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Long-term physiological effects of enhanced O_2 release by inositol hexaphosphate-loaded erythrocytes

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ABSTRACT **A continuous lysing and resealing procedure with erythrocytes permitted incorporation in these cells of inositol hexaphosphate (InsP_6), a strong allosteric effector of Hb. This leads to significant rightward shifts of the HbO_2 dissociation curves with *in vitro* P_{50} (partial pressure of O_2 at 50% Hb saturation), values increasing from 32.2 ± 1.8 torr for control erythrocytes to 86 ± 60 torr (pH 7.40; P_{CO_2} 40 torr at 37°C; 1 torr = 1.333 × 10^2 Pa). The shape of the dissociation curve was still sigmoidal, although the Hill coefficient was decreased. The life span of InsP_6-loaded erythrocytes equaled that of control erythrocytes. The long-term physiological effects of the InsP_6-loaded erythrocytes on piglets were increased O_2 release and reduced cardiac output. The reduced O_2 affinity of the InsP_6-loaded erythrocytes was still effective 20 days after transfusion in awake piglets. The electrolyte concentration appeared stable over the 5-day observation period except for a transient, but significant, hyperkalemia immediately after transfusion. The reductions in the O_2 affinity of Hb reported here are large compared with previously reported values. Introduction of InsP_6 into viable erythrocytes improves tissue oxygenation when, for any reason, normal blood flow is impaired.**

A conformational modification of intracellular Hb may considerably change its affinity for O_2, which, in turn, may have physiological implications whenever a limitation of blood flow or reduction of Hb concentration impairs systemic oxygenation (1). When O_2 uptake by erythrocytes was maintained, an *in vivo* decrease in O_2 affinity was observed to enhance tissue O_2 delivery (1). However, the consequence of a substantial increase of the *in vivo* P_{50} (O_2 partial pressure at 50% Hb saturation) is not well documented due to the difficulty in obtaining substantial and long-term low O_2 affinity. Few methods have been reported to raise P_{50} *in vivo*, and the induced P_{50} increases were always modest and/or sustained for only a short time. We previously reported a method for increasing P_{50} in anesthetized piglets using inositol hexaphosphate (InsP_6) (2). Because InsP_6, the most effective Hb allosteric effector (3), cannot diffuse through the erythrocytic membrane, InsP_6 was incorporated into erythrocytes by using a reversed osmotic–lysis process (4–6).

The aim of this study was to sustain a marked decrease in Hb affinity for several days in piglets after exchange transfusion with InsP_6-enriched homologous erythrocytes. Physiological properties and life span of lysed–resealed InsP_6-loaded erythrocytes (InsP_6–erythrocytes) were studied before exchange transfusion. The time courses of P_{50} variation and electrolyte concentrations were measured in piglets after massive exchange transfusion with InsP_6-erythrocytes suspended in porcine plasma.

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MATERIALS AND METHODS

High-P_{50} Erythrocyte Preparation. Human or porcine blood, collected on acid-citrate-dextrose solution, was stored at 4°C no longer than 5 days. After centrifugation of one blood unit (1000 × *g* for 10 min), plasma was collected, and the erythrocytes were washed with 0.15 M NaCl solution at 4°C. The supernatant and buffy coat were discarded; preliminary experiments have shown that elimination of polymorphonuclear cells avoids microaggregate formation during massive blood transfusion in the pig. This was accomplished by absorbent-cotton filtration before the washing steps (Erypur system, Organon, West Orange, NJ), thus lowering the polymorphonuclear content to <5% of basal value. The erythrocyte suspension was then washed twice with chilled 0.15 M NaCl solution. For human erythrocytes, two further washing solutions were alternatively used. (*i*) The sodium salt of InsP_6 (InsP_6-12Na; Sigma) was neutralized to pH 7.40 using 1 M HCl and finally adjusted to 260 mosmol·liter^{-1}. This solution (vol/vol) was used to wash human erythrocytes before the following lysing and resealing steps. (*ii*) To markedly increase the P_{50} (>50 torr; 1 torr = 1.333 × 10^2 Pa), the InsP_6 concentration had to be increased in the last washing step; this was done by neutralizing the InsP_6-12Na solution at pH 7.4 using an InsP_6 acidic solution instead of HCl. This InsP_6 acidic solution was prepared through ion-exchange chromatography on a Dowex 50-W column (H^+ form). Similarly, the final osmolarity was adjusted to 260 mosmol·liter^{-1}. By adjusting the InsP_6 concentration, the InsP_6 encapsulation could be varied in the human erythrocytes and P_{50} values could be obtained between 30 and 80 torr. Porcine erythrocytes were similarly modified using the same InsP_6 solutions; however, final osmolarity was adjusted to 330 mosmol·liter^{-1}.

After washing the erythrocyte suspension with one of these InsP_6 solutions, spinning erythrocytes at 1000 × *g* for 10 min, decanting the supernatant, and adjusting the final hematocrit to 70%, the lysis of the erythrocytes was done as follows. The erythrocyte suspension, cooled to 4°C, flowed continuously into the blood compartment of a Minor hemodialyzer (Gambro-France, Aulnay sous Bois, France; dialysis surface, 0.41 m^2; membrane thickness, 13.5 μm/cm) with the use of a peristaltic pump. Erythrocyte flow rate was adjusted to 20–60 ml·min^{-1}; the hemodialyzer was fed at a flow rate of 500 ml·min^{-1} using a low-ionic strength buffer, pH 7.4, at 4°C containing (in mol·liter^{-1}) phosphate (0.01), sodium bicarbonate (0.01), and glucose (0.002). The erythrocytes were carefully lysed during this dialysis step and collected at 37°C

Abbreviations: P_{50}, partial pressure of O_2 at 50% hemoglobin saturation; InsP_6, inositol hexaphosphate; InsP_6-erythrocytes, InsP_6-loaded erythrocytes.

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before being resealed through the addition (1:10, vol/vol) of a hypertonic solution containing (per liter) 1 M chloride salt with a K^+/Na^+ ratio of 8.3 to maintain a high ATP content in the resealed erythrocytes.

Resealing of the cells was done by incubation at 37°C for 30 min. The erythrocytes were then washed twice with 0.15 M NaCl/1 mM $CaCl_2$/1 mM $MgCl_2$/2 mM glucose. The last washing, done in the 0.15 M NaCl solution, was Hb free (Hb <0.05 g·100 ml^{-1}, Drabkin method). The erythrocytes were then suspended in the native autologous plasma before infusion at a chosen hematocrit.

HbO_2 dissociation curves were obtained with a dissociation curve analyzer (DCA, Radiometer) as described. Bohr factor (logarithm Po_2-to-pH ratio at constant Pco_2 = 40 torr) was calculated from HbO_2 dissociation curves for different pH values. The logarithm of Po_2 at pH 6.80, 7.10, 7.40, and 7.60 was plotted for each increment of 10% HbO_2 saturation, and the logarithm of Po_2-to-pH ratio was calculated from a straight-line relationship. A high value of the regression coefficient (r = 0.99) was obtained from these four points. The Hill constant at 50% saturation (n_{50}) was extrapolated from the slope of a straight line drawn between 30% and 70% of saturation (Sat) on a log–log graph of the Sat/(100 − Sat) vs. Po_2 relationship. O_2-binding capacity was determined by measuring O_2 content with a Lex-O_2-Con (Lexington Instruments, Delhomme, France) after reconstituted blood tonometry with a high-O_2 gas mixture (70.5% O_2/5% CO_2/24.5% N_2). Intracellular pH values of erythrocytes were determined after tonometry (5% CO_2/12% O_2) and lysing at −20°C.

Erythrocyte indices were measured for the cells subjected to the InsP_6 incorporation procedure and for the control cells—i.e., fresh untreated erythrocytes. Indices were measured in homologous plasma by means of an electronic counter (Coulter).

Exchange Transfusion in Piglets. Two days before blood exchange the piglets were anesthetized with intraperitoneal pentobarbital (20 mg/kg). A Swan–Ganz catheter was introduced into the pulmonary artery via the jugular vein, and an arterial catheter was inserted into the carotid artery. The isovolemic exchange transfusion was done in awake animals by injection of reconstituted blood into the venous catheter and withdrawal from the carotid artery as described (2). After transfusion the venous and arterial heparinized catheters remained in place, and the Swan–Ganz catheter was removed 1 day later; the jugular vein was sutured. Pulmonary and systemic arterial pressures were monitored using Statham–Gouls P23DB transducers (Ballainvilliers, France). Po_2, Pco_2, and pH were measured using an ABL_{11} measuring apparatus (Radiometer) at 37°C; O_2 saturation was measured using an OSM_2 apparatus (Radiometer), and the O_2 content was measured with a Lex-O_2-Con instrument. Blood lactate and glucose concentrations were determined enzymatically on iced arterial samples with Boehringer Mannheim kits. Cardiac output ($\dot{Q}$) was measured by thermodilution (Edwards), and O_2 consumption (V_{O_2}) was computed from $\dot{Q}$ and the measured arteriovenous difference in O_2 content.

Life spans of InsP_6-erythrocytes were unchanged from normal erythrocyte values as previously reported (6). The *in vivo* life span of lysed–resealed erythrocytes with and without InsP_6 has been determined following the recommended method for radioisotope-labeled erythrocyte-survival studies using ^{51}Cr labeling of the cells (6). The data showed that the reversed lysis process does not introduce significant perturbations of the *in vivo* life span of circulating erythrocytes.

During the first 24 hr after transfusion of the ^{51}Cr-labeled, lysed–resealed erythrocytes, a loss of 15–20% of the transfused cells is seen (6). A better recovery at 24 hr may be obtained, eliminating the most fragile cells by washing the resealed erythrocytes with a hypotonic (240-mosmol) solution before transfusion. Porcine InsP_6-erythrocytes show the same behavior (6). On a semilogarithmic scale, the half-life of the porcine erythrocytes loaded with InsP_6 by the method described is almost the same as the porcine control erythrocytes. Recovery of the InsP_6-erythrocytes was, at worst, 72%, and, at best, 85% (6). These results have been reproduced on a significant number of animals after the initial observation (6); details have been published elsewhere (6). The P_{50} of the porcine InsP_6-erythrocytes, the life spans of which were measured, was 71.5 ± 8 torr. Mean cell volumes, mean cell Hb content, and width distribution of lysed–resealed InsP_6-erythrocytes and control erythrocytes varied within acceptable limits (2, 5).

RESULTS

Eight awake piglets weighing 10.0 ± 3.5 kg were transfused by isovolemic arteriovenous exchange with InsP_6-containing blood.

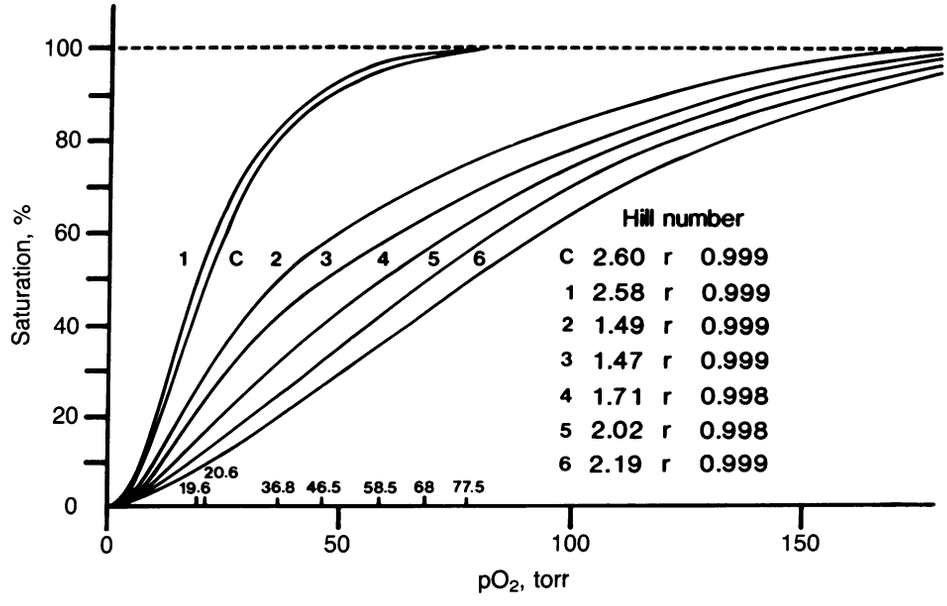

FIG. 1. HbO_2 dissociation curves and the P_{50} changes induced by InsP_6 incorporated in pig erythrocytes (curves 2–6). The technique for InsP_6 incorporation in erythrocyte has been detailed (4–6). C, Control; 1, lysed and resealed erythrocyte without InsP_6; r, regression coefficient.

Table 1. Physiological data of piglets transfused with InsP_6-erythrocytes

Physiological variables	Basal state (n = 8)	After exchange transfusion with InsP_6-erythrocytes (n = 8)	24-hr after transfusion with InsP_6-erythrocytes (n = 8)
P_{50}, torr	32.2 ± 0.8	46.7 ± 7.0*	42.5 ± 5.9*
pH			
a	7.43 ± 0.04	7.43 ± 0.07	7.44 ± 0.05
v	7.38 ± 0.03	7.34 ± 0.04	7.35 ± 0.05
Pco_2, torr			
a	40.6 ± 4.5	39.5 ± 3.2	41.5 ± 3.5
v	47.5 ± 4.9	47.0 ± 3.8	48.1 ± 4.0
Po_2, torr			
a	84.2 ± 7.5	89.2 ± 11.5	90.5 ± 8.2
v	37.4 ± 3.9	35.9 ± 5.3	36.5 ± 4.7
Hb, g/100 ml	10.4 ± 2.2	10.7 ± 1.4	9.1 ± 1.3
Saturated HbO_2, %			
a	95.0 ± 2.8	80.2 ± 2.0*	83.2 ± 1.9
v	59.0 ± 5.1	31.1 ± 6.0*	34.5 ± 5.1
$avDo_2$, ml/100 ml	5.3 ± 0.2	7.3 ± 0.3*	7.3 ± 0.2*
Arterial pressure, torr	110 ± 15	107 ± 18	120 ± 25
Mean pulmonary arterial pressure, torr	21 ± 3	22 ± 2	19 ± 3
Cardiac output ($\dot{Q}$), liter/min per kg	0.22 ± 0.04	0.17 ± 0.03*	0.17 ± 0.03*
O_2 consumption (Vo_2), ml/min per kg	11.7 ± 1.0	12.4 ± 1.1	12.4 ± 1.0

a, Arterial; $\bar{v}$, mean venous; $avDo_2$, arteriovenous O_2 difference.
*$P < 0.001$.

Fig. 1 shows the HbO_2 dissociation curves and the P_{50} changes induced by InsP_6 incorporation into porcine erythrocytes. The P_{50} value was raised from 32.5 torr (control) to a value ranging from 38.5 to 86 torr (curves 2–6). The shape of the dissociation curve was still sigmoidal, but the Hill coefficient decreased. P_{50} of blood subjected to the same experimental procedure of reversible lysis without InsP_6 was 31.3 torr (curve 1), a value close to control value, indicating that no significant loss of 2,3-bisphosphoglycerate occurred during InsP_6 incorporation into erythrocytes. Hb–O_2 binding capacity, 1.32 ± 0.06 ml of O_2 per g of Hb (n = 4) in InsP_6-erythrocytes, was near the value of 1.35 ± 0.04 ml of O_2 per g of Hb (n = 4) for fresh porcine erythrocytes.

Effects on O_2 transport of massive transfusion of InsP_6 blood in awake piglets are illustrated in Table 1. Acid–base status remained unchanged after the exchange transfusion. Because of the rightward shift of the HbO_2 dissociation curve, O_2 release increased, and cardiac output decreased. Table 2 shows the effects on piglets of the transfusion of lysed–resealed porcine erythrocytes without InsP_6.

Standardized P_{50} values (pH 7.40; Pco_2 = 40 torr at 37°C) measured on blood samples withdrawn from awake piglets after completion of exchange transfusion are illustrated in Fig. 2, P_{50} increases of 4–28 torr 8 hr after the end of the exchange transfusion were seen. Although the P_{50} increase diminished progressively with time in each experiment, the reduction in the affinity of Hb for O_2 appeared to remain effective 20 days after transfusion. The electrolyte concentration appeared stable over the 5-day period of observation, except for a transient, but significant, hyperkalemia immediately after transfusion (Table 3). All transfused animals survived after the observation period.

Table 2. Physiological data of control piglets transfused with lysed–resealed porcine erythrocytes without InsP_6

Physiological parameters for control group (n = 4)	Mean value ± SD
P_{50} (torr)	29.3 ± 1.3
Pao_2* (torr)	80.3 ± 9.4
Pvo_2† (torr)	34.5 ± 5.8
$avDo_2$‡ (ml/100 ml)	5.4 ± 0.9
$\dot{Q}$§ (liter/min per kg)	0.192 ± 0.040

*Partial pressure of O_2 in the arteries.
†Partial pressure of O_2 in the veins.
‡Arteriovenous O_2.
§Cardiac output.

Electrolyte concentrations (Na^+, K^+, Cl^-, Ca^{2+}, phosphate), urea, creatinine, protein, glucose, and Hb concentrations were determined 8, 12, 24, 48 hr, and 5 days after transfusion for five pigs and each day during 28 days for three pigs after the exchange transfusion. All values presented in Table 3 are mean ± SD; significant differences were calculated with Student's t test.

Reductions in the O_2 affinity of Hb obtained in awake animals in the present study are large; a mere 2- to 4-torr increase in P_{50} had been obtained in man by transfusion of 2,3-bisphosphoglycerate-enriched erythrocytes (7). Metabol-

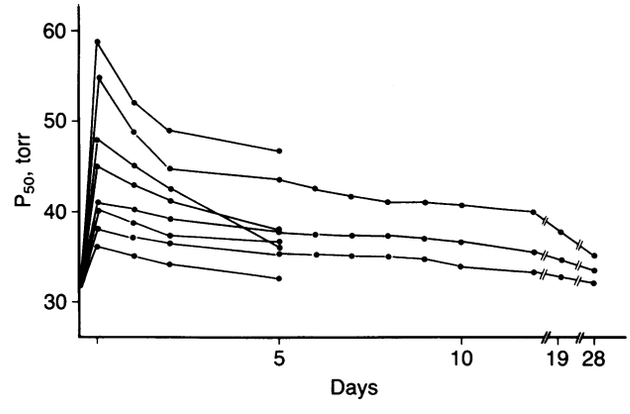

FIG. 2. Time course over 28 days of standardized P_{50} values (pH, 7.40; Pco_2 = 40 torr at 37°C) measured on blood samples withdrawn from awake piglets after exchange transfusion. P_{50} increases of 4–28 torr 8 hr after the end of the exchange transfusion were seen. Although the P_{50} increase diminished progressively in each experiment, the reduction in the O_2 affinity of Hb appeared to remain effective 19 days after transfusion. Electrolyte concentration appeared stable over the 5-day observation period (Table 2). All transfused animals survived the observation period.

Table 3. Variations of ion, protein, urea, and glucose in piglets transfused with InsP_6-erythrocytes

Analysis*	Controls (n = 8)	Time after exchange transfusion with InsP_6-containing blood, mean values ± SD (n = 8)					
		0 hr	8 hr	12 hr	24 hr	48 hr	5 days
K^+	4.2 ± 0.4	4.9 ± 0.5	4.4 ± 0.4	4.1 ± 0.3	4.1 ± 0.3	4.0 ± 0.3	4.0 ± 0.4
Na^+	141 ± 3	142 ± 4	143 ± 4	144 ± 5	142 ± 4	140 ± 4	139 ± 3
Cl^-	99 ± 3	100 ± 3	101 ± 4	98 ± 4	99 ± 5	101 ± 3	102 ± 3
Protein	11.7 ± 1.1	9.8 ± 0.7	9.7 ± 0.6	9.5 ± 0.8	10.5 ± 0.7	11.3 ± 1.2	12.2 ± 1.4
Ca^{2+}	2.55 ± 0.20	2.61 ± 0.32	2.44 ± 0.27	2.40 ± 0.22	2.53 ± 0.27	2.50 ± 0.21	2.47 ± 0.15
Phosphate	2.10 ± 0.25	2.22 ± 0.30	2.27 ± 0.25	2.30 ± 0.22	2.21 ± 0.15	2.18 ± 0.18	2.15 ± 0.25
Urea	3.6 ± 1.2	3.9 ± 1.3	4.3 ± 1.4	4.0 ± 1.2	3.2 ± 1.1	2.9 ± 1.4	3.1 ± 1.1
Creatinine	73.5 ± 15.6	75.3 ± 16.5	85.5 ± 17.3	72.2 ± 13.2	70.3 ± 13.5	69.2 ± 14.0	71.5 ± 14.3
Glucose	5.1 ± 0.8	5.6 ± 1.0	5.9 ± 1.3	4.9 ± 1.2	4.5 ± 1.3	4.8 ± 0.9	5.0 ± 1.0

*All measurements are in mmol/liter except for protein (meq/liter) and creatinine (μmol/liter).

ic regulation of intraerythrocytic concentration made it possible to sustain high P_{50} values for >6–12 hr (7, 8). Administration of glycolytic intermediates was used to stimulate synthesis of 2,3-bisphosphoglycerate in dogs, resulting in a 3-torr rightward shift in P_{50} (9). In man, phosphate, vitamin C, sodium bicarbonate (10), or phosphate fructose (11) were administered to elevate 2,3-bisphosphoglycerate levels and shift HbO_2 dissociation curves to the right. Mean standardized P_{50} of drug-treated subjects was higher than placebo-treated subjects, but the difference remained within 1.5–3.0 torr. Organic anions such as orthoiodosodium benzoate (OISB) that are unrelated to erythrocyte metabolism can enter erythrocytes to have a direct and reversible interaction with Hb (12, 13). The P_{50} increase thus obtained was of 5–7 torr in animals chronically injected. Nevertheless, OISB was not without toxic effect (13), and OISB plasma concentration had to be maintained constant to sustain high P_{50} values (13).

To explain the apparent discrepancy between the normal half-life of InsP_6-erythrocytes and the P_{50} time course observed in live animals, we studied the influence of addition of InsP_6-erythrocytes to fresh porcine blood.

Fig. 3 illustrates HbO_2 dissociation curves obtained after mixing porcine blood (P_{50} = 30.5 torr) with different volumes of InsP_6-treated porcine blood (P_{50} = 55 torr). Addition of 20% of InsP_6-treated blood to control blood increased P_{50} only 1.0–1.5 torr. In contrast, substitution of 20% of InsP_6-treated blood by control blood decreased P_{50} 8–9 torr.

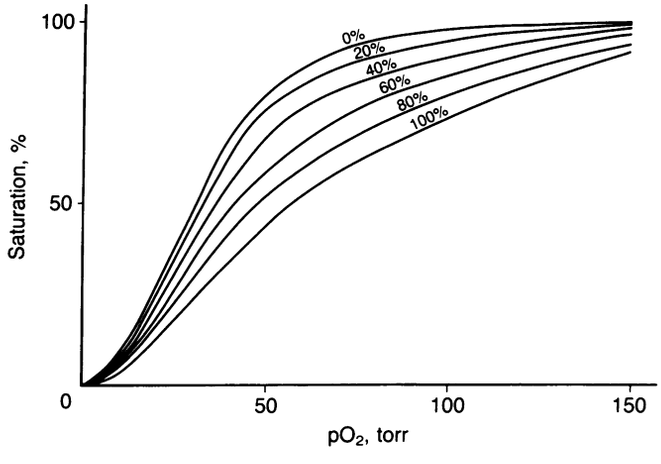

FIG. 3. HbO_2 dissociation curves obtained after mixing porcine blood (P_{50}, 305 torr) with different volumes of InsP_6-treated porcine blood (P_{50}, 55 torr). Addition of 20% of InsP_6-treated blood to control blood increased P_{50} by only 1–1.5 torr. In contrast, substitution of 20% of InsP_6 blood for control blood decreased P_{50} by 8–9 torr. These curves indicate that a relatively small loss of InsP_6-erythrocytes causes a significant leftward shift of the P_{50} value. Comparison of the *in vitro* values shown in Fig. 3 with the *in vivo* observations results in almost the same life spans for the InsP_6-erythrocytes (6).

The curves indicate that a relatively small loss of InsP_6-treated erythrocytes causes a significant leftward shift of the P_{50} value of the mixture.

Modifications in O_2 transport and delivery seen in anesthetized pigs (2) remained valid in awake animals. The low Hb–O_2-affinity erythrocytes released more O_2. O_2 consumption remained stable as the cardiac output was lowered. Several previous studies support our results. Guinea pigs after transfusion with rat erythrocytes suspended in guinea pig plasma had reduced cardiac output (12). Apstein *et al.* (14) saw increased myocardial O_2 consumption and contractile function in isolated heart perfused at fixed blood flow with low-affinity erythrocytes (high 2,3-bisphosphoglycerate content). Gross *et al.* (15) decreased HbO_2 affinity with orthoiodosodium benzoate and demonstrated improved myocardial oxygenation in isolated dog hearts. In previous work, Gersonde and Nicolau (16) have introduced InsP_6 in erythrocytes using InsP_6-loaded liposomes. This method has obtained erythrocytes with low O_2 affinity, but the technique was tedious and affected by significant hemolysis. Franco and Weiner (17) have incorporated InsP_6 in erythrocytes by a dimethyl sulfoxide shock treatment of erythrocytes, thus obtaining significant rightward shifts in the HbO_2 dissociation curve. In a recent paper Stucker *et al.* (18) indicated an inverse relationship between P_{50} and coronary blood flow. In this work a large increase in arteriovenous O_2 difference led to an increase in myocardial O_2 consumption, even though coronary blood flow was considerably reduced.

DISCUSSION

The data presented here support and widen the significance of observations we have reported previously (2, 4–6, 19) on acute physiological modifications induced by erythrocytes with increased O_2-release capacity. Indeed, the P_{50} value of InsP_6-erythrocytes is maintained in the live animal over the entire life span of these cells. These life spans, when provision is made for the rapid 24-hr lysis of the most fragile erythrocytes, is slightly longer than that of normal (control) porcine erythrocytes (rapid lysis occurs in ≈15% of the cells) (6). This rapid lysis at 24 hr is apparently the cause of the transient, post-transfusional hyperkalemia and slight loss of Hb.

The effects on electrolytes of InsP_6 incorporation are nil; the same holds for urea, creatine, and protein over the entire observational period; we were unable to see any modification in the blood of the animals transfused with InsP_6-erythrocytes, with the exception of the rightward shift in the P_{50} value.

It appears that erythrocytes capable of releasing greater amounts of O_2 within the limits of physiological Po_2 values continue to do so for their entire life span. The physiological modifications, like reduced cardiac output in the absence of any other effects detectable within this life span, suggest the

possible use of InsP_6-erythrocytes to restore normal oxygenation in case of impaired blood flow. Indeed, the heart reacts to the higher O_2-releasing capacity of InsP_6-erythrocytes by reducing its output and thus maintaining the normal O_2 consumption and arterial pressure.

The data by Stucker *et al.* (18) on isolated rat heart, showing a correlation of the coronary blood flow with the P_{50} value of the perfusing blood further supports these observations of the regulatory capacity of this organ when it is perfused by erythrocytes with high O_2-releasing capacity.

This situation is reminiscent of high muscular exertion where, when O_2 requirements are increased, nature responds by lowering the pH and increasing the organ temperature—i.e., by shifting the HbO_2 dissociation curve to the right. We have demonstrated that in the presence of high P_{50} a sufficient O_2 supply may be achieved with substantially lower values of blood flow. This may have important implications for clinical situations where blood flow is limited. By the introduction of InsP_6 in erythrocytes kept viable by our procedure (4–6) and InsP_6-erythrocytes transfusion, high P_{50} may be achieved over a protracted period.

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1. Lenfant, C., Torrance, J., English, E., Finch, C. A., Reynafarje, C., Ramos, J. & Faura, J. (1968) *J. Clin. Invest.* **47**, 2652–2656.
2. Teisseire, B. P., Ropars, C., Vallez, M. O., Hérigault, R. A. & Nicolau, C. (1985) *J. Appl. Physiol.* **58**, 1810–1817.
3. Benesch, R. E., Edalji, R. & Benesch, R. (1977) *Biochemistry* **16**, 2594–2599.
4. Ropars, C., Nicolau, C. & Chassaigne, M., inventors; Centre National de la Recherche Scientifique, Paris, France; Centre Hospitalier Regional de Tours, France; and Studiengesellscheft Kohle mbH, Mulheim Ruhr, F.R.G., assignees. Procede et dispositif pour l'encapsulation dans les erythrocytes d'au moins une substance a activite biologique, notamment des effecteurs allosteriques de l'hemoglobine et erythrocytes ainsi obtenus. European patent 0101341. November 5, 1986. 29 p., Int. Cl. A61K9/50; Appl. July 1, 1983.
5. Ropars, C., Chassaigne, M., Villeréal, M.-C., Avenard, G., Hurel, C. & Nicolau, C. (1986) *Bibl. Hematol.* **51**, 82–91.
6. Ropars, C., Teisseire, B., Avenard, G., Chassaigne, M., Hurel, C., Ginot, R. & Nicolau, C. (1985) *Ann. N.Y. Acad. Sci.* **445**, 477–491.
7. Valeri, C. R., Rorth, M., Zaroulis, C. G., Jakubowski, M. S. & Vescera, S. V. (1974) *Ann. Surg.* **180**, 106–110.
8. Dennis, R. C., Vito, L., Weisel, R. D., Valeri, C. R., Berger, R. L. & Hechtman, H. B. (1975) *Surgery* **77**, 741–747.
9. Bristow, J. D., Metcalfe, J., Krall, M. A., Welch, J. E., Black, J. A. & Oski, F. A. (1977) *J. Appl. Physiol.* **43**, 102–106.
10. Moore, L. G. & Brewer, G. J. (1981) *J. Lab. Clin. Med.* **98**, 145–154.
11. Farber, M. O., Sullivan, T. Y., Fineberg, N., Carlone, S. & Manfredi, F. (1984) *J. Lab. Clin. Med.* **104**, 166–174.
12. Laver, M. B., Jackson, E., Scherperel, M., Tung, C., Tung, W. & Radord, E. P. (1977) *J. Appl. Physiol.* **43**, 632–642.
13. Teisseire, B. P., Soulard, C. D., Hérigault, R. A., Leclerc, L. F. & Laver, M. B. (1979) *J. Appl. Physiol.* **46**, 816–822.
14. Apstein, C. S., Dennis, R. C., Briggs, L., Vogel, W. M., Frazer, J. & Valeri, R. (1985) *Am. J. Physiol.* **248**, H508–H515.
15. Gross, G. J., Warltier, D. C. & Hardman, H. F. (1977) *J. Pharmacol. Exp. Ther.* **202**, 72–81.
16. Gersonde, K. & Nicolau, C. (1979) *Blut* **39**, 1–7.
17. Franco, S. & Weiner, M. (1986) *J. Cell. Physiol.* **129**, 221–227.
18. Stucker, O., Vicaut, E., Villeréal, M.-C., Ropars, C., Teisseire, B. & Durvelleroy, M. (1985) *Am. J. Physiol.* **249**, H1224–H1227.
19. Nicolau, C., Ropars, C. & Teisseire, B. (1986) in *Phytic Acid*, ed. Graff, E. (Pilatus, Minneapolis), pp. 265–289.