Pig Reticulocytes

III. Glucose Permeability in Naturally Occurring Reticulocytes and Red Cells from Newborn Piglets

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ABSTRACT The loss of facilitated glucose transport of red cells occurring in the newborn pig was monitored in 11 density-separated cells from birth to 4 wk of age. At birth there was a threefold increase in glucose permeability from the lightest cells to the most dense, suggesting that cells having progressively less glucose permeability are released into the circulation as gestation proceeds. Because of extraordinary stimulation of erythropoietic activity, the uppermost top fraction constituting 2-3% of the total cells is composed purely of reticulocytes in the growing animal. The glucose permeability of these reticulocytes which at birth has a slow but significant rate of 3.7 μmol/ml cell × min at 25°C is rapidly decreased within 3-4 days to the level of reticulocytes produced in the adult in response to phenylhydrazine assault. Moreover, reticulocytes themselves discard their membrane permeability to glucose in the course of maturation to red cells. Thus, even though reticulocytes at birth are permeable to glucose, they will become red cells practically impervious to glucose within a few days. These findings suggest that the transition from a glucose-permeable fetal state to a glucose-impermeable postnatal state is brought about by two mechanisms: (a) dilution of fetal cells by glucose-imperious cells produced coincidentally with or shortly after birth; and (b) elimination of fetal cells, which have a shorter half-life, from the circulation.

INTRODUCTION

It has long been known that fetal red cells derived from many mammals are much more permeable to glucose than red cells obtained from adult animals (Kozawa, 1914; Widdas, 1955). After birth, the membrane permeability to glucose measured in red cells gradually decreases to the adult level within a characteristic time ranging from 3 to 4 wk in the pig (Zeidler et al., 1976) to 8 to 9 in the dog (Lee et al., 1976a). In most cases, the sluggish glucose permeability of adult red cells apparently provides a sufficient amount of the substrate to support glycolysis, the sole remnant of metabolic machinery from which mature red cells must drive essential free energy for the maintenance of cellular integrity.

The pig represents an extreme example of this phenomenon in that membrane permeability to glucose is entirely lost during the early postnatal period.
(Zeidler et al., 1976). As a result, while fetal pig red cells are capable of utilizing glucose, postnatal and adult red cells are incapable of glycolysis (Kim et al., 1973). Despite the fact that the initial observation was reported by Engelhardt and Ljubimova (1930) and Kolotilova and Engelhardt (1937) almost a half-century ago, amazingly little is known about the mechanism by which the nonglycolytic cell survives in its long journey through the circulation.

Because of the puzzling and limiting role of membrane permeability in glycolysis, a question arises as to whether the immature precursor cells are also metabolically deprived of the benefit of glycolysis. As an approach to this problem, we have examined the reticulocytes produced in the adult animals in response to phenylhydrazine assault (Kim and Luthra, 1976; Kim et al., 1976). It was found that the most immature reticulocytes possessed a glucose permeation mechanism. The salient features of this glucose transport include: (a) saturable kinetics with maximal velocity ($V_m$) ranging from 0.1 to 0.4 $\mu$mol/ml cell × min at 38°C and substrate concentration at which one-half $V_m$ occurs ($K_m$) ranging from 6.6 to 12 mM; (b) inhibition by phloretin; and (c) counter transport characteristics suggesting that glucose entry is mediated by a carrier-type transport. In immature reticulocytes, glucose consumption as high as 2.5 $\mu$mol/ml cell × h was found. As in other mammalian reticulocytes (Gasko and Danon, 1972a, b), the maturation process leading to the red cell was accompanied by a gradual shift from aerobic to anaerobic metabolism. Unlike in other mammalian reticulocytes, however, the vital membrane “carrier” responsible for glucose permeation is discarded in the course of the final stage of the cellular differentiation and maturation process, resulting in a nonglycolytic red cell.

Detailed investigation of the kinetic property of glucose entry into fetal pig red cells confirmed the early finding of Widdas (1955) who postulated the presence of a facilitated diffusion pathway for glucose (Zeidler et al., 1976). Although the transport characteristics were similar to the key features seen in reticulocytes, the $V_m$ in fetal cells was two orders of magnitude greater than that of reticulocytes.

The primary objective of this communication is to elucidate the mechanism by which glucose permeability in the red cell is discarded soon after birth. To this end, the change in glucose permeability was monitored in density-separated cells from birth to 4 wk after birth. The findings reported herein suggest that the transition from a glucose-permeable fetal state to a glucose-impermeable postnatal state is brought about by the elimination of the fetal cell population and the dilution of fetal cells by glucose-impervious cells produced at or immediately after birth.

**MATERIALS AND METHODS**

*Preparation of Reticulocytes and Red Cells*

In the newborn pig, a large number of reticulocytes begin to appear in the circulating blood within 2-3 days after birth, reaching a maximum at 1 wk and virtually disappearing by the 2nd wk. The number of reticulocytes present during this period depends greatly upon the availability of iron (which was given intramuscularly at a concentration of 100 mg/animal [iron dextran 100] about 3 days after birth). Naturally occurring maximum
reticulocytosis often amounts to as much as 15–18% of circulating blood cells. Blood samples were obtained in heparin (15 U/ml) from the anterior vena cava of restrained animals.

**Fractionation of Reticulocytes and Red Cells according to Their Density**

Fractionation of cells according to their density was performed by the modified procedure of Murphy (1973) as reported elsewhere (Kim et al., 1976). Blood samples were centrifuged at 4,000 rpm for 15 min at 4°C in a Sorvall RC-2B centrifuge (DuPont Instruments, Sorvall Operations, Newtown, Conn.). Plasma was removed and saved for later use. The white buffy coat was aspirated with caution in order not to remove the upper cell layer. Cells were resuspended in plasma at a hematocrit of 80–90% in a centrifuge tube (2.7 × 10.5 cm) and centrifuged for 30–45 min at 15,000 rpm at 30°C with the SS-34 rotor in a Sorvall RC-2B centrifuge. To obtain a horizontal surface in the top layer, the tubes were further centrifuged for 2 min in a swinging-bucket Sorvall centrifuge (model GLC-1) at 2,000 rpm at 4°C. In general, 6–10 equal fractions from the top to the bottom of the centrifuge tube were obtained layer by layer by using a pasteur pipet followed by carefully washing the side of the tube with plasma. If desired, each of these fractions can further be fractionated by utilizing a smaller centrifuge tube (1.3 × 10.0 cm). In this way, cell fractions representing as little as 2–3% of the total cells can be obtained. To determine the density, an aliquot of each fraction was centrifuged against mixtures of dibutyl phthalate and dimethyl phthalate according to the procedure of Danon and Marikovsky (1964). After centrifugation in a microhematocrit capillary tube at room temperature for 15 min in an Adams microhematocrit centrifuge (Clay Adams, Div. of Becton, Dickinson & Co., Parsippany, N. J.) the percent of the cells above each phthalate mixture was plotted against its density. The average density of each cell fraction was taken to be the specific gravity at which the cells were equally distributed from the top to the bottom of the phthalate layer. In Fig. 1, a typical result of cell separation with concomitant density measurement obtained from a 1-wk old pig is given. It was found that reticulocytes amounting to 15% of the total cells were entirely confined to the uppermost fraction. This reticulocyte-rich fraction was once more centrifuged in a smaller centrifuge tube (1.3 × 10.0 cm) and centrifuged at 4,000 rpm for 15 min at 4°C.
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10.0 cm) and six equal fractions were taken. The measurement of density of these reticulocyte-rich fractions and red cell fractions revealed that naturally occurring reticulocytes varied in density widely from 1.081 to 1.098. In sharp contrast, mature red cells ranged within narrower limits, from 1.098 to 1.104. That cell separation according to their density corresponds to the separation of cells by age has amply been established in recent years (Kim et al., 1976; Cohen et al., 1976).

3-O-Methyl-Glucose Flux Measurements

The 3-O-methyl-glucose (3-O-M-glucose) flux was carried out according to a procedure described elsewhere (Zeidler et al., 1976). Unless otherwise stated, flux measurement was performed at room temperature. An aliquot of cells was added at a hematocrit of 8% or less to flux medium consisting of 5 mM KCl, 140 mM NaCl, 10 mM Na-phosphate buffer, pH 7.4, plus 10 mM 3-O-M-glucose supplemented with 14C substrate (0.1 μCi/ml medium). 0.3-ml samples of cell suspension were rapidly mixed into 1 ml of prechilled quenching solution composed of 2 mM HgCl₂ and 2 mM KI. The mixture was quickly spun down in a Brinkmann centrifuge (model 3200, Brinkmann Instruments, Westburg, N. Y.) and the pellet was washed once with quenching solution. To extract the radioactivity, the pellet was hemolyzed by adding 0.4 ml of 1.0% saponin from which 25 μl hemolysate was taken for hemoglobin determination. To the remaining hemolysate, 0.8 ml of chloroform and methanol (2:1 vol/vol) was added. The mixture was vigorously vortexed and centrifuged for 1 min. The resultant upper phase was used for radioactivity determination. Radioactivity determination was made on a Nuclear-Chicago liquid scintillation counter (Nuclear Chicago Corp., Des Plaines, Ill.) with a counting mixture composed of PPO (2g), POPOP (100 mg), toluene (800 ml), ethanol (200 ml), and Triton X-100 (500 ml). 3-O-M-Glucose uptake was calculated from the cell radioactivity and the specific activity of 3-O-M-glucose in the medium. For each cell fraction, seven samples which were rapidly taken within 30 s were used to construct a plot of uptake vs. time from which the initial uptake rate was obtained.

Cell Tagging by ⁵¹Cr and ⁵⁹Fe

At birth, each of two newborn animals had received 100 μCi ⁵¹Cr by heart puncture. Two other litter mates were given 100 μCi ⁵⁹Fe. Combinations of 100 μCi ⁵¹Cr and 100 μCi ⁵⁹Fe were given to the remaining two litter mates. Blood samples amounting to approximately 7.0 ml were drawn from these animals at various times. The cells were subjected to density fractionation to obtain 10 equal fractions from the top to the bottom of the cell column. A portion was used for 3-O-M-glucose flux. The remainder was used for ⁵¹Cr and ⁵⁹Fe radioactivity determination which was carried out on an automatic gamma counter (model 1185, Searle Analytic Inc., Des Plaines, Ill.). The radioactivity of each fraction was expressed as the percent of the total ⁵¹Cr or ⁵⁹Fe radioactivity counts.

Sources of Materials

All pigs used in this study were purchased from the Arizona Hog Farm, Tucson, Ariz. Both ⁵¹Cr and ⁵⁹Fe and [3-O-methyl-¹⁴C]-glucose were purchased from New England Nuclear, Boston, Mass. Dimethyl and dibutyl phthalate were obtained from Eastman Kodak Corp., Rochester, N. Y. Iron dextran 100 was obtained from Franklin GND Corp., West Palm Beach, Fla.

RESULTS

The change in specific gravity of whole blood of a growing pig as measured against the known density of phthalate mixture is shown in Fig. 2. At birth, cells were distributed more or less evenly in a broad density profile ranging from
1.093 to 1.115. Within a few days, the distribution curve shifted dramatically to the left, indicating the mass emergence of newly fabricated lighter cells. As the animal aged, the distribution curve gradually moved to the right, crossing over the profile seen at birth. In 2 wk, the density profile was beginning to assume the characteristics of red cells derived from the adult animal, in which the density profile showed a steep slope, suggesting the presence of a relatively homogeneous cell population.

During this period of change in cell density, the glucose permeation mechanism is discarded. Attempts to delineate the mechanism whereby postnatal cells lose their membrane permeability to glucose have been greatly impaired by the lack of an adequate means of evaluating the complex metabolic and membranous alterations which take place within the individual cells undergoing maturation and aging. It has not been possible, for example, to arrive at a conclusion as to whether the loss of glucose permeability and metabolism is due solely to the depletion of fetal cells or to the change in fetal cell membrane permeability characteristics, or both. To address this question, we have applied the procedure of Murphy (1973) which permits the segregation of cells according to their density without grossly altering their normal physiological characteristics. This facile technique has been found to be equally effective in separating reticulocytes and red cells according to density, regardless of the mammalian species tested.

Data gathered on the glucose influx rate in density-separated red cells in several growing piglets are shown in Fig. 3. At birth the lightest cells, consisting of 2–3% of the total population, display a rapid glucose uptake amounting to 3.7 μmol/ml cells × min. With increasing cell density, glucose permeability is correspondingly enhanced and reaches a maximum of 10.8 μmol/ml cells × min for cells in the bottom 16% fraction. These results suggest that cells formed in early gestation are more permeable than are cells produced later. Because of the extraordinary stimulation of erythropoietic activity, the uppermost light fraction was entirely composed of pure reticulocytes. By the 2nd day, reticulocytes seen in the uppermost fraction had glucose permeability of less than half of what was seen in their counterpart at birth. By the 2nd wk, permeability to glucose was drastically reduced in all fractions.

A more detailed presentation of data on glucose influx in the reticulocytes representing the top 2–3% of the total cells together with those from the bottom
16% of the cells gathered from numerous growing newborn animals is shown in Fig. 4. By the 4th day, glucose permeability in the reticulocyte is all but lost. However, this residual finite permeability is always retained by the reticulocytes regardless of the age of the animal. Indeed, reticulocytes produced in the adult in response to phenylhydrazine assault have a typical carrier-mediated facilitated transport system capable of supporting glycolysis (Kim and Luthra 1976; Kim et al., 1976). These results suggest that the erythropoietic apparatus undergoes a steady alteration as gestation proceeds, resulting in the synthesis of cells whose membrane permeability to glucose is continually diminishing until the newly formed cells are nearly glucose impermeable. Concomitant with this drastic loss by newly released cells, denser cells also display a progressive reduction in membrane permeability to glucose. This observation could be due either to developmental changes occurring in each cell or to the shift in the population of denser cells each with relatively fixed glucose permeability, or both.

Another feature in Fig. 3 which should be emphasized is the kinetic parameter obtained from a 7-day old pig. Since maximum reticulocytosis, amounting to 17% of total cells, takes place at this time, cells in the top fraction, no. 1, are composed mostly of reticulocytes. Because of the low permeability of reticulo-
cytes to glucose, a small but significant change with respect to glucose permeability seen in these cells is not readily noticeable in Fig. 3. To reveal this change, the glucose permeability of cells in top fraction no. 1 from a 7-day old pig depicted in Fig. 3 is replotted on an expanded scale in Fig. 5. It is evident that reticulocytes themselves gradually lose membrane permeability to glucose in the course of maturation to red cells in much the same manner as seen in experimentally induced adult reticulocytes (Kim and Luthra, 1976; Kim et al., 1976). Thus, even though the reticulocytes seen at birth are permeable to glucose, within a few days these cells will become red cells practically impervious to glucose.

![Figure 4](image1.png)

**Figure 4.** A comparison of 3-O-M-glucose uptake in the top 2-3% and the bottom 16% cells from growing piglets.

![Figure 5](image2.png)

**Figure 5.** 3-O-M-glucose uptake by the top 16% cells which were refractionated according to their density into six fractions from a 7-day old piglet. The reticulocyte counts in each fraction are given in parentheses.
The third observation emerging from this series of studies is that the cells in the bottom 16% fraction precipitously lose membrane permeability to glucose, assuming adult cell characteristics within 2–3 wk after birth (Fig. 4). It seems as if fetal cells themselves undergo permeability changes. To unravel further the mechanism underlying this phenomenon, it was desirable to tag certain cell types in such a way that change in the membrane permeability to glucose could be monitored during aging. To accomplish this, radioisotopes $^{51}$Cr and $^{59}$Fe were injected separately into piglets at birth. While $^{59}$Fe is incorporated into the hemoglobin of newly produced cells at the time of erythropoiesis (Finch et al., 1949), $^{51}$Cr penetrates the cell membranes and combines with the globin portion of hemoglobin molecules (Cooper and Owen, 1956). Thus, the distinction between cells synthesized before (fetal cells) and after (postnatal cells) birth may be made. If the membrane permeability of these two cell types could be monitored in the growing animal, it would provide the data necessary to answer the aforementioned questions.

In Fig. 6, results on $^{59}$Fe incorporation into density-separated red cells from birth to 4 wk are shown. As expected, $^{59}$Fe incorporation has taken place only into the top 10% of the cells some 17 h after injection. 3 days thereafter, the radioactivity peak occurs at the second top fraction indicating the continual emergence of newly synthesized cells. However, as the animal ages, the $^{59}$Fe peak does not proceed serially stepwise toward the denser fraction. Rather, the peak appears abruptly at the considerably denser fraction no. 7 after 5 days.
followed by a broad distribution of radioactivity into all other fractions by the 2nd wk. Thus, although the postnatal cells just released from bone marrow or spleen are definitely lighter than the fetal cells already circulating in the bloodstream, the two cell types must undergo entirely different density changes during their aging. As a result, postnatal cells rapidly become indistinguishable from fetal cells when separated on a density basis.

The result of $^{51}$Cr incorporation is summarized in Fig. 7. In contrast to the $^{59}$Fe-labeling pattern, radioactive $^{51}$Cr was taken up by all cell fractions as expected. $^{51}$Cr incorporation, which was somewhat greater in lighter cells, gradually shifted to the right as the animal aged, indicating that the original cell population present at the time of birth was not becoming denser. However, as in $^{59}$Fe incorporation, the $^{51}$Cr-labeling pattern shown in Fig. 7 must reflect continual mixing of postnatal cells with the fetal cells. Accordingly, glucose influx rates measured in $^{59}$Fe- or $^{51}$Cr-labeled cells would not provide useful data in evaluating change in membrane permeability during fetal cell aging.

The mechanism by which the transition from the glucose-permeable fetal state to the glucose-impermeable postnatal state occurs may still be brought to light provided that the following parameters are shown: (a) the half-life of fetal cells; (b) the extent of fetal cell dilution; and (c) the time when postnatal cells without glucose permeability first appear in the circulation after birth. The half-life of fetal cells can be estimated from the results of $^{51}$Cr incorporation (Fig. 7), by taking into account the dilution of fetal cells by newly produced postnatal cells in the growing newborn pig. Of domestic mammals, the pig has one of the most rapid growth rates. Newborn pigs weighing 2-5 lbs may double their weight in 1 wk, weigh 4 times the birth weight at 2 wk, 7-8 times at 4 wk and 20 times at 8 wk (Swenson, 1964). During this rapid growth, the total blood volume per kilogram of body weight remains a relatively constant 90 ml/kg (Talbot and Swenson, 1970), so that measurement of body weight may be used for the estimation of
fetal cell dilution. The result is shown in Fig. 8 in which the 51Cr radioactivity corrected for the dilution is plotted against animal age. The half-life according to Fig. 8 is 11 days. This relatively low value might have been brought about partly by the well-known effect of chromium elution from cells, the extent of which, if it exists, is not known. Although exact measurement of the half-life of fetal pig red cells is not available, numerous estimations of the half-life of red cells of growing young pigs are available. Bush et al. (1956), using 51Cr, determined the mean half-life to be 17 days in four growing pigs. Talbot and Swenson (1963) by autologous and homologous transfusion techniques found the half-life to be 14 and 28 days, respectively. By taking into account the estimated half-life of fetal cells and the extent of fetal cell dilution and assuming that only glucose-impermeable cells are produced after birth, it is now possible to construct the rate at which membrane permeability to glucose should decay in the growing piglets. The result is shown in Fig. 9, in which the lower and upper solid lines of the shaded area are the calculated rates utilizing half-lives of 11 and 28 days, respectively. Each of three experimental animals denoted by the different symbols falls reasonably close to the predicted value from birth to 4 wk. The deviation of the two experimental points from the first part of the predicted rates may simply reflect an imperfection in the assumption employed in the calculation. To simplify the above calculations, glucose-impermeable cells are assumed to appear in the circulation after birth, although this was contrary to actual observation (Figs. 3 and 4). In any case, these findings suggest that the transitory postnatal change in membrane permeability is brought about by depletion of the fetal cell population and by simple dilution of glucose-permeable fetal cells by glucose-impermeable postnatal cells.

**DISCUSSION**

The red cells of the newborn differ in many respects from those of the adult. The spectacular change in the structure and function of cell membranes repre-
sents a much-investigated paradigm of the complex phenomenon of postnatal adaptation. The mechanism by which cells of high potassium content in newborn lamb (Tosteson, 1966; Brewer et al., 1968), calf (Israel et al., 1972), and puppy (Lee and Miles, 1972) are replaced by low potassium cells in the adult stage has been extensively investigated. Similarly, membrane permeability to glucose rapidly undergoes a reduction after birth in many mammals. Human

![Figure 9](image)

**Figure 9.** The net loss of glucose permeability in the composite population of red cells after birth. Each of three experimental animals is denoted by a different symbol. Solid lines bounding the shaded area represent the permeability change as a function of time as formulated on the basis of a model in which the extent of fetal cell dilution, the half-life of fetal cells, and the production of glucose impermeable cells after birth are taken into account. The lower and upper solid curves were calculated according to the following equations utilizing half-life values of 11 and 28 days, respectively. The fraction of fetal cell volume ($V_f$) with respect to the whole blood volume ($V_b$) is:

$$ f = \frac{V_f}{V_b} = \frac{N(t)v_t}{M(t)K}, $$

where $N(t) = N_0e^{-\text{half-life}t}$ is the number of fetal cells; $M(t) = M_0g(t)$ is the animal mass; $g(t) = 1.0 + 1.202t + (9.375 \times 10^{-2})t^2 + (5.208 \times 10^{-3})t^3$ is an empirically determined weighting function; $t$ = time in weeks after birth; $t_{1/2}$ = the cell half-life; $M_0$ = body mass at birth; $N_0$ = number of fetal cells in circulation at birth; $v_t$ = volume of individual fetal cells; $K$ = proportionality constant.

fetal cells are twice as permeable to glucose as those of the adult (Widdas, 1955). In the dog (Lee et al., 1976a) and guinea pig (Widdas, 1955), glucose permeability decreases by one and two order(s) of magnitude, respectively, to the adult level in the course of 8–9 wk after birth. In the rabbit, the permeability decreases even more drastically by three orders of magnitude (Augustin et al., 1967). These transitory permeability changes can be brought about by a number of mechanisms. Knowledge of the fetal cell life span, the rate of increase in blood volume, the extent of appearance of postnatal cells having negligible permeability, and the effect of cell aging on permeability is essential if we are to assess fully the mechanisms underlying these postnatal alterations in membrane functions.

Although there appears to be considerable uncertainty with regard to the fetal cell life span, accumulating evidence seems to favor the view that the fetal cell
has a life span shorter than that of the adult cell (Oski and Naiman, 1966). Lee et al. (1976b), ingeniously applying a technique of continuous infusion of $^{59}$Fe, concluded that the fetal dog red cell had a half-life of approximately 2 wk. Our own estimation of fetal pig cell half-life is approximately 11 days, indicating that fetal cells are rapidly eliminated from the circulation after birth. Since the extent of chromium elution from red cells is not known, this figure must represent a lower limit.

The extent of fetal cell dilution by postnatal cells can be estimated by measuring the increase in blood volume with age. Here, in view of the constancy of blood volume per unit body weight (Talbot and Swenson, 1970), body weight may be used for the first approximation of the estimation of the total blood volume after birth. In view of the exceptionally rapid growth rate, the dilution of fetal cells by the 4th wk should have reduced glucose transport to one-eighth the rate at birth.

Findings emerging from this and other laboratories (Miller et al., 1961) indicate that erythropoietic activity is extraordinarily stimulated in the newborn pig. The results presented herein demonstrate that reticulocytes having progressively less membrane permeability to glucose are released into circulation on successive days after birth. Moreover, the result shown in Fig. 5 indicates that naturally occurring reticulocytes of the newborn progressively lose their membrane permeability to glucose in the course of maturation in much the same way as do reticulocytes produced in the adult in response to phenylhydrazine injection (Kim and Luthra, 1976). Thus, even though the lightest cells (reticulocytes) constituting 2–3% of the total cells derived at birth have a slow but significant glucose influx rate (Figs. 3, 4), these cells will soon become red cells which are practically impervious to glucose. These mechanisms seem to ensure a quick transition from the glucose-dependent fetal state to the glucose-independent postnatal state.

The separation technique of Murphy (1973) employed in this study has been found to allow efficaciously cell separation representing as little as 2–3% of total cells. It has been shown elsewhere that cell separation by density corresponds to the fraction of cells according to their age (Kim et al., 1976). It is evident that the cells produced after birth are lighter than the existing fetal cells, as shown in Fig. 4 and Fig. 6. However, since postnatal cells become quickly indistinguishable from fetal cells when separated on a density basis, these cells must undergo different rates of density change in the course of cell aging. We found similar results in calf red cells in which the hemoglobin electrophoretic pattern of fetal and postnatal cells was used as a cell marker to ascertain the extent of cell mixing (Kim and Zeidler, unpublished observations). Therefore, even though this cell separation technique is enormously useful, the procedure does not permit us to monitor aging fetal cells in the growing animal. Consequently, it is not known to what extent, if any, the fetal cell aging process in itself governs membrane permeability characteristics.

The reason that pig red cells discard the glycolytic machinery adopted by other mammals in the course of evolution is not known (McManus, 1967; McManus, 1973). Unknown, too, is the in vivo metabolic substrate utilized by this nonglycolytic cell (McManus and Kim, 1969; Kim and McManus, 1974a, b).
Freshly drawn plasma cannot support red cell ATP levels. Nonetheless, it would seem that the absence of the metabolic substrate in the plasma does not necessarily exclude its role in vivo, since the extent of the substrate's input into the circulation might be delicately balanced by red cell consumption. Indeed, by infusing cells with dihydroxyacetone at a relatively constant low level of 0.01 mM, McManus (personal communication) has recently found a satisfactory maintenance of ATP levels. If such a role for dihydroxyacetone exists in vivo, it would require that some localized region in the circulation, such as liver, heart, kidney, lungs, etc., supply a low but significant level of substrate to the cells during their passage through the microcirculation. A good candidate for such a localized area could be the liver. Work is in progress to test the validity of this postulation.

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