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The effect of age and weight upon the blood volume of farm animals

William O. Butler

To the Graduate Council:

I am submitting herewith a thesis written by William O. Butler entitled "The effect of age and weight upon the blood volume of farm animals." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

Charles S. Hobbs, Major Professor

We have read this thesis and recommend its acceptance:

Sam L. Hansard, Harold J. Smith, R. L. Murphee

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

August 13, 1952

To the Graduate Council:

I am submitting herewith a thesis written by William O. Butler entitled "The Effect of Age and Weight Upon the Blood Volume of Farm Animals." I recommend that it be accepted for fifteen quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

Charles S. Hobbs
Major Professor

We have read this thesis
and recommend its acceptance:

Sam L. Hansard
Harold J. Smith
R. L. Murphree

Accepted for the Council:

E. G. Waters
Dean of the Graduate School

THE EFFECT OF AGE AND WEIGHT UPON THE BLOOD VOLUME OF FARM ANIMALS

A THESIS

Submitted to
The Graduate Council
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

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by
William O. Butler

August 1952

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INTRODUCTION

The need for a knowledge of the amount of circulating blood in man and animal has stimulated investigators in many fields of research to study and apply various methods and techniques for the quantitative determination of blood volume. This has resulted in controversies and in conflicting reports in the literature. Through these efforts to perfect methods and establish constant values for the different species much information has been acquired from which our present concepts have been formulated.

The increasing popularity and adaptability of farm animals for nutritional, radiation and therapeutic studies using labeled elements and compounds require that acceptable blood volume figures be established for use in experimental interpretations of these results. Aside from the limited early results summarized by Dukes (1947) those for the growing bovine reported by Missouri workers (1931) and the studies with swine by Hansard et al. (1951), few values have been reported on blood volume determination with farm animals.

In view of the limited information in the literature concerning the blood volume of the various species of farm animals, this study was initiated with the following objectives: (1) to determine an adaptable procedure for blood volume studies with farm animals of various ages; (2) to perfect the necessary techniques for routine determinations of blood volume for the various species; (3) to establish an acceptable value for the blood volume of the various species at definite ages and weights; (4) to determine the limitations of the accepted procedure

as affected by *in vitro* and *in vivo* studies that make for variations in values reported; and, (5) to correlate the results of this study with values and methods reported in the literature.

LITERATURE REVIEW

Interesting and detailed reports of the results and the various methods employed for the determination of blood volume in man and animals have been made by numerous investigators.

The dilution principle has been employed in the greater portion of these studies. Erlanger (1920) has reviewed and summarized the early literature and discussed the direct and indirect classification of absolute determination procedures. The shortcomings of the early direct determinations by actual collection of all blood by hemorrhage and perfusion are self-evident. The indirect method has therefore been more attractive and of particular interest to the animal physiologist and nutritionist since it is better adapted for routine investigations and does not require sacrifice of the animal.

A water dilution method for the determination of blood volume was first employed by Valentine (1838). Weber and Lehman (1850), using this method, concluded that the blood made up one-eighth of the body weight of man. Intravenous injections of salt solution as a means of blood volume determination was used by Sandler and Kronechen (1881). They substituted an equal volume of sodium chloride solution for blood and determined the degree to which it diluted the hemoglobin. The rapid disappearance of the sodium chloride, and the water in Valentine's studies proved to make both of these methods unsatisfactory.

Keith, Rowntree and Geraghty (1915) perfected a blood volume method using vital red dye. Repeated determinations by this method at short intervals with normal young dogs gave a blood volume value of

10.1 per cent of the body weight. The in vivo disappearance of the dye from the blood stream was found to be 3.0 per cent at 72 minutes after intravenous administration.

Employing the dye method, Turner and Herman (1931) found 6.25 per cent of the body weight of growing heifers to be blood, 8.11 per cent for lactating cows and 6.38 per cent for non-lactating cows.

Courtice (1949) estimated the blood volume of a variety of normal animals using the T-1824 dye procedure. Sixty rabbits, twenty-nine dogs, thirty goats, and two horses had an average blood volume of 7.0, 7.9, 7.0, and 7.2 per cent of body weight, respectively. He concluded that the blood volume varied according to body weight and not body surface area.

Hevesy (1942) introduced the isotope dilution principle for blood volume determinations and described a method whereby radioactive tri-sodium phosphate was incubated with rabbits blood for two hours at 37.5° Centigrade and reinjected into the animal. The rabbits were bled at 3, 5, 7, 9, and 15 minute intervals with almost identically calculated results for all bleedings. The corpuscle content of the animals varied between 20.5 and 25.6 grams per kilogram body weight.

Gibson et al. (1946) has described a modification of Hahn's original technique for determining circulating red cell volume by means of two radioisotopes of iron. This method was proposed to give an absolute measure of the circulating red cells, which is independent of the venous, arterial and auricular hematocrits. It provided an accurate measurement of the total circulating blood as used in conjunction with the dye plasma volume method in forty normal dogs.

Results obtained by the radioactive iron method were 15 per cent less than the results from the dye plasma procedure.

The determination of circulating red cell volume in man using radioactive phosphorus was described by Reeve and Veall (1949). The blood was incubated with P^{32}, washed free of radioactive plasma and reinjected into the subject. Approximately 10 per cent of the P^{32} disappeared from the blood per hour. The use of a Geiger-Muller tube especially designed for liquid counting greatly simplified the necessary manipulations of standards and samples.

Several other radioactive isotopes have been found useful in blood volume determinations. Potassium 42 has two properties that makes it especially adapted for blood volume studies. It decays with emission of beta particles and X-rays which are easily detectable in liquid samples. It has a short half-life of 12.4 hours which makes it possible to re-use the subjects after short intervals of time. Yalow (1951) used K^{42} tagged erythrocyte in blood volume determinations with man. The results were almost identical to those previously obtained using the P^{32} procedure.

Gray and Sterling (1950) estimated the circulating red cell volume in dogs using radioactive chromium. Ninety per cent of the Cr^{51} was taken up by the cells at two hours incubation in vitro and was retained for one day without significant loss.

Tabern (1951) of Abbott Laboratories, discussed a method for blood volume determination perfected by Storaasli, Krieger, Friedelland and Holden using I^{131} tagged serum. At the end of one hour, 90 per cent

of the injected tagged protein was present in the blood and 78 per cent was still present at four hours. The optimum time after injection for taking blood samples appeared to be 10-25 minutes.

The mean blood and packed cell volumes of twenty-two adult male rats were measured by Sharpe (1950) using cells which had been previously tagged with radioactive iron. The blood volume was found to be 4.95 milliliters per 100 grams body weight and packed cell volume was 2.32 milliliters per 100 grams body weight.

Several investigators have compared the various procedures and methods of determining blood volume with different animal species in an effort to correlate the values in the literature and to adapt the best procedures for their individual studies.

Smith et al. (1921) made a comparison of the Welcker, carbon monoxide, and dye methods on the same dog within a short interval of time. The dye method measured the plasma volume while the carbon monoxide and Welcker methods measured the red cell and hemoglobin volumes. The true total blood volume was calculated from the sum of the dye and the carbon monoxide methods.

Gibson et al. (1946) presented a comparison of the dye method with the Fe^{59} dilution procedure in determining the plasma and circulating red cell volume of rabbits. It was found that the results with the Fe^{59} tagged cells averaged about 25 per cent lower than the T-1824 dye method.

In a comparison of three methods: T-1824, P^{32}, and I^{131}, Krieger et al. (1948) found that I^{131} permitted accurate blood volume

determinations for a much longer time after injection. Ten dogs were used in the determinations and the results showed the average blood volume to be as follows: T-1824---10.5 per cent, P^{32}---9.7 per cent, and I^{131}---9.4 per cent of body weight.

Berlin et al. (1949) used the Fe^{59} and P^{32} labeled red cells in the determination of the blood volume of rats. He found essentially no difference in the blood volume as determined by the two methods. Determinations with P^{32} indicated the blood volume of normal rats to be 4.59 ± 0.57 cc per 100 grams body weight. The red cell volume averaged 2.16 ± 0.20 cc per 100 grams, and the hematocrit 45.8 ± 2.6 per cent.

Courtice and Gunton (1949) in an experiment using rabbits, compared the T-1824 method with the carbon monoxide procedure first introduced in 1900 by Haldane and Smith. These results showed that there was no marked difference between the two methods. The accuracy of the carbon monoxide method was tested by estimating the blood volume before and after the removal of a known volume of blood.

Later in the same year, Courtice and Gunton (1949) repeated the comparison of carbon monoxide and dye methods with man. The carbon monoxide values were always somewhat higher than the dye method values, probably due to the fact that the carbon monoxide is absorbed from the circulatory system or to the variation in the initial blood carbon monoxide.

A direct comparison of the blood plasma value for dogs using T-1824 and antigen distribution was made by Gregerson et al. (1950).

This experiment was designed to test for the loss of dye *in vivo* during the mixing period by comparing directly the plasma volume by the T-1824 dye method with three antigens. By means of simultaneous measurements the volume of distribution values obtained using the dye method was found to be identical with that of bovine albumin, bovine globulin, and polysaccharide S III. It was stated with considerable certainty that there was no initial rapid loss of significant amounts of T-1824 by staining or phagocytosis, and that this method was a true measure of the plasma volume.

Wasserman, in a comparison of the P^{32} and the T-1824 dye methods, reported higher values by the latter method. A 15 per cent difference between the body and venous packed cell volumes was attributed to a plasma occlusion factor as well as to inherent variation between venous blood and the blood in the circulatory system. McLain *et al.* (1951) calculated the blood and plasma volumes on dogs and rabbits by the bleeding and dye methods. The bleeding method gave the most consistent results. The results from the bleeding methods were: rabbits---43 ± 0.8 ml blood per kilogram, and dogs---62 ± 1.2 ml blood per kilogram. When plotted in the ordinary way, the results of the dye studies exceeded those of the bleeding technique by averages of 67 per cent for rabbits and 44 per cent for dogs.

Smith *et al.* (1920) outlined a method in which the dye blood volume method was adapted to repeated determinations on the same animal at short intervals. The ability to estimate blood volume changes during short intervals made it possible to study the exchange of fluids which

may go on within the body. This method was checked *in vitro* and the error was found to be less than 5 per cent in each case.

A hematocrit method using packed red cells for injection is described by Strumia *et al.* (1949). This method was based upon the red cell dilution principle. The hematocrit was taken before and after the injection of packed red cells and the blood volume calculated from the dilution factor of the increased red cells in the blood. The values obtained by the packed cell method were 10-12 per cent less than those obtained from the dye method thereby approximating more closely the values obtained using the radioactive cell dilution procedure.

Several factors have been reported by investigators as having an influence upon the blood volume values.

Berlin *et al.* (1950) made a study of the blood volume in chronic leucemia patients using the P^{32} labeled red cells. This study showed a considerable variation in the blood volume of leucemia patients due to varying plasma volumes.

Berlin *et al.* (1951) found the blood volume in women to be slightly less than in men, probably because of the increased amount of adipose tissue. It was proposed that if the blood volume was calculated on the basis of fat free body weight there would be little difference.

Courtice and Gunton (1949) found that a variation of the fluid content of the diet may affect the plasma volume significantly.

Turner and Herman (1931) reported a difference in lactating and non-lactating cows. They found the blood volume of lactating cows to be approximately 25 per cent higher than that of non-lactating cows. The stage of lactation was not mentioned.

Dukes (1933) has reported the blood volume of sheep to increase with pregnancy and to decrease with age.

Hansard et al. (1951) reported blood volume of swine to decrease with increased age and body weight, and emphasized quantitative isotope administration to be essential for compatable blood volume values.

EXPERIMENTAL PROCEDURES

General Procedure

In the initial phases of this study the dye, P^{32}, and I^{131} methods were compared in individuals of different species for blood volume determinations. The P^{32} labeled red cell method gave results compatible with published figures. The I^{131} labeled plasma procedure was inconsistent except in limited individual cases and species. In consideration of these findings and the reports of the several investigators in the literature, it was concluded that a modification of the P^{32} labeled red cell method employed by Reeve and Veall (1949) was adequate and most satisfactory for this study involving all species of farm animals.

The sheep, swine, cattle, and burros used in these studies were normal, healthy individual animals procured from the station farm flocks and herds. Each animal was oriented in a metabolism unit (Hansard, 1951) previous to the test period. Blood was drawn for labeling with activity, and immediately previous to dosing the animal was catherized (Hansard et al. 1951) to facilitate quantitative administration of the labeled red blood cells.

Phosphorus 32 Procedure

Thirty to forty milliliters of an animal's blood was drawn into a 125 milliliter Erlenmeyer flask and five to ten microcuries of isotonic P^{32} solution per milliliter of blood was added. The blood was then incubated in a Warburg shaker unit at 37.5° Centigrade for variable periods of time up to four hours. At the designated intervals,

5 milliliters of the radioactive blood was removed from the flask and centrifuged in a 40 milliliter centrifuge tube for 30 minutes at 1,800 r.p.m. (radius of 25 cm and thrust of 900). The radioactive plasma was drawn off with a suction tube after which 20 milliliters of ice-cold physiological saline was added and thoroughly mixed with the red cells. (It should be emphasized that all manipulations of the blood and red cells must be done gently) The red cell suspension was centrifuged again for 10 minutes at 1,800 r.p.m. and the saline drawn off. The red cells were washed three more times as described above, after which they were made up to the original volume with saline. One milliliter of this well-mixed cell suspension was diluted to 50 milliliters from which an aliquot was taken for measurement of activity. This was compared to a standard prepared at the same time by diluting 1 milliliter of the original incubated blood to a sufficient volume to give counts per minute comparable to the samples to be measured, and the per cent uptake by the red blood cells calculated. This was repeated at the designated intervals indicated in Table V.

Following four hours incubation period the remaining blood was removed from the water bath and a standard prepared. The red cells were then washed free from their radioactive plasma as described above, made to volume, and a 5 to 10 milliliter aliquot immediately administered quantitatively to the immobolized animal as described by Hansard et al. (1951).

At designated intervals after injection, venous blood samples were drawn from the opposite side of the animals neck for hematocrits and measurement of activity. Ten milliliters volume of the whole blood

sample was then counted, using a specially designed Geiger-Muller Counter for liquid samples (Reeve and Veal, 1949).

The total blood volume was calculated by dividing the total counts injected by the net counts per milliliter of whole blood. The per cent blood volume was then calculated by dividing the total milliliters of blood (assuming the specific gravity equal to one) by the weight of the animal expressed in grams.

Iodine 131 Procedure

Blood was drawn, defibrinated and shipped in a refrigerated container to Abbott's Laboratories where the blood serum was iodinated with I^{131} to contain 0.18 to 0.40 mc per milliliter. The experimental animals were then dosed with aliquots of the iodinated serum containing 150 to 300 /uc I^{131} depending upon the species and size of the animal. The procedures for animal management and dose administration was the same as used for the P^{32} method previously described. Blood was drawn for hematocrits and measurement of activity at 15 to 25 minute intervals. Approximately 15 milliliters of the test blood was centrifuged for 20 minutes at 1,800 r.p.m. The radioactive plasma was drawn off and an aliquot used for activity measurement as previously described for P^{32}. A standard was prepared by diluting a small portion of the original iodinated serum to a volume sufficient to give counts per minute comparable to the samples to be measured.

Calculations were made by dividing the total counts per minute injected by the counts per minute per milliliter of plasma to give the total milliliters of plasma in the animal's body. The hematocrit was

multiplied by 0.95 to correct for the plasma trapped in the packed cell column (Leeson et al., 1951). Using these corrected hematocrit values, the total milliliters of whole blood was calculated. The per cent blood volume was calculated by dividing the total milliliters of blood (assuming the specific gravity equal to one) by the weight of the animal expressed in grams.

RESULTS AND DISCUSSION

Various investigators have reported such factors as age, condition and lactation to have a definite effect upon the blood volume of man and animals. Dukes (1933) proposed the blood volume value to be independent of body weight in dogs weighing 7 to 24 pounds and in goats weighing 16 to 67 pounds. However, he noted that the blood volume in lambs decreased with increasing age between birth and 190 days with the greatest decrease occuring in the first 30 to 60 days. Berlin et al. (1951) found the blood volume in women to be slightly less than that of men. However, he proposed that if the blood volume was calculated on the basis of the fat free body there would be little sex difference. Turner and Herman (1931) reported a 25 per cent higher blood volume in lactating than in non-lactating cows.

Burros

A summary of the forty-six blood volume determinations made with thirty-seven burros is shown in Table I. Twenty-one of these determinations were based upon the P^{32} procedure and twenty-five upon the I^{131} method.

The values obtained using the P^{32} method averaged 9.52 per cent for the young burros, ranging in age from 1.5-3 months, and 6.74 per cent for mature burros as illustrated in Table II.

There was no significant difference in the values for burros obtained by the P^{32} and the I^{131} methods. The blood volume for the mature burros using the I^{131} method averaged 6.66 per cent (Table III) for the twenty-five burros studied. The same variation with age was

TABLE I

SUMMARY: EFFECT OF AGE AND WEIGHT UPON THE BLOOD VOLUME OF BURROS USING THE P^{32} AND I^{131} METHODS

Method Used	Number of Animals	Age	Weight Pounds	Per Cent Blood Volume
I^{131}	2	1 Day	49.5	19.1
P^{32}	4	1.5-3.0 Months	110.0	9.52 ± .451
I^{131}	23	Mature	336.0	6.66 ± 1.20
P^{32}	17	Mature	339.0	6.74 ± .978

TABLE II

EFFECT OF AGE AND WEIGHT UPON THE BLOOD VOLUME OF BURROS USING P^{32}

Burro Number	Sex	Age	Pounds	Cell Pack Volume	Per Cent Blood Volume
450	F	1.5 Months	90	39.4	9.46
449	F	2 Months	100	35.8	10.10
447	F	2 Months	120	39.2	9.0
446	F	3 Months	134	38.4	9.5
			110	38.2	9.52 ± .451
41	M	Mature	288	32.8	5.92
41				35.0	6.46
41				35.7	5.71
41				34.5	6.57
148	M	Mature	345	34.4	6.47
148				33.4	5.95
148				38.2	6.42
148				35.3	5.60
43	M	Mature	358	33.1	6.15
43				33.1	6.78
43				36.1	5.90
43				34.0	6.05
220	M	Mature	274	34.9	6.70

TABLE II

EFFECT OF AGE AND WEIGHT UPON THE BLOOD VOLUME OF BURROS USING P^{32} (Continued)

Burro Number	Sex	Age	Weight Pounds	Cell Pack Volume	Blood Volume
39	M	Mature	370	34.4	8.64
199	F	Mature	370	40.2	7.59
244	F	Mature	376	31.7	8.0
7	F	Mature	406	35.1	8.6
Average			339	34.8	6.74 ± .978

TABLE III

EFFECT OF AGE AND WEIGHT UPON THE BLOOD VOLUME OF BURROS USING THE I^{131} METHOD

Burro Number	Sex	Weight	Cell Pack Volume	Per Cent Blood Volume
84	M	248	29.1	7.52
184	M	248	40.6	8.60
144	M	264	32.5	6.30
54	M	282	36.6	6.60
41	M	296	35.9	7.27
137	M	300	29.7	5.10
95	M	302	41.6	7.96
235	M	306	39.0	6.50
31	M	308	33.3	4.90
223	M	318	37.5	5.40
186	M	326	31.4	5.10
197	M	347	35.3	7.45
174	M	350	40.4	9.95
65	M	353	36.4	8.03
178	M	358	30.6	4.80
28	M	360	34.3	4.60
148	M	368	35.3	6.28
160	F	368	37.9	7.60
51	M	378	41.4	7.30
43	M	380	34.2	7.07

TABLE III

EFFECT OF AGE AND WEIGHT UPON THE BLOOD VOLUME OF BURROS USING THE I^{131} METHOD (Continued)

Burro Number	Sex	Weight	Cell Pack Volume	Per Cent Blood Volume
44	M	410	38.7	7.08
249	M	420	31.3	5.96
206	F	453	38.8	7.70
Average		336	36.2	6.66 ± 1.20
160[a]	M	37	54.1	18.2
206[a]	M	62	48.8	20.0
Average		49.5	51.5	19.1

[a] One day old colts. All other animals are mature.

noted with this method as demonstrated by the P^{32} procedure. Individual determination on two one-day old burros showed an average blood volume of 19.1 per cent as compared to 9.5 per cent at 1.5 to 3 months of age by the I^{131} and the P^{32} methods, respectively. This indicates that the volume adjustment occurs very rapidly during the first few days of life and then more slowly to mature age.

In order to estimate the variation of individual animals at different time intervals, twelve blood volume determinations were made on three burros over a four week period. Table IV summarizes these data and indicates the variation between determination on the same individual to be much less than that between animals.

Five of the blood volume determinations (numbers 199, 244, and 7 in Table II, and, 160, and 206 in Table III) with mature burros were made on lactating animals. It is noted that all five of these values were at the top of the range between individuals. These data are not conclusive, but they indicate agreement with reports by Turner and Herman (1931) that the blood volume of lactating animals is calculated to be about 25 per cent higher than non-lactating animals.

The uptake of P^{32} *in vitro* by burro red cells is summarized in Table V. The mature burro red cells appear to take up the P^{32} at a faster rate during early incubation, but after three and four hour incubation there was no significant difference in the total per cent uptake at the different ages. The decrease in per cent uptake after three hours would indicate this to be sufficient incubation time. However, for all animals, four hour periods were used. The *in vivo* loss of P^{32} from the

TABLE IV

RESULTS OF REPEATED BLOOD VOLUME DETERMINATIONS AT SHORT INTERVALS ON THE SAME ANIMAL USING P^{32}

Burro Number	Sex	Age	Weight Pounds	Cell Pack Volume	Per Cent Blood Volume
41 (1)	M	Mature	288	32.8	5.92
(2)				35.0	6.46
(3)				35.7	5.71
(4)				34.5	6.57
Average				34.5	6.16 ± .415
148 (1)	M	Mature	345	34.4	6.47
(2)				33.4	5.95
(3)				38.2	6.42
(4)				35.3	5.60
Average				35.3	6.11 ± .413
43 (1)	M	Mature	358	33.1	6.15
(2)				33.1	6.79
(3)				36.1	5.90
(4)				34.0	6.05
Average				34.1	6.22 ± .392

TABLE V

ACCUMULATIVE PER CENT UPTAKE OF P^{32} IN VITRO BY THE RED CELLS OF THE VARIOUS SPECIES OF FARM ANIMALS

Species	Hours Incubated at 37.5° Centigrade				
	0.5	1.0	2.0	3.0	4.0
Mature Burro	6.8	10.1	16.0	19.6	22.6
Colt	6.4	9.1	12.4	18.6	24.0
Mature Bovine	3.3	5.2	10.4	11.9	14.1
Calf	2.2	4.3	5.9	10.2	15.0
Mature Sheep	2.9	4.7	8.0	10.1	12.8
Lamb	1.9	3.8	6.5	8.5	11.1
Mature Swine	7.3	11.5	17.1	24.7	26.0
Pig	11.3	15.5	21.0	23.7	25.0

blood is indicated by the results tabulated in Table VI. If it is assumed that the bss at 15 minutes is 0, the disappearance from the circulating blood amounts to 14.2 and 24.0 per cent at 30 minutes and one hour, respectively, or about 6 per cent for each 15 minute interval.

Cattle

A summary of the blood volume determinations made for eighteen cattle employing the P^{32} procedure is presented in Table VII. These data for the individual animals, as shown in Table VIII indicate the blood volume to range from 12.9 per cent for a two day old calf to 4.68 per cent for a nine year old cow. Like the burros, the blood volume change appears to be most rapid early in life, decreasing very rapidly during the first thirty to sixty days and more slowly toward maturity. There seems to be little difference in the per cent blood volume in the two months old calf and the mature bovine regardless of age. The four eight to ten year old animals were lactating cows. Because they were in late lactation and possibly because they were older animals, there was no significant difference in their blood volume over that of the other groups.

The accumulative per cent *in vitro* uptake of P^{32} by the red blood cells of the bovine is summarized in Table V. Unlike the burros, there was no noticeable difference in the rate of uptake by the young and mature bovine. Following administration of the labeled cells, the *in vivo* loss of P^{32} from the bovine blood was 9.8 per cent at one-half hour and 23.3 per cent at one hour. (Table VI)

TABLE VI

ACCUMULATIVE PER CENT LOSS OF P^{32}
IN VIVO

Species	15 Minutes	30 Minutes	1 Hour
Burro	0	14.2	24.0
Bovine	0	9.8	23.3
Swine	0	6.4	13.6
Sheep	0	10.3	19.5

TABLE VII

SUMMARY: EFFECT OF AGE AND WEIGHT UPON THE BLOOD VOLUME OF CATTLE

Number of Animals	Age	Weight Pounds	Per Cent Blood Volume
2	2-6 Days	60	11.65
1	3 Weeks	108	8.46
3	2-3 Months	242	6.17 ± .558
5	6-8 Months	544	5.77 ± .654
3	14-15 Months	747	5.66 ± .791
4	8-12 Years	1,045	5.68 ± .829
18			

TABLE VIII

EFFECT OF AGE AND WEIGHT UPON BLOOD VOLUME IN CATTLE

Bovine Number	Sex	Age	Weight Pounds	Cell Pack Volume	Per Cent Blood Volume
B2	F	2 Days	56	42.8	12.90
B1	F	6 Days	64	35.1	10.35
B.V.	M	3 Weeks	108	29.9	8.46
Average			76	35.9	10.57 ± 2.228
234	F	2 Months	212	41.3	6.08
220	M	3 Months	226	41.3	6.80
213	M	3.5 Months	292	50.9	5.70
Average			243	44.5	6.17 ± .559
961	M	6 Months	456	31.0	5.70
902	M	7 Months	500	44.6	4.90
985	M	8 Months	562	36.6	6.74
990	M	8 Months	570	41.7	5.70
989	M	8 Months	634	42.6	5.81
Average			544	39.3	5.77 ± .654

TABLE VIII

EFFECT OF AGE AND WEIGHT UPON THE BLOOD VOLUME IN CATTLE (Continued)

Bovine Number	Sex	Age	Weight Pounds	Cell Pack Volume	Per Cent Blood Volume
436	M	14 Months	730	41.9	5.19
470	M	15 Months	750	35.7	6.57
486	M	15 Months	760	36.0	5.21
Average			747	37.9	5.66 ± .791
18	F	8 Years	1,000	43.2	6.10
89	F	9 Years	1,095	37.1	4.68
125	F	12 Years	1,138	41.4	6.11
26	F	10 Years	1,148	40.8	6.60
Average			1,095	40.6	5.68 ± .829

When I^{131} labeled bovine and swine plasma was used in blood volume determinations with cattle and swine, invariably, the values were high and erratic. With cattle, the values ranged from 9.6 per cent to 13.6 per cent for six determinations and with swine, the values ranged from 6.4 per cent to 65.2 per cent for eight determinations. Since there were no consistent values from the use of this method and the reasons for these high values have not been formulated, no data will be reported from this procedure with cattle, sheep and swine.

Sheep

Nineteen blood volume determinations were made with sixteen animals of various ages and weights as indicated in Table IX. A summary of this study is presented in Table X. An average blood volume of 7.98 per cent was obtained with four week old lambs, 6.27 with six month old lambs, 5.51 with yearlings, 5.83 with three year old ewes and 8.0 with aged ewes. Lambs younger than four weeks were not available, consequently, the comparative rapid decrease in early life is not shown. However, it is noted that there was a slight decrease in the blood volume values of the four week old groups over the mature ewes. This difference between the four week old and the six month old groups, and the difference between the six month old and the three year old ewes was statistically significant at the 5.0 per cent level (Chamber, 1952). An average of 8.0 per cent blood volume was obtained for the three aged ewes. The only possible explanation for these higher values was the fact that these ewes were in extremely thin condition. According to Berlin et al. (1951), this absence of fat could account for the

TABLE IX

EFFECT OF AGE AND WEIGHT UPON THE BLOOD VOLUME OF SHEEP

Sheep Number	Sex	Age	Weight Pounds	Cell Pack Volume	Per Cent Blood Volume
2125	F	4 Weeks	24	45.3	8.33
2125[a]		5 Weeks	26	41.5	8.11
2100	M	4 Weeks	25	48.1	7.50
2088	M	4 Weeks	28	42.7	7.52
2088[a]		5 Weeks	33	44.7	8.46
Average			27	44.5	7.98 ± .451
93	F	6 Months	84	47.8	6.37
77	F	6 Months	88	47.8	6.27
62	F	6 Months	90	47.1	6.0
55	F	6 Months	94	43.2	6.44
Average			92	46.5	6.27 ± .193
33	F	1 Year	126	51.4	5.35
52	F	1 Year	136	40.0	5.85
32	F	1 Year	144	49.3	5.33
Average			135	46.9	5.51 ± .295

TABLE IX

EFFECT OF AGE AND WEIGHT UPON THE BLOOD VOLUME OF SHEEP (Continued)

Sheep Number	Sex	Age	Weight Pounds	Cell Pack Volume	Per Cent Blood Volume
23	F	3 Years	136	33.4	5.44
23[a]		3 Years	138	36.1	5.90
191	F	3 Years	169	33.1	6.79
24	F	3 Years	180	33.4	5.19
Average			156	34.0	5.83 ± .704
59	F	7 Years[b]	98	39.8	8.9
421810	F	7 Years[b]	106	40.2	7.6
450975	F	7 Years[b]	142	30.7	7.5
Average			115	36.9	8.0 ± .781

[a] These determinations were made one week later.

[b] These individuals were in extremely thin condition.

TABLE X

SUMMARY: EFFECT OF AGE AND WEIGHT UPON THE BLOOD VOLUME OF SHEEP

Number of Animals	Age	Weight Pounds	Per Cent Blood Volume
5	4 Weeks	27	7.98 ± .451
4	6 Months	92	6.27 ± .193
3	1 Year	135	5.51 ± .704
4	3 Years	156	5.83 ± .704
3	7 Years	115	8.0 ± .781
19			

increase in blood volume.

The rate and total per cent uptake of P^{32} *in vitro* by the red cells of sheep was greater in the mature animals than the lambs throughout incubation as indicated in Table V. However, following administration of the labeled red cells to the animal, the loss of P^{32} from the blood *in vivo* averaged 10.3 per cent at one-half hour and 19.5 per cent at one hour for all ages as shown in Table VI.

Swine

In the blood volume studies with swine, twenty-five determinations were made with sixteen animals of different ages and weights as shown in Table XI. A summary of this study is presented in Table XII. An average blood volume of 7.4 per cent was obtained for the ten pound pigs, with the values decreasing more or less regularly to an average of 4.6 for the 344 pound animals. Consideration of this data indicated that despite the variation between individuals there was no real decrease of the blood volume with increasing body size. Furthermore, as is indicated for cattle and the burro, the effect was more pronounced with the lowest weight groups. It is indicated that the blood volume of swine is somewhat lower than for the other species. This may be explained by the higher fat content of the normal growing pig over that of the other species.

The results of *in vitro* studies with swine blood is illustrated in Table V. An average of 15 per cent of the added P^{32} passed into the red cells in the first hour, 19 per cent in two hours, and 24 per cent in three hours and 26 per cent in four hours. In contrast to the burro,

TABLE XI

EFFECT OF AGE AND WEIGHT UPON THE BLOOD VOLUME OF SWINE

Swine Number	Sex	Weight Pounds	Cell Pack Volume	Per Cent Blood Volume
1	F	8	34.0	7.3
2	M	10	34.0	7.9
3	M	11	40.2	7.0
Average		10	36.0	7.4 ± .458
4	M	15	40.2	6.6
5	F	20	42.3	6.7
6	M	21	40.9	6.6
7	F	22	40.6	6.9
Average		20	41.0	6.7 ± .173
24	F	74	31.8	6.0
25	F	75	30.3	5.9
27	F	78	37.9	6.4
26	F	82	37.8	5.6
31	M	94	41.9	6.6
28	M	97	43.1	6.5
29	M	97	39.0	6.8
30	M	102	30.3	6.1
Average		98	39.1	6.3 + .44

TABLE XI

EFFECT OF AGE AND WEIGHT UPON THE BLOOD VOLUME OF SWINE (Continued)

Swine Number	Sex	Weight Pounds	Cell Pack Volume	Per Cent Blood Volume
32	M	113	39.3	5.9
33	M	118	33.2	5.3
34	M	134	37.9	6.5
Average		122	36.8	5.9 ± .6000
49	M	164	39.3	6.3
39	M	182	40.0	4.8
0	F	200	43.6	5.0
Average		182	40.9	5.4 ± .814
45	F	328	39.7	4.6
46	F	333	38.6	4.3
40	F	344	28.5	5.1
37	M	370	36.9	4.4
Average		344	38.4	4.6 ± .356

TABLE XII

SUMMARY: EFFECT OF AGE AND WEIGHT UPON BLOOD VOLUME OF SWINE

Number of Animals	Age	Weight Pounds	Per Cent Blood Volume
3	2-3 Weeks	10	7.4 ± .458
4	4-6 weeks	20	6.7 ± .173
8	3-4 Months	98	6.3 ± .404
3	4-5 Months	122	5.9 ± .600
3	7-8 Months	182	5.4 ± .814
4	2 Years	344	4.6 ± .356
25			

bovine, and sheep, the red blood cells of young pigs appeared to take up the P^{32} faster than those of older animals during the early phase of incubation, but leveled off to about the same rate as that of the older swine red cells after about three hours incubation.

Following administration of the labeled red cells to the animal, the rate of loss of P^{32} from the blood *in vivo* was 6.4 per cent at thirty minutes and 13.6 per cent at one hour. As indicated in Table VI, this is slightly lower than the rate of loss by sheep and only about half that of cattle or the burro.

For use by those interested in calculation of the plasma or red cell volumes, the hematocrit values for the various species are included along with the per cent blood volume figures. No marked age or species differences were noted in the hematocrit values. The burro with a cell pack volume of 35.8 per cent was lowest, then swine 38.4, cattle 38.8, and sheep 41.9 per cent.

SUMMARY AND CONCLUSIONS

Blood volume studies were made using the P^{32} and I^{131} methods with burros, cattle, sheep and swine. Forty-six blood volume determinations were made with burros, eighteen with cattle, nineteen with sheep, and twenty-five with swine.

The per cent blood volume for the young and old groups of the different species ranged from 19.10 to 6.66 for burros, 12.90 to 5.68 for cattle, 7.98 to 5.51 for sheep, and 7.40 to 4.60 for swine, respectively. There was some variation between individuals and between age groups but with these species there was a definite tendency toward a decrease in the per cent blood volume with increased age. This was especially marked during the first sixty days after birth.

This study indicates: (1) the P^{32} labeled red blood cell method was satisfactory for the determination of blood volume in farm animals, (2) the I^{131} procedure was satisfactory with burros, but did not appear to be satisfactory with cattle or swine, (3) a definite decrease in the percentage of blood volume was found with increased age and weight, (4) three hours incubation of the blood with P^{32} was sufficient for all species, (5) the *in vitro* uptake of P^{32} by swine red blood cells was somewhat faster than by the other species, and (6) there was no marked difference in the *in vivo* loss of the P^{32} from the red blood cells of the different species.

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