genesis are the same as in the diseases in man.

We have been unable to transmit either of the two human diseases to nonprimate hosts. We would like to infect smaller laboratory animals, but we were unsuccessful in our attempts to infect more than 20 strains of inbred and randomly bred mice and mice immunosuppressed before and after inoculation with cortisone, antilymphocyte serum, cyclophosphomide, and x-irradiation. Neonatally thymectomized and splenectomized mice have also failed to develop disease. We have not been able to confirm an earlier report that kuru was transmitted to mice that were x-irradiated after inoculation (*8*).

Other small laboratory animals, such as rats, Syrian hamsters, guinea pigs and rabbits, hens, ducks, and dogs have not been susceptible to either kuru or C-J disease. However, we have now one domestic cat, inoculated 30 months previously with brain tissue from a C-J patient, that developed the disease with accompanying pathognomic lesions of neuronal vacuolation, glial proliferation, and spongiosis in the cerebral cortical gray matter. Thus, the host ranges of the viruses of kuru and C-J disease have been extended, and there is reason to hope that the agents may eventually be adapted to mice or other small and inexpensive laboratory animals and that the disease will then have a shorter incubation period.

Note added in proof: Since this report was written we have confirmed the transmission of kuru and C-J disease to additional nonhuman primates. Histopathological lesions of kuru have been observed in the absence of clinical kuru in a gibbon ape and a mangabey which died of intercurrent illnesses 9 months and 2 months, respectively, after inoculation. Clinically recognizable C-J disease has been diagnosed and confirmed histologically in rhesus monkeys (65 to 66 months of incubation), a stump-tailed monkey (61 months of incubation), and a cynomolgus monkey (61 months of incubation); and early histopathological lesions of C-J disease have been observed in a mangabey and a pig-tailed monkey, both of which died of intercurrent illnesses 2 months after inoculation. Attempts to serially propagate kuru and C-J disease in the aforementioned species of animals have already been initiated.

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Induction of an Enzyme in Genetically Deficient Rats after Grafting of Normal Liver

Abstract. *Tissue from normal rat livers was grafted onto the livers of rats that were genetically deficient in bilirubin uridine diphosphate glucuronyltransferase activity. Twelve weeks after the grafting operation, the liver of the recipient rats had bilirubin uridine diphosphate glucuronyltransferase activity.*

The induction or transfer of enzyme activity would be an attractive mode of therapy for some of the enzyme deficiency diseases. Rugstad and his co-workers (*1*) have shown that bilirubin uridine diphosphate (UDP) glucuronyltransferase (E.C. 24.1.17) activity could be transferred into enzyme deficient homozygous Gunn rats. This was accomplished by subcutaneous transplantation of a clonal strain of rat hepatoma cells (*2*). However, transplantation of a viable neoplastic tissue holds little promise of a direct clinical application. In these studies, we grafted small amounts of normal Wistar rat liver into the liver of its enzyme deficient mutant strain, the homozygous Gunn rat, and measured the effects of the grafts upon bilirubin UDP glucuronyltransferase activity and serum bilirubin concentrations in the Gunn rats.

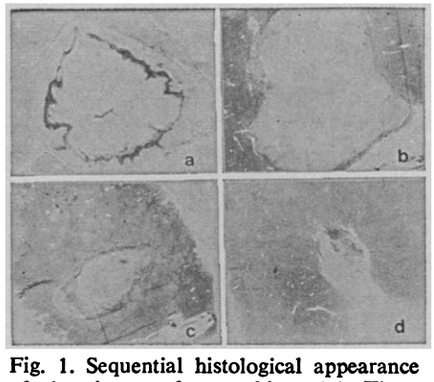

Fig. 1. Sequential histological appearance of the tissues after grafting. (a) Three weeks after the graft procedure. The pale graft is surrounded by fibrotic tissues and some inflammatory cells. (b) Six weeks after the graft procedure. The graft seems to be near the periphery. Fibrotic tissues cannot be seen. (c) Nine weeks after grafting. The size of the tissues has diminished. (d) Twelve weeks after grafting. No visible graft tissue can be identified. The location of the graft can be identified by the cleft.

Homozygous Gunn rats were identified by their yellow color shortly after birth. Their ears were punched for subsequent identification. They were weaned at 21 days of age, a blood sample was taken from the orbital sinus cavity, and serum bilirubin concentration was determined by absorbance at 450 nm (Cary model 15 recording spectrophotometer). Elevated bilirubin concentrations in homozygous animals indicated that these rats were deficient in UDP glucuronyltransferase activity. At 6 to 8 weeks, these rats were checked again for serum bilirubin concentration and were used as recipients in the grafting experiments. Donor and recipient rats were matched for age and sex, then simultaneously anesthesized by an intraperitoneal injection of 1 ml of an aqueous solution of tribromoethanol (2 percent). Approximately 5 percent of the liver of each Gunn rat was removed with a uterine punch biopsy forceps. We replaced the punch biopsies of each Gunn rat liver with identical sections from the liver of a normal Wistar rat donor.

We transplanted the donor liver from five biopsy sections into each recipient rat liver. Each plug of liver tissue from the Gunn rats was 5 mm in diameter and weighed from 250 to 300 mg. The liver samples from the Gunn rats and the remaining donor livers were put into separate weighed beakers and placed on ice for the subsequent determination of bilirubin UDP glucuronyltransferase activity. Gross morphological and histological changes in the grafted tissue were recorded every 3 weeks for 12 weeks. Four animals were killed sequentially every 3 weeks after surgery. The histological appearance of the transplanted liver is shown in Fig. 1. After 12 weeks, the transplanted livers showed no grossly visible trace of the grafted tissue and, histologically, no distortion of structure could be detected.

Rat livers were prepared as 33 percent homogenates as follows. To the weighed livers, two volumes of 0.15*M* KCl solution was added; the mixture was homogenized (Potter homogenizer with Teflon pestle). The homogenates were centrifuged at 9000*g* for 20 minutes at 4°C (Sorvall centrifuge); the supernatant was then centrifuged at 104,000*g* for 1 hour (Spinco model L2-65B ultracentrifuge). The pellet which contained the microsomes was suspended in 0.1*M* KCl, and homogenized to produce a uniform mixture; bilirubin glucuronyltransferase activity was then assayed (*3*).

Enzyme activity was measured by adding 0.1 ml of microsomal suspension to 0.15 ml of a solution containing 5×10^{-8} mole of bilirubin (the substrate); 1.5×10^{-5} mole of tris-HCl buffer, *p*H 8.0; 2×10^{-7} mole of β-mercaptoethanol; 8×10^{-6} mole of $MgCl_2$, and 5 mg of bovine serum albumin. Both test and control tubes were incubated at 37°C for 10 minutes. The reaction was started by adding 0.02 ml of 0.02*M* uridine diphosphoglucuronic acid (4×10^{-7} mole) to the test sample and 0.02 ml of water to the control tube. Incubation was continued for 30 minutes in a covered incubator. The entire procedure was carried out in a darkened room, and tubes containing bilirubin were protected from unnecessary light in order to limit photodegradation of bilirubin. The reaction was terminated by the addition of a mixture of ethyl acetate and lactic acid (5 : 8). The amount of bilirubin glucuronide formed was determined by the method of Weber and Schalm (*4*).

Total bilirubin in serum from the Gunn rat was determined by measuring absorbance at 450 nm and compared to a standard solution (Dade Reagents obtained from Scientific Products).

Table 1. Measurements of bilirubin and UDP glucuronyltransferase activity before and 12 weeks after the graft procedure. We measured UDP glucuronyltransferase activity separately in the left and right lobes of rats 6 and 7. The right lobe received grafts; the left lobe did not receive them.

Rat	Plasma bilirubin (mg/100 ml)		Enzyme activity in 30 minutes (nmole/mg protein)		
	Before	After	Before	After	
1	3.8	0.5	0.00	0.17	
2	5.9	1.0	0.00	0.54	
3	4.8	0.9	0.00	0.38	
4	3.7	1.3	0.00	0.66	
5	4.2	0.8	0.00	0.55	
				Left	*Right*
6	6.0	0.7	0.00	3.77	4.81
7	6.8	0.8	0.00	0.85	1.19

Bilirubin UDP glucuronyltransferase activity was lacking in liver microsomes from the homozygous Gunn rat. Serum bilirubin concentrations (4 to 6 mg per 100 ml of serum range) in these animals were relatively high compared to normal rats (less than 1 mg percent). These animals were killed 12 weeks after the grafting operation. Serum bilirubin concentrations were lower, and the liver microsomes showed bilirubin glucuronyltransferase activity (Table 1). Although normal Wistar rats (donors) had a mean enzyme activity of 2.9 nmole of bilirubin glucuronide formed in 30 minutes per milligram of microsomal protein, the individual variation was large. In order to determine whether or not the increased enzyme activity was present in only the grafted liver lobes or throughout the entire liver, we also determined the enzyme activity in the left lobe, which received the normal grafted tissue, and in the right lobe, which did not receive any transplant. No significant differences in enzyme activity in the separate liver lobes were observed (Table 1). In Gunn rats which had received only anesthetic and in those which were sham-operated (punched but no tissue taken out), no significant decrease in serum bilirubin concentrations nor increase in enzymic activity was observed. Transfer of liver from one Gunn rat to another Gunn rat did not alter the enzyme activity, nor did transplanting Gunn rat liver to a normal liver eliminate or decrease enzyme activity below normal levels.

These results demonstrate that it is possible to induce or transfer bilirubin UDP glucuronyltransferase activity in liver microsomes by grafting normal rat liver to Gunn rat liver.

Rugstad *et al.* (*5*) have shown that rat hepatoma cells have the ability to conjugate bilirubin in vitro. This function is retained after the cells are transplanted subcutaneously into a Gunn rat thereby providing the recipient animal with a normal pathway for bilirubin excretion. However, the enzyme activity is not induced in other tissues. Even if it were, the result would be a hepatoma that would limit the potential for survival of the recipient animals.

We did not investigate the viability or fate of the individual normal liver cells after grafting. Migration and eventual proliferation of selected cells from the normal tissue throughout the recipient Gunn rat liver could account for the appearance of enzyme activity after grafting. It is also possible that a genetic transformation takes place in the deficient liver through incorporation of informational macromolecules from the grafted liver cells. Another possibility is the presence of a stable diffusible derepressor substance in the normal liver tissue which enters the Gunn rat liver cells and enables the totally inactive genetic locus for UDP glucuronyltransferase in the Gunn rat liver to function more efficiently.

The Crigler-Najjar syndrome is the human counterpart of the hyperbilirubinemia in the Gunn rat (*6*). There have been several approaches to treatment of this disease, such as barbiturate administration (*1*, *7*, *8*) and phototherapy (*9*) in man and transplantation of functional neoplastic tissue in animals (*1*) but the effects have been at best only partially beneficial. Our experiments demonstrate a novel biological method for the induction of enzyme activity in the Gunn rat. Although this procedure holds promise for direct clinical application, extrapolation of data from the animal experiments to humans must be cautiously evaluated.

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Gelatin: A Poor Substrate for a Mammalian Collagenase

Abstract. *A rabbit tumor collagenase was purified more than 5000-fold. In this form it degrades native collagen in helical conformation at 37°C, pH 7.6, into two fragments, but it had little capacity to cleave gelatin, an indication of the importance of higher-order structure of substrate for this enzyme in pure form. It is likely that, in vivo, enzymes other than collagenase degrade gelatin polypeptides produced by primary collagenolysis.*

Collagen in native conformation is composed of three helical polypeptide (α) chains, each of which is wound around a common axis to give a coiled-coil structure (*1*). It is believed that in vertebrates collagen is degraded by specific enzymes (*2*). These collagenases cleave across the three polypeptide chains at a point three-fourths distant from the amino terminus, yielding two fragments. The data of Sakai and Gross suggest that at physiologic temperatures the two fragments spontaneously denature, and as nonhelical gelatin polypeptides they are susceptible to further degradation by this same collagenase or other proteases (*3*).

It is not known whether, after initial cleavage of the molecule, the collagenase itself has specificity to cleave further the nonhelical gelatin polypeptides it has produced. Two pieces of data suggest that, if this specificity is present, it is not strong. (i) Rheumatoid synovial tissue in culture in vitro synthesizes a neutral protease capable of degrading gelatin to small (<10,000 daltons) fragments at a rate much faster than synovial collagenase (*4*). A similar peptidase is associated with tadpole collagenase (*5*). (ii) Rheumatoid subcutaneous nodule tissue in primary culture synthesizes and releases a collagenase which at 27°C degrades collagen predominantly into two fragments corresponding to TC^A (75 percent of the α chain of collagen) and TC^B (the carboxy terminal 25 percent of the α chain) (*6*). Although 10 percent of the gelatin was broken down to small (<10,000 daltons) fragments, this was explained by the presence of contaminating protease in the enzyme. It was hypothesized that "*in vivo*, after the initial cleavage of collagen into two fragments by collagenase, . . . subsequent breakdown of these primary products of collagenolysis is accomplished by proteases" (*6*). We now present evidence to support this hypothesis. We have demonstrated that a highly purified mammalian collagenase had little specificity for cleavage of intact gelatin or TC^A or TC^B fragments at physiologic temperatures, and that the helical, secondary conformation of collagen provides needed structure for recognition and catalysis by the collagenase.

Collagenase was purified from homogenates of V_2 ascites cell carcinoma that grew after tumor cells were injected intramuscularly into the thigh muscles of adult rabbits (*7*). This rabbit tumor collagenase (RTC) was shown to be preformed in the tumor and was not synthesized in culture in vitro (*7*). When the homogenate was centrifuged, the collagenase sedimented with the nuclear fraction of the cells; it showed maximum activity at *p*H 7 to 8 and was inhibited by the same kinds of compounds (chelating agents, sulfhydryl-containing compounds, and serum) that inhibited the rheumatoid synovial collagenase. It degraded collagen in solution at 24°C into TC^A and TC^B fragments which could be precipitated to form segment-long-spacing aggregates which, when visualized by electron microscopy, confirmed that cleavage occurred at a locus 75 percent from one end of the molecule (*7*).

The enzyme was purified by extraction of frozen and thawed tumor ho-

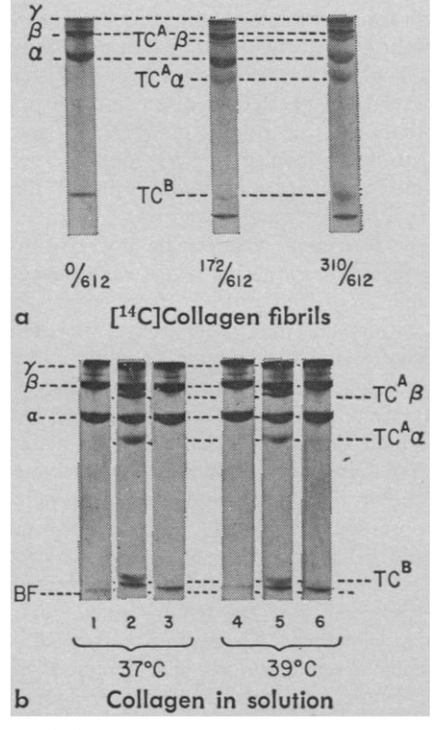

Fig. 1. (a) Polyacrylamide disc gel electrophoretic patterns of reaction mixtures of RTC and [^{14}C]collagen fibrils incubated at 37°C. Each reaction contained 50 μl of 0.4 percent [^{14}C]collagen in 0.2*M* NaCl (*p*H 7.6) (allowed to form fibrils by incubation at 37°C for 16 hours), to which was added 8 μg of RTC diluted to 200 μl in 0.1*M* tris · HCl, *p*H 7.6, 0.005*M* $CaCl_2$ (TC). At three intervals (0, 2, and 4 hours) two tubes were removed from incubation. One tube was centrifuged at 15,000 rev/min for 4 minutes at room temperature; the radioactivity in the supernatant was determined, the number of counts per minute in the supernatant of control gels was subtracted from this, and the results—0/612, 172/612, and 310/612—indicate the radioactivity in the sample supernatant expressed as a fraction of total radioactivity. The reaction in the other tube was halted by addition of ethylenediaminetetraacetate (EDTA), and remaining fibrils were solubilized by cooling to 4°C and subsequent addition of a small amount of 1.0*M* acetic acid. Portions of the solubilized reaction mixture were used for disc gel electrophoresis. (b) Results of incubation of collagen and gelatin in solution at 37°C with and without collagenase. All reactions were carried out in semimicro viscometers. Each reaction mixture contained 200 μl of gelatin (produced by 10 minutes of incubation at 45°C) or native collagen, 19 μg of RTC in 20 μl of TC or an equivalent amount of TC buffer without enzyme, 580 μl of 0.1*M* tris · HCl (*p*H 7.6), 0.2*M* NaCl, 0.005*M* $CaCl_2$, and 100 μl of 0.5*M* D-arginine (*p*H 7.6) to a final volume of 1.0 ml. After 20 minutes of incubation at 37°C or 39°C each reaction was stopped by additions of EDTA. Portions (75 μl) were subjected to electrophoresis on polyacrylamide gel and stained. Numbers correspond to samples plotted as a function of specific viscosity (η_{sp}) in Fig. 2: 1, control collagen, 37°C; 2, collagen plus RTC, 37°C; 3, gelatin plus RTC, 37°C; 4, control collagen, 39°C; 5, collagen plus RTC, 39°C; and 6, gelatin plus RTC, 39°C; *BF*, buffer front.