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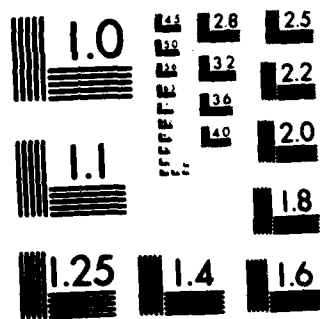
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DOMESTIC SWINE IN PHYSIOLOGICAL RESEARCH
VI. Effect of Splenic Erythrocyte Sequestration on Blood Volume Measurements in Conscious Immature Pigs

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Domestic Swine in Physiological Research. VI. Effect of Splenic Erythrocyte Sequestration on Blood Volume Measurements in Conscious Immature Pigs--Hannon, Bossone, Rodkey

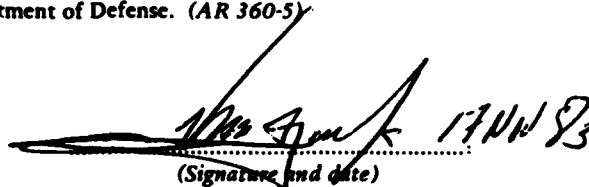
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In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

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20. ABSTRACT

When estimated by the dilution of ^{51}Cr -labelled erythrocytes under near-basal conditions, immature splenectomized pigs (N=20) had a circulating erythrocyte volume of 17.8 ± 1.64 (S.D.) ml/kg. At an assumed body to large vessel hematocrit (BH:LH) ratio of 1.0 plasma volume was 42.8 ± 2.69 ml/kg and blood volume 60.6 ± 3.30 ml/kg. At an assumed BH:LH ratio of 0.9 plasma volume was 49.6 ± 3.12 ml/kg and blood volume was 67.3 ± 3.67 ml/kg. Sham-operated pigs (N=20) had a circulating erythrocyte volume of 16.2 ± 1.39 ml/kg, and at a BH:LH ratio of 1.0 a plasma volume was 44.4 ± 2.96 and a blood volume was 60.5 ± 3.78 ml/kg; at BH:LH ratio of 0.9 plasma volume was 51.1 ± 3.42 ml/kg and blood volume was 67.2 ± 4.12 ml/kg. Kinetic analysis of early ^{51}Cr loss from the circulating blood of the sham-operated pigs indicated a splenic erythrocyte sequestration of 4.51 ± 0.89 ml/kg and a $t_{1/2}$ of 9.76 ± 1.93 minutes for splenic erythrocyte turnover. Epinephrine injection (0.025 mg/kg) caused rapid mobilization of splenic erythrocytes in sham-operated pigs (N=6) with a hematocrit rise from 0.27 ± 0.01 to 0.35 ± 0.02 . Similar results were obtained in sham-operated pigs (N=9) subjected to physical restraint. Collectively, these measurements indicated that the porcine spleen, under near-basal conditions, sequestered 20-25 percent of the total body erythrocyte volume. Volume estimates in splenectomized pigs (N=7) based on simultaneous dilutions of ^{51}Cr -labelled erythrocytes and ^{125}I -labelled bovine albumin gave circulating erythrocyte, plasma and blood volumes of 18.4 ± 2.46 , 60.7 ± 4.01 and 79.0 ± 3.51 ml/kg, respectively, and a BH:LH ratio of 0.756 ± 0.029 . The latter value, it was concluded, reflected overestimation of plasma volume by the ^{125}I -labelled albumin procedure.

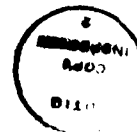
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ABSTRACT

When estimated by the dilution of ^{51}Cr -labelled erythrocytes under near-basal conditions, immature splenectomized pigs (N=20) had a circulating erythrocyte volume of 17.8 ± 1.64 (S.D.) ml/kg. At an assumed body to large vessel hematocrit (BH:LH) ratio of 1.0 plasma volume was 42.8 ± 2.69 ml/kg and blood volume 60.6 ± 3.30 ml/kg. At an assumed BH:LH ratio of 0.9 plasma volume was 49.6 ± 3.12 ml/kg and blood volume was 67.3 ± 3.67 ml/kg. Sham-operated pigs (N=20) had a circulating erythrocyte volume of 16.2 ± 1.39 ml/kg, and at a BH:LH ratio of 1.0 a plasma volume was 44.4 ± 2.96 and a blood volume was 60.5 ± 3.78 ml/kg; at BH:LH ratio of 0.9 plasma volume was 51.1 ± 3.42 ml/kg and blood volume was 67.2 ± 4.12 ml/kg. Kinetic analysis of early ^{51}Cr loss from the circulating blood of the sham-operated pigs indicated a splenic erythrocyte sequestration of 4.51 ± 0.89 ml/kg and a t-1/2 of 9.76 ± 1.93 minutes for splenic erythrocyte turnover. Epinephrine injection (0.025 mg/kg) caused rapid mobilization of splenic erythrocytes in sham-operated pigs (N=6) with a hematocrit rise from 0.27 ± 0.01 to 0.35 ± 0.02 . Similar results were obtained in sham-operated pigs (N=9) subjected to physical restraint. Collectively, these measurements indicated that the porcine spleen, under near-basal conditions, sequestered 20-25 percent of the total body erythrocyte volume. Volume estimates, in splenectomized pigs (N=7), based on simultaneous dilutions of ^{51}Cr -labelled erythrocytes and ^{125}I -labelled bovine albumin gave circulating erythrocyte, plasma and blood volumes of 18.4 ± 2.46 , 60.7 ± 4.01 and 79.0 ± 3.51 ml/kg, respectively, and a BH:LH ratio of 0.756 ± 0.029 . The latter value, it was concluded, reflected overestimation of plasma volume by the ^{125}I -labelled albumin procedure.

Key Words: erythrocyte volume, blood volume, spleen, swine.



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PREFACE

Previous Institute Reports, Letterman Army Institute of Research, on Domestic Swine in Physiological Research have included the following titles:

- I. A Biomedical Model. Report No. 91, May 1981
- II. Electrolyte Values for Arterial Serum from Young Anesthetized Pigs Maintained under Steady-State Ventilatory Conditions. Report No. 92, May 1981
- III. Blood Gas and Acid-Base Values for Arterial Blood from Young Anesthetized Pigs Maintained under Steady-State Conditions. Report No. 113, February 1982
- IV. A Blood Acid-Base Curve Nomogram for Immature Pigs. Report No. 137, February 1983
- V. Construction of Acid-Base Alignment Nomograms to Estimate Buffer Base and Base Excess Concentrations in Arterial Blood from Immature Pigs. Report No. 144, April 1983

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DOMESTIC SWINE IN PHYSIOLOGICAL RESEARCH
VI. EFFECT OF SPLENIC ERYTHROCYTE SEQUESTRATION ON BLOOD VOLUME
MEASUREMENTS IN CONSCIOUS IMMATURE PIGS

Reports on porcine blood volume measurement show a wide range of values for supposedly normal animals. Much of this variability is attributable to differences in body size, an effect first reported in 1951 by Hansard et al (1). In a limited number of animals these investigators (1) showed by means of ^{32}P -labelled erythrocyte dilution that blood volume per kilogram decreased as body weight increased. At one extreme, in 10-kg pigs, a value of 74 ml/kg was obtained while at the other extreme, in 344-kg pigs, a value of 45 ml/kg was obtained (1). Similar size-related decrements in porcine blood volume have been reported subsequently by a number of others. The available data, through 1972, have been assembled and reviewed by Steinhardt et al (2). Regression equations predicting blood volume as a function of body weight have been reported by von Engelhardt (3).

Decrements in blood volume with increasing body size are largely attributable to increases in the fat fraction of total body mass (4-6). However, widely divergent values have been reported also for plasma and erythrocyte volumes in pigs of similar body weight (2). Some of this divergency appears due to the particular indicator dilution procedure that was used in the volume estimates. Procedures based on the dilution of Evans Blue or radioiodine-labelled serum albumin produce generally higher volume estimates than procedures based on the dilution of ^{32}P - or ^{51}Cr -labelled erythrocytes. Additional variability, in the case of albumin labels, may be caused by the use of different techniques to correct for transcapillary albumin losses following indicator injection.

One variable that has been largely ignored in porcine blood volume measurements is potential sequestration, or discharge, of erythrocytes from the spleen during the measurement period. The porcine spleen is a contractile organ (7,8) which can release large numbers of erythrocytes and markedly elevate hematocrit levels when stimulated by epinephrine (7,8) or exercise (8). Steinhardt and his coworkers (8-11) have attempted to measure the impact of such splenic erythrocyte mobilization on porcine blood volume estimated by Evans Blue dilution. These attempts, however, have been less than satisfactory because the measurements did not address potential alterations in transcapillary indicator loss. In the dog at least, splenic sequestration or discharge of erythrocytes has a major effect on blood volume (12,13).

In the study described here erythrocyte storage capacity of the porcine spleen was assessed by kinetic analysis of ^{51}Cr -labelled erythrocyte dilution following intraarterial injection into conscious pigs. Storage capacity also was assessed by changes in circulating erythrocyte volume subsequent to physical restraint of the animal and by hematocrit changes subsequent to epinephrine injection. In addition, blood volume characteristics were compared in sham-operated and splenectomized pigs. Finally, vascular volumes based on the dilution of ^{51}Cr -labelled erythrocytes were compared to those based on the dilution of ^{125}I -labelled bovine serum albumin.

METHODS

Fifty-seven immature Yorkshire pigs, both barrows and gilts, were obtained from a commercial breeder (J.G. Boswell Co., Corcoran, CA) and were maintained in a common indoor holding area at Letterman Army Institute of Research until utilized for study, usually within two to four weeks after arrival. They were fed a commercial ration (Purina Pig Chow, Ralston Purina Co., St Louis, Mo) and water ad libitum. The pigs were 2 to 3 months old and weighed (mean \pm S.D.) 22.6 ± 2.21 kg when the studies were conducted.

After an overnight fast, each pig received a pre-anesthetic intramuscular injection of 2.2 mg/kg atropine sulfate 2.2 mg/kg ketamine HCl, and 2.2 mg/kg xylazine. Halothane anesthesia was induced by face mask and maintained with an endotracheal catheter. A celiotomy was performed and, in 31 animals, the spleen was removed according to standard techniques with double ligation of all vascular pedicles. All celiotomies were closed with two layers of non-absorbable surgical suture. The remaining 26 animals were sham operated, that is, adominal incisions were made, then closed immediately.

The left carotid artery of each pig was exposed and a 1.27-mm ID x 2.03-mm OD polyvinylidene catheter (Type S-54 HL Tygon, Winton Plastics and Synthetics, Akron, OH) was inserted to the level of the aorta and secured by ligatures around the carotid artery. The free end of the catheter was tunneled beneath the skin and exited on the dorsal surface of the neck. The exteriorized portion was fitted with a 17-gauge Intramedic^R Luer Stub Adapter (Clay Adams, Parsippany, N.J.) and capped with an Argyle^R Intermittent Infusion Plug (Brunswick Co., St Louis, Mo). Following wound closure the catheter was filled through the infusion plug with heparin (1000 units/ml). The catheter exit site was protected by a 5-cm x 10-cm Velcro^R (Velcro USA, New York, NY) patch sutured to the skin; a 2-cm x 7.5-cm hole was cut in the patch portion next to the skin to allow catheter access.

After a 7- to 10-day recovery period each pig, after an overnight fast, was brought into a quiet laboratory in a portable transport cage

and given a variety of fabric bedding material. After 15 to 30 minutes of rooting and bedding rearrangement, most animals voluntarily assumed a recumbent position. When so positioned, the intermittent infusion plug was removed and the stub adapter was connected to a 12-inch pressure monitoring/injection line (Cobe Laboratories, Lakewood, Co). The latter had been previously fitted with a plastic three-way stopcock (Pharmaseal^R, Inc., Toa Alta, Puerto Rico) and filled with heparinized saline (10 units/ml). The entire system was then cleansed by withdrawing 10 ml of fluid (blood plus heparinized saline) followed by flushing with fresh heparinized saline. After 15 to 30 minutes of additional voluntary recumbent rest, the experiment was started. Three experiments were conducted.

Experiment 1: Determination of Circulating and Total Body Erythrocyte Volume

Eight splenectomized and 8 sham-operated pigs were used to ascertain time-related changes in concentration of arterially injected ⁵¹Cr-tagged erythrocytes. Twenty-five milliliters of blood were withdrawn from each animal and mixed with 4 ml of ACD solution and approximately 50 uCi Na₂⁵¹Cr O₄. After standing for 30 minutes at room temperature, with occasional mixing, the blood was centrifuged at 4°C and 2500 g for 10 minutes, and the plasma was discarded. The erythrocytes were then washed twice by resuspension in cold isotonic saline followed by centrifugation. After the last wash, the erythrocytes were thoroughly mixed with 10 ml of cold saline, preparatory to injection into the pig; final volume, including erythrocytes, was approximately 13 ml. A calibrated syringe was used to inject 10 ml of the mixture rapidly into the pig. This maneuver was accomplished by means of a rubber injection bulb and pressure monitoring line attached to the carotid catheter. The injectate was flushed in with 20 ml of saline, the entire procedure required about 10 seconds. In vivo dilution of ⁵¹Cr-tagged erythrocytes was determined in blood samples (3 ml) taken at 1-minute intervals over the first 10 minutes, and at 15, 20, 30, 45, 60, 90, 120 and 150 minutes after injection. To assure the acquisition of fresh circulating blood, sample removal was immediately preceded by a 15-second period of continuous blood withdrawal from the catheter at a rate of 1 ml/sec. After sample removal, this blood was returned to the animal, and the catheter was filled with a minimal volume, about 1 ml, of heparinized saline (10 units/ml).

A Packard Auto-gamma counter, Model 5986, (Packard Instrument Co, Downers Grove, Ill), with an energy window set at 276 to 366 KEV, was used to count ⁵¹Cr-tagged erythrocyte activity. A 1-ml aliquot of each blood sample and triplicate 0.2-ml aliquots of the erythrocyte-saline injected dose were transferred to 12 x 75 mm disposable test

tubes and diluted with water to 2.0 ml preparatory to counting. Positive displacement micropipettes (Scientific Manufacturing Industries, Emeryville, CA) were used for transfers of the blood sample and injected dose. The hematocrit of each blood sample was determined with a Lourdes microhematocrit centrifuge (Vernitron Medical Products, Inc., Carlstadt, NJ), and the ^{51}Cr activity per milliliter of erythrocytes, corrected for trapped plasma, was calculated.

The foregoing data were supplemented by similar data collected from an additional 12 splenectomized and 12 sham-operated pigs to describe porcine population characteristics for circulating erythrocyte volume, total body erythrocyte volume, splenic (in the case of sham-operated pigs) erythrocyte volume, circulating plasma volume, circulating blood volume, and the kinetic characteristics of splenic erythrocyte exchange with the systemic circulation. The dilution of ^{51}Cr -tagged erythrocytes in these additional pigs was measured at 5, 10, 15, 20, 30, 45, 60, and 90 minutes after injection. In splenectomized pigs, the injected dose was divided by the average ^{51}Cr CPM/ml of erythrocytes to obtain the circulating and total body erythrocyte volumes.

Determinations of circulating and total body erythrocyte volume in sham-operated pigs was complicated by splenic sequestration of ^{51}Cr -tagged erythrocytes. Such sequestration and release of untagged previously stored erythrocytes caused a rapid decline in the circulating dilution values. This decline showed first-order kinetics with an asymptotic value being approached 30 to 150 minutes after injection. The general equation describing dilution of ^{51}Cr -tagged erythrocytes in these animals would be:

$$A_t = A_m + a_0 e^{-kt} \quad [1]$$

where A_t equals ^{51}Cr activity at any time t after injection, A_m equals the asymptotic value for complete mixing of ^{51}Cr -tagged erythrocytes in the total body erythrocyte volume, a_0 is the zero-time intercept and k is the rate constant for the fraction of circulating erythrocytes entering or leaving the spleen per unit time. A least-squares-regression procedure, employing iterations of A_m estimates, is used to calculate a_0 and k . Thus, an approximate A_m value is chosen, for example the ^{51}Cr -tagged erythrocyte CPM observed 30 minutes after injection, and this value is subtracted from all of the values recorded previously. The resultant differences are entered into Equation 1 along with their respective t values, and a_0 , k , the correlation coefficient, r , and the variance are calculated. This procedure is repeated with revised estimates of A_m until maximum r and minimum variance values are obtained. At this point the injected dose divided by A_m would equal the total body erythrocyte volume. The injected dose divided by $A_m + a_0$ would equal the circulating erythrocyte volume, and the difference between total body and

circulating erythrocyte volumes would equal the volume of erythrocytes sequestered in the spleen. In addition, the value can be used to determine the half-time of splenic erythrocyte turnover. Calculation of A_m , a_0 and k values can be a time-consuming procedure when done by hand, but fortunately readily available computer programs expedite the task.

In both sham-operated and splenectomized pigs arterial hematocrit values were used to estimate circulating plasma and blood volumes. Two procedures were used. In one, equality of arterial and total body hematocrit values was assumed. Thus,

$$PV = EV \frac{1 - (0.97)(Hct)}{(0.97)(Hct)} \quad [2]$$

where PV and EV represent circulating plasma and erythrocyte volumes, respectively, Hct represents the arterial hematocrit and 0.97 is a correction factor for trapped plasma. In the second procedure, the total body hematocrit value was assumed to be 90 percent of the arterial hematocrit value. Thus,

$$PV = EV \left[\frac{1 - (0.9)(0.97)(Hct)}{(0.9)(0.97)(Hct)} \right] \quad [3]$$

With either procedure, the sum of circulating erythrocyte and plasma volumes was used to estimate circulating blood volume.

Experiment 2: Measurement of Splenic Erythrocyte Mobilization

Two procedures were used to assess porcine capacity for circulatory mobilization of erythrocytes sequestered in the spleen. In one, control values for hematocrit, and circulating erythrocyte, plasma and blood volumes were measured with ^{51}Cr -tagged erythrocytes in 8 splenectomized and 8 sham-operated pigs under conditions of voluntary recumbent rest. These measurements were obtained during the course of the Experiment One. Immediately after the last blood sample was withdrawn in the first experiment the recumbent pig was grabbed by the front legs, raised to a vertical position and placed on its buttocks. This led to considerable agitation and struggle on the part of the animal, but it was physically restrained in such a position for one minute. Then, an additional 3-ml blood sample was withdrawn for hematocrit determination, and ^{51}Cr counting, from which the changes in circulating erythrocyte, plasma and blood volumes, relative to earlier control values, were calculated; body to venous hematocrit ratios of 0.9 and 1.0 were assumed.

In the other procedure, a series of 4 control hematocrit values were determined at 5 minute intervals in 4 splenectomized and 6 sham-operated pigs not used in the other experiments. Again, the pigs

remained in a voluntary recumbent position while control blood samples were obtained. After the last sample was removed the pig was injected intraarterially with 0.5 ml of epinephrine (1 mg/ml), and blood samples for hematocrit determination were taken at 2.5, 5, 10, 20, 40, and 80 minutes after injection. Epinephrine caused an immediate arousal of the animal, but in all instances recumbency was resumed after 10 or 15 minutes. The changes in hematocrit following epinephrine were used as an index of splenic erythrocyte mobilization.

Experiment 3: Simultaneous Determination of Circulating Erythrocyte and Plasma Volumes

The erythrocyte volume of 7 splenectomized pigs was estimated by the dilution of ^{51}Cr -tagged erythrocytes, as in Experiment One. In addition, plasma volume was estimated simultaneously by the dilution of ^{125}I -tagged bovine serum albumin. In the latter instance, approximately 10 uCi of ^{125}I -albumin was diluted in 15 ml of the animal's plasma and 10 ml was injected through the carotid artery catheter and flushed in with 20 ml of saline. The entire procedure required about 10 seconds. Samples for erythrocyte ^{51}Cr and plasma ^{125}I counting were withdrawn, as in Experiment One, at 1-minute intervals for the first 10 minutes, and at 15, 20, 30, 45, 60, 90, 120 and 150 minutes after injection. Plasma was collected following sample centrifugation and 1-ml aliquots, obtained with a positive displacement pipette, were counted in the Model 5986, Packard Auto-gamma counter at an energy window of 18 to 80 KEV. Radioactivity in the injected dose was determined by triplicate counts of 0.2-ml aliquots obtained with a positive displacement micropipette. A semilogarithmic, least-squares extrapolation of plasma ^{125}I concentration between 30 and 150 minutes after injection was used to calculate the zero-time plasma ^{125}I dilution and this value in turn was used to calculate plasma volume. Blood volume was calculated as the sum of ^{51}Cr -determined erythrocyte and ^{125}I -determined plasma volumes. Blood volume so measured was divided into ^{51}Cr -determined erythrocyte volume to obtain the total body hematocrit value, and then this value was divided by the arterial hematocrit to obtain the total body to large vessel hematocrit ratio. Finally, the arterial hematocrit value and an assumed total body to large vessel ratio of 1.0 were used to calculate plasma and blood volumes from ^{51}Cr -determined erythrocyte volume, and erythrocyte and blood volume from ^{125}I -determined plasma volume.

Accuracy of the pipettes used in this study was assessed gravimetrically first with distilled water and subsequently with blood. In the latter instance, blood density was first obtained by weighing exactly 100 ml in a volumetric flask and using the same blood to determine the blood weight, and calculated volume, delivered by the

0.2-ml and 1.0-ml positive displacement pipettes and the 10 ml calibrated injection syringes.

In addition, hematocrit values used in the calculation of erythrocyte and plasma volumes were corrected for trapped plasma, as determined by a procedure similar to that reported by Baker (14). Briefly, an accurate, gravimetrically measured volume (30 to 70 ml) of porcine blood was placed in a beaker along with an accurately measured dose of ^{125}I -bovine albumin or $\text{Na}_2^{35}\text{SO}_4$. The blood and isotope were gently, but thoroughly, mixed for 10 minutes with a magnetic stirrer, at the end of which 5 or more microhematocrit capillary tubes were filled and centrifuged at 12,000 g for 5 minutes. The remaining blood was centrifuged at 2500 g for 10 minutes, plasma was collected and the activity of ^{125}I -bovine albumin or $\text{Na}_2^{35}\text{SO}_4$ was determined in 5 or more 1-ml plasma aliquots. Average isotope dilution was used to calculate the beaker plasma volume, and, by difference from the original beaker blood volume, the beaker erythrocyte volume and hematocrit. Finally, the difference between beaker hematocrit and the slightly lower average capillary tube hematocrit was used to calculate the plasma fraction trapped in the erythrocyte column packed by the microhematocrit centrifuge.

RESULTS

As indicated in Table 1, a high degree of volume measurement accuracy was obtained with the positive displacement pipettes and injection syringes. No significant differences were observed between the volume of water and blood delivered by each type of the pipette or syringe.

Trapped plasma contained in the packed erythrocyte column following microhematocrit centrifugation ranged from 1.2 to 5.0% (Table 2). There was no significant difference between the percentage estimated by ^{125}I dilution and the percentage estimated by ^{35}S dilution. The composite average was $2.8 \pm 1.26\%$ (mean \pm S.D.).

Experiment 1: Circulating and Total Body Erythrocyte Volume

Figure 1 shows the normalized dilution characteristics of ^{51}Cr -tagged erythrocytes over the first 45 minutes after injection in sham-operated and splenectomized pigs; the data were normalized by assigning a value of 100 to the extrapolated zero-time dilution value. Beyond 45 minutes, values in the splenectomized pigs showed no statistically significant change from those obtained several minutes after injection. Values obtained 1 or 2 minutes after injection tended to be higher than those recorded subsequently. These apparent elevations, and suggestions of cycling over the first 10 minutes after injection, may have been due to recirculation of blood through vascular circuits close to the point of injection and sampling.

Table 1. Measurement accuracy of 0.2 and 1.0 ml positive displacement sample transfer pipettes and 10.0 ml calibrated injection syringes

Trial*	0.2 ml		1.0 ml		10 ml	
	H ₂ O	Blood	H ₂ O	Blood	H ₂ O	Blood
1	0.1989	0.2000	0.9951	0.9993	9.9915	9.9906
2	0.1996	0.1968	1.0040	1.0033	10.0078	10.0253
3	0.2002	0.1974	0.9964	1.0004	10.0041	9.9990
4	0.1994	0.1999	0.9978	0.9930	9.9935	9.9836
5	0.1993	0.1993	1.0048	0.9968	9.9985	10.0070
Mean	0.1995	0.1987	0.9996	0.9986	9.9991	10.0011
S.D.	<u>+0.00048</u>	<u>+0.00066</u>	<u>+0.00200</u>	<u>+0.00174</u>	<u>+0.00308</u>	<u>+0.00722</u>

* Each trial value, in ml, represents the average of triplicate measurements of distilled water or blood. Replacement 0.2 and 1.0 plastic tips and different 10 ml syringes were used for each trial.

Table 2. Trapped plasma (T.P.) as determined by the ¹²⁵I-albumin or Na₂³⁵SO₄ contained in the erythrocyte column packed by ³⁵S-rohematocrit centrifugation

Trial*	¹²⁵ I-albumin		Na ₂ ³⁵ SO ₄	
	Hct	T.P.(%)	Hct	T.P.(%)
1	0.22	3.2	0.26	2.0
2	0.28	2.7	0.33	2.2
3	0.29	2.3	0.30	4.7
4	0.30	1.2	0.28	1.2
5	0.30	2.1	0.33	4.1
6	0.29	5.0	0.29	3.4
Mean	0.28	2.7	0.30	2.9
S.D.	<u>+0.030</u>	<u>+1.28</u>	<u>+0.028</u>	<u>+1.35</u>

*Blood from separate pigs (N=12) was used in each trial.

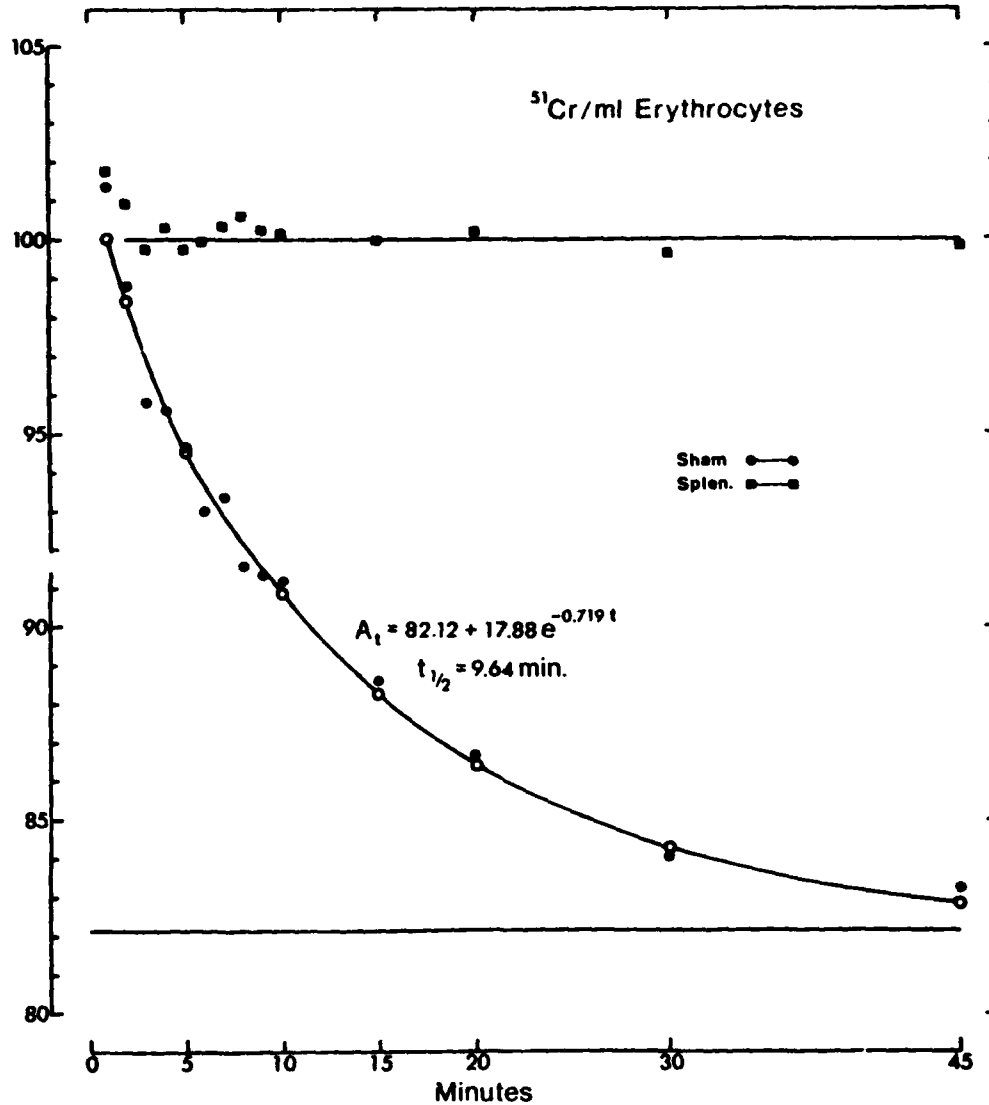


Figure 1. Alterations in the concentration of ^{51}Cr -tagged erythrocytes as a function of time after injection. Upper horizontal line indicates average value in splenectomized pigs (N=8). Lower horizontal line indicates asymptotic value reflecting complete body mixing in sham-operated pigs (N=8); open circles indicated points calculated by the indicated regression equation. Ordinate values are normalized such that concentration at $t=0$ is assigned a value of 100.

Splenectomized pigs had slightly but significantly higher hematocrit values and circulating erythrocyte volumes and slightly lower-circulating plasma volumes compared to the values found in sham-operated pigs (Table 3). Total circulating blood volume was essentially equal in both groups. Sham-operated pigs had a calculated splenic erythrocyte volume of 4.51 ± 0.89 (S.D.) ml/kg which represented about 22% of the total body erythrocyte volume.

Table 3. Blood volume characteristics of sham-operated and splenectomized pigs as estimated by ^{51}Cr -labelled erythrocytes

Measurement	Sham-operated Pigs	Splenectomized Pigs
	N = 20	N = 20
Hematocrit	0.267 ± 0.0153	$0.293 \pm 0.022^*$
Circ Erythro	16.16 ± 1.386	$17.76 \pm 1.644^*$
Total Erythro Vol	20.67 ± 1.658	$17.76 \pm 1.644^*$
Splenic Erythro Vol	4.51 ± 0.894	0
Circ Plasma Vol (A)	44.33 ± 2.964	$42.85 \pm 2.694^*$
Circ Plasma Vol (B)	51.09 ± 3.416	$49.59 \pm 3.118^*$
Circ Blood Vol (A)	60.48 ± 3.776	60.60 ± 3.301
Circ Blood Vol (B)	67.24 ± 4.198	67.34 ± 3.668

All volume measurements expressed as mean ml/kg \pm S.D.
Volume calculations after (A) assume a total body to large vessel hematocrit ratio of 1.0, after (B) a ratio of 0.9.

*Significant ($P < 0.05$) difference between groups.

Because of this splenic component, total body erythrocyte volume was significantly greater in sham-operated than in splenectomized pigs. The average $t_{1/2}$ value for splenic red cell turnover in 20 pigs was 9.76 ± 1.93 minutes. Application of regression Equations 1 for ^{51}Cr -labelled erythrocyte concentration in individual pigs showed that values of λ_0 , as anticipated, varied in direct proportion to the injected dose and $t_{1/2}$. More specifically λ_0 , on the average, equaled $0.213A_m$.

The following average equation, therefore, reflected the population characteristics of the sham-operated immature domestic pigs used in this study:

$$A_t = A_m(1 + 0.213 e^{-0.739 t}) \quad [4]$$

Experiment 2: Splenic Erythrocyte Mobilization

Table 4 summarizes the effects of 1-minute of physical restraint, subsequent to a control period of recumbent rest, on the circulating blood volume and related variables of sham-operated and splenectomized pigs. In both groups, physical restraint was associated with a significant increase in hematocrit, but the effect was significantly (by analysis of variance) more pronounced in the sham-operated animals. This difference, reflecting splenic erythrocyte mobilization, caused the sham-operated animals to have elevated circulating erythrocyte volumes following physical restraint. In both groups restraint led to comparable decrements in calculated circulating plasma volume which in the splenectomized pigs led to a significant decrease in calculated blood volume. Physical restraint had no effect on the blood volume of sham-operated pigs; the decrement in plasma volume was offset by splenic erythrocyte mobilization.

Table 4. Effects of physical restraint on the blood volume characteristics of sham-operated and splenectomized pigs

Measurement	Sham-Operated Pigs		Splenectomized Pigs	
	Control	Restrained	Control	Restrained
Hematocrit	0.269+0.078	0.347+0.013*	0.281+0.021	0.302+0.022*
⁵¹ Cr/ml Erythro	17.2+3.54	17.1+3.42	17.3+2.72	17.4+2.77
Circ Erythro Vol	16.3+0.88	21.1+1.70*	17.1+1.61	17.1+1.63
Circ Plasma Vol(A)	44.1+2.68	39.7+0.86*	43.8+3.01	36.4+2.57*
Circ Plasma Vol(B)	51.0+3.10	46.5+1.00*	50.5+3.47	45.8+3.23*
Circ Blood Vol(A)	60.4+3.34	60.8+3.81	60.9+3.69	56.4+3.26*
Circ Blood Vol(B)	67.3+3.72	67.6+4.24	67.6+4.10	62.9+3.64*

All measurements expressed as mean ml/kg ± S.D. for 8 sham-operated and 8 splenectomized pigs. ⁵¹Cr C.P.M. expressed in thousands. Volume calculations after (A) assume a total body to large vessel hematocrit ratio of 1.0, after (B) a ratio of 0.9.

*Indicates a significant (P<0.05) difference between control and restrained values.

Similar hematocrit effects were observed when resting sham-operated and splenectomized pigs received intraarterial injections of epinephrine (Fig 2). No attempt was made to assess the effects of epinephrine on vascular volumes because the rate and extent of splenic erythrocyte storage during the recovery phase could not be estimated in the sham-operated pigs. Nevertheless, the hematocrit increments observed immediately after injection were nearly identical to those observed after physical restraint.

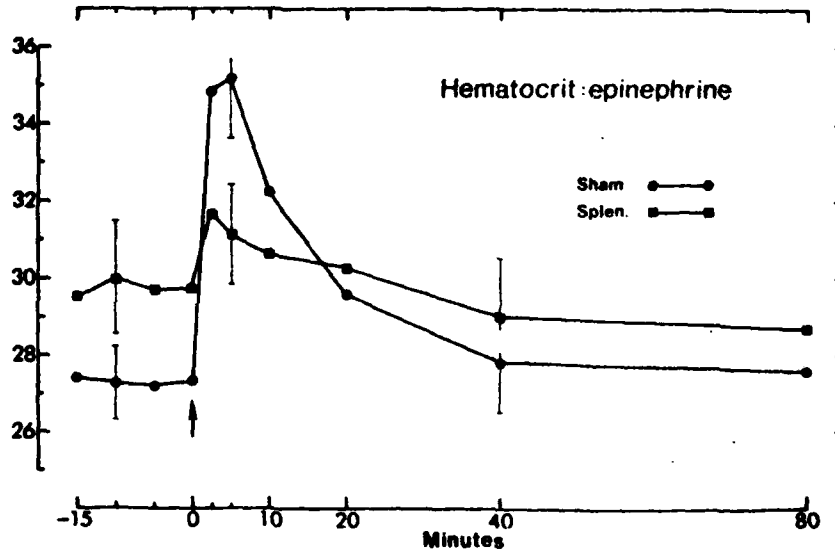


Figure 2. Effect of a 0.5 ml, 1:1000 dilution epinephrine injection (arrow) on the hematocrit of splenectomized (N=4) and sham-operated (N=6) conscious pigs.

Experiment 3: Simultaneous Erythrocyte and Plasma Volume Measurements

Attempts to measure circulating plasma volume by the dilution of ¹²⁵I-labelled bovine albumin dilution were complicated by disappearance of the label from the circulation. Kinetic evaluation of this disappearance revealed two components, suggesting at least two pools of albumin dilution (Fig 3). The larger pool which became apparent about 20 minutes after injection had a $t_{1/2}$ of 6.26 hours; the smaller pool which became apparent about 2 minutes after injection had a $t_{1/2}$ of 4.87 minutes. Plasma volume estimates were based on least-squares extrapolation of the larger pool to zero time as indicated in Figure 3. The blood volume characteristics of splenectomized pigs as estimated by the dilution of ⁵¹Cr-labelled erythrocytes and ¹²⁵I-labelled albumin are summarized in Table 5. These data show that

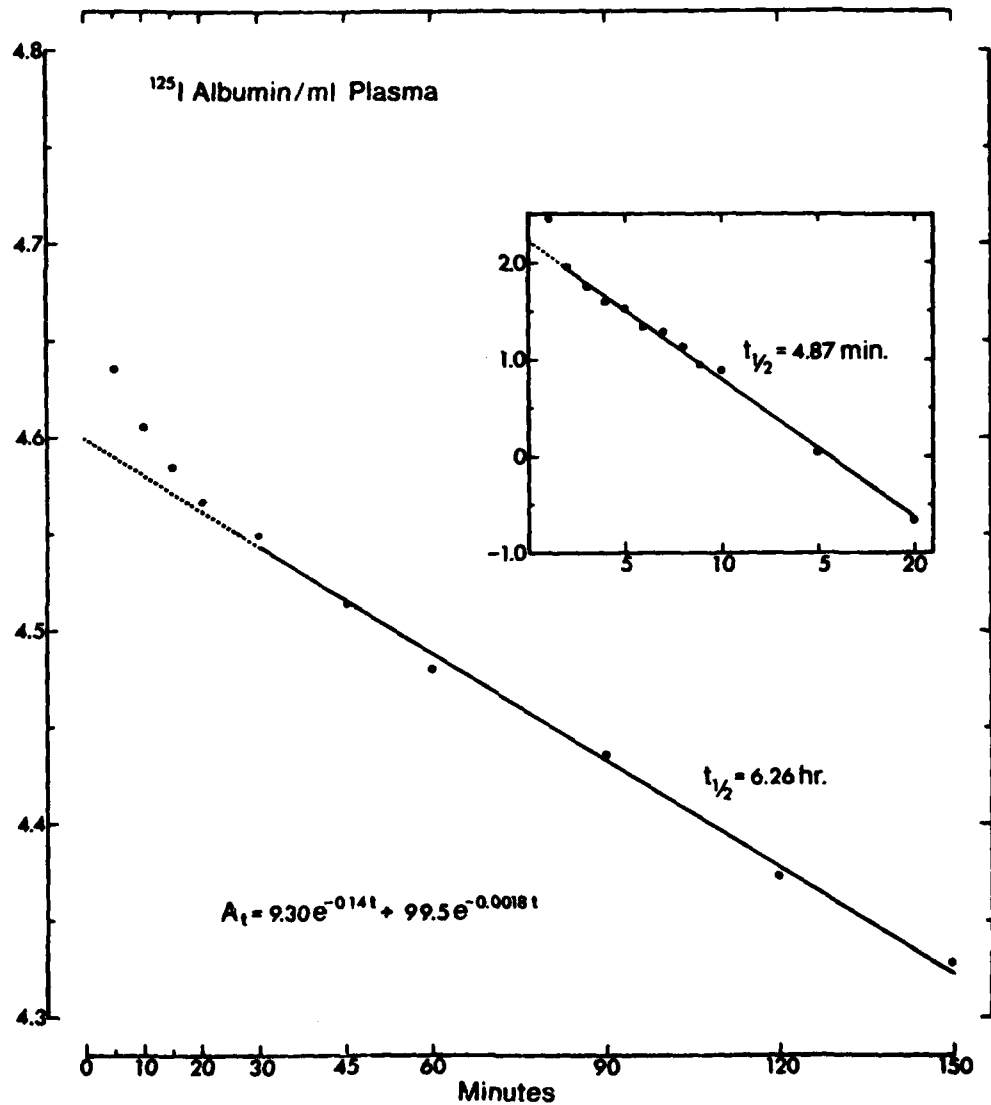


Figure 3. Alterations in the plasma concentration ¹²⁵I-tagged albumin as a function of time in conscious splenotomized pigs (N=7). Ordinate values expressed as the natural logarithm of the normalized ¹²⁵I activity, a value of 100 being assigned to the activity recorded 10 minutes after injection.

^{125}I -tagged albumin dilution gives a significantly greater plasma, erythrocyte and blood volume than ^{51}Cr -tagged erythrocyte dilution. As would be expected, blood volume determinations based on the two radiolabels were intermediate to those obtained by either label alone. The average body hematocrit was considerably lower than the arterial hematocrit, the ratio of the two being 0.756 ± 0.029 .

Table 5. Porcine blood volume characteristics as estimated by the dilution of ^{51}Cr -labelled erythrocytes and ^{125}I -labelled plasma albumin

Measurement	^{51}Cr -Erythro. Dilution	^{125}I -Albumin Dilution	Diff.
Erythrocyte Volume	18.4 \pm 2.46	27.0 \pm 3.62	8.6*
Plasma Volume	41.3 \pm 3.33	60.7 \pm 4.04	19.4*
Blood Volume	59.7 \pm 3.43	87.7 \pm 4.46	28.0*
$^{51}\text{Cr}/^{125}\text{I}$ Blood Volume		79.0 \pm 3.51	
Body Hematocrit		0.233 \pm 0.024	
Arterial Hematocrit		0.308 \pm 0.022	
Body/Arterial Hct Ratio		0.756 \pm 0.029	

Indicated values represent the mean ml/kg \pm S.D. for 7 animals

*Significant ($P < 0.05$) difference.

DISCUSSION

The results of this study show that the spleen of the immature, near-basal pig sequesters 20 to 25 percent of the total body erythrocyte volume. Under these conditions, the turnover of sequestered splenic erythrocytes averages about 7 percent per minute. Under conditions other than basal, however, splenic contraction can cause a rapid transfer of stored cells to the circulating blood. In the present experiments, such erythrocyte mobilization was observed following the intraarterial injection of epinephrine or following a brief period of physical restraint which, presumably, led to endogenous catecholamine release. Splenic contraction and erythrocyte release, as evidenced by increased venous hematocrit levels, has been studied extensively by Steinhardt and his coworkers (2,8,10,11) in domestic pigs subjected to treadmill exercise or to intravenous epinephrine injections. Epinephrine-induced splenic contraction in minipigs has also been investigated by Wachtel and McCahan (7).

Interpretational difficulties are encountered when the results of these studies are compared to those recorded here, largely because experimental conditions associated with data collection were not equivalent. For instance, Steinhardt et al (8) investigated the hematocrit responses of intact and splenectomized pigs intravenously injected with epinephrine (0.008 mg/kg) while the animals were restrained in a lateral recumbent position. Not only was their dosage of epinephrine lower than that used in the present study (about 0.025 mg/kg) but also their use of physical restraint could have produced elevated control hematocrit values. Either of these factors could explain the lower epinephrine-induced hematocrit increments reported by Steinhardt et al (8), relative to those observed here.

Failure to recognize, or adequately account for, the erythrocyte storage and autotransfusion functions of the porcine spleen also would seem to be a major factor contributing to the wide variations in the hematocrit values and circulating erythrocyte volumes reported for normal pigs. In animals of the same general body size as those used in the present study, normal hematocrit values range from about 0.30 to about 0.39 (15-18). In all of these reports (15-18), hematocrits were measured in venous blood samples obtained by vena cava puncture while the animals were being physically restrained. They are substantially higher than those recorded here for arterial blood taken from pigs under near-basal conditions but are similar to those obtained after one minute of physical restraint. Splenectomy also induces a slight elevation in resting hematocrit level, as seen in Tables 3 and 4, and in data reported by Steinhardt et al (8) and by Wachtel and McCahan (7). These increments may be due to splenic contraction during the course of the splenectomy procedure itself. In support of this contention, arterial and splenic venous hematocrit values measured in 10 pigs during the early stages of spleen removal averaged 0.28 ± 0.024 and 0.50 ± 0.110 , respectively. Alternatively, the splenectomized pigs may have responded to the lack of erythrocyte reserves by increased erythropoietic activity.

In the intact pig, splenic blood flow and erythrocyte sequestration can lead to overestimated values for circulating erythrocyte volume when the measurements are based on the dilution of labelled erythrocytes. Thus, following injection, the spleen acts as a "sink" sequestering the injected cells and replacing them, via splenic venous outflow, with endogenous unlabelled cells. This process leads to a rapid decline in labelled erythrocyte concentration shortly after injection but continues for an hour or more before the ratio of labelled to unlabelled cells becomes equal in the circulating blood and spleen. The sequestration-dilution effect seen here in pigs (Figure 1) has been observed also in dogs (13,19) and cats (20). Unless appropriate corrections are made, it will lead to an apparent increase in calculated volume of circulating erythrocytes as a function of time after injection. One-time measurement of label dilution, therefore, usually produces an erroneous over-estimation of

circulating erythrocyte volume, the magnitude being dependent upon the time after injection at which dilution is determined. Extrapolation of dilution values to zero time will minimize or eliminate such errors, but only if the extrapolation procedure is based on a two-compartment model which segregates total body erythrocyte volume into circulating and splenic components. Extrapolation is rarely, if ever, attempted, possibly because of the difficulties encountered in obtaining an adequate series of dilution samples shortly after labelled erythrocyte injection. Inadequate or no correction for splenic erythrocyte sequestration may have compromised measurement accuracy in many studies (1,4,5,21-27) in which circulating erythrocyte volume of swine was determined by the dilution of labelled cells. Accordingly, reported values for pigs of comparable size invariably exceed the circulating values, but approximate the total body values seen here in conscious near-basal pigs. In some instances (5,21,22) splenic discharge of sequestered erythrocytes, induced by the use of physical restraint during the course of measurement, could have elevated the values.

Indicator loss, in this instance to the interstitial fluid, can also present problems when circulating plasma volume is estimated by the dilution of serum albumin labelled with radioisotopes or T-1824 dye. Some (2,8-11,24,25,28,29) but not all (1,6,21-23,26,27,30-32) of the investigators who have used these labels in swine have based their volume estimates on the extrapolation of dilution values to zero time, as was done in the present study with ^{125}I -labelled bovine serum albumin. With albumin labels, one-time dilution samples do not appear to alter seriously the magnitude of plasma volume estimates, provided they are taken within 10 or 15 minutes after injection. The radioisotope concentration in such samples (Figure 3) approximates the extrapolated zero-time values. Either procedure, therefore, yields about the same plasma volume value, and in pigs of comparable size reported values (24,25,28) approximate those observed here.

The present study does raise serious questions about the validity of plasma volume measurements based on the dilution of labelled albumin. Plasma volumes so obtained were markedly greater than those based on labelled erythrocyte dilution and large vessel hematocrit. The plasma volume estimates, furthermore, produced elevated values for circulating erythrocyte and blood volume when the latter are calculated on the basis of large vessel hematocrits. In this respect, similar values have been obtained by others in pigs of equivalent size (23-25,28). These elevated plasma volume values also produced elevated blood volume values when the erythrocyte component of blood volume was determined simultaneously by the dilution of cells labelled with ^{51}Cr . The elevated plasma volume values, however, led to a calculated total body hematocrit which was considerably lower than arterial hematocrit. In the present experiment with splenectomized pigs, the ratio of the two averaged 0.756--a value slightly greater than those reported in 6-week-old pigs by Talbot and Swenson (24) and

lower than the values for newborn pigs by Deavers et al (26) and Linderkamp et al (27) and for newborn to 12-week-old pigs by Setiabudi et al (25). Autotransfusion of splenic erythrocytes, and a resultant increase in the erythrocyte volume component, may have been responsible for the higher ratio values reported by some of these workers (25).

Total body to large vessel hematocrit ratios below 1.0 are commonly attributed to relative plasma excess in small vessels, including arterioles, capillaries, and venules (12,14,32). Indeed, direct estimation of small vessel hematocrits has yielded values ranging down to 0.1 or lower (33). In humans and dogs such reduced values in small vessels potentially could account for total body to large vessel hematocrit ratios of about 0.9, a commonly calculated value (12,32). In pigs, however, the ratio observed here does not seem compatible with this explanation. Thus, the contributions of small and large vessel hematocrits to total body hematocrit can be expressed by the following equation:

$$H_B = H_S + H_L(1 - V_S) \quad [5]$$

where H_B , H_S , and H_L are the total body, average small vessel, and large vessel hematocrits, respectively, and V_S is the small vessel fraction of total blood volume. When H_L and H_B are measured, as in the present experiment with splenectomized pigs, a curve relating to various values of H_S can be constructed. Figure 4 shows the results of such an effort in which the H_L and H_B values recorded here in pigs are compared to commonly reported H_L and H_B values for humans (32). In pigs, but not in humans, these curves show that one would have to assume unreasonably large values for V_S to account for observed H_L and H_B values. For example, if the average H_S is assumed to be 0.1 (Fig. 4), then V_S would be 0.125, a reasonable value, in humans but 0.36, an unreasonable value, in pigs. In fact, if the average porcine small vessel hematocrit is assumed to be zero, small vessels would account for 24 percent of the circulating blood volume. In view of these calculations, it seems reasonable to conclude that porcine plasma volume was overestimated by the ^{125}I -dilution procedure used in this study. Alternatively, circulating erythrocyte volume may have been underestimated by the dilution of ^{51}Cr -labelled erythrocytes. This alternative seems highly unlikely since it would imply labelled-erythrocyte sequestration somewhere in the splenectomized pig. Decreasing label concentration following injection, which was not observed in this experiment, should have been seen if the labelled erythrocytes were so sequestered.

What could account for the overestimation of porcine plasma volume with the ^{125}I dilution procedure? Errors in isotope counting or sample volume measurement do not seem likely.

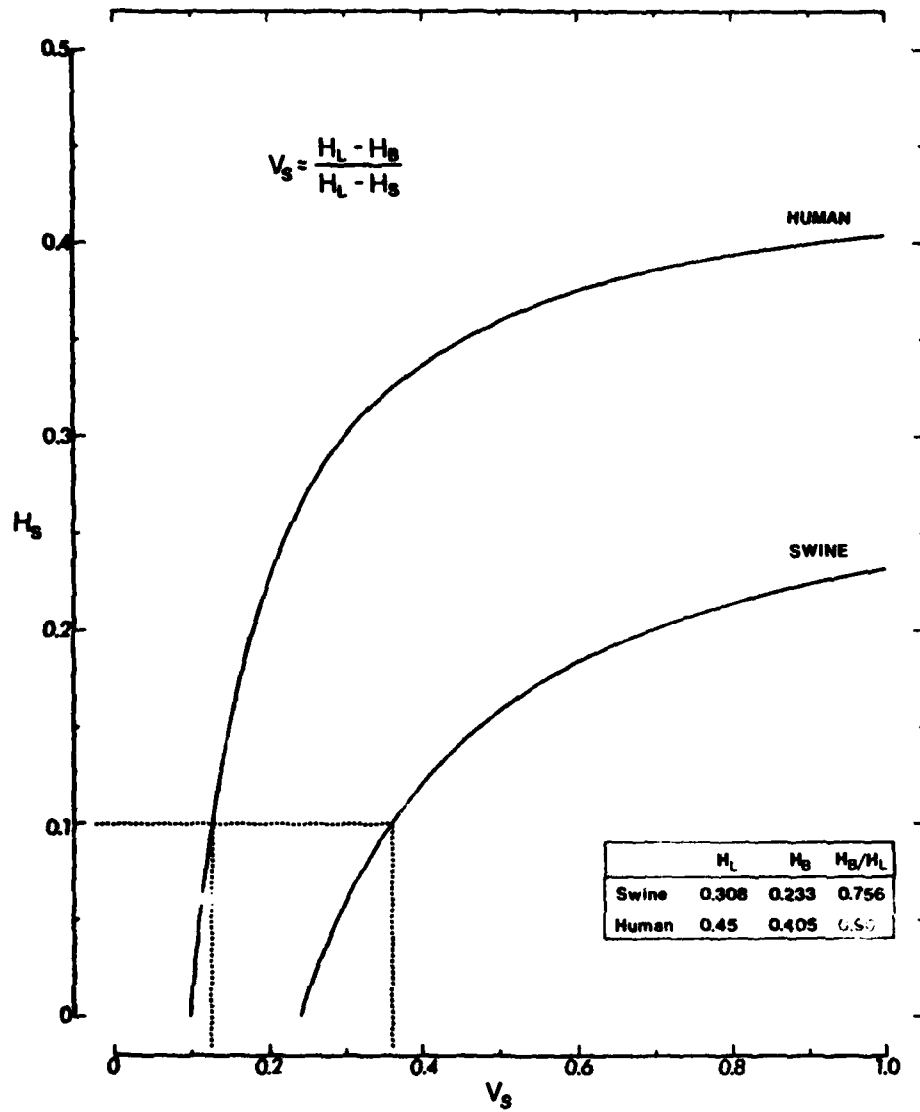


Figure 4. Interrelationship of average small vessel hematocrit (H_s) to average small vessel fraction (V_s) of total blood volume as estimated by rearrangement of the equation [5].

Accuracy evaluations of both procedures showed potential errors should not exceed 1 percent. Furthermore, the same sampling pipettes and the same type of calibrated injection syringe were used in the ^{51}Cr and ^{125}I procedures. Potential errors, therefore, would have had an equal effect on both procedures. Contamination of radioiodine-labelled albumin with inorganic ^{125}I , which would rapidly leave the circulation following injection, could account for a small fraction of the overestimation of plasma volume. Determinations of the inorganic iodine fraction following precipitation of the albumin fraction with cold trichloroacetic acid or following separation with a molecular sieve showed that 95 percent or more of the ^{125}I was bound to albumin. Transcapillary loss of the inorganic fraction may have contributed to the early, more rapid decrease in plasma ^{125}I concentration seen here, but other investigators (34,35) have attributed this effect to delayed mixing of the albumin-labelled fraction. The most likely explanation for the overestimation of plasma volume would be a rapid early loss of radioiodinated albumin during the first minute or two after injection. Direct evidence for such a loss in dogs has been presented by Vidt and Saperstein (36), who concluded that circulating plasma includes a fraction which extends beyond the anatomical boundaries of capillary beds. The implications of, and support for, this concept in terms of microcirculatory hydrodynamics has been elegantly discussed by Howe and Sheaffer (37).

CONCLUSIONS

- Under near-basal conditions the porcine spleen sequesters 20 to 25 percent of total body erythrocytes.
- Splenically sequestered erythrocytes are rapidly discharged into the circulating blood following intravascular epinephrine injection or physical restraint of the animal. Both procedures lead to a marked increase in large vessel hematocrit.
- Kinetic analysis of ^{51}Cr -labelled erythrocyte dilution following intravascular injection provides an accurate procedure for estimating circulating, total body, and splenic erythrocyte volumes. Simultaneous hematocrit measurements allow estimation of circulating plasma and blood volumes.
- Plasma, erythrocyte, and blood volume estimates based on the dilution of ^{125}I -labelled bovine albumin markedly exceed those based on ^{51}Cr -labelled erythrocyte dilution.
- Low values for the total body to large vessel ^{125}I ratio in pigs indicates overestimation of plasma volume by the ^{125}I -labelled albumin procedure.

RECOMMENDATIONS

- Experiments should be conducted to determine the factor(s) responsible for overestimation of porcine plasma volume when procedures based on the dilution of plasma albumin are used.
- The relationships of total body mass and lean body mass to circulating erythrocyte, plasma and blood volumes of swine should be investigated.
- Alterations, if any, in splenic erythrocyte storage capacity as a function of body size should be determined.

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