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NEONATAL ANEMIA AND GROWTH IN SABLE ANTELOPE (HIPPOTRAGUS NIGER)<sup>†</sup>

By M. Bush, U. S. Ssal, E. Smith, M. D. Lewis and L. M. Bush

Observations on hematology of zoo-born sables and other bovids during the postpartum period have demonstrated a decline in hematocrit during the first 10 days after birth. The possibility of a pathological process of postnatal blood loss in sable calves was suspected because of initial hematocrit values below 30 % which continued to decline, positive occult blood test on stool specimens, and the loss of two of six calves during the first month postpartum.

The declines in hematocrit or "physiological anemia" observed in young laboratory animals and domestic animals occurs later in the postnatal period, after a two-fold or greater increase in weight (Halvorsen and Halvorsen, 1973) and thus may have a different mechanism than the rapid changes observed in the antelope. Plans were made to study sable calves with respect to hematology, blood cell indices, blood volume, growth, serum proteins, erythropoiesis, hemolysis, blood loss, and clinical signs of disease in order to determine the basis for this anemia.

An unusual opportunity was presented with the birth during April 1974 of two calves within one day of one another. They were sired by the same mals. One of the dams was the daughter of the other female and had been sired herself by the same male (Buechner, et al., 1974). One of the calves, a female, was left with her dam, the oldest female. The other calf, a male, was rejected by the dam and was hand-reared. The results of two months' studies on these animals are described here.

Materials and methods

Blood samples were taken from the jugular vein using manual restraint. Autologous red blood cells drawn shortly after birth were labeled with chromium-51 (<sup>51</sup>Cr) (Gray and Sterling, 1950; Mollison and Veall, 1955; Zehr, et al., 1969). Plasma was separated from the cells and they were washed twice with saline prior to incubation with the <sup>51</sup>Cr since significant amounts of this material are bound to plasma proteins when the incubation is carried out with whole blood from ruminant species. The labeled red cells were washed and resuspended in sterile saline. A 5.0 ml aliquot was injected intravenously into the recurrent tarsal vein using a 19 gauge catheter at the times indicated in Table 1. This was accomplished within 6 hours of birth in the female and about 18 hours postpartum in the male. Both calves were given a prophylactic injection of a long-acting penicillin preparation at the time of injection of the labeled cells. The remaining portions of the labeled red cells were used for determination of hematocrit and radioactivity for calculation of total injected counts and counts per minute per ml of injected red blood cells. The blood sampling schedule followed is indicated in the text figures and included samples at 90 minutes and 12-14 hours after injection of the labeled red cells for estimations of blood volume and red blood cell mass. Portions of each blood sample were used for cell counts by the Unopette method (Becton-Dickson Co.) and with a Coulter Counter Model B, microhematocrit, hemoglobin as cyanmethemoglobin, reticulocyte counts using a fresh methylene blue stain, and differential white cell count. Serum protein fractions were measured by cellulose acetate electrophoresis using the Beckman Microzone apparatus and a Beckman densitometer for scanning the stained strips. Total protein was

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measured by the UV spectrophotometric method of W a d d e l l (1956) and in a Goldberg refractometer. Assays for blood clotting factors were done on samples collected at 15 and 21 days postpartum through the courtesy of Dr. W. S w a i m , Minneapolis VA Hospital. Body weights were measured each time the animals were handled. Stool and urine samples were collected from the hand-reared animal for measurement of  $^{51}\text{Cr}$  radioactivity excretion and for occult blood tests using Hematest<sup>®</sup> tablets (Ames Lab.). Several stool specimens were also obtained from the mother-reared calf. Radioactivity was measured in a Nuclear-Chicago autogamma counter. All counts were corrected for decay to a standard time using both a standard and table. Statistical calculations were performed on a Wang calculator, model 462-1.

The hand-reared male animal was fed several diets during the initial phase of the study. An analysis of his mother's milk revealed 81.3 % water, 13.3 % protein, 4.0 % fat and 0.57 % of carbohydrate. The composition and quantity of the diet was adjusted to meet the growth needs of the animal as reflected in the weight gain data when compared to the mother-reared calf. An oral iron supplement was added from the 10th day postpartum. Ampicillin was given the 11th through 13th day for a fever and navel infection. The mother-reared animal had access to soil and consumed some on occasion, thus providing a probable source of exogenous iron.

### Results

The weight gain of the female, dam-reared calf, Fig. 1, was linear over the 50-day period shown in the graph. The regression equation ( $y = a + b x$ ) for weight gain over time for the first 56 days is  $y = 13.6 \text{ kilos} + (0.49 \text{ kilo/day}) (x \text{ days})$  with  $r = +.9992$ ,  $F = +.6059$ ,  $p < 0.0001$ . The linear regression analysis for the male yielded  $y = 10.6 + 0.47x$  with  $r = +.9933$ ,  $F = 814$  and  $p < 0.0001$ . The erratic early weight gains for the hand-reared male reflect the limitations of the initial diet and the results of the changes made to improve the caloric and protein intake. With these changes the gain after 10 days showed an approximately linear character and nearly paralleled that of the female. The male eventually at 60 days surpassed the female in weight.

The data for hematocrit, Fig. 2, illustrate the pattern of changes which prompted this study. There was a progressive decline from the immediate postpartum levels of 29 and 32 % to a minimum reached within 24 h and maintained for seven days in the female and reached at four to nine days and maintained until 20 days in the male with the levels in both animals then increasing until they plateaued at 40-50 days. The hematocrit in the female began increasing sooner, increased more rapidly in the earlier phases, and reached a plateau sooner than in the male. These results can be readily correlated with the weight gain data shown in Fig. 1, except for the initial decline in hematocrit. Regardless of the weight gain pattern, there was an initial decline in hematocrit. The decline occurred more slowly and persisted longer in the male, who was having growth difficulties during this period. The upturn in hematocrit began in both animals when they had gained 2.5 kilos.

The patterns of decline and subsequent rise in hematocrit appear to be mirrored by the changes in red blood cell count and hemoglobin, Figs. 3 and 4. The single deviation in red cell count at seven days in the male perhaps may be attributed to a technical error. Body weight in the female was increasing linearly during the decline in red blood cell count. The general pattern in the first two days postpartum is that of a dilution or loss of red cell mass. A comparison of the red cell and hematocrit curves indicates that the decline of red blood cells persisted past the time when the hematocrit began to increase in both animals. This would suggest increasing red cell size.

The red blood cell indices are shown in Figs. 5 and 6. The values for mean corpuscular volume (MCV) in the male fluctuated around  $26 \mu^3$  with two peaks at the times of maximum reticulocyte counts. The MCV values in the female showed a significant elevation around 10 days also corresponding to the period of highest reticulocyte levels. The mean corpuscular hemoglobin concentration (MCHC) in both animals increased from 30-31 % to adult levels of

Table 1. Vital Statistics and Initial Conditions for Blood Studies of Sable Calves.

	<u>Male</u>	<u>Female</u>
Animal Number	M00900	M00899
Date of Birth	4/27/74	4/26/74
Time of Birth	0100	0005
Dam ID	M00197	33,385
Weight (kilos)	12.5	13.9
Time of initial sample	1240	0100
Time of labeled red cell injection	1810	0517
Total cpm <sup>51</sup> Cr injected	4.5 x 10 <sup>6</sup>	6.2 x 10 <sup>6</sup>

Figure 1. Body weight curves of the mother-reared female and hand-reared male sable calves from birth to 50 days.

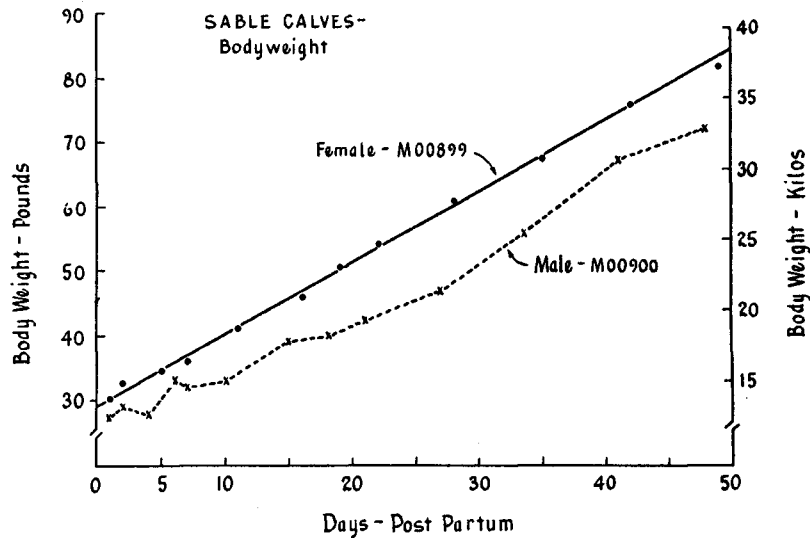


Figure 2. Hematocrits of the sable calves from birth to 50 days.

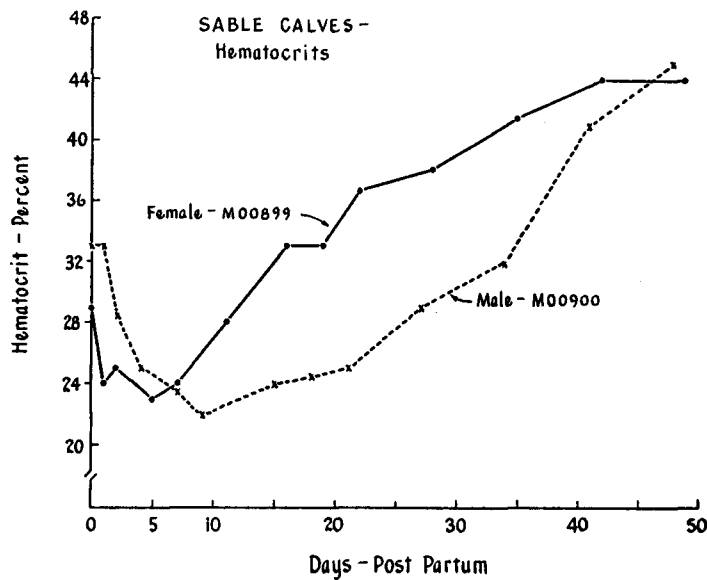


Figure 3. Red blood cells of the sable calves from birth to 50 days.

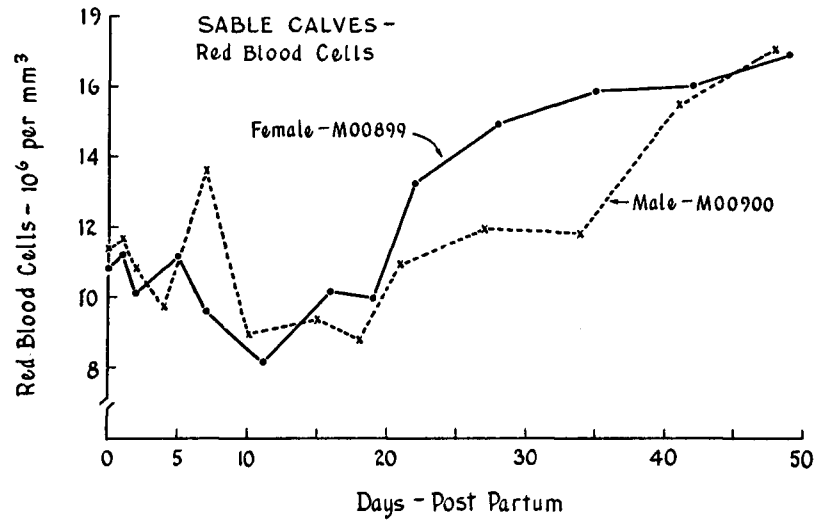


Figure 4. Hemoglobin levels of the sable calves from birth to 50 days.

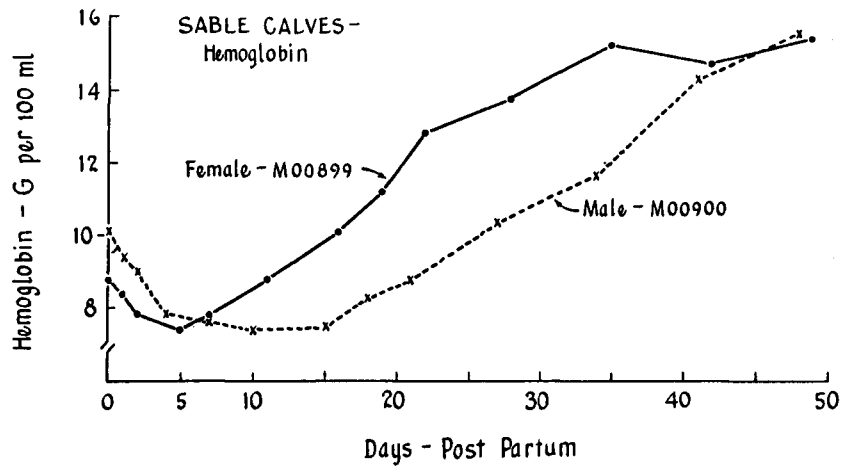


Figure 5. Mean corpuscular volume of red blood cells of the sable calves from birth to 50 days.

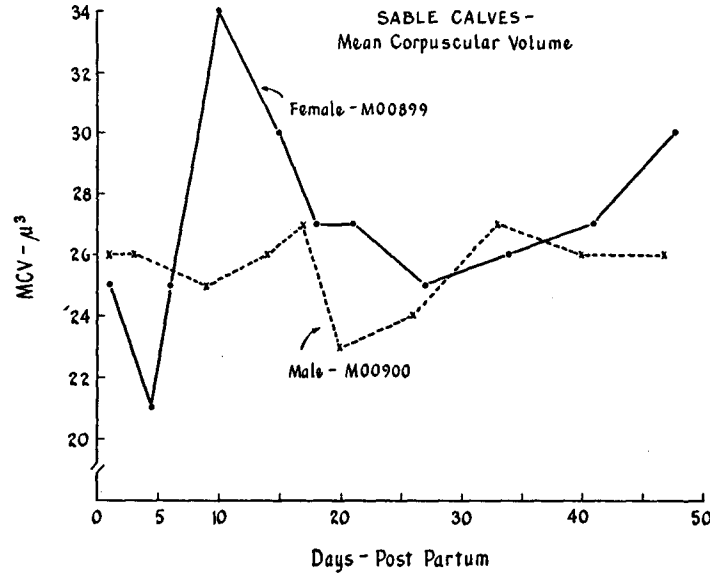
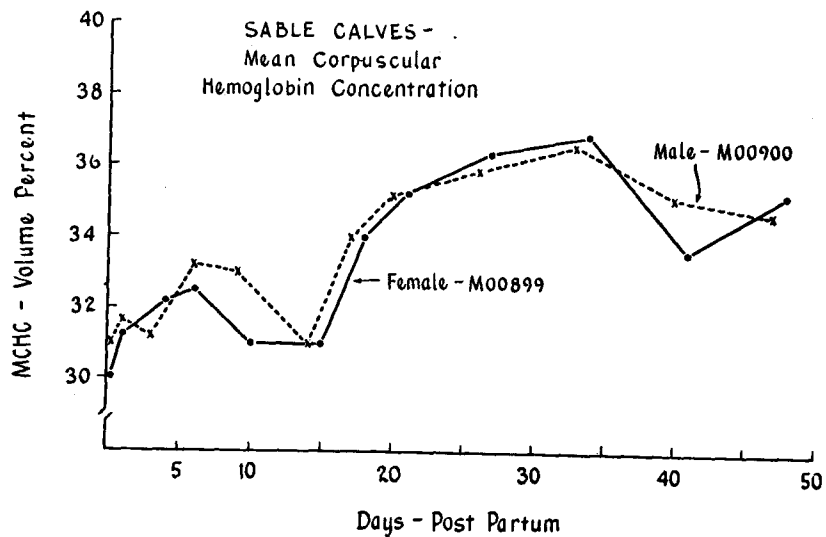


Figure 6. Mean corpuscular hemoglobin concentration of the sable calves from birth to 50 days.



36-37 % over a four-week period following the pattern shown in Fig. 6. The decrease in MCHC at 10-15 days is coincident with the increase in MCV and corresponds to the reticulocyte peak. Thus, the reticulocytes were larger cells with a lower content of hemoglobin. It is also notable that, in contrast to many species (Seal et al., 1967; Seal and Erickson, 1969; Wintrobe and Shumacher, 1936), the red cells of these newborn sable were smaller, rather than larger, than cells of the adult animals.

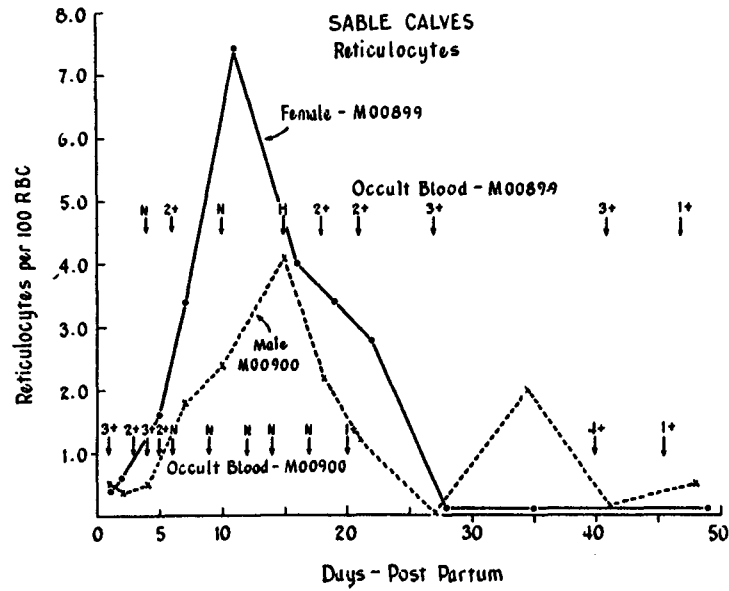
The reticulocyte count data, Fig. 7, showed an increase in the female by five days postpartum which peaked at about 11 days at 7.5 % and then declined to adult levels by 28 days. The response in the male was delayed, peaking in 15 days at 4.0 % and dropping to adult levels by 27 days. The reticulocyte counts peaked about 5-6 days after the nadir of the hematocrit. Results of tests for occult blood in the feces, also shown in Fig. 7, indicated a period of positive tests prior to the increase in reticulocytes in the male but not in the female.

The white blood cell count in the female calf, Fig. 8, showed a rapid increase during the first 8 h, a subsequent decline over five days, followed by another increase during the period of reticulocytosis and finally a decline to approximately adult levels. A similar pattern occurred in the male calf except that the initial elevation was less striking. Differential white cell counts showed that the lymphocyte counts were less than 1000/cu.mm for the first five days and then fluctuated around 2000/cu.mm in the female until a sharp rise to 4000 at 49 days which was still present at 56 days and not followed further. Thus, the white blood cell peaks during the first 15 days were due entirely to increases in neutrophils. The same relationships were present in the samples from the male and the elevation in lymphocytes also occurred in the period after 50 days and persisted for at least 40 days more.

Total serum proteins, Fig. 9, showed an interesting pattern of changes with striking differences between the two animals. Levels in the female increased rapidly during the first 48 h to 9 g% followed by a gradual decline over the next three weeks to around 7 g%. Her total serum proteins increased from 4.7 to 5.9 g% during the first 7 h postpartum. The hematocrit declined from 29.5 to 23.7 % during this period. These results are consistent with a plasma and blood volume expansion as a result of globulin absorption from colostrum obtained by nursing during this time. The male showed a very small increase in plasma proteins from 4.6 to 5.3 g% during the first 48 h and remained about 3 g% lower than the female over the next three weeks and then began a gradual increase toward levels similar to those of the female. Electrophoretic analysis of the serum protein fractions, Fig. 10, demonstrated that the entire difference between the two animals may be attributed to the elevated levels of gamma globulins which were responsible for the early rapid increase in the female and account for the 3 g% difference between the two animals. The essential absence of the electrophoretic gamma globulin fraction in the male, upon direct examination of the cellulose acetate strips, is consistent with the fact that he did not nurse and that immunoglobulins are obtained from maternal colostrum in the early postnatal period with little or no transfer across the placenta during pregnancy in ruminant species with nonhemochorial placentation (Brambell, 1970). The navel infection in the male calf may have been a result of this lack of circulating immunoglobulins.

Blood volume and red cell survival times were estimated using  $^{51}\text{Cr}$  labeled autologous red blood cells. The radioactivity data, plotted in terms of percent of dose per liter of blood or percent of dose per liter of red blood cells, yielded the curves shown in Figs. 11 and 12. The values for red blood cells were obtained by counting a known volume of whole blood, determining the hematocrit and calculating the results in terms of the cells. Counts of separated plasma were not significantly different from background. The curves for the female were essentially linear for the first 10 days with an estimated  $t_{1/2}$  of 10.5 or 11 days. The curves in the male are different from one another and from those found in the female, the RBC curve shows a change in slope at about 10 days. The initial curve yielded a  $t_{1/2}$  of eight days. Since the animals were not in a steady state with respect to body weight, blood volume or red blood cell production and destruction, these  $t_{1/2}$  numbers do not have a definable

**Figure 7.** Reticulocyte counts of the sable calves from birth to 50 days. Fecal occult test results are also shown with the arrow indicating the day on which the sample was collected. An "N" indicates a negative result.



**Figure 8.** White blood cell counts of the sable calves from birth to 50 days.

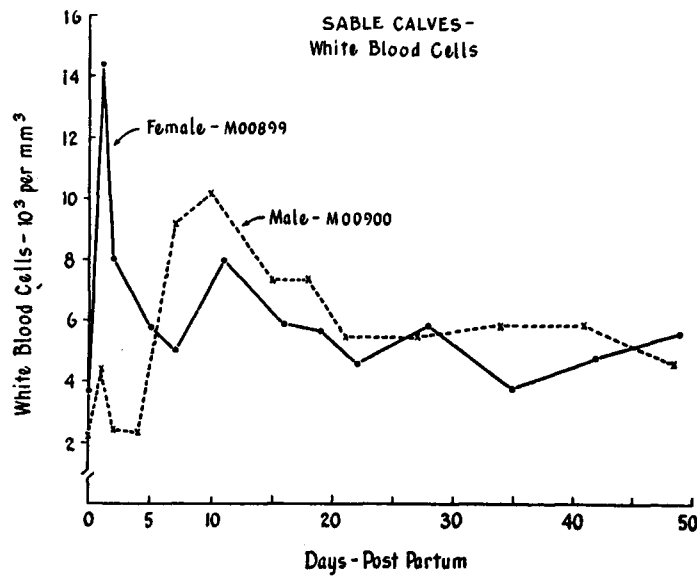




Figure 9. Total serum proteins of the sable calves from birth to 50 days.

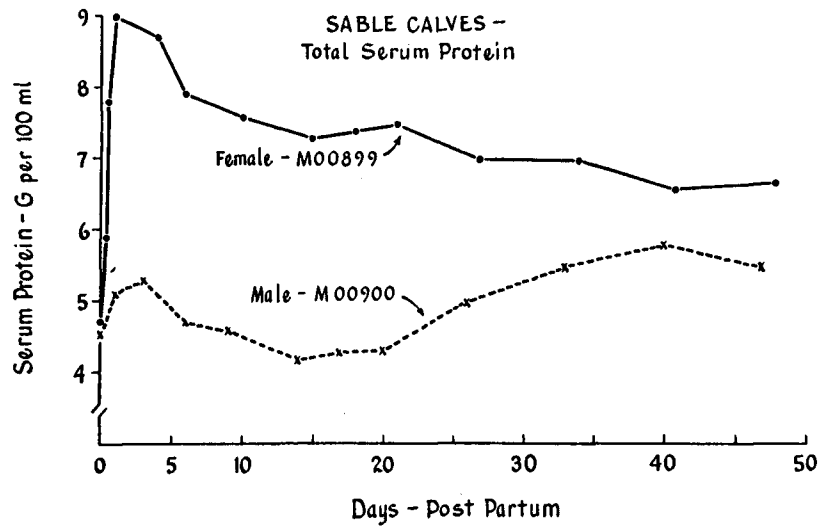


Figure 10. Albumin and gamma globulin serum protein fractions of the sable calves from birth to 10 days.

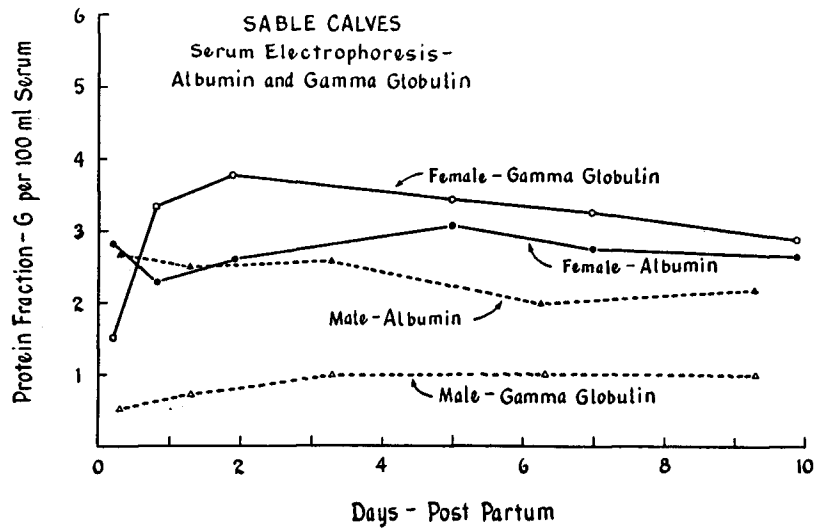


Figure 11. Red blood cell specific activity curves with  $^{51}\text{Cr}$  labeled red blood cells in the female sable calf from birth to 50 days.

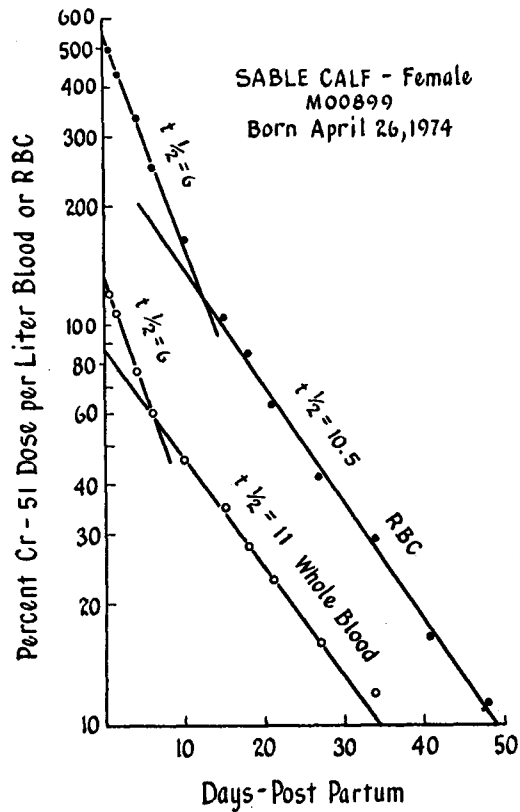
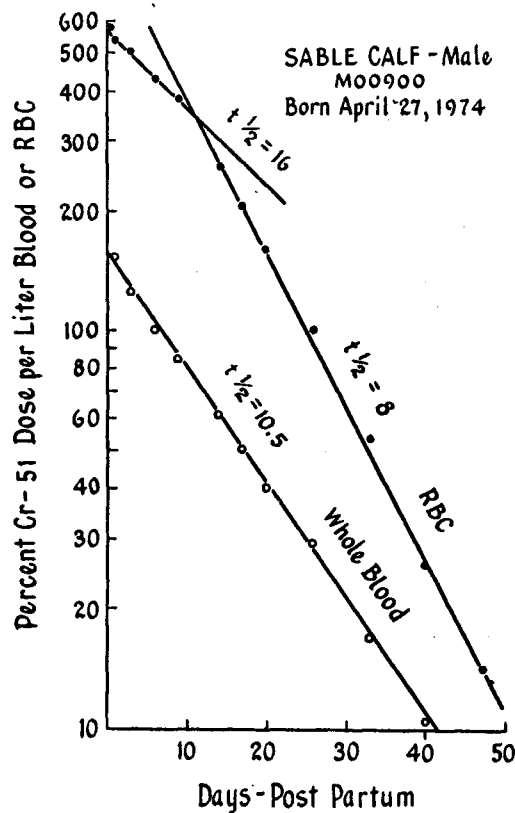


Figure 12. Red blood cell specific activity curves with  $^{51}\text{Cr}$  labeled autologous red blood cells in the male sable calf from birth to 50 days.



significance with respect to red blood cell survival (Greger sen and Rawson, 1959; Swan and Nelson, 1971). They are an expression of the red blood cell bound radioactivity. Also, it is not possible to correct the data for  $^{51}\text{Cr}$  elution from the red cells since the rate is now known for these cells. However, the rapid early decline in red blood cell  $^{51}\text{Cr}$  in the female suggests the occurrence of a rapid elution or random destruction process in this early period.

Whole blood volume and red cell mass were calculated from the data of the 90 minute post-injection sample and from the plotted curves, Table 2. The single point estimates yielded blood volumes, as percent of body weight, of 5.1 % for the female and 4.3 % for the male. However, the RBC volumes were the same for both animals. These results were calculated with the hematocrits of the 90 minute post-injection sample. During the eight hours after birth, the hematocrit of the female declined from 29.5 to 23.7 % but that of the male did not change. This change in the female is almost certainly due to volume expansion as the result of globulin absorption from ingested colostrum and not loss of red blood cells during this time. Recalculation of the data in terms of the 29.5 hematocrit in the immediate postpartum sample yields a blood volume of 585 ml which is 4.2 % of the female's body weight and the same as that calculated for the male. One may estimate the amount of protein absorbed and the resultant increase in plasma volume from these data. The increase in protein is  $5.9 - 4.7 = 1.2 \text{ g\%}$  and the plasma volume (at 8 h) is  $726 - 172 = 554 \text{ ml}$ . The increase in plasma volume is  $726 - 583 = 143 \text{ ml}$  which is a  $(143 \div 411) \times 100 = 35$  percent increase in plasma volume or a 25 % increase in blood volume during this time. The absorbed protein is  $(1.2 \times 5.54) + (1.43 \times 4.7) = 13.2 \text{ g}$ . Similar increases of blood volume with ingestion of colostrum have been reported for piglets (McCance and Widdowson, 1959).

These data also allow calculation of the minimum quantity of red blood cells being produced each day. Thus, given a blood volume of 5.5 % and an increase in body weight from 14.1 to 16.4 kilos, there are 21 ml of whole blood being added each day for the increase in mass. During days 1-7 when there is no significant change in hematocrit, this indicates the synthesis of 5.1 ml of red blood cells per day in the female for a minimum total of 30-32 ml of new red blood cells produced during this period. This estimate does not include replacement of cells being lost. Direct estimates of these losses cannot be made from the data available because too many kinds of changes are occurring simultaneously.

The data for the hand-reared male, Fig. 12, show a slower decline in red blood cell radioactivity during the first seven days when essentially no volume expansion due to growth could have occurred. There is no indication of an increase in the extrapolated whole blood or red blood cell volumes from this early red blood cell curve. There was also a delay in the peak of reticulocytes. These observations indicate that the delayed body growth was accompanied by a delay in increase of erythropoiesis.

The isoelectric focusing patterns of serial hemolysates from these calves, Fig. 13, demonstrate the presence of fetal hemoglobins in this species, which are progressively replaced by the adult proteins. The concentration of the fetal form decreased 3-4 times more rapidly in the female calf than in the male during the first two weeks postpartum. There was approximately 15 % of the adult form present at birth in both animals and it appears likely that synthesis of the fetal form had ceased at this time as observed in bovine calves (Tumbleson and Kalish, 1972). If this is true, the relative concentrations of the two forms provide another means for estimating the disappearance of the fetal red blood cells as a cohort. Examination of Fig. 13 indicates that the pattern of appearance of the adult hemoglobin corresponds to the pattern of change seen in the isotope disappearance curves. Calculation of the amount of hemoglobin synthesized during the period from April 26 to May 2 may be estimated from body weight, blood volume and hemoglobin level data. The proportion of adult hemoglobin in samples from these two dates was

Table 2. Sable Calves Blood Volume Data

Data Sources and Values	ANIMAL	
	M00899	M00900
Single Point Estimates*		
CPM per ml blood	8571	8328
Hematocrits	23.7	32.0
% Dose per liter blood	138	185
% Dose per liter RBC	582	578
Blood Volume (ml)	726	541
RBC Volume (ml)	172	173
Blood Volume - % Body Weight	5.1	4.3
Whole Blood Time Curve		
t $\frac{1}{2}$ (days)	6	10.5
Intercept (% dose/L)	130	155
Blood Volume (ml)	769	645
Blood Volume - % Body Weight	5.5	5.2
Early RBC Curve		
t $\frac{1}{2}$ (days)	6	16
Intercept (% dose/L)	520	560
RBC Volume (ml)	192	179

\* Blood sample taken at 90 minutes after injection of  $^{51}\text{Cr}$  labeled autologous red blood cells. This was approximately 8 hours after birth.

estimated from densitometer scans of electrophoretic patterns. Thus, for the female calf on April 26: (Hgb 8.1 g/100 ml) (766 ml blood) = 62.9 g Hgb of which 14 % or 8.9 g was of the adult form; On May 2: (Hgb 7.8 g/100 ml) (902 ml blood) = 70.4 g Hgb of which 23.6 % or 16.6 g was of the adult form. The total net increase in hemoglobin was 7.5 g and the calculated increase in the adult form was 717 g. Since all of the increase in hemoglobin can be accounted for in terms of adult hemoglobin, it is likely that no fetal hemoglobin synthesis occurred after birth.

The loss of  $^{51}\text{Cr}$  in the feces and urine was measured in specimens collected over a 10-day period in the male calf, Table 3. The loss by the urinary route may represent elution from the red cells or hemolysis or both. The fecal loss appears to have been gradually increasing over this time period and may represent degradation of red cells or perhaps blood loss into the intestine. The presence of isotope in the feces is indicative of blood loss into the intestine in humans but the occult blood test was negative at this time. This test requires the presence of 0.5 to 2 ml of blood per 100 g of feces to yield a 1+ positive result (Christensen et al., 1974). Unfortunately, samples were not collected during the period the test was positive. An estimate of the amount of fresh blood that would be required to yield the observed cpm in the feces can be calculated from the blood specific activity at that time and the quantity of feces produced. It varies from 1.5 to 5 ml and thus the occult blood test should have been positive during this period if the isotope was attached to intact hemoglobin with peroxidase activity. It is doubtful if this test for occult blood in feces has any validity when applied to animals whose diet may contain other peroxidase active substances. Fecal samples were also collected from the female calf on two occasions; May 11 (15 days postpartum) 4400 cpm/100 g (blood, 2196 cpm/ml) and on May 14 (18 days postpartum) 7900 cpm/100 g (blood, 1738 cpm/ml). This was during a period of positive occult blood test. However, comparable levels of radioactivity in the male calf were associated with a negative test result. Since the composition of the diet can influence the occult blood test, it appears likely that it cannot be used for detection of intestinal bleeding in these circumstances. During this 10-day period, a total of 5 % of the  $^{51}\text{Cr}$  appeared in the feces and urine of which one-third was in the urine. Since elution from the red cells should have been about 9 % during this time and red cell degradation should be about the same amount, it appears likely that the isotope in urine represents excretion of eluted label and the isotope in the feces may represent some blood loss or excretion of label from destroyed cells.

Measurement of direct and total serum bilirubin on samples drawn 10 days postpartum yielded values for the female of 0 and 0.2 mg/100 ml and for the male values of 0.4 and 0.8 mg/100 ml. These values, at the nadir of the hematocrit, are within the normal range and well below the 3 mg% or greater levels usually associated with hemolytic diseases (Osaki and Stockman, 1974).

The possible occurrence of a bleeding syndrome, reflected in clotting factor levels, was examined at 15 and 21 days postpartum with the appearance of positive occult blood test in the feces of the female calf. These results, Table 4, yielded no differences between the two animals except a lower factor VII in the male whose fecal blood tests were negative at this time. The activated partial thromboplastin time (APTT) was long compared to values for human samples but was similar in both animals and may be characteristic of this species under these assay conditions which are designed for human samples. Platelets in the female were  $900,000/\text{mm}^3$  and were  $963,000/\text{mm}^3$  in the male at this time. None of these results support the presence of a clotting or fibrinolytic disorder.

Hematocrit data during the neonatal period from other wild ruminant species at National Zoological Park are tabulated in Table 5 with some similar data from pigs and dogs. The neonatal hematocrit of sable calves is substantially lower than the five other wild species for whom data are available. An early fall in hematocrit occurred in all the wild species but never below 30 %.

Table 3. Male Sable Calf, M00900: Fecal and Urinary Content of <sup>51</sup>Cr Label \*

<u>Sample</u>	<u>Days Post-</u>	<u>Blood</u>	<u>Feces</u>	<u>Occult</u>	<u>Urine</u>
<u>Date (1974)</u>	<u>Partum</u>	<u>cpm/ml</u>	<u>cpm/65 gm</u>	<u>Blood</u>	<u>cpm/430 ml</u>
May 7	10	3770 (5/6)	5720	--	--
May 8	11	--	5135	Neg.	--
May 9	12	--	5135	Neg.	29,670
May 11	14	2750	6630	Neg.	14,620
May 13	16	--	6695	Neg.	10,190
May 14	17	2240	6825	Neg.	15,050
May 15	18	--	7995	--	10,880
May 16	19	--	8710	--	
May 17	20	1800	9685	1+	29,670

\* The average fecal output during this 10 day period was 65 g/day and the average daily urine output 430 ml.

Figure 13. Isoelectric focusing electrophoresis of the hemolysates from the sable calves showing the change from fetal to adult hemoglobins.

Table 4. Selected Clotting Factor Assays In Young Sables with Early Anemia.

<u>Animal and Age</u>	<u>Assay</u>					
	<u>PT*</u>	<u>APTT*</u>	<u>TT*</u>	<u>Factors</u>		
				<u>V</u>	<u>VII</u>	<u>VIII</u>
Sec.	Sec.	Sec.	% of N	% of N	% of N	
<u>MO0899</u>						
15 Days	10.4	75.1	16.9	520	275	570
21 Days	10.2				290	
<u>MO0900</u>						
14 Days	11.0	95.7	16.7	580	140	610
20 Days	10.5				120	
<u>MO0829</u>						
1 $\frac{1}{2}$ Years	10.6				245	

The samples were collected into buffered citrate, cooled, centrifuged at 20,000 g to assure removal of platelets and the plasma shipped on dry ice to the laboratory for assay. All tests were performed with reagents used for assay of human samples. The units are either seconds or percent of a human normal standard as indicated.

\*Normal human 95% confidence limits for the test procedure used for the prothrombin time (PT) were 10.5-12.5 sec.; Activated partial thromboplastin time (APTT) 26-41 sec.; Thrombin time (TT) 13-19 sec.

Table 5. Hematocrits During Neonatal and Early Post-Partum Period of Some Domestic and Wild Ungulate Species.

<u>Species*</u> (N)	Neonatal	Lowest	Days
	<u>Hematocrit (%)</u>	<u>Hematocrit (%)</u>	<u>Postpartum</u>
Sable (7)	30	14	3-9
Kudu (2)	55	48	4-8
Bongo (2)	57 (1 day)	42	4
	47 (2 days)	33	5
Reindeer (3)	43	32	5-8
Gnu (1)	42	35	8
Dorcas Gazelles (2)	50	42	3-8
Pigs (20) <sup>†</sup>	33.0 ± 1.6	22.1 ± 0.9	14
Dogs (15) <sup>§</sup>	41.2 ± 1.0	26.5 ± 0.9	35

\* Sable (Hippotragus niger); Kudu (Tragelaphus strepsiceros); Bongo (Taurotragus eurycerus); Reindeer (Rangifer tarandus); Gnu (Connochaetes gnou); Dorcas Gazelle (Gazella dorcas); Pigs (Sus scrofa); and Dogs (Canis familiaris).  
 † Talbot and Swenson, 1970.      § Deavers et al. 1971.



## Discussion

The decline in hematocrit occurring during the first 24-48 h postpartum in these sable calves differs from the "physiological" anemia of infancy described in humans (O s k i and S t o c k m a n , 1974), rats (C o n s t a b l e , 1963; G a r c i a , 1957; M a s t e r s et al., 1972), rabbits (H a l v o r s e n and H a l v o r s e n , 1973; L i t t l e , 1970), guinea pigs (C o n s t a b l e , 1963), pigs (M c C a n c e and W i d d o w s o n , 1959; T a l b o t and S w e n s o n , 1970; T u m b l e s o n and K a l i s h , 1972), dogs (D e a v e r s et al., 1971) goats (R i e g e l et al., 1961), and cats (M o t t , 1968). The hematocrit decrease in sable antelope occurred sooner and more rapidly, within hours postpartum in the female, than has been reported for other species, except the pig (M c C a n c e and W i d d o w s o n , 1959; T u m b l e s o n and K a l i s h , 1972). Thus, it appears likely that the mechanism is different from the more slowly developing and persistent anemia described in the other species, which has been attributed to the rate of iron utilization outstripping the ability of the animal to mobilize iron or the available supply (H a l v o r s e n and H a l v o r s e n , 1973; H i l l m a n and H e n d e r s o n , 1969). The data on the female indicate that a rapid plasma volume expansion occurred during the period of initial nursing. The serum electrophoresis data demonstrate the rapid appearance of a large gamma globulin mobility protein fraction which would be derived by absorption from colostrum. The osmotic effects of this absorbed protein would result in the plasma volume expansion and subsequent decline in hematocrit with no decrease in total circulating red blood cell mass. A similar phenomenon occurs in pigs which are born with a serum protein level of 3.0 g% which increases to 7 g% by 8 h postpartum after nursing. This was shown to be due entirely to an increase in gamma globulins (T u m b l e s o n and K a l i s h , 1972). Thus, pigs have an early rapid decline in hematocrit followed by a more slowly developing decline if they are not adequately supplemented with iron (B u s h et al., 1955; F u r u g o u r i , 1974a, 1974b; M c C a n c e and W i d d o w s o n , 1959; T a l b o t and S w e n s o n , 1970; T u m b l e s o n and K a l i s h , 1972).

Comparison with the other wild ruminant data available does indicate that the initial hematocrit of the sable antelope is lower. The data on neonatal blood volumes further suggest that the newborn calves have about one-half the blood volume observed in other species, including cattle (H a x t o n et al., 1974; P a y n e et al., 1967) and sheep, for whom detailed data are available in the neonatal period (C r e a s y et al., 1970; P i p k i n and K i r k p a t r i c k , 1973). The sables thus have a substantially lower red cell mass per unit of body weight at birth than has been recorded for any other mammalian species. Similar hematology data on newborn sable calves at three other zoos indicate that the phenomenon is not peculiar to a particular management program. The animals also are from different wild stock, so an isolated genetic event is not involved. No evidence could be found for the occurrence of hemolysis either intravascular or extravascular. The loss of the <sup>51</sup>Cr label by way of the feces and urine was only a fraction of the expected red cell destruction rate and hence did not demonstrate significant blood loss. The newly produced red cells were normochromic and hence there was no evidence for iron availability limiting erythropoiesis. Iron was also available in the diet of both animals. Reticulocytes peaked at 10 days in the female and her hematocrit increased steadily concurrently with the increase in body weight indicating an increasing rate of erythropoiesis until near adult levels were reached at 40 days of age when about 20 ml of red blood cells per day were being produced. However, it is possible the reduced red blood cell mass in the neonatal animal was the result of limited iron availability during pregnancy since the red blood cells were smaller than adult cells and relatively hypochromic.

There was a close association of nutritionally based growth problems with a delay in erythropoiesis in the male calf as reflected in the prolonged low hematocrit to 20 days of age and the delay in reticulocytosis. It would be valuable to determine the levels of 2,3-diphosphoglyceric acid in the red blood cells of sable calves to determine if changes are correlated with the shift in hematocrit and the onset of reticulocytosis (P a p a d o p o u l o s et al., 1974). The delays in growth and reticulocytosis and the

resulting delay in expansion of the red cell mass and blood volume account for the different specific activity curves in the male. However, despite the lack of colostrum the blood volume of this animal gradually increased to about the same as the female over a 4-5 day period, apparently by an increase in albumin level. These treatments and interpretations of the isotope data were made without applying corrections for elution of label and cell destruction since the assumption of a steady state does not apply (Cline and Berlin, 1963; Hughes, Jones and Mollison, 1956; International Committee for Standardization in Hematology, 1971; Mollison, 1961). Virtually all parameters of the system are changing including blood volume, rate of synthesis, type of red blood cell, and body weight. Also, neither elution rate nor rate of cell destruction are known. Thus the  $t_{1/2}$  numbers are solely an assistance in comparisons of the curves.

The sum of the data of this study indicate that there was no pathological process occurring postpartum in these animals resulting in an anemia. Therefore, therapeutic measures such as iron and vitamins are not indicated for dam-reared calves with access to dirt. Also, the use of blood transfusions in the face of the low hematocrits are not indicated and may well delay the onset of the calf's reticulocytosis. The data do demonstrate that these antelope had a lower blood volume and a smaller red cell mass per unit body weight than has been described previously for any mammalian species. The characterization of this phenomenon requires further information as to whether it is characteristic of the species and, hence, genetic or the result of some feature of sable pregnancy in captivity. It does not seem to be shared by the few other captive ruminants we have examined.

Summary:

Neonatal Anaemia in Antelopes (*Hippotragus niger*).

An account is given of neonatal anaemia in newborn antelopes. They differed from other wild ruminants by lower blood quantity and erythrocyte count at the time of birth.

Zusammenfassung:

Neonatale Anämien bei Rappenantilopen (*Hippotragus niger*).

Es wird über Anämien bei neugeborenen Rappenantilopen berichtet. Im Vergleich zu anderen Wildwiederkäuern wurden eine geringere Blutmenge sowie eine niedrigere Erythrozytenzahl zur Zeit der Geburt festgestellt.

Résumé:

Anémies néonatales chez *Hippotragus niger*.

En comparant avec d'autres ruminants sauvages on a pu observer après l'accouchement une masse sanguine moins importante ainsi qu'un nombre plus réduit d'Erythrocytes.

Резюме:

Неонатальная анемия у раппенантилопы (*Hippotragus niger*).

Сообщается об анемии у новорожденной раппенантилопы. По сравнению с другими жвачными у животного обнаружено пониженное количество крови и низкое число эритроцитов.

Literature:

- BRAMBELL, F. W. R. (1970): The transmission of passive immunity from mother to young. Amsterdam: North-Holland Publishing Company.
- BUECHNER, H. K., STROMAN, H. R. and W. A. XANTEN (1974): Breeding behavior of sable antelope (*Hippotragus niger*) in captivity. Int. Zoo. Yb. 14, 133-137.

- BUSH, J. A., BERLIN, N. E., JENSSEN, W. N., BRILL, A. B., CARTWRIGHT, G. E. and M. M. WINTROBE (1955): Erythrocyte life span in growing swine as determined by glycine- $^{14}\text{C}$ . *J. Exp. Med.* 101, 451-459.
- CHRISTENSEN, F., ANKER, N. and M. MONDRUP (1974): Blood in faeces. A comparison of the sensitivity and reproducibility of five chemical methods. *Clin.Chim.Acta* 57, 23-27.
- CLINE, M. J. and N. I. BERLIN (1963): The red cell chromium elution rate in patients with some hematologic diseases. *Blood* 21, 63-69.
- CONSTABLE, B. J. (1963): Changes in blood volume and blood picture during the life of the rat and guinea-pig from birth to maturity, *J. Physiol.* 167, 229-238.
- CREASY, R. K., DROST, M., GREEN, M. V. and J. A. MORRIS (1970): Determination of fetal, placental and neonatal blood volumes in the sheep. *Circ. Res.* 27, 487-494.
- DEAVERS, S., SMITH, E. L. and R. A. HUGGINS (1971): Changes in red cell volume, venous hematocrit, and hemoglobin concentration in growing beagles. *Proc. Soc. Exp. Biol. and Med.* 137, 299-303.
- FURUGOURI, K. (1974a): Kinetics in iron metabolism in piglets. *J. Anim. Sci.* 38, 1249-1256.
- FURUGOURI, K. (1974b): Nonheme iron mobilization from the liver in piglets. *Jap. J. vet. Sci.* 36, 255-261.
- GARCIA, J. F. (1957): Changes in blood, plasma and red cell volume in the male rat, as a function of age. *Am. J. Physiol.* 190, 19-24.
- GRAY, S. J. and K. STERLING (1950): The tagging of red cells and plasma proteins with radioactive chromium. *J. Clin. Invest.* 29, 1604-1613.
- GREGERSEN, M. I. and R. A. RAWSON (1959): Blood volume. *Physiol. Rev.* 39, 307-342.
- HALVORSEN, K. and S. HALVORSEN (1973): The "early anaemia"; its relation to postnatal growth rate, milk feeding, and iron availability. Experimental study in rabbits. *Arch. Dis. Child.* 48, 842-849.
- HAXTON, J. A., SCHNEIDER, M. D. and M. P. KAYE (1974): Blood volume of the male Holstein-Friesian calf. *Am. J. vet. Res.* 35, 835-837.
- HILLMAN, R. S. and P. A. HENDERSON (1969): Control of marrow production by the level of iron supply. *J. Clin. Invest.* 48, 454-460.
- HUGHES JONES, N. C. and P. L. MOLLISON (1956): The interpretation of measurements with  $^{51}\text{Cr}$ -labelled red cells. *Clin. Sci.* 15, 207-218.
- INTERNATIONAL COMMITTEE FOR STANDARDIZATION IN HEMATOLOGY (1971): Recommended methods for radioisotope red cell survival studies. *Blood* 38, 378-386.
- LITTLE, R. A. (1970): Changes in the blood volume of the rabbit with age. *J. Physiol.* 208, 485-497.
- MASTERS, A. M., LESLIE, A. J. and I. KALDOR (1972): Red cell and plasma volume development in newborn rats measured with double label. *Am. J. Physiol.* 222, 49-54.
- McCANCE, R. A. and E. M. WIDDOWSON (1959): The effect of colostrum on the composition and volume of the plasma of newborn piglets. *J. Physiol.* 145, 547-550.
- MOLLISON, P. L. (1961): Further observations on the normal survival curve of  $^{51}\text{Cr}$ -labelled red cells. *Clin. Sci.* 21, 21-36.
- MOLLISON, P. L. and N. VEALL (1955): The use of the isotope  $^{51}\text{Cr}$  as a label for red cells. *Br. J. Haematol.* 1, 62-74.
- MOTT, J. C. (1968): The effect of haemorrhage on haemoglobin concentrations, blood volume and arterial pressure in kittens and cats. *J. Physiol.* 194, 659-667.
- OSKI, F. A. and J. A. STOCKMAN, III. (1974): Anaemia in early infancy. *Br. J. Haematol.* 27, 195-199.

- PAPADOPOULOS, M. D., MARTENS, R. J., FORSTER, R. E., II and F. A. OSKI (1974): Postnatal changes in oxygen-hemoglobin affinity and erythrocyte 2, 3-diphosphoglycerate in piglets. *Pediat. Res.* 8, 61-66.
- PAYNE, E., RYLEY, J. W. and R. J. W. GARTNER (1967): Plasma, blood and extraoellular fluid volumes in grazing Hereford oattle. *Res. Vet. Sci.* 8, 20-26.
- PIPKIN, F. B. and M. L. KIRKPATRICK (1973): The blood volumes of fetal and newborn sheep. *Q. J. exp. Physiol.* 58, 181-188.
- RIEGEL, K., HILPERT, P. and H. BARTELS (1961): Vergleichende Untersuchungen der Erythrocytenmorphologie des fetalen Hämoglobins und der Sauerstoffaffinität des Blutes von Säuglingen, Zicklein und Lämmern. *Acta haemat.* 25, 164-183.
- SEAL, U. S., SWAIM, W. R. and A. W. ERICKSON (1967): Hematology of the Ursidae. *Comp. Biochem. Physiol.* 22, 451-460.
- SEAL, U. S. and A. W. ERICKSON (1969): Hematology, blood chemistry and protein polymorphisms in the white-tailed deer (*Odocoileus virginianus*). *Comp. Biochem. Physiol.* 30, 695-713.
- SWAN, H. and A. W. NELSON (1971): Blood volume measurement: Concepts and technology. *J. Cardiovas. Surg.* 12, 389-401.
- TALBOT, R. B. and M. J. SWENSON (1970): Blood volume of pigs from birth through 6 weeks of age. *Am. J. Physiol.* 218, 1141-1144.
- TISDALL, M. and J. P. CROWLEY (1971): The pattern of disappearance of foetal haemoglobin in young calves. *Res. vet. Sci.* 12, 583-584.
- TUMBLESON, M. E. and P. R. KALISH (1972): Serum biochemical and hematological parameters in crossbred swine from birth through eight weeks of age. *Can. J. comp. Med.* 36, 202-209.
- WADDELL, W. (1956): A simple ultraviolet spectrophotometric method for the determination of protein. *J. Lab. Clin. Med.* 48, 311-314.
- WINTROBE, M. M. and H. B. SHUMACHER (1936): Erythrocyte studies in the mammalian fetus and newborn. *Am. J. Anat.* 58, 313-328.
- ZEHR, J. E., JOHNSON, J. A. and W. W. MOORE (1969): Blood volume measurements in sheep by chromium-51-labeled red blood cells. *Am. J. vet. Res.* 30, 1699-1704.

Address of authors: M. B u s h , D. V. M.,  
National Zoological Park, Smithsonian Institution  
Washington 9, DC 20009 (USA)