some suspension remains active even after weeks of storage is advantageous for serial experiments.

Lipid vesicles are already important in medicine as drug carriers in vivo (*20*), and it is possible that liposome-mediated gene transfer might become applicable to experiments in vivo.

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15. Mouse LTK^- cells were seeded 24 hours before transformation (5×10^5 cells per 10-cm petri dish). Before adding the liposome suspension the medium ws changed to Dulbecco's minimum essential medium (DMEM) without serum. Ten to five hundred nanomoles of phospholipid encapsulating 25 to 1000 ng of pAGO were added directly into the medium. After incubation for 30 minutes at 37°C, the cells were treated with 25 percent glycerol for 4 minutes; 24 hours later the cells were incubated either with [^{3}H]thymidine for autoradiographic analysis or with HAT medium as described (*6*) for selection of stable transformants.
16. For the autoradiographic assay cells were exposed to [^{3}H]thymidine (25 μCi per milliliter of DMEM containing 10 percent fetal calf serum) overnight, fixed with methanol, extracted with 5 percent trichloroacetic acid and overlaid with Kodak NTB-2 emulsion (*17*). The dishes were developed 48 to 72 hours later, and TK^+ positive cells (showing radioactivity in the nucleus) were counted under a Leitz light microscope.
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Group B Erythrocytes Enzymatically Converted to Group O Survive Normally in A, B, and O Individuals

Abstract. *With an α-galactosidase, B erythrocytes can be converted to blood group O under conditions that neither impair their viability in vitro nor affect their ability to survive normally after transfusion to individuals of groups O, A, and B. Such an approach has the potential for producing enzymatically converted group O cells for use in transfusion therapy. It should also be possible to convert A cells to group O by using the appropriate* α-N-*acetylgalactosaminidase.*

Individuals of blood groups A, B, and O expresss A and H, B and H, and H antigens, respectively, on their erythrocytes. The antigenic molecule consists of one or more straight or branched carbohydrate chains attached to ceramide or a peptide backbone, which in turn is embedded in the lipid bilayer of the cell membrane. Blood group specificity is determined by the nature and linkage of the monosaccharides at the ends of the carbohydrate chains. For H antigenic activity the immunodominant sugar fucose is bound in an α-1,2-glycosidic linkage to the penultimate galactose residue. Blood group A or B specificity occurs when *N*-acetylgalactosamine or galactose is attached in an α-1,3 linkage to the same residue as fucose (*1*). Given such structures, it is possible to convert A or B antigenic determinants into H by using specific exoglycosidases to hydrolyze only the *N*-acetylgalactosamine or galactose linked through a terminal α-glycoside, thus producing group O cells. Our interest is in effecting this conversion under conditions that yield cells of transfusable quality. Using an α-galactosidase previously reported to remove group B activity (*2*), we converted B erythrocytes to group O and transfused the converted cells back to the donor and to O and A individuals, where they survived normally.

To achieve this, we have developed treatment conditions that maintain the integrity and viability of the erythrocyte while providing reasonable enzyme kinetics. For these studies coffee bean α-galactosidase (E.C. 3.2.1.22) (Boehringer Mannheim) was obtained and purified to yield a specific activity of the order of 25 U (*3*) per milligram of protein. No detectable sialidase activity was found and proteases were present in only trace amounts. Contaminating exoglycosidases, when present, were at levels never greater than 2×10^{-3} percent of the specific activity of α-galactosidase.

We have extensively studied enzymatically converted cells in vitro (*4*). No more than 0.5 to 1 percent of cells are lysed by the conversion process, and fragility tests demonstrate that enzyme treatment neither increases the cells' susceptibility to osmotic shock nor renders any part of the cell population more fragile. Adenosine 5′-triphosphate and 2,3-diphosphoglycerate concentrations as well as partial oxygen pressures and oxygen dissociation curves remain normal during the treatment to remove B antigenicity and there is an increase in methemoglobin formation of only 1 to 3 percent. These results indicate that cell deformability and normal oxygen binding and exchange are unimpaired. Under the light microscope, treated cells in phosphate-buffered saline (*p*H 7.3) are seen as a mixture of discocytes and spiculated discocytes in about equal numbers. They do not exhibit any membrane stickiness, since after centrifugation they resuspend in the same manner as untreated cells and are not agglutinated by their own serum or by the serum of A or B individuals.

We have searched for changes in membrane structure which could result from enzyme treatment. For example, we tested the membrane surface for perturbation caused by the removal of terminally α-linked galactose residues from group B antigens, the P_1 antigen (if present), and trihexosyl ceramide, which is a surface constituent of all erythrocytes. We used as markers acetylcholinesterase, which is bound to the outer membrane surface (*5*), and cholesterol, which is involved in the maintenance of proper membrane fluidity (*6*). Their levels were unchanged following enzyme conversion. Converted cells were also subjected to antigen profiling as another measure of membrane alteration. Among the antigens tested for were A, B, H, Rh (D, C, and E), MNS, P_1, Lewis, Kell, Lu-

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theran, Duffy, and Kidd. Several batches of untreated and treated cells containing different permutations of these antigens were examined. Antigens that were originally present on untreated cells remained on their enzyme-treated counterparts. Similarly, antigens that were absent did not appear after enzyme treatment. The antigenicities of only B and P_1, whose activity is also due to a terminally α-linked galactose residue, were lost after conversion, and H activity was increased to levels found in group O erythrocytes.

We performed the same in vitro studies with B and AB-like gibbon erythrocytes and obtained the same results. We then performed in vivo studies with ^{51}Cr-labeled, enzymatically converted gibbon erythrocytes and found that they had normal survival rates after being returned to the donor animal (*7*). Formation of antibody to enzyme-treated cells was not detected after such cells were transfused to the same donor animal three times, indicating that they were not immunogenic under the conditions of the experiment. Also, antibodies to the enzyme preparations were not detected in the animals.

Our latest results concern the survivability of enzymatically converted cells in humans. Three healthy volunteers of blood groups A, B, and O were selected on the basis of Rh compatibility (Rh+), the lack of high-titer antibodies to minor blood group red cell antigens, and the absence of detectable antibody activity toward the α-galactosidase preparation used to remove group B antigenicity. A few cells were obtained from our group B volunteer and treated with α-galactosidase. These converted cells were tested against the group B donor's fresh serum and against serum from the O and A individuals. Standard pretransfusion serological procedures were used at room temperature and 37°C (*8*). We could not detect any agglutination or hemolysis, indicating that compatability had been achieved in vitro and that the volunteers could be used to determine the survival of enzymatically treated cells in vivo.

Approximately 30 ml of blood was collected, with citrate phosphate and dextrose used as an anticoagulant (sterile procedures and solutions were used throughout). The blood was centrifuged and the plasma and buffy coat (leukocytes and platelets) were removed. The packed erythrocytes were resuspended in normal saline (0.15*M* NaCl) and centrifuged, and the topmost layer of erythrocytes was aspirated to remove any remaining white cells and platelets. This procedure was repeated once with 4.2 ml of cells, which were then resuspended in 10 ml of normal saline, labeled with ^{51}Cr (200 μCi; Squibb), and incubated at room temperature for 15 minutes (*9*). The cells were washed three times by centrifugation and resuspension in normal saline and then three times, at 5-minute intervals, in a buffer of sodium phosphate, sodium citrate, and saline (PCS) (*p*H 5.7) (*10*) to adjust their *p*H to that of the buffer. Packed cells (3.2 ml) were then mixed with 2000 U of α-galactosidase in PCS to a final volume of 11.2 ml, and 350 μl was removed and placed in a pilot tube to provide samples for monitoring the removal of B antigenicity. Both reaction mixtures were incubated at 26°C. We removed samples from the pilot tube at various intervals, washed them, and tested them for agglutinability by using one drop of a 5 percent suspension of cells and two drops of commercial antiserum to human B cells.

The mixture was allowed to stand at room temperature for 10 minutes, centrifuged for 1 minute at 3200 rev/min, and then examined microscopically for agglutination. After 60 minutes the cells were no longer agglutinable. However, since the limit of detection of the agglutination reaction is believed to be at approximately 2000 antigenic sites per cell

Table 1. Blood volume and survival time measurements for ^{51}Cr-labeled, enzymatically converted group B erythrocytes after transfusion to O, A, and B individuals. Values are uncorrected for ^{51}Cr elution.

Blood group of recipient	Blood volume (mg/kg)*	Cell survival (%)			T_{50}§ (days)
		30 minutes†	60 minutes†	24 hours‡	
O	59	99	98	97	31
A	60	98	99	97	30
B	58	98	100	96	33

*Blood volume was determined by taking the total radioactivity of the transfused cells (obtained by multiplying the radioactivity of the standard by the weight of the transfused erythrocytes and dividing by the weight of the standard) and dividing by the radioactivity of the whole blood sample taken at 15 minutes divided by the body weight. †Calculated by using the value for the sample taken at 15 minutes to represent 100 percent survival. ‡Calculated by using the average of the values for the samples taken at 15, 30, and 60 minutes to represent 100 percent survival. §T_{50} is the period during which at least 50 percent of the converted cells are still present in the circulation.

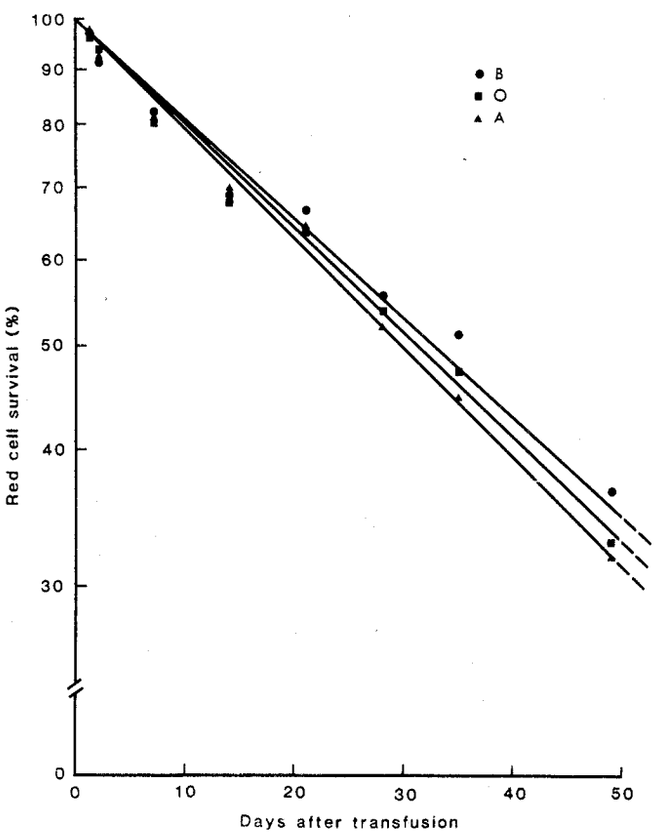

Fig. 1. Survival of ^{51}Cr-labeled, enzymatically converted B erythrocytes after transfusion to O, A, and B individuals. Values are uncorrected for ^{51}Cr elution.

(*11*), the incubation was continued for 30 minutes to ensure that all susceptible group B antigens were converted to H activity. We then tested cells from the primary reaction mixture and obtained the same results. The enzyme solution was removed after centrifugation and the packed cells were washed four times with 45 ml of phosphate-buffered saline (PBS) to remove any remaining traces of the enzyme preparation. The cells were left in the last wash for 15 minutes to ensure equilibration to *p*H 7.3. After centrifugation, the cells were resuspended in 12.5 ml of PBS to a final volume of 15.5 ml. Three 5-ml portions, each containing about 1 ml of converted cells, were taken up in preweighed syringes, and the weight of cells to be administered was measured. Transfusion was performed quantitatively by several back-and-forth rinsings of each syringe with the recipient's blood. A portion of the remaining 0.5 ml of cells was used as a standard to determine the total amount of radioactivity administered.

During the ensuing 49 days blood samples were taken and their radioactivity was measured to determine the survival of the transfused cells. Using data on the first blood samples, which were taken 15 minutes after transfusion, we calculated the blood volumes of the three participants (Table 1) and found them to be within the normal range (*12*). The results, which reflect the amount of radioactivity initially administered as compared with the amount found in the circulation, are very similar for all three recipients. This indicates that there was no selective destruction of enzymatically converted cells by the immune systems of the O and A individuals in the first few minutes after transfusion. The amount of radioactivity was essentially unchanged in samples of whole blood taken at 15, 30, and 60 minutes (Table 1), and the plasma in these samples was devoid of ^{51}Cr, demonstrating that rapid destruction of converted cells was not occurring at these time points. These values were averaged and taken to represent 100 percent survival.

Over 95 percent of the converted cells were present in the circulation after 24 hours (Table 1). At least 50 percent of the converted cells remained in the circulation for 30 to 33 days. This is in accord with published values for normal survival (*13*). Finally, the three survival curves (Fig. 1) have essentially the same shape. They did not show a marked downward shift in slope to indicate an increase in the rate of cell destruction due to an immune response (*14*).

These results are the first indication that small quantities of enzymatically converted group B erythrocytes can survive normally in recipients whose immune systems would not tolerate untreated cells.

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Luteal Phase Defects Induced by an Agonist of Luteinizing Hormone–Releasing Factor: A Model for Fertility Control

Abstract. *Subcutaneous injection of 50 micrograms of a luteinizing hormone–releasing factor agonist (LRF agonist) for three successive days at the time of menstruation in normal cycling women induces a shortened luteal phase with suboptimal concentrations of circulating estradiol and progesterone. This luteal phase defect follows a reduced concentration of follicle-stimulating hormone during the follicular phase and a resulting inadequate follicular maturation. Since a short luteal phase is associated with an endometrium not conducive to implantation, administration of the LRF agonist at the onset of menstrual cycle may prove to be a practical and novel approach to fertility control.*

The "spontaneous" occurrence of short or inadequate luteal phases of the menstrual cycle in humans (*1*) and monkeys (*2*) has received increasing recognition as a significant cause of infertility and repeated early abortions (*3*). The endometrium in women with such luteal phase defect is known to develop inadequately with respect to both its morphology and function as a result of impaired secretion of estradiol (E_2) and progesterone. A normal predecidual reaction usually does not occur and normal nidation is thereby impeded (*4*).

In monkeys there is evidence that such luteal defects are the consequence of a deficiency in follicle-stimulating hormone (FSH) which results in suboptimal folliculogenesis. However, there is no direct evidence in women that FSH deprivation during the early follicular phase of the menstrual cycle is causally related to the sequence of impaired follicular maturation followed by luteal dysfunction.

Since infertility is a dominant feature of luteal phase dysfunction (*1–4*), pharmacologically induced luteal defects would appear to offer considerable potential as a method of contraception. Prolonged infusion of luteinizing hormone–releasing factor (LRF) or daily administration of long-acting LRF agonists is known to induce pituitary desensitization resulting in lowered concentrations of circulating gonadotropin (*5*). We therefore explored the possibility that the administration of an LRF agonist very early in the follicular phase might cause inadequate folliculogenesis and subsequent luteal dysfunction.

Five normal women (ages 22 to 39 years) with menstrual cycles of 28 days volunteered for this study. In each subject, a control cycle and a treatment cycle were studied by daily measure-

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