Some of the gametes are incompletely separated (Fig. 2c, arrows) and are organized into multilobed masses of foraminiferal cytoplasm containing two or more nuclei. These probably correspond to the clumps of cytoplasm seen with the light microscope. Higher magnifications (Fig. 2d) show that the incipient, undivided gametes are derived from parent cytoplasm. The small vacuoles filled with translucent masses of fibrillar material are characteristic of parent cytoplasm (*2*). Within the cytoplasmic clumps (Fig. 2d) the nuclei are being separated from the cytoplasm and possess double membranes.

The gametes are evidently in their terminal stage of development. Sequential sections were taken through the entire specimen, but no residual parent nucleus was observed. We suspect that the single large nucleus of the parent cell (*2*) had already undergone repeated division to yield the numerous nuclei producing the gametes. This is consistent with many observations in benthic Foraminifera (*3*), in which the gamete-producing (gamont) cell contains a single large nucleus, whereas the agamont stage is multinucleate.

Flagella are present in various stages of development. We have observed no more than two flagella in cross sections of individual gametes. The cytoplasm of the gametes contains tubular mitochondria and single-membrane organelles with a granular matrix and a dense inclusion body. These organelles resemble peroxisomes. Golgi bodies and occasional lipid droplets were observed. Biflagellated gametes have been reported in several species of benthic Foraminifera (*4–6*). The cytological events during gametogenesis in planktonic Foraminifera are similar to those seen in some species of benthic Foraminifera—*Myxotheca* sp. (*4*), *Boderia turneri* (*5*), and *Nemogullmia* sp. (*6*).

We have estimated the total number of gametes within the shell by counting the number in a single section of the specimen. There were 4295 gametes in a cross-sectional area of 5×10^4 μm^2 (Fig. 2b). The section's volume was determined by assigning a thickness equivalent to the mean diameter of the gametes (5 μm). This volume was divided by the number of gametes to yield a volume-to-gamete ratio of 60 μm^3 per gamete. The volume of the entire shell was 17×10^6 μm^3, which, when divided by the foregoing ratio of volume to gamete, yielded the total number of gametes in the shell (2.8×10^5 gametes). The total number of gametes produced exceeds this estimate as a sizable proportion of them had already been released.

The combined evidence from light microscopy and electron microscopy confirms that sexual reproduction (gametogenesis) occurs in the life cycle of these planktonic Foraminifera. The large number of gametes released from the shell suggests that these species are gametogamous (fusion outside the shells of parent organisms) and not gamontogamous (fusion within the paired shells of parent organisms) (*3*). Hence we know that one part of their life cycle is a sexual phase, but we have not yet observed fusion of gametes. We do not have evidence as yet that there is asexual reproduction, although multiple nuclei have been reported in *Globigerina bulloides*, which was presumed to be an agamont stage (*7*). Further observations of reproduction in isolated cells and in cultures of two or more gamonts of the same species are required to determine whether planktonic Foraminifera are monoecious or dioecious. Such studies may allow us to deduce their complete life cycle.

The prodigious number of gametes in planktonic Foraminifera is frequently observed in lower organisms that release their gametes into the environment. Such large numbers would be expected if the foraminiferal species were dioecious. Although these organisms are abundant in the ocean, they are so widely spaced that only the production of myriads of gametes would ensure fusion of sufficient numbers to yield enough offspring to maintain the population. If sexual reproduction occurs in planktonic Foraminifera, as indicated by our evidence, this would allow genetic diversification and may help to explain their great abundance in widely diversified habitats.

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Hepatocellular Transplantation for Metabolic Deficiencies: Decrease of Plasma Bilirubin in Gunn Rats

Abstract. *A sustained decrease of plasma bilirubin concentrations occurred in homozygous recessive Gunn rats lacking the enzyme uridine diphosphate glucuronyltransferase following infusion into the portal vein of hepatocytes from heterozygous nonjaundiced Gunn rats possessing the enzyme. Transplantation of cells capable of continuous enzyme production could be an effective mode of therapy for congenital enzyme deficiency diseases.*

There is currently no effective treatment for many congenital enzyme deficiency diseases. Enzyme infusion therapy has only a temporary effect (*1*), and whole organ transplantation is associated with considerable morbidity and mortality (*2*). An excellent model of congenital enzyme deficiency disease is provided by the Gunn rat, a noninbred mutant Wistar rat. The homozygous recessive Gunn rat has a deficiency of the liver enzyme uridine diphosphate glucuronyltransferase (UDPGT), which is necessary for the conjugation of bilirubin to bilirubin diglucuronide. Affected animals are jaundiced at birth, and all bilirubin is unconjugated. Heterozygous animals have decreased enzyme levels but are unable to maintain a normal concentration of plasma bilirubin unless they are challenged with a bilirubin load.

Previous reports have described the lowering of serum bilirubin in jaundiced Gunn rats by subcutaneous implantation of rat hepatoma cells (*3*) and by direct implantation of pieces of Wistar rat liver into punch biopsy sites in the livers of Gunn rats (*4*). Results of these studies stimulated us to develop a technique of hepatocellular transplantation that would be easily applicable to human congenital enzyme deficiency disease. Two

series of experiments in Gunn rats are described.

In the first series, 25 homozygous recessive jaundiced Gunn rats weighing 280 to 350 g were divided into four groups. Total bilirubin concentrations were measured three times preoperatively in all animals, determinations being made with the AO No. 10200 bilirubinometer (American Optical). Group 1 (eight rats) received a hepatocellular transplant via the portal vein; heterozygous Gunn rats were used as cell donors. Donor hepatectomy was performed and the liver was immediately sliced and rapidly cooled to 4°C in Hanks solution. Two grams of liver were passed through a 100-mesh stainless steel screen and washed three times in Hanks solution at 4°C. The hepatocytes were resuspended in 5 ml of Hanks solution for transplantation. Histologic study of the preparation showed solitary hepatocytes and aggregates of hepatocytes, as well as free nuclei. More than 90 percent of the hepatocytes excluded trypan blue.

Recipients received 100 units of sodium heparin (Upjohn) subcutaneously 1 hour preoperatively. At the time of operation the animals were anesthetized with ether, and a midline incision was made. The portal vein was cannulated with a 23-gauge butterfly needle and the hepatocytes, suspended in Hanks solution, were infused during a 5-minute period. After infusion the needle was withdrawn and hemostasis was obtained by pressure over a piece of oxidized regenerated cellulose (Johnson & Johnson) for 2 minutes. In preliminary histological studies, examination with a light microscope showed that the transplanted hepatocytes localized in the recipient liver sinusoids.

Group 2 (eight rats) underwent a similar operative procedure; however, only Hanks solution was infused into the portal vein. Group 3 (five rats) received a portal vein infusion of a suspension of spleen cells from heterozygous Gunn rats. Suspensions of spleen cells were prepared in a manner similar to that described above for the liver. In group 4 (four rats) heterozygous hepatocytes were dispersed into the peritoneal cavity. Postoperatively, all animals received 1 ml of rabbit anitserum to rat lymphocytes (Microbiological Associates) subcutaneously 6 days per week for 6 weeks and 1 ml per week thereafter. Plasma bilirubin concentrations were determined daily for 2 weeks and twice weekly thereafter; the mean concentrations for each group are given in Table 1.

Bilirubin concentrations rose in the immediate postoperative period in all rats.

Table 1. Preoperative and postoperative bilirubin concentrations in homozygous jaundiced Gunn rats subjected to heterozygous hepatocellular transplantation via the portal vein (group 1), Hanks solution infusion via the portal vein (group 2), heterozygous spleen cell infusion via the portal vein (group 3); and heterozygous hepatocyte disperson intraperitoneally (group 4). S.E.M., standard error of the mean.

Time	Mean plasma bilirubin ± S.E.M.			
	Group 1 (N = 8)	Group 2 (N = 8)	Group 3 (N = 5)	Group 4 (N = 4)
Preoperative	10.0 ± 0.5	9.3 ± 0.6	9.1 ± 0.6	8.1 ± 0.6
Postoperative				
Week 1	11.3 ± 1.0	9.7 ± 0.7	10.2 ± 0.9	9.1 ± 1.3
Week 2	8.6 ± 1.0	8.9 ± 0.7	7.2 ± 1.3	8.3 ± 0.9
Week 3	6.5 ± 0.6*†	8.1 ± 0.7	7.3 ± 0.6	8.7 ± 1.5
Week 4	6.0 ± 0.5‡	8.4 ± 0.4	7.4 ± 0.3	7.6 ± 0.9
Week 6	5.5 ± 0.8‡	7.6 ± 0.4	7.9 ± 0.2	7.0 ± 0.7
Week 8	5.9 ± 0.8‡	6.7 ± 0.3§	7.1 ± 0.6	8.3 ± 1.4
Week 10	7.0 ± 0.7*	7.7 ± 0.6	8.9 ± 0.4	8.3 ± 0.9
Week 12	6.4 ± 0.1§	9.0 ± 0.7	8.7 ± 0.2	7.8 ± 0.7

*Significance of difference from the preoperative value (paired t-test). †$P < .025$. ‡$P < .005$. §$P < .01$.

In the rats receiving hepatocellular transplants via the portal vein (group 1) concentrations of bilirubin fell gradually, and at 3 weeks postoperative values were significantly lower than preoperative values (paired t-test). This effect persisted for the duration of the study and has lasted for 30 weeks in two animals. Postoperative concentrations of bilirubin in the other animals (groups 2, 3, and 4) were variable but not significantly different from preoperative values except in group 2 at the eighth postoperative week (paired t-test). This decrease was only temporary. The mean decrease in concentrations of plasma bilirubin at each week was significantly greater in group 1 at all times ($P < .05$).

In a second series of experiments, heterozygous Gunn rat hepatocytes were infused into the portal vein of eight homozygous rats. In this series of experiments, both conjugated and unconjugated plasma bilirubin was measured according to the alkaline azobilirubin blue reaction method as modified by Tchida *et al.* (*5*). Again, a significant reduction in total plasma bilirubin occurred (preoperative, 10.45 ± 1.58 mg/100 ml; 5 weeks postoperative, 5.5 ± 2.1) ($P < .01$ by paired t-test) in these animals. All of the decrease in plasma bilirubin concentration after transplantation was in the unconjugated fraction. There was no measurable change in circulating conjugated bilirubin, suggesting that newly conjugated bilirubin was either excreted in the bile or rapidly cleared by the kidneys. Bile duct cannulation with 24-hour collection of bile could not be accomplished in animals that had received transplants because of the previous surgery within the porta hepatis. Currently there is no quantitative laboratory technique that can accurately measure urinary excretion of bilirubin (*6*).

Demonstration of increased levels of UDPGT in homozygous Gunn rats following hepatocellular transplantation of heterozygous cells would provide further indirect evidence that conjugation of bilirubin is responsible for the decrease in total bilirubin concentrations. Direct measurement of the levels of UDPGT in hepatic tissue needs to be made. However, it may be difficult to demonstrate a difference in enzyme levels between rats that have received transplants and those that have not received them, since each individual transplanted cell will contain a relatively small quantity of the enzyme, and these cells or clumps of cells will have been dispersed throughout the liver.

Allogeneic hepatocellular transplantation via the portal vein is capable of lowering concentrations of plasma bilirubin in congenitally jaundiced Gunn rats receiving small amounts of immunosuppression. The effect could not be duplicated by spleen cell infusion into the portal vein, which suggests that liver cells are required to supply the missing enzyme; nor could the effect be duplicated by intraperitoneal dispersion of hepatocytes, indicating that the intraportal route may be required. Portal blood "hepatotropic factors" have previously been shown to be necessary for liver allograft survival (*7*). In fact, if portal blood is diverted away from the host liver and through the allograft, transplant atrophy is prevented but host liver atrophies (*8*). Failure to observe a significant effect when hepatocytes were dispersed intraperitoneally (in this study) and when hepatocyte or liver slice implantation was carried out to subcutaneous tissue, meso-

testis, anterior chamber of the eye, or to lymph nodes [in other studies (*9*)] may be due to a lack of hepatotropic factors in the blood perfusing these sites.

The portal vein route has previously been utilized successfully for transplantation of islets of Langerhans for the amelioration of diabetes in this and other laboratories (*10, 11*). Less islet tissue is required for cure when the portal vein route is used than when transplantation to other sites is performed. In addition, allogeneic transplantation has been performed with minimal immunosuppression, and it has been suggested that the portal vein and liver may be immunologically privileged sites (*11*). Data from studies on liver transplantation or from studies in which other organ allografts (heart, kidney) have been transplanted with venous drainage to the portal circulation support this hypothesis (*12, 13*). The mechanism of prolonged allograft survival is unknown, but it has been proposed that the liver inactivates or alters histocompatibility antigens (*13, 14*). In preliminary studies in our laboratory, however, hepatocyte transplantation via the portal vein without immunosuppression was not associated with lowering of recipient plasma bilirubin concentrations.

Bilirubin concentrations did not completely return to normal in any of the homozygous animals receiving intraportal hepatocytes, possibly because of the decreased enzyme levels in the hepatocytes obtained from the heterozygous animals used as donors, or perhaps because of insufficient immunosuppression. In our experiments, heterozygous Gunn rats were chosen as donors to minimize histocompatibility differences. In very similar experiments, performed recently and independently, Groth *et al.* (*15*) have observed a transient but unsustained fall in concentrations of bilirubin in nonimmunosuppressed homozygous Gunn rats infused via the portal vein with pure allogeneic hepatocytes (Wistar donors). Improved immunosuppression of homozygous recessive Gunn rats receiving a hepatocellular transplant from allogeneic donors with normal liver enzyme levels may result in lowering the concentration of plasma bilirubin of recipient animals even further.

Enzyme replacement therapy by cells capable of continuous enzyme production could be an effective mode of treatment of human enzyme deficiency diseases. The successful transplantation of UDPGT-synthesizing hepatocytes to Gunn rats lacking this enzyme provides a rational basis for such treatment. Technical application of this model would not be difficult. Only a small midline incision is necessary to isolate a loop of bowel and cannulate a mesenteric vein. Hepatocytes could be slowly infused and portal pressure measured before, during, and after infusion. The entire procedure could be performed with a local anesthetic. With current advances in prenatal diagnosis of congenital enzyme deficiency disease, it is possible to contemplate prenatal therapy. Enzyme-producing cells could be infused in utero or via the umbilical vein at birth. In utero transplantation could even result in tolerance to the transplanted cells, thus obviating the need for immunosuppression.

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Gnathotrichus sulcatus: Synergistic Response to Enantiomers of the Aggregation Pheromone Sulcatol

Abstract. *In laboratory and field bioassays,* Gnathotrichus sulcatus *responded to sulcatol (6-methyl-5-hepten-2-ol) only when both enantiomers were present. Response was greater to racemic sulcatol than to a mixture (65 : 35) of* S-(+) *and* R-(−) *enantiomers, the naturally occurring isomeric ratio. Enantiomer-specific active sites on receptor proteins in the same or different cells are implicated.*

The ambrosia beetle, *Gnathotrichus sulcatus* Le Conte (Coleoptera: Scolytidae) produces its aggregation pheromone, sulcatol (6-methyl-5-hepten-2-ol), in a mixture (65 : 35) of *S*-(+) and *R*-(−) enantiomers (*1*). We report that *G. sulcatus* responds to sulcatol only when both enantiomers are present, the first demonstration of synergistic response by insects to enantiomers of an attractive pheromone.

The ability of insects, particularly Lepidoptera, to distinguish between geometric isomers of volatile pheromones is well established (*2*). Only recently has research disclosed that insects are capable of olfactory discrimination between optical isomers. Oviposition by female spruce budworm moths, *Choristoneura fumiferana* (Clem.), is stimulated by exposure to *S*-(+)-α-pinene, but not to its *R*-(−) enantiomer (*3*). The ant, *Atta tex-*