Red blood cell life span in the ovine fetus

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Brace, Robert A., Christiane Langendörfers, Tae-Bok Song, and Donald M. Mock. Red blood cell life span in the ovine fetus. Am J Physiol Regulatory Integrative Comp Physiol 279: R1196–R1204, 2000.—Red cell life span within the fetal circulation has not been reported, although erythrocyte life span has been studied in the adult and newborn. The present study quantified red cell life span in 12 chronically catheterized fetal sheep at 97–136 days gestation (term = 150 days) with the use of autologous red cells labeled with [14C](cyanate). Cyanate forms a permanent covalent bond with hemoglobin and acts as a permanent red cell label. In the fetuses, blood 14C activity decreased in a curvilinear fashion with time and reached 50% of the initial activity at 16.4 ± 1.6 (SE) days. In contrast, 14C activity of autologous red cells in two adult ewes decreased linearly with time as expected, reached 50% of the initial 14C activity in 59 days, and yielded life spans of 117 and 121 days. Computer modeling and parameter optimization taking into account growth and skewed life span distribution were used to analyze the 14C disappearance curve in each fetus. The mean life span of all red cells in the fetal circulation was 63.6 ± 5.8 days. Mean red cell life span increased linearly from 35 to 107 days as fetal age increased from 97 to 136 days (r = 0.83, P < 0.001). Life span of cells produced at the time of labeling was significantly greater than the mean life span. Fetal growth rate estimated from parameter optimization was 3.28 ± 0.72%/day; this compared well with the rate of 3.40 ± 0.14%/day calculated from fetal weights at autopsy. Mean corpuscular volume decreased as a function of gestational age, but the decrease was small compared with the large increase in red cell life span. We conclude the following: 1) red cell life span in the fetal circulation is short compared with the adult; 2) red cells in younger fetuses have shorter life spans than in near-term fetuses; 3) the curvilinear disappearance of labeled red cells in the fetus appears to be due primarily to an expanding blood volume with fetal growth; and 4) red blood cell life span in a growing organism will be significantly underestimated unless the expansion of blood volume with growth is taken into account.

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IN MAMMALS, MANY MILLIONS of newly produced red blood cells are released into the circulation each day as older red cells are lost or removed from the bloodstream. The average time that an erythrocyte remains in the circulation is termed the red cell life span. Because red cell life span is a potentially important determinant of oxygen delivery to the body’s tissues, the theoretical basis for determining red cell life span has been well validated in adults, and methods based on theory have been applied frequently to measurements in animals including humans (7, 13, 15). The random labeling method is the most commonly used technique for determining red cell life span; typically, an aliquot of autologous or homologous red blood cells is radiolabeled and infused into the circulation. The disappearance of the labeled cells from the circulation is measured as a function of time, and the resulting disappearance curve is interpreted in terms of life span by performing a straight line extrapolation to the time when no labeled cells remain in the circulation (13). The same method of life span determination has been used when red cells with compatible yet unique surface antigens rather than cells labeled with radioisotopes were injected (17). Mammalian red cell life span is ~120 days in adults of different species, including humans and sheep (7, 13, 15, 20). The life span of fetal red cells within the fetal circulation has not been previously reported. Furthermore, methods for accurately interpreting disappearance curves of labeled red cells in rapidly growing organisms such as fetuses have not been published. Past studies have attempted to estimate red blood cell life span in the fetus by studying either the newborn or “fetal” red cells isolated from the umbilical cords and/or placentas at the time of delivery. These studies have generally suggested that red cell life span in the fetus and newborn is shorter than in the adult (6, 10, 11). However, most of the previous studies used interpretation methods for the adult and hence do not provide an accurate estimate of fetal erythrocyte life span for several reasons including the following: 1) dilution of the label due to the rapid expansion of blood volume with growth generally was not taken into account (17); 2) variable elution of the radiolabel from the red cells occurred, and this can be especially large in the fetus.
(9, 20); and 3) the skewed distribution of the life span of cells in the fetal circulation was not considered (6). Furthermore, preferential removal of the fetal cells from the newborn or adult circulation may have occurred because fetal cells are larger and express different surface antigens. Preferential removal has been reported in both humans (9) and sheep (20) even after matching of blood types. Most of these factors would cause the curve appearance in the fetus to be curvilinear rather than linear. Furthermore, when using the straight line extrapolation method that works well in the adult, each of the above factors would cause the estimated red blood cell life span to underestimate the true life span in the fetus and newborn. Another complicating factor is that some studies have found fetal red cell life span to be the same as in the adult (8, 11). There presently exists no explanation for this discrepancy with studies suggesting that fetal red cells have a short life span.

The purposes of the present study were threefold: 1) to develop a quantitative method that can be used to accurately determine red blood cell life span from disappearance curves in growing organisms; 2) to determine erythrocyte life span in the ovine fetus as a function of gestational age; and 3) to use both the new method and the observed fetal red blood cell life span values to interpret potential errors and discrepancies in previously reported fetal erythrocyte life spans.

We used three techniques to determine red cell life span in the fetus while avoiding the problems of past studies. First, the chronically catheterized fetal sheep was used as an animal model so that the life span of autologous cells could be determined directly within the fetal circulation. Second, 14C-labeled cyanate was used as a red cell label; the permanent covalent bond between cyanate and hemoglobin renders loss of the label due to elution negligible (12, 13, 16). Third, computer-modeling and parameter-optimization techniques that take into account fetal growth and changes in the red cell life span during gestation were used to analyze the curvilinear disappearance of the labeled fetal cells, yielding precise estimates of red cell life span as a function of fetal age and an independent, verifiable estimate of fetal growth rate.

METHODS

Animal studies. Twelve chronically catheterized fetal sheep and two nonpregnant adult ewes were studied. The protocol was approved by the Animal Subjects Committee of the University of California, San Diego. The National Research Council’s Guide for the Care and Use of Laboratory Animals was followed.

Each of the 12 pregnant ewes carried a single fetus. Gestational age at the time of surgical catheter implantation ranged from 92 to 131 days (term = 145–150 days). The surgical preparation, animal care, and maintenance have been described in detail elsewhere (3, 4). Briefly, under inhalation anesthesia with the use of aseptic techniques, polyvinyl catheters were placed in fetal and maternal femoral arteries as well as attached to the fetal skin for accessing the amniotic fluid. In fetuses with a gestational age <100 days at the time of surgery, a vascular catheter was placed in a fetal carotid artery rather than a femoral artery. The catheters were passed subcutaneously into a pouch sewn to the flank of the ewe. Propylactic antibiotics were administered at the time of surgery to the ewe and over the first 5 postsurgical days to the fetus (4).

Experiments began on postoperative day 5. Plasma was removed and saved from 1 ml of fetal blood, the red cells were washed three times to remove white cells and platelets, and the red cells were labeled with [14C]cyanate by incubation for 30 min as previously described (14, 16). The labeled cells were washed four times to remove unbound [14C]cyanate and resuspended in the saved plasma before injection into the fetal circulation (14, 16).

Fetal arterial blood was sampled at 1-h postinjection and at 24-, 48-, or 72-h intervals thereafter for a maximum of 26 days at gestational ages ranging from 97 to 150 days. Sample volume (0.25 ml in fetuses <112 days gestation at the start of the study and 0.5 ml in fetuses ≥112 days) was chosen to minimize the amount of blood and radioactivity removed (<2% of that injected). On the basis of studies in newborn sheep in which fetal hemoglobin was present in high concentrations at birth and disappeared completely from the circulation within 2–3 wk after birth (1, 21), a short red cell life span was anticipated. Thus initial studies were designed to sample for only 14 days. However, preliminary analyses of samples from the first six animals revealed that [14C]cyanate-labeled red cells remained in the fetal circulation at 2-wk postinjection. Thus the sampling period was extended until catheter failure or delivery to better characterize red cell disappearance.

To compare fetal with adult values measured by the same technique in the same laboratory, two nonpregnant adult ewes aged 2–3 years were studied. The femoral artery was catheterized chronically; red cells from 2 ml of blood were removed and labeled with [14C]cyanate, washed four times, and reinfused. Blood samples (1 ml) were taken at 1-h postinjection, three times weekly for the first month, and weekly thereafter for 101 days.

From each 0.5-ml fetal blood sample, 0.05 ml were used for determination of hematocrit in triplicate (3), and 0.2 ml were used for determination of blood gases and pH (Nova Stat Profile Ultra analyzer). The remaining 0.25 ml of blood were divided into two equal aliquots, weighed, and stored for 14C counting. For the 0.25-ml blood samples from the younger fetuses, hematocrit and blood gases were not determined to minimize the effect of sampling on blood and 14C loss from the fetus. Plasma protein concentration was determined refractometrically from the plasma in the hematocrit tubes (3). For the two adult sheep, hematocrit was determined in triplicate, and the remaining blood was weighed and stored in two aliquots. Stored samples were anticoagulated with heparin and refrigerated until analyzed.

Mean corpuscular volume (MCV) (i.e., the average volume per red cell) was determined in four fetuses from this study and in 23 fetuses in other concurrent studies with the use of a Coulter Multisizer model IIe. Calibration of the Multisizer was performed with the use of human red cell standards (4 C tri pack; Coulter, Miami, FL).

From the stored blood samples, hemoglobin with the bound 14C was isolated with the use of an acid-acetone-water extraction procedure as detailed elsewhere (14, 16). The protein precipitate was dissolved in liquid scintillation counting fluid (Ecolume; ICN Biomedicals, Irvine, CA) and counted for 10 min per sample. The radioactivity per gram of blood was normalized with respect to time with the day 0 value taken as 100%.
In the fetus, the circulating red cell label is diluted over time as blood volume expands with growth. As an independent measure of growth, body weights were measured at autopsy in 46 fetuses over the age range of 100–147 days gestation, and average growth rates were calculated.

**Life span determination.** In the adult, the method of determining red cell life span from disappearance curves is well established and is straight forward (13). In the fetus, the method for interpreting disappearance curves has not been established, and there are a number of complicating factors. These factors are typically assumed constant in the adult but had to be taken into account in development of the method for determining red cell life span in the fetus: 1) an increasing absolute rate of red cell production; 2) increasing life spans of newly produced red blood cells as the fetus ages; and 3) somatic growth that leads to increases in blood volume with time. To illustrate development of the method for determining red cell life span in the fetus, the following description compares the effects of these factors in the adult and fetus.

Each day, the adult releases a large number of new erythrocytes into the circulation. Figure 1 illustrates the distribution of life spans of the cells released on a given day assuming the newly released cells have a life span of 120 ± 2 (SD) days. At steady state, the adult continues to release the same number of new erythrocytes each day as the previous day, and the distribution of life spans is the same (Fig. 1A). Each day, the oldest erythrocytes are removed from the circulation due to senescence, and the cells remaining in circulation have 1 day less remaining in their lives. Hence, the distribution of the remaining life of all red cells in the circulation is flat as illustrated in Fig. 1B, with a drop to zero cells for any time greater than the mean life span (plus ~2 SD) of the newly released cells. To determine life span in the adult with the use of the random labeling method, a sample of blood is labeled and injected into the circulation. Because the remaining life distribution is flat, the disappearance of these labeled cells from the circulation declines linearly with time (Fig. 1C). Due to spread in life span distribution of the newly released erythrocytes, the disappearance curve deviates slightly from linear near the intersection of the linear portion and the time axis. Red cell life span is then determined analytically by using least-squares linear regression to define the point at which the regression line intersects the time axis. The shape of the distribution of the life span of the newly released cells (Fig. 1A) affects the disappearance curve only near the intersection with the time axis and thus typically has no effect on the calculated life span because disappearance curves typically are followed for only 50–80% of the life span.

In the fetus, red cell production, life span distribution, and disappearance kinetics are much different. First, the number of erythrocytes produced each day increases as the fetus grows. Figure 2A illustrates this for the ovine fetus, which grows an average of ~3.5% per day, as used in this study. Over a period of 3 wk, the fetus more than doubles the number of erythrocytes produced daily. This has a dramatic effect on the distribution of the remaining life of the circulating red cells and results in a skewed distribution as illustrated by the solid line in Fig. 2B. Second, as also illustrated in Fig. 2A, the mean life span of newly produced red cells increases as the fetus ages. This changes the distribution of the remaining life so that it is slightly flatter as illustrated by the dotted line in Fig. 2B.

If fetal blood with the remaining life distributions of Fig. 2B is labeled and injected into a growing fetus, then the disappearance curves are concave with respect to the time axis as illustrated by the curves in Fig. 2C. This concave shape occurs primarily because the expansion of vascular volume with growth dilutes the concentration of the labeled cells and additionally is affected by the skewed distribution of the remaining life of the cells in the circulation. With changes in the absolute rate of red cell production, changes in mean red cell life span with gestational age, a skewed life span distribution, and expansion of blood volume with growth all occurring simultaneously, an analytic determination of red cell life span from a disappearance curve is not possible. To take into account all of these factors, a computer model of fetal red cell kinetics was developed. Kinetic parameters included the life span of newly released red cells, expansion of blood volume with fetal growth, and changes in life span as the fetus ages (Fig. 2A). The computer simulation allowed for addition of newly released red cells, aging of previously released red cells, and removal of cells at the end of their life span. These functions were integrated across time to calculate distribution curves as a function of gestation age (Fig. 2B). Integration was performed with simple Euler integration techniques, with accuracy of the integration being verified by maintaining the value of the time increment sufficiently low so that it had no effect on overall

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**Fig. 1.** A: theoretical comparison of the number (arbitrary scale) and life span of newly released red cells in the circulation of the adult. The standard deviation was assumed to be 0.2 days. B: distribution of remaining life for all red cells in the circulation of the adult. Curve computed by summing daily productions illustrated in A. C: disappearance of labeled red cells after injection into the circulation. Curve calculated from B.
outcome. From these distribution curves and fetal growth rate, disappearance curves were computed (Fig. 2C). The mean life span of all cells in the circulation was then calculated from the distribution curves and the life span of the cells at the time they were released into the circulation. The model also included the day 0 radioactivity as a parameter. This was included to take into account the small amount of $^{14}$Ccyanate that may be lost over the first 24 h after injection due to incomplete binding within the red cell (16).

To determine experimental values from model parameters, parameter-optimization techniques were used to obtain the best statistical fit between the computer-generated and observed disappearance curves. The extent of agreement between the experimental- and model-generated disappearance curves was assessed by least-squares regression. Each of four parameter values in each individual fetus was varied independently to minimize the sum of the squared differences between the computed and experimental values for the entire disappearance curve. Day 0 values were excluded from the regression analyses in 9 of 12 fetuses because the values were discontinuous with values on subsequent days; we assume this is caused by a small loss of label during the first 24 h (16). By setting both growth rate and the daily increase in life span of newly released red cells to zero, the computer model produced the same value for life span as the linear interpretation method used in the adult. The linear interpretation method was also applied to data from each fetus for comparative purposes.

Data presentation and statistical analysis. Experimental values are presented as the mean ± 1 SE. The two values of fetal blood $^{14}$C activity for each day were averaged before analysis. Bivariate and multivariate correlation and least-squares regression were used to determine relationships between optimized parameter values and fetal gestational age. A lower case $r$ represents bivariate and upper case $R$ represents multivariate correlation coefficients. $^{14}$C disappearance curves were considered curvilinear if second or higher order terms from polynomial regression were statistically significant. Hematocrit changes with time in the two adult ewes were analyzed with a two-factor repeated-measures ANOVA. Fetal weights at autopsy were log transformed to linearize before regression analysis. A paired $t$-test was used to determine the statistical significance of the difference between mean life span for all cells in the circulation and the life span of newly released cells as well as the difference between the mean life span and the life span as determined by linear extrapolation. Changes in MCV with gestational age were analyzed with polynomial regression. For all tests, statistical significance was chosen as $P \leq 0.05$.

RESULTS

In the 12 fetuses, red cell labeling occurred at 118.1 ± 3.5 (SE) days gestation; range = 97–136 days. The $^{14}$C label disappeared from the fetal circulation in a curvilinear fashion ($P < 0.01$) and reached 50% of the day 0 value at an average of 16.4 ± 1.6 days (Fig. 3).

Red cell life span as determined from computer modeling and parameter optimization for mean data is illustrated in Fig. 4 and is compared with the linear extrapolation method as used in the adult. For the 12 fetuses, the mean correlation coefficient between experimental values and the simulated disappearance curve was 0.946 ± 0.010. The average life span for all red cells in the fetal circulation was 63.6 ± 5.8 days (range = 29.6–95.8 days). This is 5.5 ± 1.4 days shorter ($P = 0.0028$) than the life span of red cells released at the time of labeling (69.1 ± 6.3 days; range = 34.6–104.8 days). Using the straight line extrapolation method as used in the adult yielded a life span of 36.5 ± 4.7 days, a value significantly less than the mean life span ($P < 0.0001$).

Both the life span of all red cells in the circulation and the life span of newly released red cells exhibited a significant positive relationship with fetal gestational age (Fig. 5). The slopes of the two regression equations were 1.37 ± 0.30 and 1.54 ± 0.03 days/day, respectively. These slopes both differ significantly from zero ($P < 0.001$) but are not significantly different from each other ($P = 0.58$). Extrapolation from the regression equations to term (150 days) yields a life span for all red cells of 107.4 ± 3.8 days compared with 118.3 ± 3.9 days.
days for the newly released cells. This difference approached statistical significance ($P = 0.058$).

From the optimized parameter values, the daily increase in life span with advancing gestation in the 12 fetuses was $0.52 \pm 0.22$ days/day. From regression analysis, this parameter was not significantly related to gestational age.

Mean values for fetal hematocrit, plasma protein concentration, arterial pH, and blood gas values over the gestational age range of 112–150 days are listed in Table 1. Hematocrit, plasma protein concentration, and arterial carbon dioxide tension correlated positively and significantly with gestational age, whereas oxygen tension and pH were not correlated significantly with age (Table 1).

From measured fetal weights at autopsy, there was an exponential increase in fetal weight as a function of gestational age (Fig. 6). The regression equation was best described as a power function ($weight = 35.421 \times 1.034^{Age}$) where weight is in grams and gestational age is in days. This corresponds to a growth rate of $3.40 \pm 0.14\%$/day. Fetal growth rate as determined from the disappearance curves with the use of parameter optimization was $3.28 \pm 0.72\%$/day. These values do not differ statistically ($P = 0.79$). Furthermore, fetal growth rate as determined from modeling was not significantly related to gestational age.

MCV (red cell) in the fetuses decreased with increasing gestational age (Fig. 7); the polynomial regression relationship is $MCV = 99.4 - 0.8345 \times Age + 0.0028 \times Age^2$. The most rapid age-related decrease occurred in the midgestation fetuses; by contrast, MCV decreased only $7 \mu l$ (from $45 \mu l$ to $38 \mu l$) over the age range for which red cell life spans were determined. With the use of MCV calculated from the regression equation, there was a negative correlation between red cell life span and MCV ($r = -0.595$, $P = 0.042$). With the use of multivariate regression, both gestational age and MCV were significantly related to red cell life span ($R = 0.892$, $P < 0.00001$).

The disappearance curves and red cell life span determinations for the two adult sheep that were studied are shown in Fig. 8. Both disappearance curves were linear with time. Blood $^{14}C$ concentration was reduced to $50\%$ of its initial value at 59 days. Average red cell life span was 119 days. Hematocrit did not change significantly with time in these animals ($P = 0.51$).

**DISCUSSION**

The present study was designed to determine the life span of red blood cells in the circulation of the ovine fetus from labeled red cell disappearance curves. On the basis of our findings, we conclude the following: 1) Red cell life span in the ovine fetus is less than that in the adult; 2) Fetal red cell life span increases with gestational age; 3) The mean life span of all erythrocytes in the fetal circulation is less than the life span of the newly released red cells; 4) Methods used in the adult for interpreting disappearance curves significantly underestimate red cell life span in the growing fetus; and 5) To appropriately interpret curvilinear fetal disappearance curves, a computer model with parameter optimization was necessary so that the expansion of fetal blood volume with growth, increases in life span with gestational age, and a skewed life span distribution could be taken into account simultaneously.
The conclusion that fetal red cell life span is less than that in the adult is on the basis of the present observation that erythrocyte life span in the ovine fetus during the last third of gestation averaged 64 days, which is about half of the adult values that averaged 119 days. Although we studied only two adult sheep, their values agreed well with a much larger body of data indicating that erythrocyte life span in adult sheep is \textbf{120} days (14, 20). Other investigators have concluded that fetal red cell life span is less than adult red cell life span, so it may be questioned what is new about our study. However, none of the previous studies examined red cell survival in the fetal circulation. Furthermore, the previous studies generally used interpretation methods that were theoretically un-

sound because the effects of growth were not taken into account (6, 7, 9, 10, 17, 20). The current study significantly improves the quality of the knowledge base underlying the conclusions that erythrocyte life span in

![Fig. 5. Regression analysis of red cell life span in the fetus as a function of gestational age for all cells in the circulation (left) and for newly released red cells (right). The regression line (solid line) and the 95\% confidence interval about the regression line (dotted line) are shown. Each dot represents a different fetus. The circles show values in 2 adult nonpregnant ewes.](http://ajpregu.physiology.org/)

![Fig. 6. Ovine fetal weight as a function of gestational age. Regression line (solid line) and the 95\% confidence interval about the regression line (dotted line) are shown.](http://ajpregu.physiology.org/)
the fetal circulation is short and that it increases with gestational age because the methodology used allowed proper interpretation of the disappearance curves in terms of both fetal growth and the changing distribution of red cell life span with gestational age. Both of these factors can have large effects on the shape of the disappearance curve and hence on the estimated life span. For example, with application of the commonly used straight line interpretation method to the disappearance curves from the present study, mean fetal red cell life span would be underestimated by almost 50%. It might be suggested that even though the fetal disappearance curves were slightly curvilinear, they are sufficiently close to linear so that the straight line interpretation as used in the adult is appropriate. However, linear interpretation is on the basis of the assumption that no growth is occurring so that blood volume is constant, and it is also on the basis of the assumption that all red cells have the same life span when released into the circulations. Neither of these assumptions is valid in the fetus. Thus not only is the basis of the straight line method not applicable to the fetus, but the logical flow is strikingly emphasized by the underestimation of the life span in the growing organism.

The curvilinear nature of the disappearance curves found in the present study has been observed in previous studies in young and growing animals and humans. However, past studies have interpreted such data as indicating either that there exists two populations of red cells with some having a short life span or that some cells were damaged during the labeling process and thus were disappearing from the circulation more quickly than normal (6, 14). Although the latter remains a possibility for some labeling methods, the present study suggests that the primary cause of the curvilinear nature of the disappearance curve in the growing animal is the dilution of labeled red cells due to expansion of blood volume with growth. In fact, the estimate of fetal growth rate in the present study agreed so well with the growth rate determined from measured body weights that the curvilinearity can be almost attributed entirely to growth because the increase in life span with gestational age had only a small effect as illustrated in Fig. 2. This conclusion is on the basis of the assumption that ovine fetal blood volume expands at the same rate as body weight. Two observations support this assumption: 1) blood volume in the chronically catheterized ovine fetus expands at a rate of 3.5 ± 0.5%/day (19), a value essentially the same as the 3.28 ± 0.72%/day determined from computer modeling and 3.40 ± 0.14%/day calculated from fetal weights at autopsy in the present study; and 2) weight-normalized blood volume in the chronically catheterized ovine fetus is independent of fetal body weight over a wide range of fetal weights (3).

The present study also detected a significant increase in fetal red cell life span with gestational age. This results in a mean life span of all red cells in the fetal circulation that is significantly less than the life span of the newly released red cells. This difference aids in evaluating conflicting results of previous studies. For example, although most previous studies have concluded that the life span of fetal red cells is shorter than that of the adult, a few studies have concluded that fetal and adult red cell life spans are the same (8, 11). The latter studies used the tracer incorporation method that labels the newly produced red cells,
The conclusions from the present study regarding fetal red cell life span are dependent on a number of other assumptions. First, the $^{[14]C}$cyanate had no effect on life span. This is likely as the use of the $^{[14]C}$cyanate label in the adult produces the same life span as determined with other labels and other methodologies (14, 16). Second, catheterization of the fetus may have in itself led to a shortened red cell life span due to factors such as an allergic reaction to the catheter material or a thrombotic response to the catheter. Although possible, these are unlikely in that we know of no support for the ideas in the thousands of papers that have been published using this animal model. Furthermore, both the immune and thrombotic systems of the fetus are immature and lack the responsiveness of the adult.

One inconsistency in our analysis merits discussion. For the parameter red cell life span change with gestational age, computer modeling with parameter optimization yielded an average value of 0.52 ± 0.22 days/day for the 12 fetuses. However, regression analysis of life span as a function of gestational age yielded a slope of 1.54 ± 0.30 days/day. We currently do not have an explanation for this apparent conflict. Notwithstanding, both analyses suggest that the life span of newly released red cells increases by ~1 day/day of gestation over the last third of gestation in the ovine fetus.

In summary, the present study suggests that red cell life span in the ovine fetus over the last third of gestation increases at a rate of ~1 day/day gestation and reaches adult values at term. Furthermore, we conclude that labeled red cell disappearance curves in the growing organism can be interpreted properly only if the expansion of blood volume with growth and potential changes in red cell life span with age are taken into account.

**Perspectives**

It has been widely accepted that the life span of red cells within the fetus is less than adult values even though red cell life span in the fetal circulation has not been previously reported. Although this conclusion appears to be correct, it was based largely on an inappropriate interpretation of data. That is, neither the expansion of blood volume with growth nor the skewed distribution of life spans within the circulation nor the increase in life span with gestational age were taken into account in most studies. The present study shows that these errors led to a substantial underestimation of the true life span. In fact, the error is so large that the true life span of “fetal” red cells isolated at the time of birth may be little different from adult values. Furthermore, there has been no previous explanation for the few studies that found the life span of fetal red cells to be the same as adult values. The latter studies may be accurate in that they were performed in the new-
born with correction for growth, and the methodology (tracer incorporation) used determined the life span of newly produced cells. The present study suggests not only that the life span of newly produced fetal erythrocytes is longer than the mean life span, but also that the newly produced cells at term have the same life span as the adult. More importantly, the present study provides a methodology for studying erythrocyte life span in any growing organism when using the random labeling method.

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