into particles during viral morphogenesis. A plausible model for this is specialized transduction of Tn5. Alternatively, one might imagine a mechanism of generalized transduction involving the packaging of random host DNA fragments into virus particles. However, the fact that ten Tn5 insertions selected independently as transducible all map to a common DNA region argues against this model.

Because transducing particles transfer genes between cells, they must interact with the surface of the recipient cell and deliver genetic material into the cell. Nevertheless, we did not detect a lytic cycle when testing for plaques on an agar overlay of vegetative YS cells. Although insertion of Tn5 within a particle may render it defective, the supernatant from normal YS bacteria lacking Tn5 also failed to produce plaques on an overlay of vegetative YS cells. We have tested, in addition to strain YS, other wild-type *Myxococcus* strains for sensitivity, including strains of *M. xanthus*, *M. virescens*, and *M. fulvus*. The reason that these particles fail to form plaques is unknown. It is possible that vegetative cells are immune or that these particles may not cause cell death or cell lysis in cells that produce them.

Our assay for particles is based on genetic selection of a drug resistance marker and therefore has the sensitivity to detect very low levels of particles in culture supernatants. Unlike classical schemes for detecting latent viruses based on plaque formation, our assay may detect viruses that never cause cell death. This approach could lead to the isolation of new viral-like elements that move horizontally between cells within a population. The potential importance of such elements remains to be explored, particularly in developmental systems where only a rare subset of cells within the population may be sensitive to viral infection.

References and Notes

1. J. W. Wireman and M. Dworkin, *Science* **189**, 516 (1975).
2. E. Rosenberg, Ed., *Myxobacteria Development and Cell Interactions* (Springer-Verlag, New York, 1984).
3. D. E. Berg *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3628 (1975).
4. D. Kaiser and M. Dworkin, *Science* **187**, 653 (1975).
5. J. M. Kuner and D. Kaiser, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 425 (1981).
6. E. Sodergren *et al.*, *Genetics* **105**, 281 (1983).
7. J. M. Campos, J. Geisselsoder, D. R. Zusman, *J. Mol. Biol.* **119**, 167 (1978).
8. J. M. Kuner *et al.*, in *Microbiology 1981*, D. Schlessinger, Ed. (American Society for Microbiology, Washington, D.C., 1981).

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Enzymatic Removal of Bilirubin from Blood: A Potential Treatment for Neonatal Jaundice

Abstract. *Current treatments for severe jaundice can result in major complications. Neonatal jaundice is caused by excessive accumulation of bilirubin in the blood. A small blood filter containing immobilized bilirubin oxidase was developed to reduce serum bilirubin concentrations. When human or rat blood was passed through the enzyme filter, more than 90 percent of the bilirubin was degraded in a single pass. This procedure may have important applications in the clinical treatment of neonatal jaundice.*

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All human newborns accumulate bilirubin to levels greater than those in adults, and 20 percent accumulate enough to stain their skin, resulting in jaundice (*1*). Bilirubin binds to cellular and mitochondrial membranes, causing cell death in a variety of tissues; clinically, bilirubin toxicity may lead to mental retardation, cerebral palsy, deafness, seizures, or death (*1*).

The two most common treatments for jaundiced infants are phototherapy and exchange transfusion (*1, 2*). In phototherapy, the infant is exposed to blue light that converts bilirubin to a less toxic photoisomer (*2*). Since only 15 percent of total body bilirubin can be photoisomerized through the skin, phototherapy does not control cases of severe jaundice (*2*). In those cases, infants undergo exchange transfusion, which requires staged removal of the infant's blood and its replacement with bilirubin-free adult blood. This procedure may result in hypoglycemia, hypocalcemia, acidosis, coagulopathies, graft-versus-host disease, transmission of infectious diseases (for example, hepatitis or acquired immune deficiency syndrome), or death (*1*). Although these treatments have reduced the occurrence of bilirubin toxicity, the risks associated with exchange transfusion remain a serious problem. Chromatography has been tested as a technique to replace exchange transfusions. Numerous resins have been used to adsorb bilirubin (*3*); however, they removed not only bilirubin but also essential compounds such as thyroxine, cortisol, and aldosterone (*3*).

Here, we report the use of a highly specific enzyme to remove bilirubin from the bloodstream. With this method, a small reactor containing an immobilized bilirubin-detoxifying enzyme is placed in an extracorporeal circuit. A prototype device markedly reduced serum bilirubin concentrations both in human blood and in genetically jaundiced rats.

Bilirubin oxidase from *Myrothecium verrucaria*, which catalyzes the oxidation of bilirubin with O_2, was used in this study (*4*). Results from electronic absorption spectroscopy (*5*) and thin-layer chromatography (*4, 6*) have shown that the initial product of the enzymatic reaction appears to be biliverdin, which is much less toxic than bilirubin (*7*). Bilirubin oxidase further oxidizes biliverdin to as yet unidentified products. We tested biliverdin and its enzymatic oxidation products for teratogenic (*8*), cytotoxic, and mutagenic (*9*) effects; none of these was detected, even at concentrations 60 times greater than the maximum physiological concentration.

Kinetic parameters of bilirubin oxidase were determined with solutions of bilirubin in 0.05*M* phosphate buffer (*10*). Enzymatic oxidation of bilirubin may be described by the Michaelis-Menten model with a Michaelis constant, K_m, of 50 μM. Bilirubin forms a noncovalent complex with human serum albumin (HSA), which has at least two binding sites for bilirubin, a primary site with an association constant K_a of $5.5 \times 10^7 M^{-1}$ (in defatted HSA at *p*H 7.4 and 37°C) and a secondary site with a K_a of 4.4×10^6 M^{-1} (*11*). Measuring enzymatic oxida-

tion (*10*) with bilirubin and HSA at concentrations that occur in neonatal jaundice (*1*), we found that bilirubin oxidase also oxidized bilirubin in the presence of HSA.

Bilirubin oxidase was covalently attached (*12*) to agarose beads and thus immobilized. Therefore, blood was exposed to the enzyme only in the reactor and was returned to the reservoir free of the enzyme. One gram of the agarose support bound 8 units (*13*) of enzymatic activity. The stability of bilirubin oxidase was greatly enhanced by immobilization: at physiological temperature and *p*H, free bilirubin oxidase lost half of its activity in 12 hours, whereas the half-life of immobilized enzyme was 60 hours.

To determine the effectiveness of the immobilized enzyme in blood, we tested a column containing 5 g of agarose with the active enzyme (Fig. 1). Control columns contained the same amount of either untreated agarose or agarose containing denatured bilirubin oxidase. Samples were periodically taken from the column influent and effluent and assayed for serum bilirubin and albumin (*14*). For the experiments in vivo, Gunn rats (body weight, 300 g) on lipid-free or regular diets were used (*15*). For the experiments in vitro, the blood reservoir was 24 ml of human umbilical-cord blood containing 2000 units of heparin; HSA and bilirubin were added to bring their concentrations to 1.3 m*M* and 340 μ*M*, respectively.

The experimental columns, both in vitro and in vivo, cleared 90 to 95 percent of the bilirubin in the blood in a single pass (Fig. 2), converting the serum color from bright yellow to dark green. Control columns had no appreciable effect on serum bilirubin concentrations (Fig. 2). Serum bilirubin concentrations in the treated rat and reservoir fell less rapidly than effluent concentrations, presumably because of mixing with untreated blood. In a series of six experiments in vitro, we found that the serum bilirubin concentration in the blood reservoir decreased by 61 percent within 30 minutes (Fig. 2A). In a series of six experiments in vivo, serum bilirubin concentration in the rats decreased by 50 percent within 30 minutes and remained low even after the enzyme treatment, indicating that a short period of exposure rapidly lowers the bilirubin level in rats (Fig. 2B).

Blood sampled after the trials revealed no statistically significant change in platelet or white blood cell counts and a 20 percent decrease in hematocrit in both experiments and controls. Our experience with immobilized heparinase reactors suggests that further refinements in reactor design may eliminate hemolysis (*16*). The electrolyte balance was unaffected, and the concentrations of calcium, phosphorus, albumin, blood urea nitrogen, creatinine, uric acid, cholesterol, triglyceride, and liver enzyme remained within normal limits. The glucose concentration increased during the experiments, as expected in a postoperative, restrained animal (*17*). The decrease in thyroxine we observed was less than one-third of that seen in an anion-exchange reactor tested for removal of bilirubin (*3*).

An immobilized bilirubin oxidase reactor therefore reduced the serum bilirubin concentration by at least half within 30 minutes, making it more rapid than exchange transfusions. The undesirable side effects of the latter procedure, due to significant volume shifts and exposure to adult donor blood, are avoided. Also, the enzyme reactor is smaller than proposed anion-exchange reactors (*3*), presumably because of the specificity and high catalytic activity of bilirubin oxidase. Further, the anion-exchange reactor cleared only 33 percent of reservoir bilirubin in a half hour (*3*), in contrast to the enzymatic reactor, which cleared 50 to 60 percent of the bilirubin in the same time. Since current treatments already expose an ill newborn to umbilical vessel catheterization, the required extracorporeal circuit for enzyme therapy would impose no additional invasive procedure. This new technique may be useful in the treatment of severe jaundice in newborns, and it lends further support to

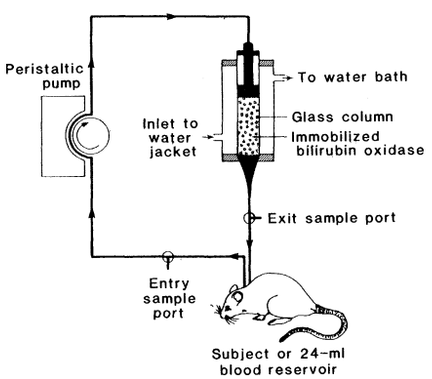

Fig. 1. The experimental apparatus. A column (Bio-Rad Econo-Column; 1.5 cm in diameter, 10 cm in length) was jacketed with a water jacket (Lab-Crest) filled with flowing water at 37°C. A Silastic catheter [0.51 mm internal diameter, 0.94 mm external diameter (Dow-Corning)] from a blood reservoir was attached to a three-way plastic stopcock (entry port) by a 23-gauge needle (Becton-Dickinson). Silicon tubing (Silastic, Dow-Corning) attached to the other side of the entry port carried the blood through a peristaltic pump (Cole-Palmer) to the top of the column. Blood passed through the column (1 ml/min) and exited through silicon tubing and the exit port to return to the reservoir through another Silastic catheter. Sepharose 4B-CL beads (5 g, Pharmacia) were packed into the column to form the reactor. Active columns contained beads with bilirubin oxidase immobilized on them. Before each experiment, the column was rinsed with 100 volumes of sterile isotonic saline containing 500 units of heparin per milliliter (Elkins-Sinn). All samples were taken from either entry or exit ports with a 1-ml syringe.

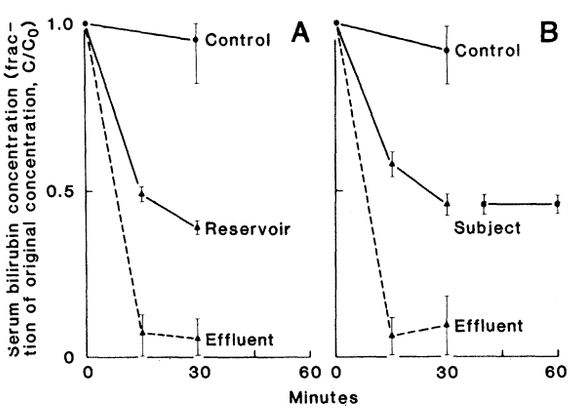

Fig. 2. Enzymatic clearance of bilirubin from blood. Serum bilirubin concentrations as a fraction of the original concentration (C/C_0) are plotted as a function of time. Control points (●) represent the mean serum bilirubin concentrations of four experiments after passage over beads without the enzyme present. Experimental points (▲) represent serum bilirubin concentrations of six experiments after passage over beads with bilirubin oxidase (Amano) immobilized on them (about 40 units of enzymatic activity per column). The solid lines represent the reservoir data sampled from entry ports, and the dotted lines represent the column effluent data sampled from exit ports. The standard deviations are depicted for each point. Bilirubin and HSA assays had a mean error of 6 and 4 percent, respectively. (A) Enzymatic clearance of bilirubin from human umbilical-cord blood in vitro. The blood reservoir was 24 ml of human cord blood from mature and premature infants to which heparin, bilirubin (Porphyrin Products), and HSA (Sigma) were added to achieve initial concentrations of 83 unit/ml, 340 μ*M*, and 1.3 m*M*, respectively. Blood was used within 12 hours of collection. (B) Enzymatic clearance of bilirubin in vivo from six male Gunn rats (blood volume of 80 ml/kg). The blood reservoir is the rat. Initial bilirubin concentrations ranged from 250 to 320 μ*M*. In three out of six rats, blood samples were taken 10 and 30 minutes after the experiment; the squares represent the mean serum bilirubin concentrations in these samples.

the concept that small enzyme reactors may provide a rapid, selective means for removing toxic substances from blood (*16, 18*).

References and Notes

1. M. J. Maisels, in *Neonatology*, G. B. Avery, Ed. (Lippincott, Philadelphia, 1981), pp. 473–544.
2. A. N. Cohen and J. D. Ostrow, *Pediatrics* **65**, 740 (1980).
3. S. Sideman *et al.*, *Am. Soc. Art. Intern. Org. J.* **4**, 164 (1981); B. F. Scharschmidt, P. H. Plotz, P. D. Berk, J. G. Waggoner, J. Vergalla, *J. Clin. Invest.* **53**, 786 (1974).
4. S. Murao and N. Tanaka, *Agric. Biol. Chem.* **46**, 2031 (1982); N. Tanaka and S. Murao, *ibid.*, p. 2499.
5. The absorbed energies and their relative amplitudes of absorption for electronic absorption spectroscopy were identical to those cited for biliverdin by A. F. McDonagh, in *The Porphyrins*, D. Dolphin, Ed. (Academic Press, New York, 1979), pp. 293–491.
6. Biliverdin (Porphyrin Products) and the initial product of enzymatic oxidation of bilirubin were dissolved in ethyl acetate. On silica plates with a mixture of methanol and water (20:80 by volume) as a solvent, both samples migrated with a retardation factor, R_f, of 0.75.
7. M. L. Cowger, *Biochem. Med.* **5**, 146 (1971).
8. A. G. Braun, C. A. Buckner, D. J. Emerson, B. B. Nichinson, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2056 (1982).
9. T. R. Skopek, H. L. Liber, J. J. Krowlewski, W. G. Thilly, *ibid.* **75**, 410 (1978).
10. These experiments were done in air at *p*H 7.4 and 37°C.
11. R. Brodersen, *CRC Crit. Rev. Clin. Lab. Sci.* **11**, 305 (1980).
12. J. Kohn and M. Wilchek, *Biochem. Biophys. Res. Comm.* **107**, 878 (1982).
13. A unit of enzymatic activity is defined as the activity that results in oxidation of one micromole of bilirubin to biliverdin per minute at 37°C and *p*H 7.4.
14. B. T. Doumas, W. A. Watson, H. G. Biggs, *Clin. Chim. Acta* **31**, 87 (1971); B. Billing, R. Haslam, N. Wald, *Ann. Clin. Biochem.* **8**, 21 (1971).
15. D. I. Whitmer and J. L. Gollan, *Semin. Liver Dis.* **3**, 42 (1983). The Gunn rats (Blue Spruce Vendors) were all male and homozygous for lack of glucuronyl transferase. After the rats were anesthetized, the left carotid artery and jugular vein were cannulated with Silastic catheters, and 2000 units of heparin were injected. While the rats were still unconscious, they were placed in restraining cages; the catheters were then attached to the apparatus as shown in Fig. 1. All the rats regained full consciousness before the experiments began.
16. R. Langer *et al.*, *Science* **217**, 261 (1982).
17. J. F. Vondruska and R. A. Greco, *Bull. Am. Soc. Vet. Clin. Path.* **2**, 3 (1973).
18. T. M. S. Chang, *Kidney Int.* **7**, 5387 (1975); L. Olanoff, K. Venkatasubramanian, F. R. Bernath, R. Joyeuse, *J. Biomed. Mater. Res.* **8**, 125 (1977); C. M. Ambrus *et al.*, *Science* **201**, 837 (1978).

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Vision Guides the Adjustment of Auditory Localization in Young Barn Owls

Abstract. *Barn owls raised with one ear plugged make systematic errors in auditory localization when the earplug is removed. Young owls correct their localization errors within a few weeks. However, such animals did not correct their auditory localization errors when deprived of vision. Moreover, when prisms were mounted in front of their eyes, they adjusted their auditory localization to match the visual error induced by the prisms, as long as the visual and auditory errors were within the same quadrant of directions. The results demonstrate that, during development, the visual system provides the spatial reference for fine-tuning auditory localization.*

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Sensory space does not project directly onto the sensory surface of the ear in the way that it does onto the eye or the body surface. As a consequence, the auditory system must derive spatial information indirectly from a variety of acoustic cues. These spatial cues depend on the size and shape of the head and ears and hence change as the head and ears grow. How then, might an animal maintain accurate sound localization during maturation? Young barn owls adjust their interpretation of auditory spatial cues on the basis of experience (*1*). Owls raised with one ear occluded learn, within 4 to 6 weeks, to localize sounds accurately using the altered auditory cues imposed by the earplug. When the earplug is removed, young owls make large localization errors, which they correct over a period of weeks.

We have investigated the signal that guides the adjustment of auditory localization in maturing barn owls. Of all senses, vision provides the most detailed spatial information to the brain. Even in owls, in which hearing is highly developed and visual acuity is relatively poor, the spatial resolving power of vision is still superior to that of audition (*2*). Moreover, in humans, vision strongly influences the perception of sound source location (*3*). Therefore, we hypothesized that vision plays an important role in adjusting errors in auditory localization in barn owls.

We monaurally occluded nine baby barn owls aged 26 to 44 days (*4*). The animals remained monoaurally occluded for 41 to 97 days (Table 1). During this time they were trained to orient their heads toward auditory (noise burst) and visual (light-emitting diode) stimuli, which were presented at random locations in a darkened, sound-attenuating chamber (*5*). One week before an earplug was removed, we attached spectacle frames to each owl (*6*). The mean of more than 100 responses to visual stimuli with the spectacle frames empty defined the head-centered spatial origin for each owl. During the experiments, the accuracy of auditory and visual localization was computed on the basis of this reference value.

The experiments began on the day the earplug was removed. Immediately after earplug removal, the responses of the owl to auditory and to visual stimuli were measured. Because auditory and visual stimuli were presented independently and in a darkened chamber, visual capture did not influence these responses (*3*). Owls that had been raised with the right ear plugged oriented to the right and above the auditory stimulus, whereas owls that had been raised with the left ear plugged oriented to the left and below the auditory stimulus (*7*). Two animals were allowed normal vision and served as controls; five animals were fitted with Fresnel prisms (*8*), which deviated vision by 10°, and two animals were fitted with opaque occluders which prevented vision totally (Table 1). After 28 days, the opaque occluders were replaced with prisms. Most of the owls were exposed to several different prism orientations, each orientation being maintained for periods of 22 to 68 days. The final experiment in every case was to remove the prisms and follow the recovery of accurate auditory localization.

Owls 1 and 2, which were permitted normal vision, adjusted their auditory errors rapidly (Fig. 1, A and B). The errors of both birds diminished at average rates of 0.7° per day, and after 28 days these birds were localizing sounds with mean errors of less than 3°, our criterion for normal localization accuracy (*1*). In contrast, owls 3 and 4, which had their eyes covered with opaque occluders after the earplugs were removed, maintained constant auditory errors for periods of 28 days (Fig. 1C). Since without vision these animals did not adjust their auditory errors, vision must be essential to trigger the adjustment process, to guide the adjustment, or both.

The role of vision was clarified by the first experiment done on owl 5. As shown in Fig. 1D, the bird had an initial auditory error of right 6.4° and up 8.4°. Prisms were mounted on this owl to