficiency diseases, we repeated the experiment using similar animals and procedure. In addition, bile duct cannulation was performed in order to study the effect of liver grafting on the chemical nature of bile pigments.

Table 1. *Serum bilirubin concentrations in recipient and sham-operated Gunn rats*[1]

	Recipient Gunn rats							Sham-operated Gunn rats			
	A	B	C	D	E	Mean		F	G	H	Mean
Days after transplantation											
0	4.6	5.5	4.6	4.1	5.8	4.9 ± 0.7	NS[2]	5.6	5.2	5.2	5.3 ± 0.2
21	3.7	4.6	2.8	2.8	3.2	3.4 ± 0.8	NS	3.2	4.5	2.6	3.4 ± 1.0
42	3.1	4.4	2.8	2.7	2.9	3.1 ± 0.7	NS	3.1	2.8	2.6	2.8 ± 0.6
69	3.6	3.8	2.9	3.4	3.7	3.5 ± 0.4	NS	4.0	3.8	4.2	4.0 ± 0.2
90	3.7	4.1	3.8	3.5	5.0	4.0 ± 0.6	NS	6.2	2.3	4.2	4.2 ± 2.0
99–113	2.7	3.3	4.2	3.1	[3]	3.3 ± 0.8	NS	5.3	[3]	2.8	4.0 ± 1.8

[1] See text for further details.
[2] Statistically nonsignificant difference from control at $P < 0.05$.
[3] Death.

Table 2. *Hepatic UDP glucuronyl transferase and UDP glycosyl transferase activities (bilirubin) in recipient and sham-operated GUNN rats*[1]

	Recipient Gunn rats						Sham-operated Gunn rats			
	A	B	C	D	E	Mean	F	G	H	Mean
Enzyme activity										
UDP glucuronyl transferase	0	0	0	0	[2]	0	0	[2]	0	0
UDP glycosyl transferase	0	0	0	0	[2]	0	0	[2]	0	0

[1] Mean enzyme activities in normal rat liver were 320 ± 25 nm/g·20 min and 68 ± 9 nm/g·20 min, respectively.
[2] Death.

MATERIALS AND METHODS

Homozygous Gunn rats of either sex, weighing 150–250 g, were donated by Dr. Joseph Krasner, State University of New York Medical Center, Buffalo, New York. Their identity was confirmed by detection of unconjugated hyperbilirubinemia. Donor Wistar rats of either sex and of similar weight were obtained from the Animal Institute, Albert Einstein College of Medicine.

Donor Wistar rats and recipient Gunn rats were matched for sex and weight and were anaesthesized with ether. With an uterine punch biopsy forceps, a small piece of recipient liver was removed and replaced by a small piece of donor liver, identical in size. Depending on the size of the liver, three to four pieces of liver tissue were grafted in different lobes of recipient liver. The amount of transplanted liver tissue ranged from 180–250 mg/rat. Three rats were sham-operated; the liver was punched in one rat and the two other rats received two pieces of liver from each other. After 3.5 months, each rat was anesthesized with 2.5–5.0 mg Nembutal subcutaneously for 35 hr. The common bile duct was cannulated with polyethylene tubing no. 10. Rats were kept in a heated cage at 42° while bile was collected on ice in the dark for 2 hr after which the rat was killed; the liver was removed and perfused with 40–50 cc ice-cold saline, and a biopsy of liver from the graft areas was placed in 10% formaldehyde for histologic examination. The liver was immediately frozen and stored in liquid nitrogen (−180°) for 1–3 days. When necessary, bile was also stored −180° before chromatographic study of pigments was performed.

Microsomal suspensions were prepared by differential centrifugation of liver homogenates (4) and were stored at −15° overnight or in liquid nitrogen (−180°) for 1–3 days. Enzymatically catalyzed glucuronidation and glucosidation of bilirubin were studied. A digitonin-activated liver microsomal suspension, 0.2 ml, was added to 0.8 ml Tris buffer, 0.1 M, pH 8.1, containing 33 mM $MgCl_2$ and 3 mM UDP glucuronic acid (UDPGA) or UDP glucose (UDPG). Control tubes lacked UDPGA or UDPG. Microsomal suspension were activated by mixing 3:1 with digitonin suspension (25 mg/ml). The reaction was started by adding, 0.2 ml bilirubin solution and was stopped after 20 min. Bilirubin solution was made by dissolving 4.4 mg bilirubin in 0.5 ml 0.05 M NaOH, and diluting this solution with phosphate buffer, pH 7.8 (1:30, v/v). The reaction was performed in a dark room at 37° in a shaking waterbath. After incubation, the mixtures were submitted to the diazo reaction described by Van Roy and Heirwegh (8). Azo pigments were extracted with methylpropylketone-*n*-butyl acetate (17:3) and color in the extract was determined spectrophotometrically at 530 mM (conjugated bilirubin was calculated assuming E_{530} 44.4 10^3 M^{-1} cm^{-1}) (8). Protein was determined by the method of Lowry *et al.* (5) with bovine serum albumin as reference. Bilirubin in bile was analyzed according to Heirwegh *et al.* (2). Blood samples, 50 μl, were taken from the orbital sinus every 3 weeks during which these rats were under light ether anesthesia. Total serum bilirubin concentrations were determined according to the technique of Evelyn and Malloy (6).

RESULTS

Table 1 presents serum total bilirubin concentrations which were determined every 3 weeks after the grafting procedure. Serum total bilirubin concentrations of recipient rats were not significantly lower (Student's *t*-test) than those of sham-operated rats.

Liver microsomal suspensions from recipient and sham-operated rats lacked UDPGT (bilirubin) or UDP glycosyl transferase (bilirubin) activities. Microsomal suspensions from control Wistar rats prepared by the same procedure had both enzyme activities (Table 2).

Thin layer chromatography of azo pigments from bile of recipient and sham-operated rats showed α_0, α_2, and β_2 bands, but no γ band (glucuronide-containing band).

The transplantation site in recipient livers was recognized by light microscopy as a cleft between the liver lobes which was probably caused by necrosis of transplanted tissue. The transplantation site contained macrophages laden with bile pigments, plasma cells intermixed with connective tissue, newly formed capillaries, and structures resembling bile ductules. In most animals, there was a reactive inflammatory granuloma in the area adjacent to the transplantation site. In one sham-operated rat which received liver biopsies from another Gunn rat, the transplantation site contained proliferated bile ducts with small islands of hepatocytes and macrophages intermixed with lymphocytes.

After the grafting procedure, all rats remained healthy and gained weight.

DISCUSSION

Transplantation of normal rat liver into Gunn rat liver was associated with a decrease in total serum bilirubin concentration (Table 1). This is probably due to factors other than the grafting of Wistar rat liver tissue, because the serum bilirubin concentrations similarly decreased in sham-operated rats as well.

Histologic examination revealed that the grafted tissue disappears, as reported by Mukherjee and Krasner (3); however, UDPGT activity (bilirubin), UDPG glucosyl transferase activity (bilirubin), and bile analysis failed to reveal evidence of bilirubin glucuronide or bilirubin glucoside formation.

The operative techniques were identical. The total amount of grafted liver tissue was 180–250 mg whereas, in the earlier study (3), transplanted plugs of liver tissue weighed 200–250 mg.

SUMMARY

Grafting of liver from normal Wistar rats into the liver of homozygous Gunn rats did not result in hepatic UDPGT activity in recipient rats or in the presence of bilirubin glucuronide in their bile.

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Glucose newborn
marasmic kwashiorkor protein
marasmus

Metabolic and Hormonal Responses to a Protein-Glucose Meal in Normal Infants and in Marasmus and Marasmic Kwashiorkor

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Extract

Blood sugar and plasma free fatty acids (FFA), immunoreactive insulin (IRI), and growth hormone (GH) responses to a protein-glucose meal were determined in normal infants and those with marasmus and marasmic kwashiorkor. Among the normal subjects, fasting blood sugar (BS), peak BS and IRI, and the IRI/BS ratio tended to decrease as age increased. Peak IRI was at least 13 μU/ml above fasting in 21 of 24 infants. Fasting GH levels were high, 38.5 ± 13.6 (SD) and 26.3 ± 14.0 ng/ml, in the two youngest groups (under 1 year) and were comparable with those of the late newborn period. They were slightly lower, 20.8 ± 22.1, in those 12.5–18.5 months of age. Suppressions of FFA and subsequent rebounds were in close temporal relation to BS and IRI peaks and lows, but not the GH levels. GH was promptly suppressed by the meal, and in most infants secondary elevations were seen.

Untreated marasmic infants had normal or low BS, correspondingly normal or low IRI, markedly elevated FFA (1,821 ± 588 μEq/liter), and GH levels comparable with those of the control subjects. There was some delay in BS elevation and disappearance and poor insulin release after the meal, with only two of nine having elevations of at least 13 μU/ml. The BS elevations and IRI responses, however, were adequate to block FFA release. GH levels were poorly suppressed by the meal but some infants had further elevations, possibly in response to protein. After partial rehabilitation, fasting BS and FFA and BS elevations after the meal were normal. A slight improvement in insulin release was apparent. Fasting GH levels and responses to the meal were normal.

Fasting, minimally treated children with marasmic kwashiorkor (MK) had normal or low BS, normal or low IRI, normal FFA, and probably normal GH levels. There was considerable delay in BS elevation, moderately delayed glucose disappearance, and very poor or unmeasurable insulin release after the test meal; FFA and GH were poorly suppressed. After partial rehabilitation, fasting BS was