BLOOD VOLUME STUDIES ON DOMESTICATED ANIMALS

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Ph.D. Thesis Summary

Blood Volume Studies on Domesticated Animals

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Methods for determination of blood volume in man and common laboratory animals have been the subject of many investigations over the past twenty years. The availability of isotopic markers for red blood cells and plasma proteins made such studies technically easier and more accurate. By comparison these methods and measurements have been applied relatively seldom to the domesticated animals.

The aim of the work described in this thesis was to apply to the domestic animals blood volume techniques which had proved to be successful in man. Early on in this work it became clear that because of species differences modifications of existing methods would have to be made, e.g. it was found that the uptake of ^{32}P by red cells showed very wide species differences. Perhaps the most important species anomaly was found in the measurement of "trapped plasma" in the haematocrit. This is a very important correction which has to be applied in all precise work, particularly where efforts are being made to measure true blood volume by simultaneous estimation of red cell volume and plasma. This species anomaly had not been formerly reported.

A critical approach to the technicalities of measuring trapped plasma was made, and a method developed which overcomes many of the difficulties involved in the 'direct' procedure. Using this method a substantial number of determinations of trapped plasma

were carried out on the bloods of normal horse, cow, sheep, pig and dog, under centrifugation conditions suitable for each species. While centrifuge conditions were being investigated an important temperature effect on trapped plasma was observed. A study was also made of the distribution of trapped plasma in the red cell column, thus allowing variations in haematocrit to be taken into account in correcting for trapped plasma.

In the estimation of trapped plasma in the foregoing experiments centrifugation was carried out in polythene tubes or Wintrobe haematocrit tubes at an RCF of 1500 *g* for varying periods of time. Chien, Dellenback, Usami and Gregersen (1965) compared trapped plasma in the haematocrit of various species when the blood was spun at 1500 *g* for 30 minutes in Wintrobe tubes and when spun at 15000 *g* for five minutes in micro-haematocrit capillary tubes. Though the latter method of centrifugation resulted in a much lower proportion of trapped plasma, a species difference was still quite obvious. Even after centrifugation at the higher impulse, the red cell columns of goat and sheep bloods were found to contain 9 per cent and 4 per cent trapped plasma respectively.

Marked species differences were found in the uptake of ^{32}P by red blood corpuscles. Red cells of the ruminants, cow and sheep, and the cells of the horse labelled relatively slowly with this isotope. Some species differences of this nature had been noticed previously by Hansard et al (1953) and Schumbye (1952a).

It was found that removal of plasma from the cells before addition of the isotope increased the amount and rate of uptake of ^{32}P and in the larger species this was of marked practical value. In studying the persistence of ^{32}P-labelled cells in the circulation the radioactivity of samples was related to a common haematocrit, but although this was done with a correct allowance for trapped plasma the disappearance curves showed significant fluctuations. The rate of disappearance however was less than that described by Hansard et al (1953).

When ^{51}Cr became available it was used for labelling red cells simultaneously with ^{32}P and the uptake and loss from the circulation of the two isotopes was compared. It was found that the red cells of all the species studied took up ^{51}Cr rapidly and effectively. The loss of ^{51}Cr from the circulation was less rapid than that of ^{32}P but it was found that in the farm animals the rate of loss of ^{51}Cr was distinctly greater than the loss rate described in man and the dog. Blood volumes based on labelled cells in general were in agreement with the results reported in the literature of the last fifteen years. Blood volumes based on ^{51}Cr-labelled cells were smaller than those based on ^{32}P-labelled cells in all the species examined. As the red cells of species which labelled poorly with ^{32}P labelled rapidly and effectively with ^{51}Cr, and as the loss of ^{51}Cr from the circulation was less, it was considered to be the label of choice for blood volume estimations based on labelled cells.

It was considered that the results obtained by the use of labelled cells were not quite satisfactory and that the use of plasma protein labelled with ^{131}I might give better results and was worthy of further investigation.

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BLOOD VOLUME STUDIES ON DOMESTICATED ANIMALS

CONTENTS

CONTENTS (Contd.)

CONTENTS - PART 1

DETERMINATION OF THE INTERCELLULAR PLASMA OF THE PACKED CELL COLUMN

CONTENTS - PART 1 (contd.)

CONTENTS - PART 2

BLOOD VOLUME MEASUREMENTS ON DOMESTICATED ANIMALS

CONTENTS — PART 2 (Contd.)

GENERAL INTRODUCTION

Estimates of blood volume in man and animals can be made use of in a number of ways. They give a quantitative indication of the requirements for blood or fluid replacement in some disease states and their accurate assessment is essential for the elucidation of the mechanisms involved in the regulation of blood volume (Gregersen and Rawson, 1959). Clinicians are enabled to take a broader view of blood chemistry by estimating blood constituents in terms of the total amount in the circulation and not simply as a concentration (Ravdin, Walker and Rhoads, 1953). Blood volume bears a close relationship to the lean body mass (Allan, Peng, Chen, Huang, Chang and Fang, 1956). The latter is a useful parameter for the definition of other functions, and in animals reared for food, knowledge of lean body mass is of marked economic importance.

Gregersen and Rawson (1959) stated that more accurate methods of estimation revealed errors in previously accepted values, and prior to assessment of changes in pathological conditions it was necessary to study further normal ranges. Mayerson (1965) noted that while there was fairly general agreement that available methods gave a reasonable measure of the blood volume the accumulation of data in animal species other than man and the dog was slow.

All modern methods of estimation are based on the introduction into the blood stream of a measured quantity of some

test substance, and the estimation of its dilution in a blood sample withdrawn after a time sufficient for mixing and insufficient for the substance to have changed character or escaped from the circulation to a significant degree. The test substance in the circulation is associated with either the plasma or red cell portion. Before radioactive isotopes became available for physiological use the most standard procedure involved the injection of the blue dye known as T1824 or Evans' blue (Dawson, Evans and Whipple, 1920; Gregersen, Gibson and Stead, 1935), which became firmly and selectively bound to plasma albumin (Rawson, 1943), and this method is still in common use. Radioactive iodine (^{131}I) is now used to label plasma albumin (Fine and Seligman, 1943), and to some extent the chromium isotope ^{51}Cr (Sterling and Gray, 1950), is used for the same purpose. The red blood corpuscles are labelled with the phosphorus isotope ^{32}P, (Hevesy and Zerahn, 1942) or ^{51}Cr (Gray and Sterling, 1950).

Some other test substances, not in common use, are carbon monoxide gas (Root, Roughton and Gregersen, 1946; phenylhydrazine (Nizet, 1948); brilliant vital red (Keith, Rowntree and Geraghty, 1915); ^{55}Fe (Hahn and Hevesy, 1940) and ^{59}Fe (Anderson, 1942).

After injection of the labelled cells or plasma protein a blood sample is usually taken from a superficial vein and the concentration of test substance in it determined by the analysis of its plasma when labelled albumin has been injected, or analysis

of whole blood when labelled cells have been used. Since whole blood volume may be calculated from measurements made with either labelled plasma or labelled cells and since the values obtained differ to some extent, the following conventions have been adopted.

BV = The total circulating blood volume.

This can only be estimated when plasma volume and cell volume are measured simultaneously, and equals PV + CV.

PV = The circulating plasma volume based on measurements using labelled plasma.

CV = The circulating red cell volume based on measurements with labelled cells.

Bv_p = The blood volume based on measurement with labelled plasma.

Bv_c = The blood volume based on measurement with labelled cells.

Pv = Plasma volume calculated in experiments when measurement is made with labelled cells.

Cv = The red cell volume calculated in experiments when measurement is made with labelled plasma.

Consider the case where labelled plasma protein has been injected in an amount corresponding to 50,000 counts of radioactivity per second. Suppose that after mixing the concentration in the plasma is 200 counts per second per ml, the plasma volume (PV) by the dilution principle is $\frac{50,000}{200}$ = 250 ml and the blood

volume estimated by labelled plasma (Bv_p) can be determined only if the proportion of plasma to red cells in the blood is known. This proportion is usually determined by centrifuging a sample of venous blood under standard conditions in a haematocrit tube to separate cells and plasma and recording the volume of cells as a percentage of the whole sample. This percentage is referred to as the haematocrit (Ht) of the sample. Blood volume may then be calculated according to the formula:

$$Bv_p = PV \times \frac{100}{(100-Ht)}$$

Let it be assumed that the observed Ht is 40

then $Bv_p = 250 \times \frac{100}{100-40} = 416.7$ ml

As PV = 250 ml by subtraction the figure for indirectly obtained red cell volume (Cv) is found to be 166.7 ml.

The above calculation of blood volume and red cell volume is based on the assumption that the haematocrit consists of undiluted red cells, but Reeve (1948) pointed out that the observed haematocrit reading for the blood of man is too large, as some plasma is trapped among the cells of the packed red cell column.

Let it be assumed that the haematocrit of the sample after correction for 'trapped plasma' is 38.0 then the calculation of blood volume derived from plasma is as follows:-

$$Bv_p = PV \times \frac{100}{(100-\text{Corrected Ht})}$$

$$= 250 \times \frac{100}{(100-38)}$$

$$= 403.2 \text{ ml}$$

As PV = 250 ml then Cv = 153.2 ml.

It can be seen that by failing to allow for 5% of the volume of the observed haematocrit being due to trapped plasma, the blood volume is calculated to be 416.7 ml instead of 403.2 ml, an overestimate of 3.3%, and the cell volume is calculated to be 166.7 ml instead of 153.2 ml, an overestimate of 8.8%.

When labelled cells have been injected, the blood volume based on labelled cells (Bv_c) may be estimated on analysis of a sample of withdrawn whole blood according to the formula:

$$Bv_c = \frac{\text{Total radioactivity injected}}{\text{Radioactivity/ml of whole blood withdrawn}} \text{ ml}$$

Assuming that 50,000 counts per second was the total injected and 100 counts per second per ml was the amount found in the sample, then Bv_c = 500 ml. From the estimated blood volume and the haematocrit reading, direct estimation of cell volume (CV) and indirect estimation of plasma volume (Pv) can be made. Assuming an observed haematocrit of 40

$$CV = Bv_c \times \frac{Ht}{100}$$

$$= 500 \times \frac{40}{100}$$

$$= 200 \text{ ml}$$

$$Pv = Bv_c \times \frac{(100-Ht)}{100}$$

$$= 500 \times \frac{60}{100}$$

$$= 300 \text{ ml}$$

If allowance is made for 5% of the observed haematocrit being due to trapped plasma, the estimated cell volume and plasma volume becomes:-

$$CV = 500 \times \frac{38}{100}$$

$$= 190 \text{ ml}$$

$$Pv = 500 \times \frac{62}{100}$$

$$= 310 \text{ ml}$$

If no allowance is made for trapped plasma it can be seen that the red cell volume is overestimated by 10.0 ml or 5.3% and the plasma volume is underestimated by 10.0 ml which is 3.2% less than the value obtained by use of the corrected haematocrit.

The blood volume based on labelled cells may be calculated by first assessing red cell volume then using the haematocrit to derive plasma volume and blood volume. The direct red cell volume is calculated from the formula:-

$$CV = \frac{\text{Total radioactivity injected}}{\text{Radioactivity/ml of red cells withdrawn}} \text{ ml}$$

$$= \frac{\text{Total radioactivity injected}}{\text{Radioactivity/ml whole blood} \times \frac{100}{Ht}} \text{ ml}$$

The blood volume based on labelled cells is then calculated from the formula:-

$$Bv_c = CV \times \frac{100}{Ht}$$

The cell, plasma and blood volumes derived in this way are exactly the same as those derived by the method used in the last example, and the errors due to lack of correction for trapped plasma are identical.

It is technically very difficult to obtain a measurable volume of red cells quite free of fluid, so in practice it is necessary to measure the cells in the presence of that fluid and make an allowance for it. The results of failure to do this have been shown. However it is possible to err in the opposite direction by using a factor which over-corrects for trapped plasma. This has its greatest effect on blood volume and red cell volume estimations based on labelled plasma. In the recent example of calculation of blood volume based on labelled plasma, assuming the allowance for trapped plasma of 5% of the red cell column was correct, the following results were obtained - Bv_p = 403.2 ml, PV = 250 ml and Cv = 153.2 ml. If the calculations are now made using an allowance for trapped plasma of 10% of the observed haematocrit the following results are obtained:-

$$Bv_p = PV \times \frac{100}{(100-\text{Corrected Ht})}$$

$$= 250 \times \frac{100}{(100-36)}$$

$$= 390.6 \text{ ml}$$

$$PV = 250 \text{ ml} \quad \therefore \quad Cv = 140.6 \text{ ml}$$

It can be seen that the calculated blood volume is too low, due to a false reduction in the calculated cell volume of approximately 6%.

In estimations of blood volume based on labelled cells, if an over-correction is made for 'trapped plasma' in the haematocrit, the cell volume becomes falsely small, plasma volume becomes falsely large, and the total blood volume remains the same.

It is obvious therefore, that while plasma volume may be measured directly by means of labelled plasma, blood volumes and cell volumes can only be estimated by methods which involve haematocrit determinations, and if the calculations are to be reasonably accurate, the correct allowance must be made for 'trapped plasma' in the red cell column of the haematocrit tube.

Even when an accurate allowance has been made for trapped plasma, in general published results on blood volume estimation in man and laboratory animals have indicated higher volumes when these have been based on plasma volume estimations than when they have been derived from red cell volume estimations, i.e. $Bv_p > BV > Bv_c$. The cause of this, which may be of great importance, is not yet fully understood, but it appears that the difference

between the haematocrit of blood in the peripheral vessels and the haematocrit of the blood in the body as a whole may be an important factor (Gregersen and Rawson, 1959). It is often tacitly assumed that the peripheral haematocrit has cells and plasma in the same proportion as in the remainder of the blood of the body, though the work of Fahraeus (1929) and Fahraeus and Lindqvist (1931) indicates that there is a lesser proportion of cells in relation to plasma in the small capillaries than in larger vessels and it follows that the ratio of cells to plasma of the whole body, the 'body haematocrit' must also be less than that in the larger vessels.

When a test substance is injected into the circulation, it does not diffuse homogeneously, but is associated with either the plasma or the corpuscles and its dilution really measures either plasma volume or red cell volume. Gregersen and Rawson (1959) point out that some investigators appear to think that they are measuring total blood volume directly by analysing their test substance in a sample of whole blood. True blood volume (BV) can only be measured directly if cells and plasma are labelled separately and the volume of each determined simultaneously. Such simultaneous estimations of total red cell mass and total plasma in man appear to show that the body red cell mass is only about 90% of the proportion of red cells shown by the peripheral haematocrit reading. It follows that in a calculation of blood volume from a knowledge of the true total red cell volume (CV)

and the peripheral haematocrit, blood volume will be underestimated because there is a relatively higher proportion of plasma in the body than in the peripheral haematocrit. Similarly in a calculation based on determined true total plasma volume (PV) and peripheral haematocrit, total blood volume will be overestimated because the haematocrit indicates a higher proportion of red cells to plasma than is present in the total circulation.

From simultaneous estimations of plasma volume and red cell volume the relationship body haematocrit/venous haematocrit can be determined. It has been found that this factor, termed 'F cells', does not vary with changes in the venous haematocrit level. When it is applied to the venous haematocrit it computes the body haematocrit. If this factor is found to be constant in a species it becomes possible to obtain a fairly accurate estimation of blood volume in that species by measuring either red cell volume or plasma volume and calculating blood volume from the peripheral haematocrit adjusted by 'F cells'. 'F cells' is rather constant in normal man and the average value is 0.91 (Reeve, 1952). In infants it has been found to be 0.87 (Mollison, Veall and Cutbush, 1950). In normal dogs 'F cells' may vary from 0.87 to 1.1 depending on the proportion of blood in the spleen and a constant 'F cells' cannot be assumed in calculating blood volume in this species unless the spleen has been removed. In the splenectomised dog the figure of 0.9 has been found (Reeve, Gregersen, Allen and

Sear, 1953); Rawson, Chien, Peng and Dellenback, 1959). Hodgetts (1961) found 'F cells' to be very variable in the sheep unless the animals were splenectomised or given adrenaline to empty the spleen at the start of the estimation. When this was done the figure of 0.81 was obtained.

It is obvious then that an accurate venous haematocrit reading is an essential and important factor in all estimations of blood volume and an accurate haematocrit reading can only be made if the percentage of the red cell column which is due to 'trapped plasma' is known. The amount of plasma trapped in the red cell column of the centrifuged blood of man has been well investigated and there is general agreement that after centrifugation for 30 minutes at 1500 g the observed haematocrit can be corrected for trapped plasma by multiplying by a factor of 0.96 (Gregersen, 1951), if the observed haematocrit is not abnormally low or high. When the work described in this thesis began, there was a tendency to assume that the amount of 'trapped plasma' in the centrifuged blood of animals was similar to that in man. Hansard, Butler, Comar and Hobbs (1953), in calculating sheep plasma volume from blood volume based on labelled cells, allowed for 5% trapped plasma. They had centrifuged the blood at only 906 g for 30 minutes and according to the work described later in this thesis the amount of trapped plasma was probably about 20% of the packed cell volume. Assuming an estimated sheep blood volume of 3000 ml,

an observed haematocrit of 40.0 and an allowance for trapped plasma of 5%, plasma volume is calculated as follows:-

$$Pv = Bv_o \times \frac{(100-\text{Corrected Ht})}{100} \text{ ml}$$

$$= 3000 \times \frac{(100-38)}{100}$$

$$= 30 \times 62$$

$$= 1860 \text{ ml}$$

If a trapped plasma of 20% of the red cell column is allowed for, the calculated plasma volume is

$$3000 \times \frac{(100-32)}{100} \text{ ml}$$

$$= 30 \times 68$$

$$= 2040 \text{ ml}$$

Plasma volume has therefore been wrongly calculated as 1860 ml instead of 2040 ml, equivalent to an underestimation of 9%. If the same faulty allowance for trapped plasma is applied to calculation of cell volume this is overestimated by a factor of 18.75%.

When the work described in this thesis was started there was a large amount of information on the use of isotopic methods in blood volume studies on man and the common laboratory animals, and comparatively little on the application of these techniques to farm animals generally. The aim of the present investigation was to study the use of isotopic red-cell labelling methods for

blood volume measurement on a range of domesticated animals. No attempt to establish so called 'normal values' was intended but simply an assessment of the advantages and limitations of these methods when applied to the different species.

Early on in the work it became clear that there was a marked species difference in the amount of plasma trapped in the red cell column during the haematocrit determination by conventional methods of centrifugation. Because of the importance of the haematocrit measurement in blood volume studies, a large part of the work described here was performed in relation to this heretofore unrecognised species difference, and is detailed in Part I. Studies on the estimation of blood volume in different species are described in Part 2.

PART 1

DETERMINATION OF THE INTERCELLULAR PLASMA OF THE PACKED RED CELL COLUMN

INTRODUCTION

Hirota (1926) centrifuged blood samples from various species of animals until the packed red cell column would reduce in size no further. Other conditions of centrifugation being equal he reported that the number of revolutions per minute required for the different species was:- horse, 2000; rabbit, 2200; man, 2600; goat, 2900 and ox, 3000. Miller (1925) showed that the packing of red cells was closely related to the relative centrifugal force applied during centrifugation. Hooper, Smith, Belt and Whipple (1920) in common with many earlier workers, assumed that plasma trapped in the cell interstices of packed haematocrits was negligible and pointed out that hypertonic anticoagulant could reduce the size of red cells, so that the volume of red cells might appear less than it should. Leeson and Reeve (1951) found that the haematocrit was consistent if anticoagulant, force and time of centrifugation and the form of centrifuge tube were standard. They found that haematocrit estimations in 3.0 and 9.0 mm bore haematocrit tubes averaged 1.0% more red cells than estimations in 1.0 mm bore tubes after centrifugation of identical samples under standard conditions. They calculated that assuming that the red blood corpuscles were rigid flat discs, the most efficient packing would leave about 9 per cent space for plasma. As a smaller proportion of plasma was found by experiment, the conclusion was that cells must be deformed by the centrifugal force

to fill the interstices in part at least. They also showed that a smaller proportion of plasma is trapped in the lower sections of the red cell column.

Owen and Power (1953) remark that in the literature the method of recording trapped plasma has not been uniform. The more accurate method is to record the amount as a percentage of the packed red cell column, but some authors have recorded it as a percentage of the total blood in the centrifuge tube, and others have not made it clear to which standard they were referring. Hlad and Holmes (1953) defined the physical variations of centrifugation affecting the proportion of trapped plasma but they did not specify the temperature of their centrifuge although it was stated to be refrigerated. They used a basic mathematical theoretical approach to their experiments and concluded that the amount of plasma trapped was directly related to 'impulse', which depends on force and time of centrifugation. As force = $0.010966 \times R^2 \times r \times m$ (dynes) when R = revolutions per minute, r = radius and m = mass, it is obvious that the number of revolutions per minute of the centrifuge is the major factor affecting force.

The height of the observable haematocrit and the amount of plasma trapped within it are related (Chaplin and Mollison, 1952). Hodgetts (1959) studied the effect of varying forces and times of centrifugation on the haematocrit and trapped plasma of the blood of sheep. She found that the same impulse (i.e. force x time of

centrifugation) did not always produce the same effect on the haematocrit, and that a small force for a long time was less effective in reducing the haematocrit than the same impulse produced by a greater force acting for a shorter time. She pointed out that it was difficult to compare her work with others because so often the conditions of centrifugation were not fully specified. Time and revolutions per minute might be given, but the radius of the centrifuge omitted, so that the relative centrifugal force (RCF) could not be calculated. The RCF is expressed in terms of gravity (g) and is derived from the formula

$$RCF = 1.119 \times R^2 \times r \times 10^{-5}$$

when R = revolutions per minute and r = radius of centrifuge, measured to the bottom of the haematocrit tube in cm.

Although it is now generally agreed that for human blood under standard conditions of centrifugation (1500 g for 30 minutes) about 4 to 5 per cent of the packed cell column consists of plasma, widely different estimates have been proposed by different workers. The differences may be largely due to differences in the methods employed. Most determinations of intercellular plasma depend on the addition to the blood sample of some readily measurable test substance, such as Evan's blue or ^{131}I-labelled albumin, mixing, and centrifugation of a portion under standard conditions. In

the so-called 'direct' procedure, after centrifugation both the supernatant plasma and the packed cells are analysed directly for the test substance, i.e. the packed cells are analysed directly for plasma. In the 'indirect' procedure the true volume of plasma in the sample is estimated by the dilution principle. The proportion of red cells is calculated either by comparing the assay of the test substance in whole blood with its concentration in the plasma, or by subtracting the plasma volume from the volume of the original blood sample.

As an example of the latter method let it be assumed that the volume of the blood sample is 80.0 ml and that the test substance is ^{131}I-labelled plasma which has a total activity of 10,000 counts/minute and is of negligible volume. After addition and mixing, a sample is centrifuged and the plasma assayed. Assuming an activity of 200 counts/minute/ml is obtained, the volume of plasma in the blood must be 10,000 divided by 200 = 50.0 ml. The red cell volume must therefore be 30.0 ml and the percentage of red cells 37.5. Supposing the reading in the haematocrit tube is 39.5, the difference between this and the red cell percentage should theoretically be due to trapped plasma, i.e. 2 in 39.5 or 5.1%.

The grave disadvantage of this indirect method is that it attempts to determine a relatively small volume by finding the difference between two relatively large volumes and a small

percentage error in determining either or both of the large volumes will have a great effect on the small volume. Assuming a 2% error in the dilution technique, the red cell percentage might have been found to be 36.75, and assuming a 1.0% error in reading the haematocrit, it might have been recorded as 39.9. The difference then becomes 3.15, indicating a trapped plasma of approximately 7.9%, a figure 55% greater than that found previously. Mathematical aspects of the errors involved in this method are dealt with by Vazquez, Newerly, Yalow and Berson (1952). Nevertheless some workers still hold to the indirect procedure on the grounds that there are also serious objections to the use of the direct method.

The two main criticisms levelled at the direct procedure are (a) the technical difficulty of obtaining for analysis packed cells which are uncontaminated by supernatant plasma, and (b) the fact that as the trapped plasma is not evenly distributed throughout the packed cell column, any method in which only part of the cell column is analysed is likely to give misleading results. Leeson and Reeve (1951) used a special haematocrit tube with a rubber diaphragm in its base through which a needle was inserted to withdraw packed cells. Hlad and Holmes (1953) used a plastic centrifuge tube through the base of which an 18 gauge needle was inserted and the packed cells allowed to drip out at the rate of one drop per thirty seconds. Owen and Power (1953) froze the

centrifuged haematocrit tube in a dry ice-ethanol bath then broke it into sections and recorded that the proportion of trapped plasma increased the further the section was from the bottom of the tube. Hodgetts (1959) obtained packed cells by pipetting off plasma from above them and washing the inside of the tubes above the packed cells by repeated careful rinsing with pipetted saline.

The difficulties in the above techniques might be avoided by a method which makes use of the fact that while plasma conducts electricity, red cells do not, and the amount of conductivity in the packed red cell column should therefore estimate the plasma content. Within the range of normal human haematocrits the percentage of red cells determined by such a method is very similar to that found by standard centrifugation methods (Kernen, Wurzel and Okada, 1961).

Little work on haematocrit determination or evaluation of the amount of trapped plasma has been recorded on the blood of farm animals. In the burro Hansard et al (1953) assumed a trapped plasma of 5% after centrifugation at 906 *g* for 30 minutes. In the cow Reynolds (1953a) estimated trapped plasma as 6% after centrifugation for 30 minutes at 2500 r.p.m. with a centrifuge head of 18 cm. radius, i.e. an RCF equal to 1260 *g*. She used a dye dilution method and both direct and indirect methods of estimation.

In the sheep Kennedy and Millikan (1938) noted that an

RCF of approximately 19000 g for 25 minutes was required to obtain maximum packing of red cells in the haematocrit and that packing was incomplete within 4 hours at 2760 g. Schambye (1952b) used T1824 and an indirect method of determination and obtained a result of 5% trapped plasma in this species. The conditions of centrifugation were not fully specified. Hodgetts (1959) used homologous ^{131}I-labelled albumin and a direct method of estimation. She estimated trapped plasma after varying forces and times of centrifugation and also artificially altered the proportion of red cells in the blood samples to produce haematocrits beyond the normal range. The amount of centrifugation which she ultimately recommended was 2756 g for 60 minutes, which was associated with an estimated trapped plasma of 3.2 per cent.

In the goat, Klement, Ayer and Rogers (1955) used a correction factor for trapped plasma which indicated they believed the amount to be nearly 20%. Anderson and Rogers (1957) used ^{131}I-labelled albumin to determine the proportion of trapped plasma in the blood of eight goats. Centrifugation was carried out at 1800-2000 g for 30 minutes in 3mm Wintrobe tubes. The direct method of estimation resulted in a figure of 19.8% and by the indirect method a figure of 17.8% was arrived at. Haematocrit determinations in capillary tubes spun at 13000 to 14000 g for 8 minutes were 85-87% of those of duplicate samples spun as for the trapped plasma experiment.

Chien, Dellenback, Usami and Gregersen (1965) used ^{131}I and an indirect method of calculation to estimate the trapped plasma in the red cell column of the blood of man, dog, elephant, sheep and goat after centrifugation in Wintrobe tubes at 1500 g for 30 minutes and after centrifugation in microhaematocrit capillary tubes at 15000 g for 5 minutes. Their results are included in those shown in Table 1 and Table 2.

Figures from the literature on trapped plasma in human blood are shown in Table 1 and figures from the literature on the bloods of some animal species in Table 2. Many of the figures shown in Table 2 are not the results of determinations of trapped plasma by the authors, but are figures compiled by Owen and Power (1953) from the information available in the literature. They assumed that the difference between the observed haematocrit and the calculated haematocrit was due to trapped plasma and accepted, not without reserve, some dubious criteria which authors had used for the calculation of haematocrit values.

The work described in this section of the thesis represents the first systematic attempt to measure intercellular plasma in the packed red cell column of bloods of different species of animals under standard conditions using reliable analytical methods. A method is described for obtaining packed red cells uncontaminated by supernatant plasma, so that it is possible to use the direct method of estimation of trapped plasma, with its

TABLE 1

ESTIMATIONS OF TRAPPED PLASMA IN THE RED CELLS OF THE HAEMATOCRIT OF MAN

FIGURES FROM THE LITERATURE

Author	No. Determinations	Conditions of Centrifugation	Plasma % of Red Cell Column	Method	Remarks
Chapin & Ross (1942)	31	1800 g 1 hour	8.5	T-1824	Direct
Leeson & Reeve (1951)	9	1500 g 30 mins.	5.1	^{131}I-albumin	Direct
	3		3.6	^{131}I-albumin	Direct and sedimentation rate increased by fibrinogen
Jackson & Mutt (1951)	19	1000 g	10.5	T-1824	Indirect
		1500 g	4.0	T-1824	Indirect
		2300 g	2.5	T-1824	Indirect
		3100 g	2.3	T-1824	Indirect
		4200 g	2.1	T-1824	Indirect
		5600 g	2.0	T-1824	Indirect
Vazquez, Newerly	20	2000 g 30 mins.	3.1	^{131}I-albumin	Direct
Yalow & Berson (1952)	5		2.1	^{131}I-albumin	Direct (dialysed)
	8		3.9	^{24}Na	
	15		3.5	^{131}I-albumin	Indirect
Owen & Power (1953)	39	2200 g 60 mins.	3.8	^{131}I-albumin	Indirect Range -2.9 to + 7.9
Chien, Dellenback	6	1500 g 30 mins.	4.0	^{131}I-albumin	Indirect
Usami & Gregersen (1965)	6	15000 g 5 mins.	1.0	^{131}I-albumin	Indirect

TABLE 2

ESTIMATIONS OF TRAPPED PLASMA IN THE RED CELLS OF THE HAEMATOCRIT IN SOME ANIMAL SPECIES

FIGURES FROM THE LITERATURE

Author		Species	No.	Conditions of Centrifugation	Plasma % of Ht	Method	Remarks
Hirota	1926	Horse	25	Unknown 30 mins.	1.5	Centrifugation and time	* Method suspect
Hirota	1926	Ox	3	Unknown 30 mins.	5.9	"	* Method suspect
Lindhard	1926	Ox	11	3000 rpm 75 mins.	51.3	Vital red	* Method suspect
McLain & Ruhe	1949	Ox	13	1500 g	7.0±4.7	T-1824	Indirect. Defibrinated blood
McLain & Ruhe	1949	Ox	10	1500 g	3.4±1.0	T-1824	Direct. Defibrinated blood
Reynolds	1953a	Ox	6	1800 g 30 mins.	6.0	Dye	Direct and Indirect
Ponder & Saslow	1930	Sheep	3		3.9	Haemoglobin	* Method suspect
Kennedy & Millikan	1938	Sheep		1500 g 30 mins.	10.0-12.0	Centrifugation to limit	* Method suspect
Schambye	1952b	Sheep		6000 rpm 20 mins.	5.0	T-1824	Indirect
Hodgetts	1959	Sheep	14	1712 g 30 mins.	14.3	^{131}I-plasma	Direct
Hodgetts	1959	Sheep	14	1712 g 60 mins.	8.3	^{131}I-plasma	Direct
Hodgetts	1959	Sheep	14	4756 g 60 mins.	3.2	^{131}I-plasma	Direct

* Figure for trapped plasma calculated by Owen & Power (1953) from the evidence available, which was not necessarily accurate.

TABLE 2 - Continued

Author	Species	No.	Conditions of Centrifugation	Plasma % of Ht	Method	Remarks
Chien et al 1965	Sheep	5	1500 g 30 mins.	9.0	^{131}I-plasma	Indirect
Chien et al 1965	Sheep	5	15000 g 5 mins.	4.0	^{131}I-plasma	Indirect
Hirota 1926	Goat	5	Unknown 30 mins.	6.9	Centrifugation and time	* Method suspect
Anderson & Rogers 1957	Goat	8	2000 g 30 mins.	19.8	^{131}I-plasma	Direct
Anderson & Rogers 1957	Goat	8	2000 g 30 mins.	17.8	^{131}I-plasma	Indirect
Chien et al 1965	Goat	9	1500 g 30 mins.	18.0	^{131}I-plasma	Indirect
Chien et al 1965	Goat	9	15000 g 5 mins.	9.0	^{131}I-plasma	Indirect
Lindhard 1926	Dog	13	3000 rpm 75 mins.	31.6	Vital red	* Method suspect
Gregersen & Schiro 1938	Dog	5	1500 g 30 mins.	4.2	Vital red	Indirect
Gregersen & Schiro 1938	Dog	8	1500 g 30 mins.	4.2	T-1824	Indirect. 2 human bloods included
Gregersen, Boyden & Allison 1950	Dog	4	1500 g 30 mins.	3.8	T-1824	Indirect
Gregersen, Boyden & Allison 1950	Dog	4	1500 g 30 mins.	3.5	Antigens	Indirect
Chien et al 1965	Dog	5	1500 g 30 mins.	5.0	^{131}I-plasma	Indirect
Chien et al 1965	Dog	5	15000 g 5 mins.	3.0	^{131}I-plasma	Indirect
Leeson & Reeve 1951	Rabbit	10	1500 g 30 mins.	5.5±0.46	^{131}I-plasma	Direct
Chien et al 1965	Elephant	4	1500 g 30 mins.	6.0	^{131}I-plasma	Indirect
Chien et al 1965	Elephant	4	15000 g 5 mins.	4.0	^{131}I-plasma	Indirect

* Figure for trapped plasma calculated by Owen & Power (1953) from the evidence available, which was not necessarily accurate.

obvious advantages, and avoid the major criticisms which have been levelled against this procedure.

MATERIALS AND METHODS

In the experiments which follow the direct procedure was used throughout. The test substance was in each case homologous serum albumin trace labelled with ^{131}I. A small volume of labelled albumin was added to the heparinized blood sample. After thorough mixing the sample was centrifuged and radioactivity determinations were carried out on the packed cells and on the supernatant plasma. The difficulty of obtaining packed cells for analysis uncontaminated by supernatant plasma was overcome by carrying out centrifugation in polythene tubes (Randolph and Ryan, 1950), which could be clamped, or frozen and cut, to separate the portion containing red cells. These methods are more fully described below.

The Preparation of ^{131}I-Labelled Albumin

^{131}I was obtained from the Radiochemical Centre, Amersham, Buckinghamshire in the form of sodium iodide. Whole blood samples were obtained from the various species with aseptic precautions and the serum removed after clotting had occurred. About 30.0 ml of serum was usually dealt with at one time. Anhydrous sodium sulphate was added to the serum to a final concentration of 18%, and this precipitated most of the globulins. The mixture was then centrifuged to separate the precipitate, and the supernatant

fluid was transferred to a dialysis sac and dialysed against repeated large volumes of 0.85% NaCl until the dialysate appeared to be free of sulphate when tested with Ba Cl_2. This 'albumin' preparation was then trace labelled with ^{131}I by the method described by Francis, Mulligan and Wormall (1951). The albumin solution was made alkaline by the addition of 0.1 ml of 5N NH_4OH to every 1.0 ml of protein solution. The iodine solution was prepared by saturating a 0.1 N solution of potassium iodide with finely powdered elementary iodine. This solution was allowed to stand for 48 hours, with occasional shaking, after which the excess iodine was filtered off, leaving a solution containing approximately 12.7 mg of pure iodine per ml, i.e. a solution which was approximately 0.1 N. From it a volume containing the appropriate amount of pure iodine was measured and to this was added a solution of sodium ^{131}I. The amounts of I_2 in KI and sodium ^{131}I required to give a labelled protein of a certain I_2 content and specific radioactivity can be calculated since in such an iodination reaction about 1/3 of the free I_2 and 1/6 of the ^{131}I becomes attached to the protein. The protein solution was iodinated by adding the radioactive iodine solution drop by drop with constant stirring. The solution was then brought to pH 7.0-7.5 by the careful addition of 2N followed by 0.5N acetic acid and the use of universal indicator paper. The labelled

protein was then transferred to a dialysis sac and dialysed for at least 48 hours at 5°C against several changes of 0.85% NaCl to remove any unbound ^{131}I. The solution was then removed from the sac and centrifuged at 1500 g for one hour to remove any particulate material which might have been present. The radioactivity of the labelled protein in the supernatant fluid was shown to be more than 99% precipitable with trichlor-acetic acid.

The Preparation of Polythene Tubes

Polythene tubes suitable for the following experiments could not be obtained commercially and they were therefore prepared from polythene tubing. "Portex" polythene tubing (7-8 mm internal diameter) was cut into lengths of approximately 20 cm, and sealed at one end in the following manner. A piece of solid polythene was placed on a nickel planchette of the type used for radioactive samples. The planchette was placed on an electric hot plate until the polythene had completely melted. One end of the tube to be sealed was now immersed in the melted polythene and held there until it had completely fused with the polythene in the planchette. After cooling the planchette was removed with pliers and the tube trimmed with a sharp knife or scalpel. In order to give support during centrifugation these tubes were placed inside a pyrex test tube (15 cm x 15 mm).

Centrifugation

Blood samples in the prepared polythene tubes or Wintrobe haematocrit tubes were centrifuged at a relative centrifugal force (RCF) of 1500 g for varying periods of time. In the preliminary experiments an M.S.E. medium centrifuge without temperature control was used, but in later experiments an M.S.E. refrigerator centrifuge was used.

Determination of Radioactivity

Determination of Radioactivity

After centrifugation, measured amounts of supernatant plasma and packed cells were made up to a volume of 10.0 ml for counting in the M6 liquid Geiger-Müller tube (20th Century Electronics). The latter is constructed mainly of glass and is designed to measure the radioactivity in 10.0 ml of liquid held in the outer chamber of the tube and surrounding the gas-filled central core. The counter tube was shielded from extraneous radiation by being placed within a cylindrical lead 'castle' (Panax Ltd.). All counting was performed at a voltage suitable for the counter used. Usually each sample was counted for a time sufficient to record at least 2500 counts. Duplicate counts were made of each sample and if these varied by more than 5% additional counts were made. Before counting the first sample and between the determinations of successive samples a count on the empty M6 tube was undertaken to eliminate the possibility of the presence of residual activity from previous samples. If excess activity was found to be present

the tube was rinsed repeatedly with distilled water containing a little carrier iodide and dried by the insertion and manipulation of a piece of filter paper. This process was repeated if necessary until contamination was eliminated. The counting mechanism was switched on simultaneously with a stop watch and switched off at the full minute reading which first followed a total count sufficient for a statistical accuracy of 1.0-2.0 per cent. The radioactivity of each sample was recorded as counts per minute.

Corrections to observed count rates

(a) Background activity: A certain amount of radioactivity is always present in the environment and is responsible for a proportion of the impulses recorded even when the tube is enclosed in its 'castle'. At the beginning and end of each counting session a count was performed with the tube empty and clean, and the mean 'background' rate subtracted from all other readings.

(b) Correction for radioactivity decay: ^{131}I decays with a half-life of 8.1 days. In the following experiments it was always possible to relate the activity of plasma to the activity of packed cells of the same sample within such a short period of time that no correction needed to be made for decay.

EXPERIMENTAL

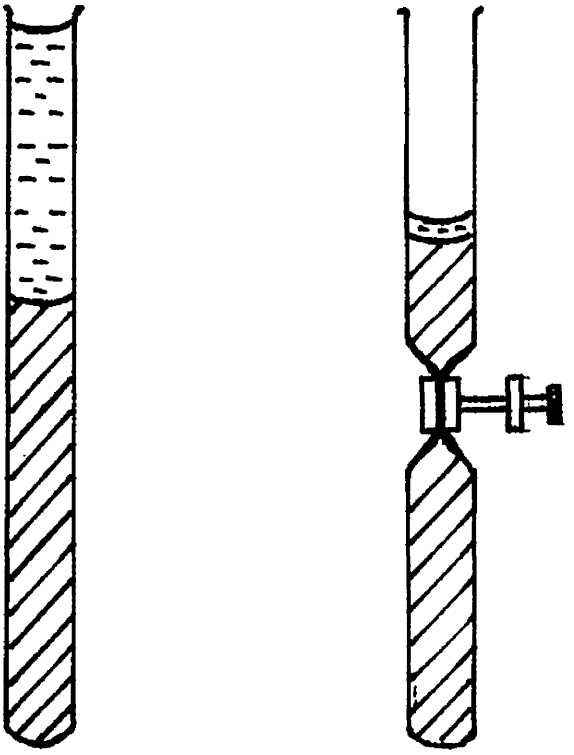
A. Preliminary Experiment Without Temperature Control

The determination of trapped plasma in rabbit, dog, horse and bovine bloods after centrifugation at 1500 g for 30 minutes, temperature unknown

About 10 ml of blood from each of the experimental animals was collected in a stoppered bottle containing heparin. About 0.2 ml of a solution of homologous serum albumin trace-labelled with ^{131}I was added to each sample which was then mixed thoroughly by repeated inversion for at least one minute. Each sample was divided and transferred to two polythene tubes and two Wintrobe haematocrit tubes by means of a teat pipette. All the tubes were then centrifuged in the M.S.E. medium centrifuge for 30 minutes at an RCF of 1500 g. After centrifugation most of the supernatant plasma was removed carefully with a teat pipette and the polythene tube clamped below the top of the red column as in Figure 1. The tube was then cut off just below the clamp and the packed cells transferred to a graduated centrifuge tube which was spun for a short time to facilitate reading of the volume of cells. The cells were then lysed by addition of 0.02N NaOH and made up to 10.0 ml for counting. Before the samples were shaken, a drop of capryl alcohol was added to prevent foaming. Duplicate

FIGURE 1

Separation of packed cells free from plasma



0.2 ml samples of the supernatant of each blood sample were made up to 10.0 ml with 0.02N NaOH. Radioactivity determinations were carried out in the M6 liquid counter. By comparing the count rates of the cell samples with those of the corresponding supernatant plasma it was possible to calculate the amount of plasma in each cell sample and hence the percentage of trapped plasma. The results are shown in Tables 3,4,5 and 6 and summarised in Table 7.

In the foregoing experiments on various species the conditions of centrifugation used were those generally accepted for estimations of human blood, and the results obtained in the rabbit, dog and horse are comparable to the figure of 4-5% now generally agreed for human blood under these conditions (Gregersen, 1951). The result for bovine blood is obviously in a different category, ranging from 6% to 24% with a mean of 12.5% in the 22 samples studied. Because of the very wide range in the results obtained for bovine samples it would obviously be unwise in experimental work to apply the mean correction of 12% indiscriminately to all samples and it seemed that the results on bovine bloods, which were surprising, warranted further investigation.

It seemed likely that closer packing of bovine cells could be obtained by increasing centrifugation either by using a greater RCF for the same time or the same RCF for a longer period of time and it was decided to try the latter method. As a preliminary

TABLE 3 - RABBIT

THE DETERMINATION OF TRAPPED PLASMA IN BLOOD AFTER CENTRIFUGATION AT 1500 *g* FOR 30 MINUTES, TEMPERATURE UNKNOWN

Rabbit No.	Vol. Cells ml	Corrected Counts/min. Cells	Corrected Counts/min/ 0.2 ml Plasma	Vol. Plasma in Cells ml	% Plasma in Cells
1	0.80	48.0	328.2	.029	3.7
2	1.15	99.4	453.3	.044	3.8
3	1.30	87.4	440.0	.040	3.1
4	1.35	103.4	481.6	.043	3.2
5	2.50	354.3	642.0	.110	4.4
6	1.00	77.7	458.1	.034	3.4
7	1.07	67.7	361.6	.037	3.5
8	1.45	131.6	471.4	.056	3.9
9	1.10	74.4	403.1	.037	3.4
10	1.17	122.3	537.1	.046	3.9
11	1.10	61.5	363.4	.034	3.1
12	1.17	94.5	441.3	.043	3.7
13	1.10	60.6	361.4	.034	3.0
14	1.40	97.2	436.7	.045	3.2
15	1.30	69.6	388.4	.036	2.8
16	1.05	63.4	438.8	.029	2.8
17	1.50	79.4	355.0	.045	3.0
18	1.25	73.3	465.0	.032	2.5
19	1.27	63.9	396.1	.032	2.5
20	1.58	138.2	527.5	.052	3.3
21	1.45	80.2	345.7	.046	3.2
22	1.45	97.7	431.3	.045	3.1

Mean 3.3 ± 0.47

Range 2.5 - 4.4

TABLE 4 - DOG

THE DETERMINATION OF TRAPPED PLASMA IN BLOOD AFTER CENTRIFUGATION AT 1500 g FOR 30 MINUTES, TEMPERATURE UNKNOWN

Dog No.	Vol. Cells ml	Corr. Counts/Min. Cells	Corr. Counts/Min. 0.2 ml Plasma	% Plasma in Cells
1	1.70	448.7	1644.1	3.21
2	1.40	280.6	1259.0	3.19
3	1.40	331.9	1408.4	3.07
4	1.50	436.6	1577.2	2.83
5	1.70	561.2	1834.2	3.60
6	1.35	291.6	1794.2	3.24
7	1.40	312.7	1630.2	2.91
8	1.40	364.5	1658.9	3.14
9	1.40	306.0	1433.5	3.22
10	1.27	236.8	1318.2	2.83

Mean 3.12 ± 0.26

Range 2.83 - 3.60

TABLE 5 - HORSE

THE DETERMINATION OF TRAPPED PLASMA IN BLOOD AFTER CENTRIFUGATION AT 1500 g FOR 30 MINUTES, TEMPERATURE UNKNOWN

Horse No.	Vol. Cells ml	Vol. Plasma ml	Plasma %
1	1.00	0.028	2.8
2	1.03	0.025	2.4
3	1.24	0.035	2.8
4	0.95	0.027	2.8
5	0.95	0.025	2.5
6	1.00	0.025	2.5
7	1.10	0.033	3.0
8	1.00	0.031	3.1
9	0.90	0.022	2.4
10	1.20	0.035	2.9
11	1.22	0.030	2.4
12	1.18	0.032	2.7
13	1.45	0.044	3.0
14	1.10	0.031	2.8
15	0.95	0.023	2.4
16	1.08	0.034	3.2
17	1.42	0.026	1.9
18	1.35	0.032	2.4
19	1.30	0.025	1.9
20	1.50	0.041	2.7
21	1.45	0.037	2.5
22	1.50	0.036	2.4
23	1.55	0.034	2.1
24	1.50	0.033	2.2
25	1.60	0.063	4.0
26	1.80	0.064	3.5
27	1.65	0.060	3.7
28	1.80	0.069	3.8
29	1.78	0.068	3.8
30	1.70	0.071	4.2
31	1.65	0.068	4.1
32	1.68	0.070	4.2

Mean 2.9 ± 0.67

Range 1.90 - 4.20

TABLE 6 - COW

THE DETERMINATION OF TRAPPED PLASMA IN BLOOD AFTER CENTRIFUGATION AT 1500 g FOR 30 MINUTES, TEMPERATURE UNKNOWN

Cow No.	Vol. Cells ml	Vol. Plasma ml	Plasma %
1	1.40	0.097	6.9
2	1.36	0.208	15.3
3	1.55	0.229	14.8
4	1.50	0.090	6.0
5	1.30	0.101	7.8
6	1.35	0.139	10.3
7	1.35	0.120	8.9
8	0.60	0.037	6.1
9	1.35	0.142	10.5
10	1.10	0.075	6.8
11	1.22	0.138	11.3
12	1.32	0.161	12.2
13	1.30	0.211	16.2
14	0.90	0.105	11.7
15	2.00	0.488	24.4
16	1.60	0.259	16.2
17	1.90	0.319	16.8
18	1.53	0.298	18.5
19	1.55	0.229	14.8
20	2.00	0.180	9.0
21	1.72	0.206	12.0
22	1.75	0.305	17.4

Mean 12.5±4.84

Range 6.0 - 24.4

TABLE 7

SUMMARY OF DETERMINATIONS OF TRAPPED PLASMA IN RABBIT, DOG, HORSE AND COW BLOODS AFTER CENTRIFUGATION AT 1500 g FOR 30 MINUTES, TEMPERATURE UNKNOWN

Species	No. of Animals	Intercellular Plasma	Range
Rabbit	22	3.3 ± 0.47	2.50 - 4.40
Dog	10	3.1 ± 0.26	2.83 - 3.60
Horse	32	2.9 ± 0.67	1.90 - 4.20
Cow	22	12.5 ± 4.84	6.00 - 24.40

to estimating trapped plasma under new conditions of centrifugation it was decided to estimate the effect of these conditions on the normal haematocrits of various species. Because of the tendency of ordinary centrifuges to heat with prolonged use it was decided to use a machine with temperature control. All subsequent experiments were carried out using an M.S.E. refrigerator centrifuge. Initially this machine was run at 15°C, i.e. a little below room temperature.

Summary

Trapped plasma was estimated in the haematocrits of 22 rabbits, 10 dogs, 32 horses and 22 cows under conditions of centrifugation acceptable for estimations on man. The results in rabbit, dog and horse were comparable to those of man, but in the bovine a mean of 12.5% was obtained with a range 6.0-24.0%.

It was decided to investigate the effect of longer centrifugation on the percentage of trapped plasma. As a preliminary it was decided to determine the effect of longer centrifugation on the normal haematocrit and for this purpose to use a centrifuge with temperature control.

B. Experiments in a Temperature Controlled Centrifuge

(1) The effect of increasing the time of centrifugation on Haematocrit determinations of bovine, sheep, dog, rabbit, pig and horse bloods. Centrifugation at 1500 g and 15°C

Heparinised blood samples were collected from at least ten individuals of each of the above species and Wintrobe haematocrit tubes were set up in triplicate for each sample. These tubes were centrifuged for a total of 3½ hours. Readings were taken at 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, and 210 minutes. In order to facilitate comparison between different samples the haematocrit readings at the different time intervals were all expressed as a percentage of the final reading at 210 minutes. The results are shown in Table 8 and Figure 2. It is obvious that the species studied fall into two groups. In the dog, rabbit, pig and horse the cells have packed down fairly well after one hour's spinning, whereas in bovine and sheep 2-3 hours spinning appears to be necessary. Since the trapped plasma is a minor part of the red cell column the species differences which have been shown to become apparent simply on determination of the haematocrit, must correspond to relatively large differences in the proportion of plasma trapped among the red cells.

TABLE 8

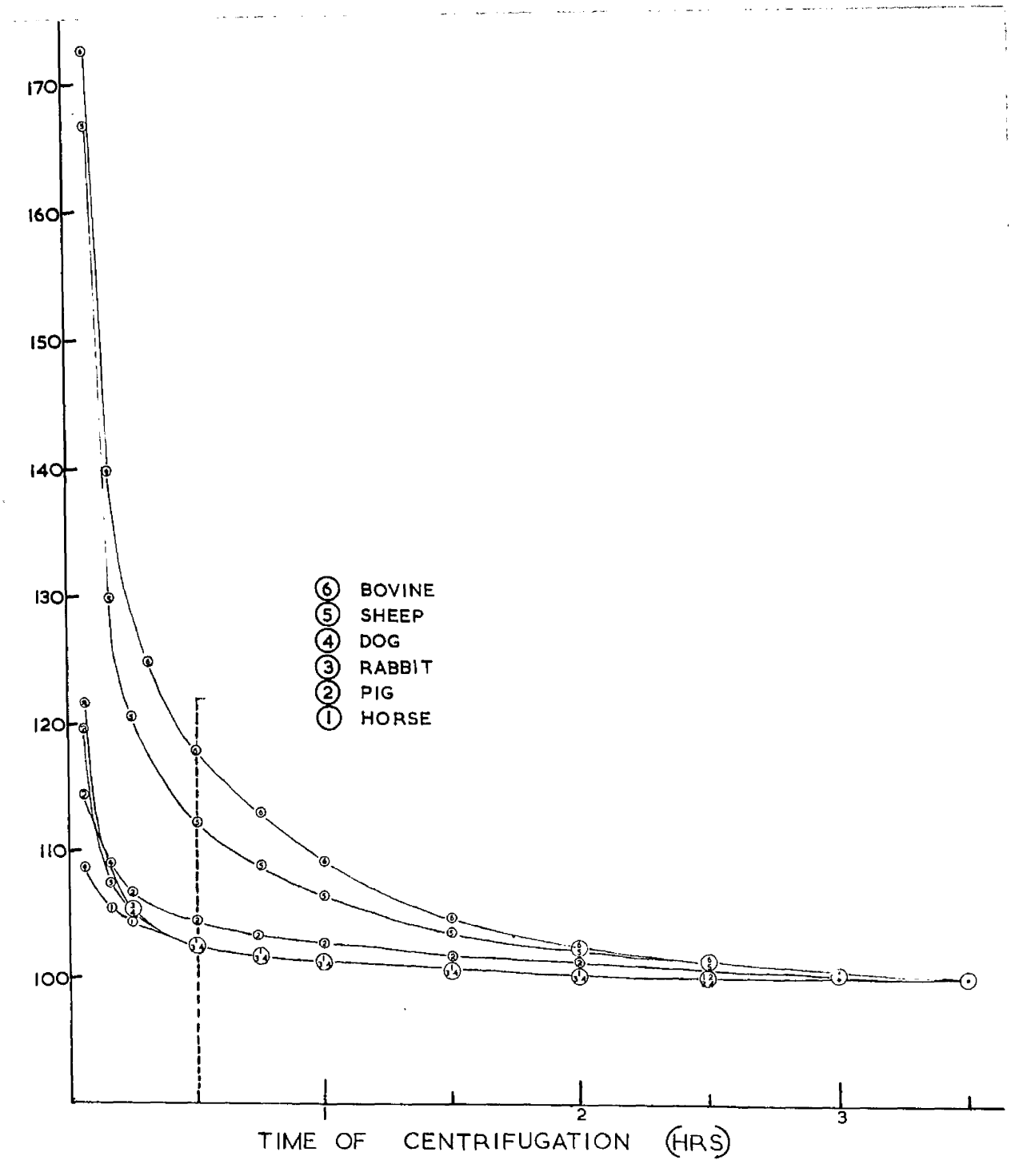
THE EFFECT OF INCREASING THE TIME OF CENTRIFUGATION ON HAEMATOCRIT DETERMINATIONS ON THE BLOOD OF VARIOUS SPECIES. CENTRIFUGATION AT 1500 g AND 15°C

THE PERCENTAGE COLUMN EXPRESSES THE Ht VALUE AS A PROPORTION OF THE FINAL READING AT 210 MINUTES

Time Min.	Bovine		Sheep		Dog		Rabbit		Pig		Horse	
	Ht	%	Ht	%	Ht	%	Ht	%	Ht	%	Ht	%
5	54.9	172.6	57.7	160.8	56.1	121.7	49.5	119.6	47.0	114.4	37.4	108.7
10	44.5	139.9	44.9	129.8	50.2	108.9	44.4	107.2	44.8	159.0	36.3	105.5
15			41.7	120.5	48.6	105.4	43.5	105.1	43.8	106.6	35.9	104.4
20	39.7	124.8										
30	37.5	117.9	38.8	112.1	47.3	102.6	42.5	102.7	42.9	104.4	35.3	102.6
45	35.9	112.9	37.6	108.7	46.9	101.7	42.1	101.7	42.3	102.9	35.2	102.3
60	34.7	109.1	36.8	106.4	46.7	101.3	41.9	101.2	42.2	102.7	34.9	101.5
90	33.3	104.7	35.8	103.5	46.4	100.1	41.6	100.5	41.8	101.7	34.6	100.6
120	32.6	102.5	35.4	102.3	46.2	100.2	41.6	100.5	41.6	101.2	34.6	100.6
150	32.2	101.3	35.1	101.4	46.1	100.0	41.4	100.0	41.7	100.7	34.6	100.6
180			34.8	100.6	46.1	100.0	41.4	100.0	41.3	100.5	34.4	100.0
210	31.8	100.0	34.6	100.0	46.1	100.0	41.4	100.0	41.1	100.0	34.4	100.0

FIGURE 2

The effect of increasing the time of centrifugation on haematocrit determinations on the blood of various species. Centrifugation at 1500 g and 15°C. The earlier haematocrit readings are expressed as a percentage of the final reading at 3½ hours.



Summary

Haematocrit determinations were made at different times of centrifugation at 1500 g and 15°C in various species. Bovine and sheep red cells were found to pack poorly if spun for under 2 hours. This was in line with the previous experiment in which trapped plasma in the bovine haematocrit was found to be high when centrifugation had been carried out for 30 minutes.

(2) Determination of the Intercellular Plasma in Bovine Blood after Centrifugation for 2 hours at 1500 g and 15°C

66 samples of bovine blood were obtained in heparinised bottles from that number of animals when they were being slaughtered at an abattoir. Trapped plasma was estimated in duplicate samples by the method outlined in the preliminary experiment (A), but with the centrifugation time increased to two hours. The results are shown in Table 9.

As it had been demonstrated that bovine blood showed a large drop in haematocrit between 30 minutes and 2 hours spinning at 1500 g and 15°C, it was expected that the determination of trapped plasma after 2 hours centrifugation under these conditions would give a result lower than that obtained when the conditions of centrifugation were 1500 g for 30 minutes, temperature unknown. However, it was found that the amount of trapped plasma was 11.4% with a very wide range, similar to the result in the preliminary experiment.

TABLE 9

DETERMINATION OF INTERCELLULAR PLASMA IN 66 BOVINE BLOODS AFTER 2 HRS. CENTRIFUGATION AT 1500 g AND 15°C

Cow No.	% Trapped Plasma	Cow No.	% Trapped Plasma	Cow No.	% Trapped Plasma
1	15.9	23	9.9	45	12.1
2	9.8	24	8.9	46	10.8
3	7.2	25	15.6	47	12.3
4	8.8	26	10.4	48	17.4
5	7.1	27	8.0	49	6.8
6	14.4	28	7.8	50	16.0
7	11.7	29	7.9	51	13.2
8	13.4	30	9.2	52	12.1
9	11.5	31	15.5	53	17.4
10	6.0	32	12.9	54	9.5
11	9.8	33	14.7	55	13.0
12	5.5	34	17.7	56	18.3
13	6.1	35	17.1	57	12.8
14	5.8	36	10.2	58	13.6
15	16.2	37	14.5	59	12.9
16	8.3	38	18.6	60	16.2
17	6.4	39	8.8	61	4.7
18	6.4	40	15.8	62	11.6
19	9.9	41	11.6	63	7.6
20	8.9	42	13.7	64	20.5
21	8.5	43	15.4	65	14.0
22	8.2	44	16.3	66	17.4

Mean 11.9 $\pm$ 3.9 Range 4.7 - 20.5

This result was surprising and seemed to throw doubt on the validity of the method for estimating trapped plasma. It suggested that the drop in haematocrit of bovine blood between 30 minutes and 2 hours centrifugation was not simply due to closer packing of cells with the appropriate decrease in intercellular plasma. It seemed that there might be some other factor involved, e.g. the extrusion of fluid from the cells as a result of the prolonged centrifugation.

An experiment was therefore carried out to test the validity of the trapped plasma correction over a wide range of haematocrit values of the same blood sample. This is described below.

(3) Experiment to test the validity of the method of estimation of trapped plasma

About 5.0 ml of ^{131}I labelled albumin was added to approximately 300 ml of heparinised bovine blood which was then thoroughly mixed. From this blood 48 samples were made up in polythene tubes and from each tube two Wintrobe haematocrit tubes were filled, and all tubes were spun at 1500 g and 15°C. Four polythene tubes and the corresponding haematocrit tubes were removed after 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165 and 180 minutes centrifugation. The haematocrits were read, the polythene tubes clamped, volumetric and radioactivity determinations performed on cells and supernatant, and the percentage of trapped

plasma calculated as described in the preliminary experiment (A). The volume of trapped plasma was then subtracted from the observed haematocrit of each sample to determine the true red cell volume and the results are shown in Table 10 and Figure 3.

It is clear from the results in Table 10 and Figure 3 that the drop in haematocrit which occurs with prolonged centrifugation of bovine blood is associated with a corresponding drop in the trapped plasma. When correction is made for the trapped plasma at different times throughout the experiment a fairly constant figure for red cell volume is obtained. This seems an adequate vindication of the method.

Summary

Trapped plasma was estimated in bovine blood after centrifugation for an increased time. The temperature of the centrifuge was 15°C. The proportion of trapped plasma was little less than that determined in experiment (A).

The method of estimation of trapped plasma was tested and considered to be valid.

TABLE 10

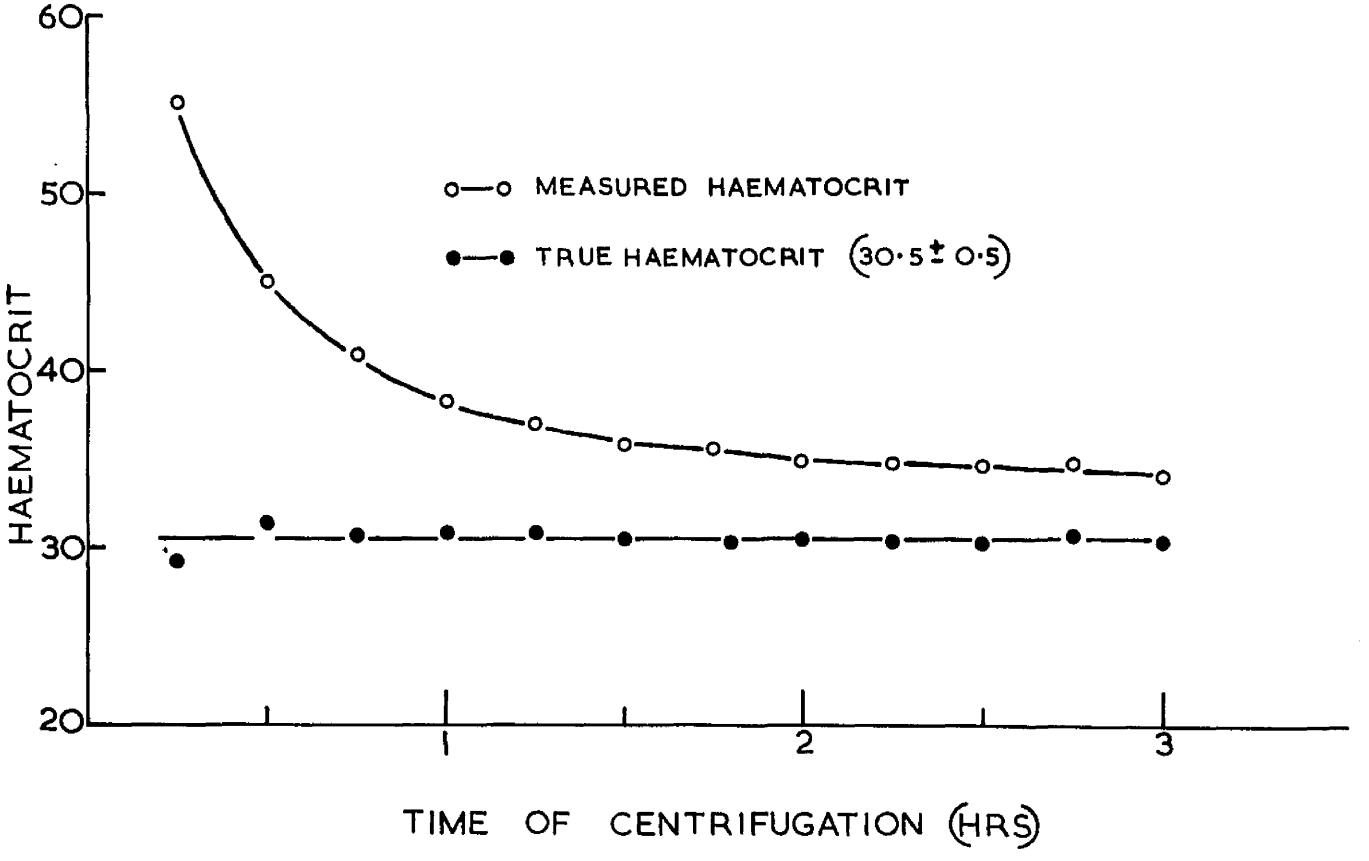
EXPERIMENT TO TEST THE VALIDITY OF THE METHOD OF ESTIMATION OF TRAPPED PLASMA. CENTRIFUGATION OF SIMILAR BOVINE BLOODS IN POLYTHENE TUBES AT 1500 *g* AND 15°C. FOUR TUBES REMOVED AT EACH TIME INTERVAL

Time of Centrifugation Mins.	Measured (Observed) Ht	% Trapped Plasma	Actual Trapped Plasma	True Ht (Corrected for Trapped Plasma)
15	55.4	47.3	26.2	29.2
30	45.0	30.3	13.6	31.4
45	40.9	24.9	10.2	30.7
60	38.3	14.2	7.4	30.9
75	37.0	16.6	6.1	30.9
90	35.8	14.7	5.3	30.5
105	35.7	15.0	5.4	30.3
120	34.9	12.2	4.3	30.6
135	34.8	12.7	4.4	30.4
150	34.7	12.3	4.3	30.4
165	34.8	11.6	4.0	30.8
180	34.0	10.6	3.6	30.4

Mean 30.5 ± 0.5

FIGURE 3

The comparison of measured (observed) and true haematocrits of bovine blood after varying periods of centrifugation at 1500 g and 15°C.



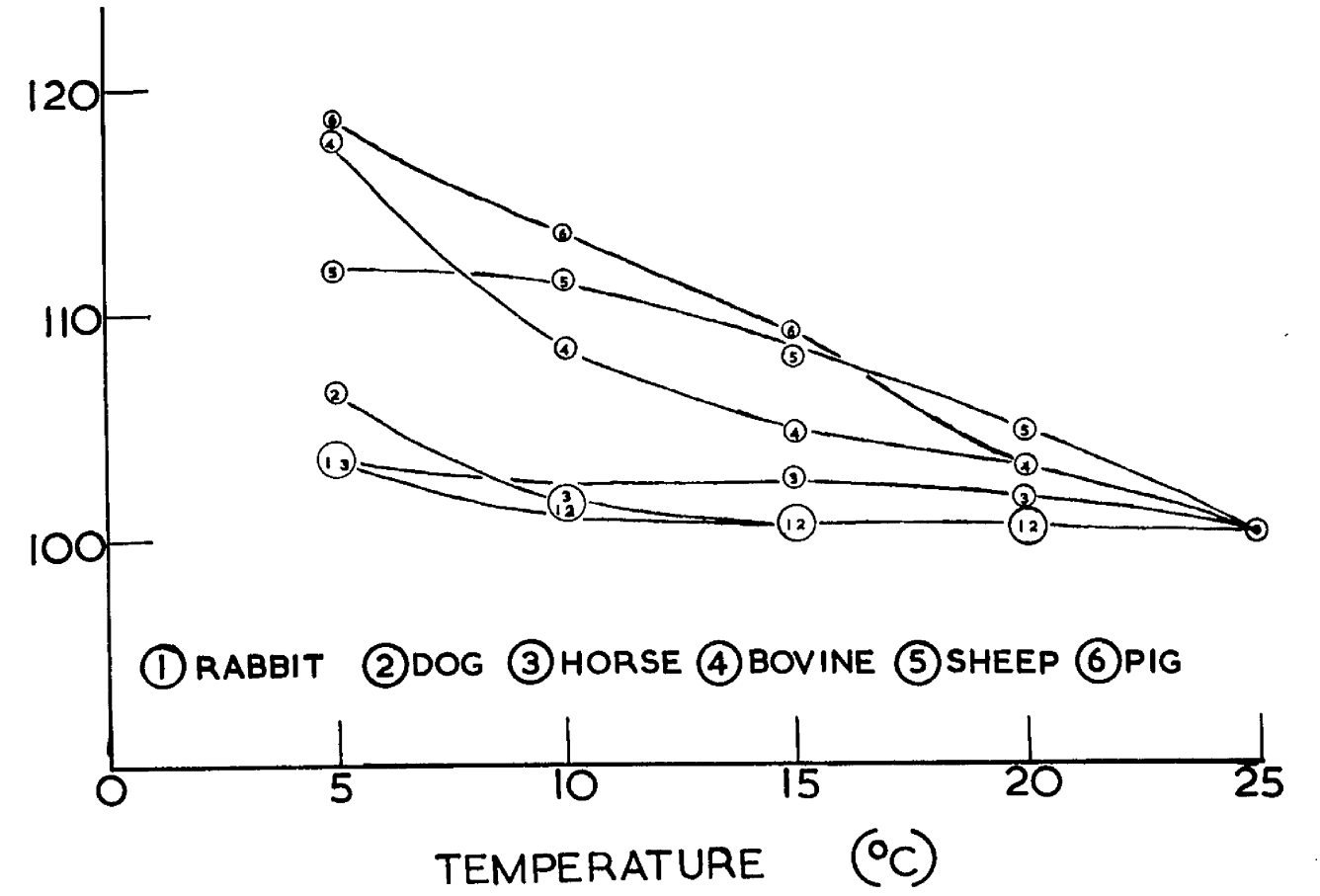
Following the last experiment a different explanation had to be sought for the apparent lack of decrease in bovine trapped plasma even after prolonged centrifugation at 1500 *g* and 15°C. It was noted (Table 10) that after 30 minutes spinning at 15°C the proportion of trapped plasma was 30.3%, an even higher figure than that obtained in the preliminary experiment (A), when the centrifuge without temperature control was used. This suggested that the temperature of the centrifuge might be an important factor in influencing the result of determination of trapped plasma, as up to 30 minutes spinning time, conditions of centrifugation in those two experiments had only differed in that respect. It was decided to determine the effect of varying temperatures on the haematocrit readings of the blood of various species as a preliminary to studying the effect of temperature on trapped plasma distribution.

(4) The Effect of the Temperature of Centrifugation on the Haematocrits of the Blood of Various Species

Quadruplicate samples of blood of each species were spun in standard Wintrobe haematocrit tubes for 30 minutes at 1500 g at 5, 10, 15, 20 and 25°C. The average haematocrit reading at each temperature below 25°C was expressed as a percentage of the reading at 25°C which was taken as 100%. The results are shown in Figure 4. It is clear that the red cells of all species pack

FIGURE 4

The effect of the temperature of the centrifuge on the haematocrit of the blood of various species.



more effectively with increase in temperature of the centrifuge and this is most marked with the red cells of bovine, sheep and pig. To confirm this finding it seemed desirable to determine if it was reflected in the amount of plasma trapped at different temperatures.

(5) The Effect of the Temperature of Centrifugation on the Percentage of Plasma Trapped in the Haematocrit

Homologous ^{131}I-labelled albumin was added to heparinised blood samples from four pigs and mixed well. Duplicate samples of each pig's blood were placed in polythene tubes and centrifuged at 1500 g for 30 minutes, one sample in the M.S.E. Medium Centrifuge at a temperature varying from 20-35°C, and its duplicate in the refrigerated centrifuge at 15°C. The amount of trapped plasma was estimated as before and the results are shown in Table 11.

Summary

The results of experiment 5 confirm those of the previous one and explain the apparent anomaly between the results of experiments (A) and (B)1 and (B)2, i.e. failure to obtain better packing of red cells when the time of centrifugation was greatly increased. The increased time had been offset by the poorer packing obtained at lower centrifuge temperatures in experiments

TABLE 11

THE EFFECT OF THE TEMPERATURE OF THE CENTRIFUGE ON THE PROPORTION OF PLASMA TRAPPED IN THE HAEMATOCRIT OF THE BLOOD (PIG) 1500 g FOR 30 MINS.

Animal No.	Intercellular Plasma %	
	Centrifuge Temperature 15°C	Centrifuge Temperature 20-35°C
1	13.8	5.6
2	11.4	5.9
3	14.2	8.7
4	17.9	5.9

(B)1 and (B)2. Under the conditions of centrifugation studied, the temperature of the centrifuge was shown to be of marked importance, and it would appear that for present purposes, 25°C is a more suitable temperature than 15°C. All further experiments were therefore carried out at the higher temperature, and an RCF of 1500 g.

(6) Determination of the Amount of Trapped Plasma at Different Levels of the Red Cell Column.

Modified Analytical Method

It has already been mentioned that in the past "direct" determinations of intercellular plasma had been rendered difficult by the technical problem of obtaining packed cells uncontaminated by supernatant plasma, and representative of the whole column, as the proportion of trapped plasma varies at different heights of the column. Other workers have shown that much more plasma is trapped in the proximal than in the distal part of the haematocrit tube. As haematocrits vary, those in the lower range have a relatively low proportion of trapped plasma.

It was decided to study the distribution of the plasma trapped in the red cell column of the various species of domesticated animals so that graphs might be constructed from which the amount of trapped plasma could be estimated for different volumes of red cell column in standard tubes under standard conditions of centrifugation.

Materials and Methods

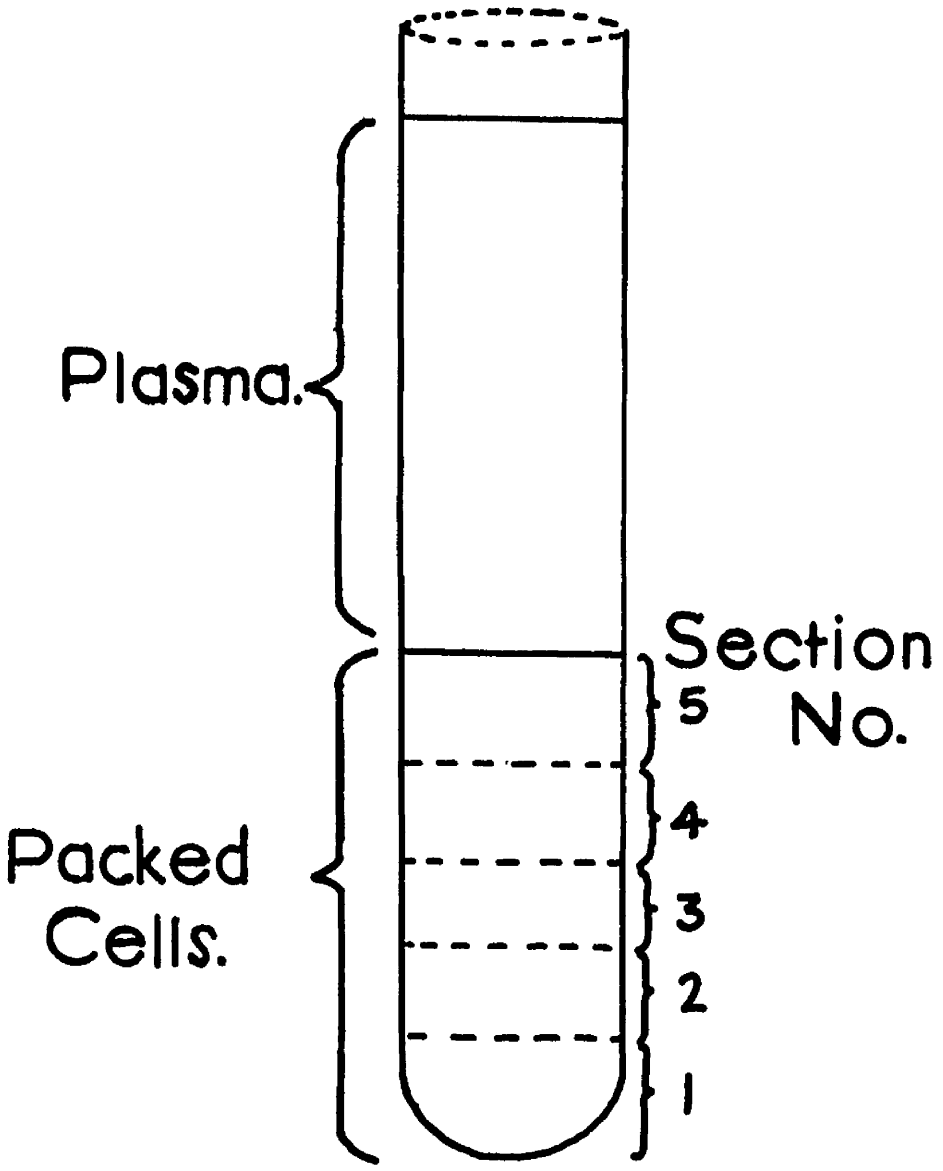
Until now the method of clamping a polythene tube just below the upper surface of the red cell column had been used. The disadvantages were that the uppermost layer was excluded from examination, and although the operation was performed quickly there was a short time when some movement of cells and plasma took place around the site of clamping and their proportions may have thereby been altered. To overcome these difficulties a more accurate method was devised.

After centrifugation and removal of most of the plasma the polythene tube was plunged into a beaker containing acetone to which had been added a block of solid CO_2. The blood in the tube was solidified almost instantaneously and that part of it consisting of red cells was cut into approximately 1 cm lengths by a small mechanical saw. For convenience numbers were given to each section, beginning with the most distal, as in Figure 5.

The polythene surrounding each 1 cm section was cut longitudinally in three places and stripped off the frozen blood, which was placed in a graduated centrifuge tube, allowed to melt, and the volume read off (a more precise method based on weighing was introduced later). The cells were then made up to 10.0 ml with water containing a little 0.1N NaOH and 2-3 drops of capryl alcohol to prevent foaming, and radioactivity estimated in the M6 liquid counter. Plasma samples (usually 0.2 ml) were diluted

FIGURE 5

The Red Cell Column. Method of Numbering Sections.



with 0.01N Na OH to 10.0 ml for counting. As the radioactivity in a known volume of plasma was determined it was possible to calculate the volume of plasma trapped among the red cells of a section, as the radioactivity of the section was due solely to trapped plasma.

Measurement of the volume of a section of packed red cells in a centrifuge tube was thought to be a somewhat inaccurate method of measuring such a comparatively small amount and it was decided to find the specific gravity of packed bovine red cells so the volume of such a specimen could be calculated by weighing the tube before and after blood was put in it and using the specific gravity factor.

Heparinised blood samples were obtained from 8 bovines by jugular venepuncture and duplicate samples prepared. One of the samples from each animal was spun for 10 minutes, the plasma removed and its specific gravity determined by means of a specific gravity bottle. The other sample of whole blood was used for the determination of its specific gravity by the same method and samples were also spun in standard Wintrobe haematocrit tubes for $2\frac{1}{2}$ hours at 1500 *g* and $25^{\circ}C$. The specific gravity of packed cells was calculated according to the formula

$$\text{S.G. Packed Cells} = \frac{\text{S.G. whole blood} \times 100 - \text{S.G. plasma } (100-\text{Ht})}{\text{Ht}}$$

The results are shown in Table 12.

TABLE 12

THE DETERMINATION OF THE SPECIFIC GRAVITY OF PACKED BOVINE RED CELLS

Cow No.	Ht	S.G. Plasma	S.G. Whole Blood	S.G. Packed Red Cells
1	37.4	1.039	1.063	1.103
2	38.7	1.041	1.065	1.103
3	38.3	1.042	1.064	1.099
4	30.2	1.040	1.059	1.103
5	36.3	1.041	1.063	1.102
6	36.5	1.040	1.062	1.100
7	37.9	1.040	1.064	1.103
8	35.1	1.041	1.064	1.107
				Mean 1.103

Summary

A method of freezing the red cell column and obtaining sections for analysis of trapped plasma is described, overcoming previous criticisms of the 'direct' method of analysis.

The specific gravity of packed red cells was determined, so that the volume of packed cells could be estimated more accurately than before, by weighing.

In the study of the amount of plasma trapped at different levels of the red cell column, normal bovine haematocrits usually provided only 3-4 sections and it was decided to increase this number by using blood which had the proportion of red cells increased by removal of 50 to 60% of the plasma before the addition of labelled albumin. It was thought that increase in the height of the packed red cell column might possibly alter the amount of plasma trapped in the lower section and to determine if this was so the following experiment was carried out.

(7) The Effect of Increase in Height of the Red Cell Column on the Proportion of Plasma Trapped in the Lower Sections

Heparinised bovine blood was divided into two portions. One portion was centrifuged for 10 minutes at 1500 *g* and about half of the plasma was discarded, the remainder being termed an 'enriched' sample. The other portion was termed an 'ordinary'

sample and both ordinary and enriched samples had ^{131}I-labelled bovine albumin added and effectively mixed. Octuplicate samples of 'ordinary' and 'enriched' blood were placed in polythene tubes, centrifuged at 1500 *g* for 2 hours at 25°C and frozen. The red cell columns were cut into approximately 1 cm sections and the trapped plasma determined. The results are shown in Table 13.

Those determinations were made on the blood of one bovine and it was decided to make a similar investigation using blood from 6 bovines.

From each animal 'ordinary' and 'enriched' heparinised samples of blood were prepared. From each type of sample duplicate specimens were placed in polythene tubes, centrifuged and frozen as described previously. The tubes containing the ordinary samples were cut at the level of the top of the red cell column, excluding the buffy coat, and the block of cells distal to that point was used for the estimation of plasma as described previously. The tubes containing the 'enriched' blood which of course had a higher haematocrit were cut at the level which was equivalent to the top of the red cell column of the ordinary sample and only the block of cells distal to this point was used for the estimation of trapped plasma contained therein. The results are shown in Table 14.

TABLE 13

THE ESTIMATION OF TRAPPED PLASMA IN SECTIONS OF THE HAEMATOCRIT OF NORMAL AND CELL ENRICHED BOVINE BLOODS

Haematocrit Section No.	1	2	3	4	5	6
Trapped Plasma% Ordinary Blood	5.8	7.0	8.5	11.2		
Mean			8.2			
Trapped Plasma % Enriched Blood	5.7	7.3	9.1	10.8	12.5	18.5
Mean			8.1			

TABLE 14

THE PROPORTION OF PLASMA TRAPPED IN THE RED CELL COLUMN OF ORDINARY BOVINE BLOOD COMPARED WITH THAT IN THE EQUIVALENT SECTION OF THE HAEMATOCRIT OF ENRICHED BLOOD

Cow No.	% Trapped Plasma Ordinary Blood	% Trapped Plasma Enriched Blood
1	5.43	5.14
2	6.46	6.48
3	7.77	7.08
4	6.59	6.32
5	7.12	7.09
6	4.19	3.85
7	6.06	5.73
8	4.14	4.19
Mean	5.97	5.74

Summary

Heparinised bovine blood samples were each split into two portions. One of these had the red cell content artificially augmented by the removal of plasma. In all samples trapped plasma was then estimated in that part of the lower sections equal in length to the red cell column of the corresponding untreated portion. Good agreement was obtained between the results from augmented and non-augmented blood. These results indicate that it should be possible to make accurate estimation of trapped plasma gradation in the red cell columns of bloods of various species using augmented samples and construct graphs to show how trapped plasma varies with the haematocrit reading.

(8) The Accurate Determination of the Distribution of Trapped Plasma in the Red Cells of Different Species After Centrifugation at 1500 *g* and 25°C

(a) The Estimation of Trapped Plasma in Bovine Blood After 2 Hours Centrifugation

Heparinised samples from each of 20 bovines had the red cell column "enriched" by removal of plasma, and duplicate samples were centrifuged in polythene tubes which were then frozen. That part containing red cells was cut in sections approximately 1 cm long and the trapped plasma in each section estimated as described previously. The results are shown in Table 15.

TABLE 15

THE DISTRIBUTION OF TRAPPED PLASMA IN THE ENRICHED RED CELL COLUMNS OF THE HAEMATOCRITS OF BOVINES AFTER CENTRIFUGATION AT 1500 g AND 25°C FOR 2 HOURS

Cow No.	% Trapped Plasma in Haematocrit Section No.					
	1	2	3	4	5	6
1	3.9	4.7	5.2	5.8	6.4	6.9
1 duplicate	3.6	4.3	5.2	5.5	6.2	6.6
2	6.1	7.0	7.6	8.3	8.9	8.9
2 "	5.4	7.4	7.9	9.1	9.4	9.7
3	3.3	4.4	5.0	5.5	6.3	6.6
3 "	3.4	4.4	5.0	5.6	6.1	6.7
4	5.4	6.7	7.0	7.9	8.9	9.5
4 "	5.4	6.6	5.9	7.9	8.5	9.1
5	5.6	6.6	7.3	8.1	8.8	9.5
5 "	5.7	6.9	7.5	8.1	8.7	9.2
6	5.6	6.8	7.6	8.5	8.8	9.3
6 "	4.8	6.7	7.5	7.9	8.8	9.3
7	6.1	7.3	7.7	8.5	9.5	10.3
7 "	6.0	7.1	7.9	8.5	9.3	10.3
8	5.0	6.0	6.6	7.2	7.7	8.4
8 "	5.0	5.9	6.5	6.9	7.5	8.0
9	3.4	4.6	5.1	5.6	6.3	
9 "	3.4	4.2	4.9	5.5	5.9	
10	5.0	6.2	7.0	7.6	7.4	
10 "	-	-	-	-	-	

TABLE 15 - Continued

Cow No.	% Trapped Plasma in Haematocrit Section No. 1	2	3	4	5	6
11	5.6	6.8	7.9	8.5	9.2	
11 duplicate	5.7	6.7	7.9	8.4	9.4	
12	5.3	6.5	7.2	7.8	8.6	
12 "	5.4	6.5	7.1	7.7	8.7	
13	6.1	7.4	7.7	8.0	9.2	
13 "	6.1	7.0	7.9	8.2	8.7	
14	6.2	7.5	8.4	9.1	9.8	
14 "	5.9	7.1	7.8	8.8	9.3	
15	3.5	4.2	4.8	5.4	5.9	
15 "	3.5	4.3	4.8	5.4	6.1	6.6
16	5.0	6.5	7.1	7.8	8.4	
16 "	-	-	-	-	-	
17	5.0	5.9	6.7	7.0	7.4	
17 "	5.1	5.9	6.6	7.1	7.4	
18	2.4	2.9	3.3	3.8	4.0	
18 "	2.3	2.9	3.4	3.7	4.2	
19	3.5	4.2	4.8	5.3	5.6	
19 "	3.6	4.5	5.0	5.4	5.8	
20	4.9	6.1	6.7	7.3	7.6	
20 "	4.8	6.0	6.8	7.2	7.7	
Mean	4.8	5.8	6.5	7.1	7.7	8.4

It was necessary to determine the plasma trapped in sections 1 + 2, 1 + 2 + 3, etc., and this was done by totalling the overall volumes for these sections, then the plasma volumes and expressing the latter as a percentage. As an example, the figures for one sample from one cow are shown in Table 16. The average figures for the 20 cows are shown in Table 17.

As the volume of each section was determined and the internal diameter of the polythene tube was known, the length of each section was calculated and the proportion of trapped plasma related to the height of the red cell column. Average figures determined on the 20 cows, and individual maximum and minimum figures, are recorded in Table 18 and in graphical form in Figure 6. The lower minimum figure was recorded but considered to be outwith the normal range. Before each bovine blood sample had been enriched, duplicate Wintrobe haematocrit tubes had been filled and were centrifuged with the polythene tubes. Using these haematocrit determinations and reading off the mean graph (Figure 6), the results shown in Table 19 were obtained.

As there was a marked difference between the maximum and minimum lines on the mean graph, individual graphs were drawn for each of the duplicate samples from each bovine (Figures 7, 8, 9, 10 and 11). By reading the haematocrits against individual graphs the results shown in Table 20 were obtained.

TABLE 16

THE DISTIRBUTION OF TRAPPED PLASMA IN THE ENRICHED RED CELL COLUMN OF THE HAEMATOCRIT OF 1 COW

Tube Section No.	Weight Cells gm	Volume Cells ml	Volume Trapped Plasma ml	% Trapped Plasma
1	0.4458	0.404	.02	3.9
2	0.4504	0.408	.02	4.7
3	0.4488	0.407	.02	5.2
4	0.4220	0.383	.02	5.8
5	0.4389	0.398	.03	6.4
6	0.4380	0.397	.03	6.9
1		.404	.02	3.9
1 + 2		.912	.04	4.3
1 + 2 + 3		1.219	.06	4.6
1 + 2 + 3 + 4		1.602	.08	4.9
1 + 2 + 3 + 4 + 5		2.000	.11	5.2
1 + 2 + 3 + 4 + 5 + 6		2.397	.14	5.5

TABLE 17

THE DISTRIBUTION OF TRAPPED PLASMA IN THE ENRICHED RED CELL COLUMN OF THE HAEMATOCRITS OF 20 COWS

Tube Section No.	Mean Vol. Cells ml	Mean Vol. Trapped Plasma ml	Mean % Trapped Plasma
1	0.424	.02	4.8
1 + 2	0.841	.04	5.3
1 + 2 + 3	1.237	.07	5.7
1 + 2 + 3 + 4	1.629	.10	6.0
1 + 2 + 3 + 4 + 5	2.018	.13	6.4
1 + 2 + 3 + 4 + 5 + 6	2.403	.17	6.9

TABLE 18

THE DISTRIBUTION OF TRAPPED PLASMA IN THE ENRICHED RED CELL COLUMN OF THE HAEMATOCRITS OF 20 COWS RELATED TO THE LENGTH OF THE CELL COLUMN

	Tube Section	Volume (ml)	Length (mm)	% Trapped Plasma
Average (20 Cows)	1	0.42	9.6	4.8
	1 + 2	0.84	19.0	5.3
	1 + 2 + 3	1.24	28.0	5.7
	1 + 2 + 3 + 4	1.63	36.9	6.0
	1 + 2 + 3 + 4 + 5	2.02	45.7	6.4
	1 + 2 + 3 + 4 + 5 + 6	2.40	54.4	6.9
Maximum (Cow No. 7)	1	0.45	10.1	6.1
	1 + 2	0.88	19.8	6.7
	1 + 2 + 3	1.27	28.7	7.0
	1 + 2 + 3 + 4	1.66	37.6	7.3
	1 + 2 + 3 + 4 + 5	2.05	46.3	7.8
	1 + 2 + 3 + 4 + 5 + 6	2.48	56.0	8.2
Minimum (Cow No. 3)	1	0.44	9.9	3.4
	1 + 2	0.85	19.3	3.8
	1 + 2 + 3	1.23	27.8	4.2
	1 + 2 + 3 + 4	1.64	37.1	4.5
	1 + 2 + 3 + 4 + 5	2.02	45.7	4.8
	1 + 2 + 3 + 4 + 5 + 6	2.39	54.0	5.1
Sub-minimum (Cow No. 18)	1	0.42	9.5	2.3
	1 + 2	0.79	17.9	2.6
	1 + 2 + 3	1.20	27.0	2.9
	1 + 2 + 3 + 4	1.58	39.7	3.1
	1 + 2 + 3 + 4 + 5	2.00	44.7	3.3

FIGURE 6 .

The proportion of trapped plasma in relation to the height of the red cell column.

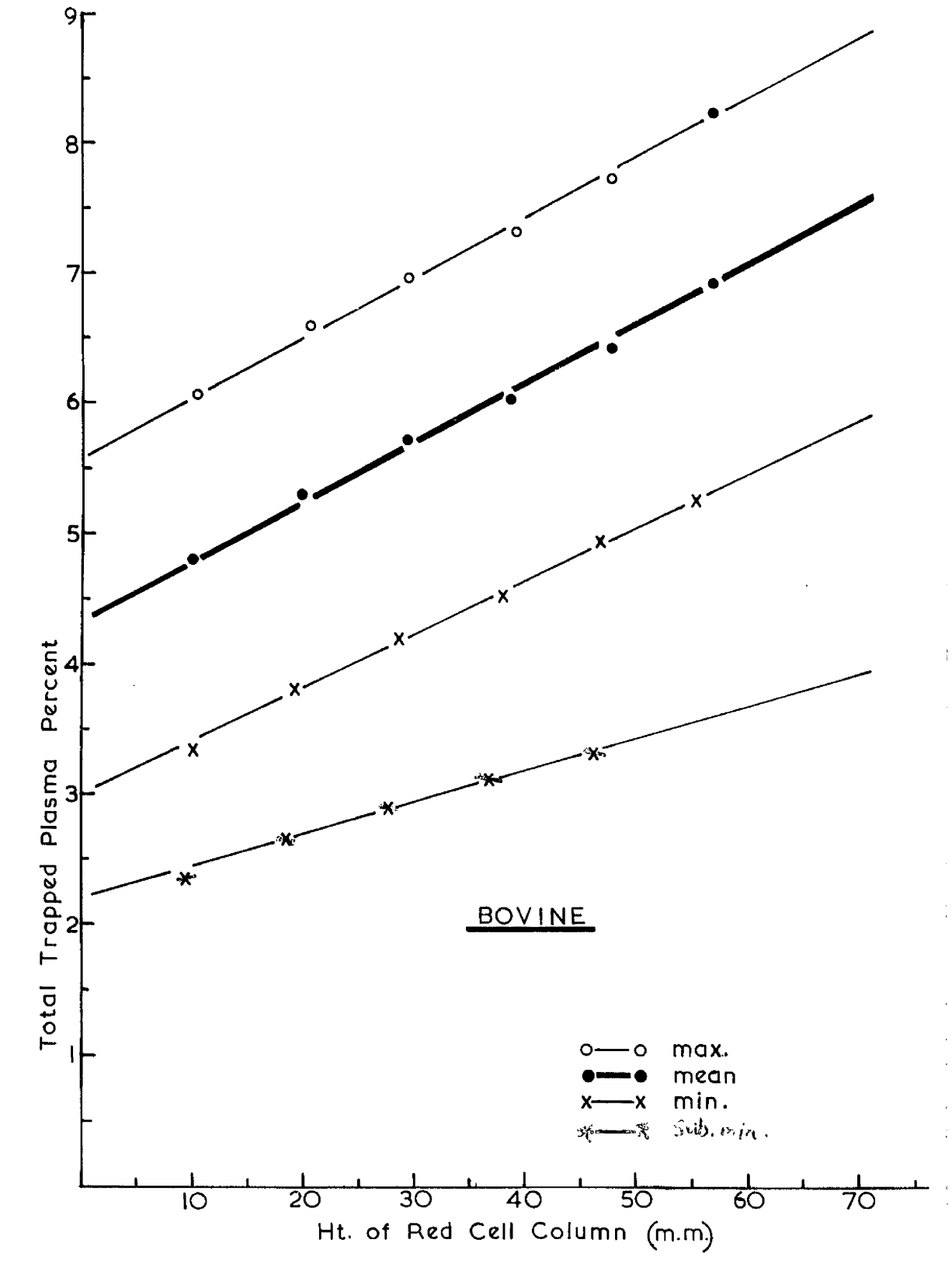


TABLE 19

THE PERCENTAGE OF TRAPPED PLASMA IN THE RED CELL COLUMN OF

THE PERCENTAGE OF TRAPPED PLASMA IN THE RED CELL COLUMN OF

RESULTS OBTAINED BY READING INDIVIDUAL HAEMATOCRITS AGAINST

THE MEAN GRAPH

Bovine No.	Ht	% Trapped Plasma	Bovine No.	Ht	% Trapped Plasma
1	30.6	5.8	11	38.9	6.2
1*	30.5	5.8	11*	38.9	6.2
2	30.5	5.8	12	36.8	6.2
2*	31.9	5.9	12*	36.8	6.1
3	32.7	5.9	13	38.2	6.1
3*	32.4	5.9	13*	38.2	6.1
4	34.8	6.0	14	40.0	6.2
4*	34.5	6.0	14*	40.0	6.2
5	36.2	6.0	15	39.9	6.2
5*	37.0	6.1	15*	39.9	6.2
6	35.4	6.0	16	40.3	6.3
6*	35.4	6.0	17	37.0	6.1
7	35.2	6.0	17*	37.0	6.1
7*	35.5	6.0	18	39.1	6.2
8	38.6	6.2	18*	39.1	6.2
8*	38.8	6.2	19	36.9	6.1
9	37.9	6.1	19*	36.9	6.1
9*	37.9	6.1	20	43.4	6.4
10	36.7	6.1	20*	43.4	6.4

* = Duplicate

Mean 6.1 ± 0.2

Range 5.8 - 6.4

FIGURE 7

The proportion of trapped plasma in relation to the height of the red cell column.

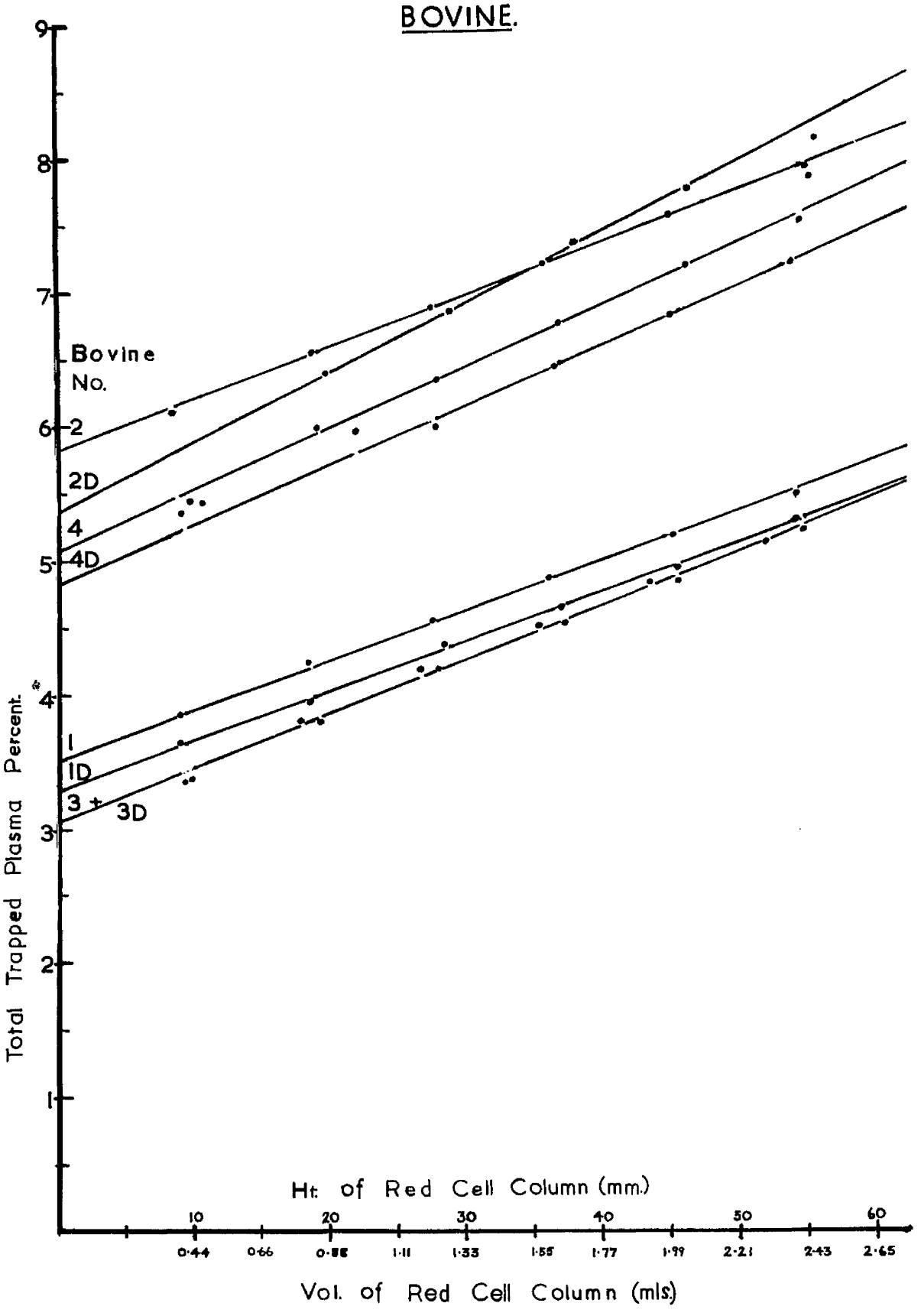


FIGURE 8

The proportion of trapped plasma in relation to the height of the red cell column.

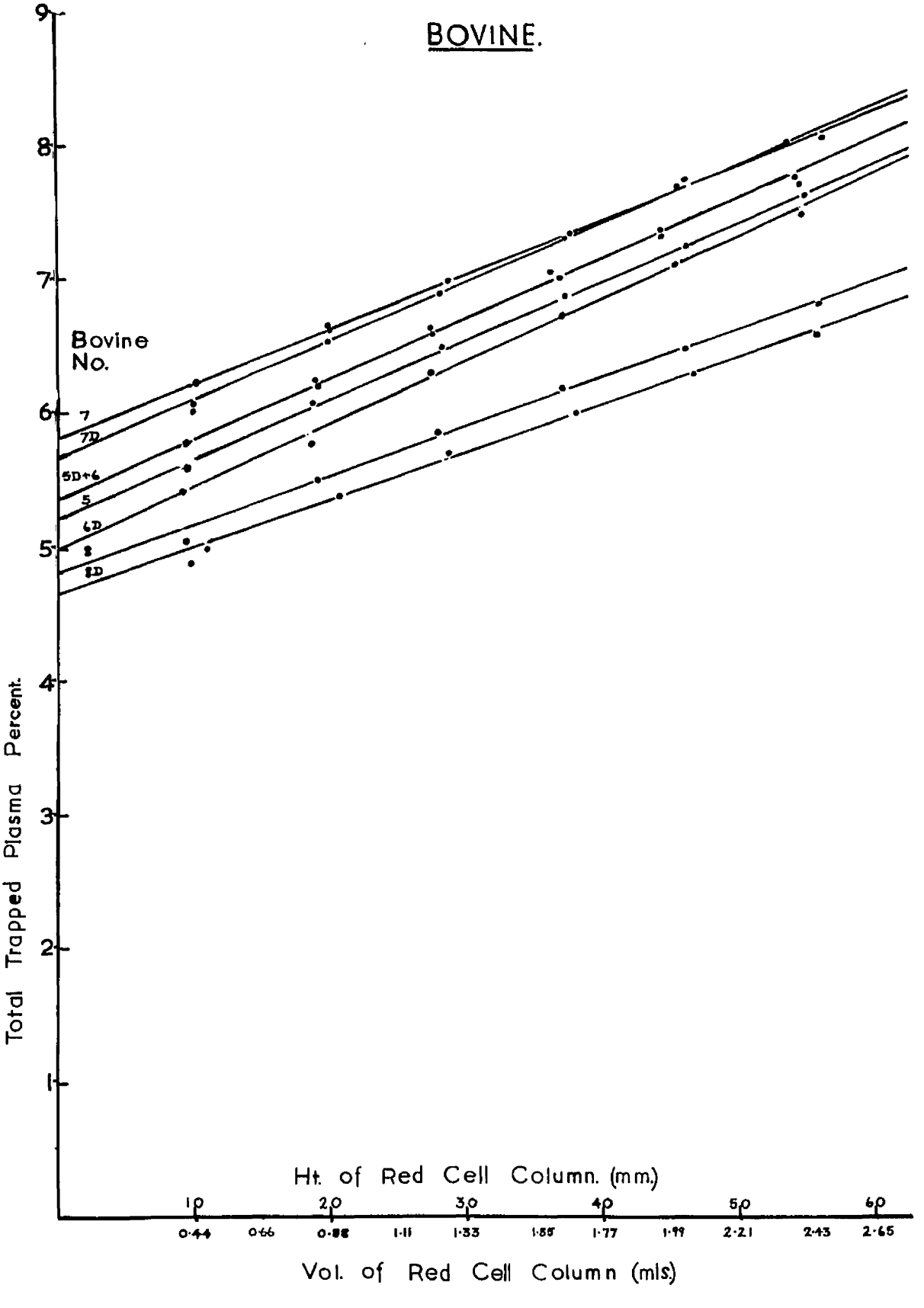


FIGURE 9

The proportion of trapped plasma in relation to the height of the red cell column.

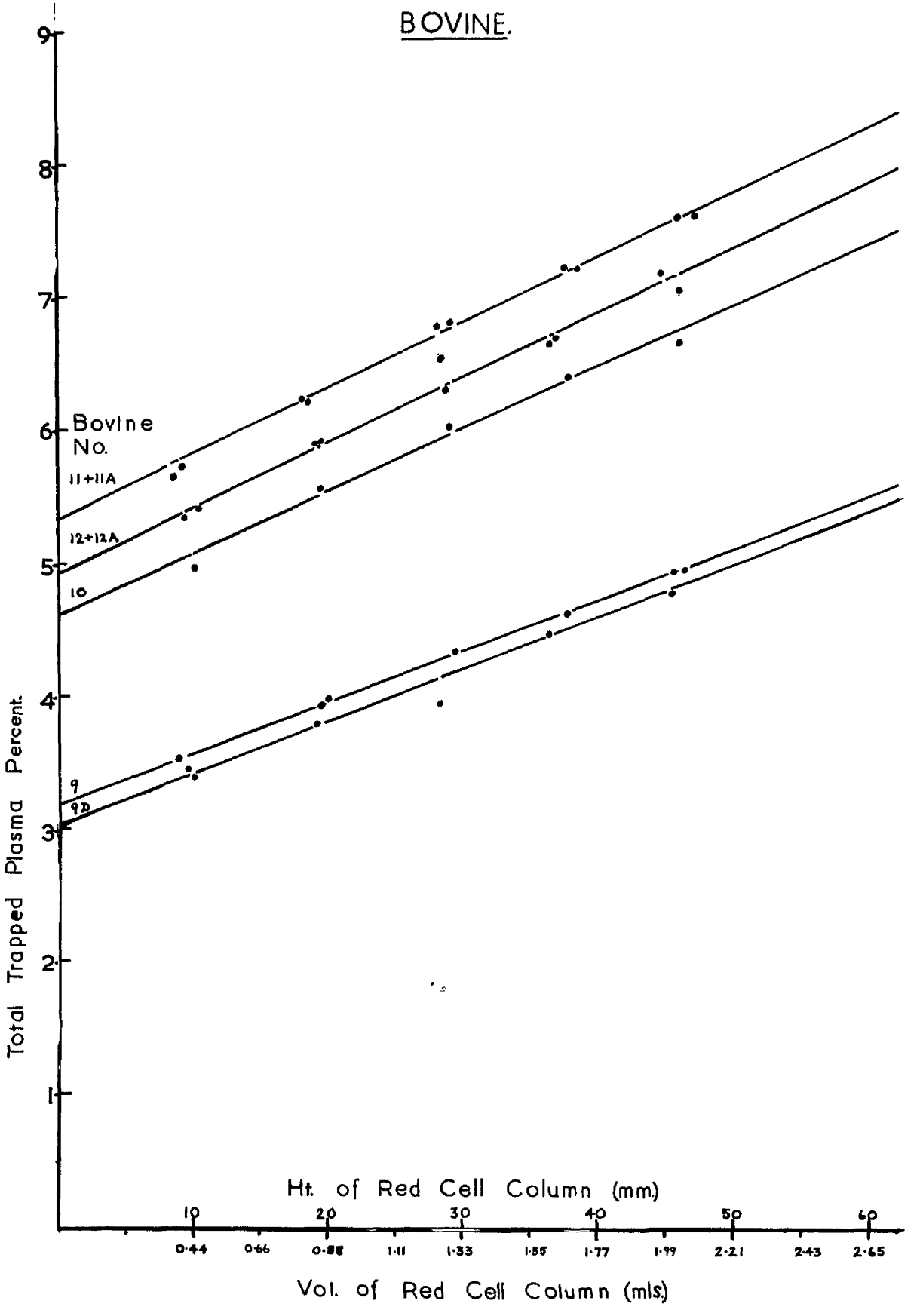


FIGURE 10

The proportion of trapped plasma in relation to the height of the red cell column.

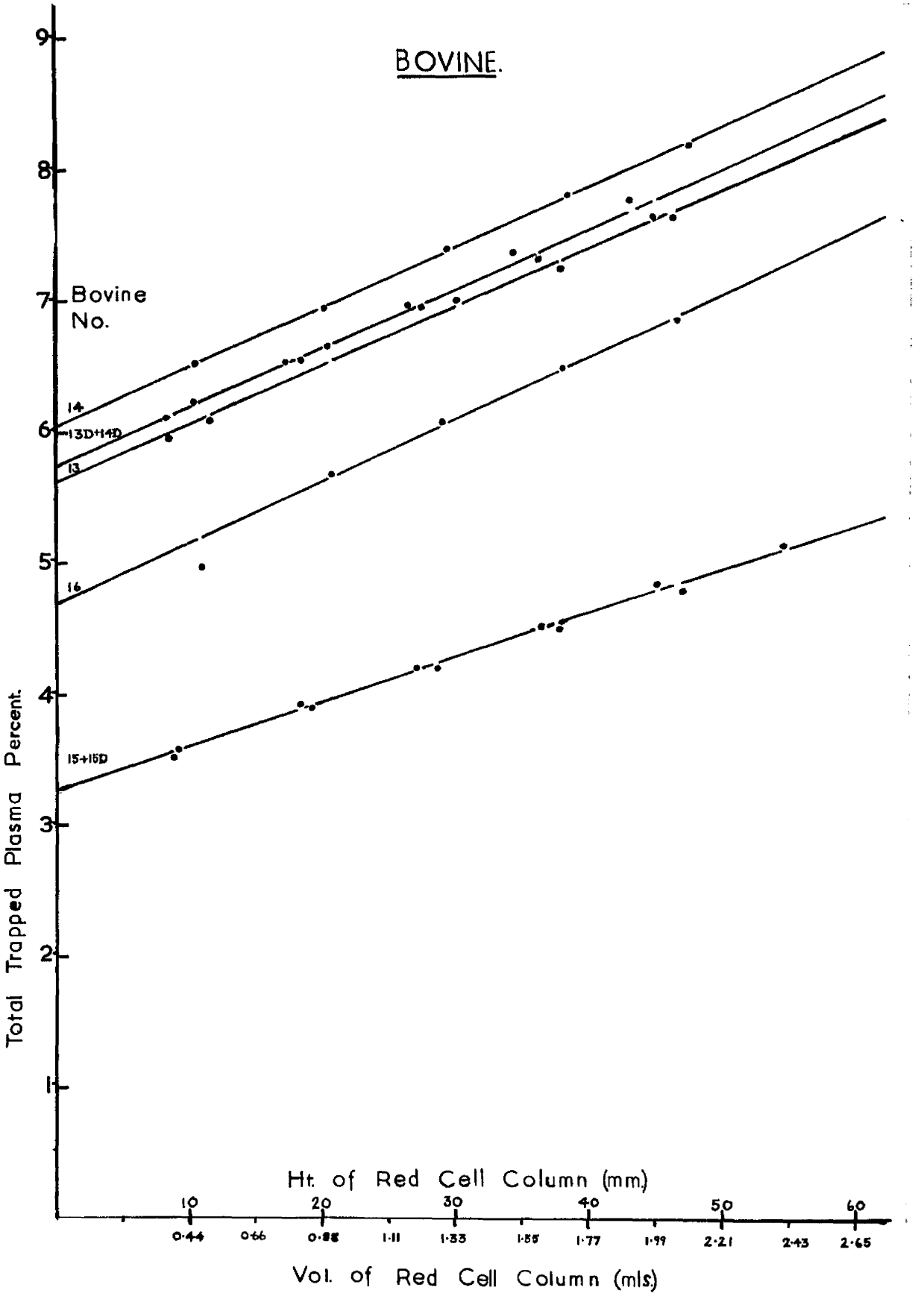


FIGURE 11

The proportion of trapped plasma in relation to the height of the red cell column.

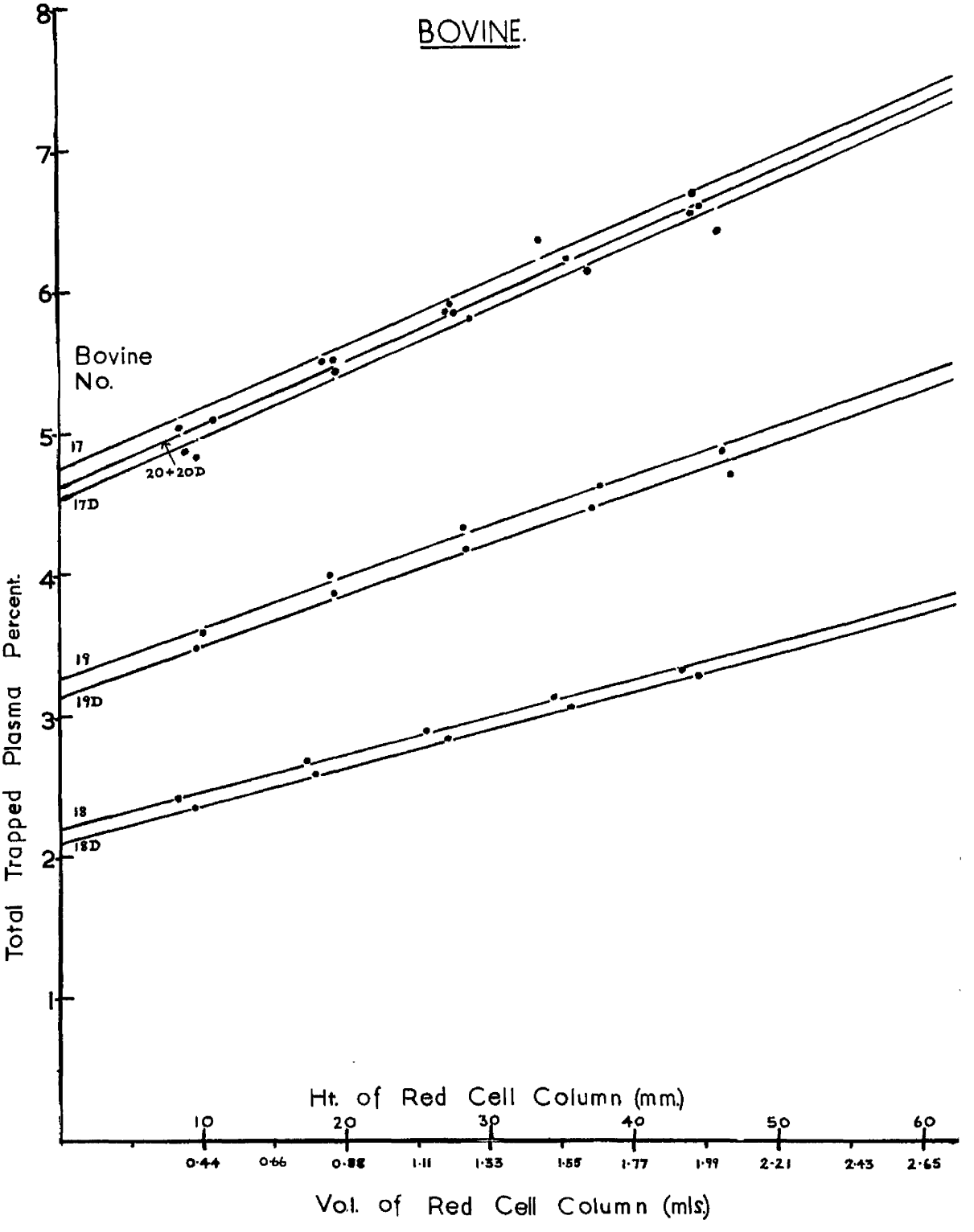


TABLE 20

THE PERCENTAGE OF PLASMA IN THE RED CELL COLUMN OF THE BLOOD OF 20 COWS

RESULTS OBTAINED BY READING INDIVIDUAL HAEMATOCRITS AGAINST INDIVIDUAL GRAPHS

Bovine No.	Ht	% Trapped Plasma	Bovine No.	Ht	% Trapped Plasma
1	30.6	4.6	11	38.9	7.3
1*	30.5	4.5	11*	38.9	7.3
2	30.5	6.8	12	36.8	6.5
2*	31.9	7.1	12*	36.8	6.5
3	32.7	4.4	13	38.2	7.3
3*	32.4	4.5	13*	38.2	7.5
4	34.8	6.7	14	40.0	7.9
4*	34.5	6.4	14*	40.0	7.5
5	36.2	6.8	15	39.9	4.6
5*	37.0	7.1	15*	39.9	4.6
6	35.4	7.0	16	40.3	6.6
6*	35.2	6.6	17	37.0	6.4
7	35.2	7.3	17*	37.0	6.2
7*	35.5	7.2	18	39.1	3.2
8	38.6	6.2	18*	39.1	3.2
8*	38.8	6.1	19	36.9	4.5
9	37.9	4.6	19*	36.9	4.5
9*	37.9	4.5	20	43.4	6.6
10	36.7	6.3	20*	43.4	6.6

* = Duplicate

Mean 6.0 ± 1.3

Range 3.2 - 7.9

Summary

The proportion of trapped plasma in sections of the haematocrits of 'cell enriched' bovine bloods was determined after centrifugation for 2 hours. Mean and individual graphs were constructed by plotting the proportion of trapped plasma against the height of the red cell column.

Haematocrits of equivalent 'ordinary' bloods were determined. Trapped plasma was estimated by reading the haematocrits against both the mean graph and individual graphs. There was fairly good correlation between those estimations, but the results obtained by reading off individual graphs showed rather a wide range and it was thought that longer centrifugation might reduce this.

(b) The Estimation of Trapped Plasma in Bovine Blood After 3 Hours Centrifugation

Duplicate samples of heparinised blood from 28 bovine animals were prepared for centrifugation as in the last experiment, but centrifugation was continued for 3 hours. It was decided to estimate the total trapped plasma in the red cell column, but not to estimate its distribution throughout the column. Accordingly, after quick-freezing the red cell column was cut immediately below the buffy coat and the radioactivity in the distal block of cells estimated and compared with that in the corresponding supernatant plasma. The results are shown in Table 21. The mean and range are not significantly different from the figures determined when

TABLE 21

BOVINE % TRAPPED PLASMA 1500 g 25°C 3 HOURS

Cow No.	% Trapped Plasma	Cow No.	% Trapped Plasma	Cow No.	% Trapped Plasma
1	5.4	11	4.4	21	7.4
2	6.5	12	6.6	22	7.8
3	7.8	13	6.9	23	4.6
4	6.6	14	6.9	24	6.8
5	7.1	15	7.3	25	6.2
6	4.2	16	6.3	26	3.2
7	6.1	17	4.6	27	4.5
8	4.1	18	6.3	28	6.5
9	4.5	19	7.2		
10	7.0	20	6.0		

Mean 6.1 ± 1.3

Range 3.2 - 7.8

centrifugation was carried out for only 2 hours.

(c) The Estimation of Trapped Plasma in Sheep Blood After 2½ hours Centrifugation

Trapped plasma gradation and total trapped plasma in the red cell column of the blood of sheep was studied in the same way as had been done in the bovine. Heparinised blood samples were obtained from 13 sheep and duplicate standard haematocrit tubes prepared for each animal. The remaining blood of each animal was 'enriched' by removal of plasma and duplicate samples in polythene tubes centrifuged with the Wintrobe tubes. After centrifugation the haematocrits were read and the polythene tubes frozen, cut into 1 cm sections, and measured as has been described for the bovine. An example of the range of figures obtained is given in Table 22 and Figure 12. Again the length of each section was calculated and graphs constructed by plotting the percentage of trapped plasma found in a section against the height of the haematocrit. An individual graph was prepared for each animal and a mean graph prepared from the average figures. Estimations of trapped plasma were made by reading off individual graphs and the mean graph according to the haematocrit of each sheep and the results are shown in Table 23. The figures derived from mean and individual graphs are so similar that no gross error would occur with the use of a correction factor for 'trapped plasma' if that was obtained by reading off the 'mean' graph. The average

TABLE 22

DISTRIBUTION OF TRAPPED PLASMA IN THE RED CELL COLUMN OF THE ENRICHED BLOOD OF THE SHEEP AFTER CENTRIFUGATION AT 1500 g AND 25°C FOR 2½ HOURS

	Tube Section No.	Volume (mls)	% Trapped Plasma
Average of 13 sheep duplicate samples	1	0.41	2.8
	1 + 2	0.84	3.3
	1 + 2 + 3	1.25	3.7
	1 + 2 + 3 + 4	1.65	4.1
	1 + 2 + 3 + 4 + 5	2.05	4.5
Maximum (Sheep 13A)	1	0.41	3.0
	1 + 2	0.85	3.6
	1 + 2 + 3	1.30	4.1
	1 + 2 + 3 + 4	1.70	4.5
	1 + 2 + 3 + 4 + 5	2.10	4.8
	1 + 2 + 3 + 4 + 5 + 6	2.37	5.1
Minimum (Sheep 2A)	1	0.35	2.5
	1 + 2	0.78	2.8
	1 + 2 + 3	1.19	3.2
	1 + 2 + 3 + 4	1.62	3.6
	1 + 2 + 3 + 4 + 5	1.99	3.9
	1 + 2 + 3 + 4 + 5 + 6	2.24	4.2

FIGURE 12

The Proportion of trapped plasma in Relation to the height of the red cell column.

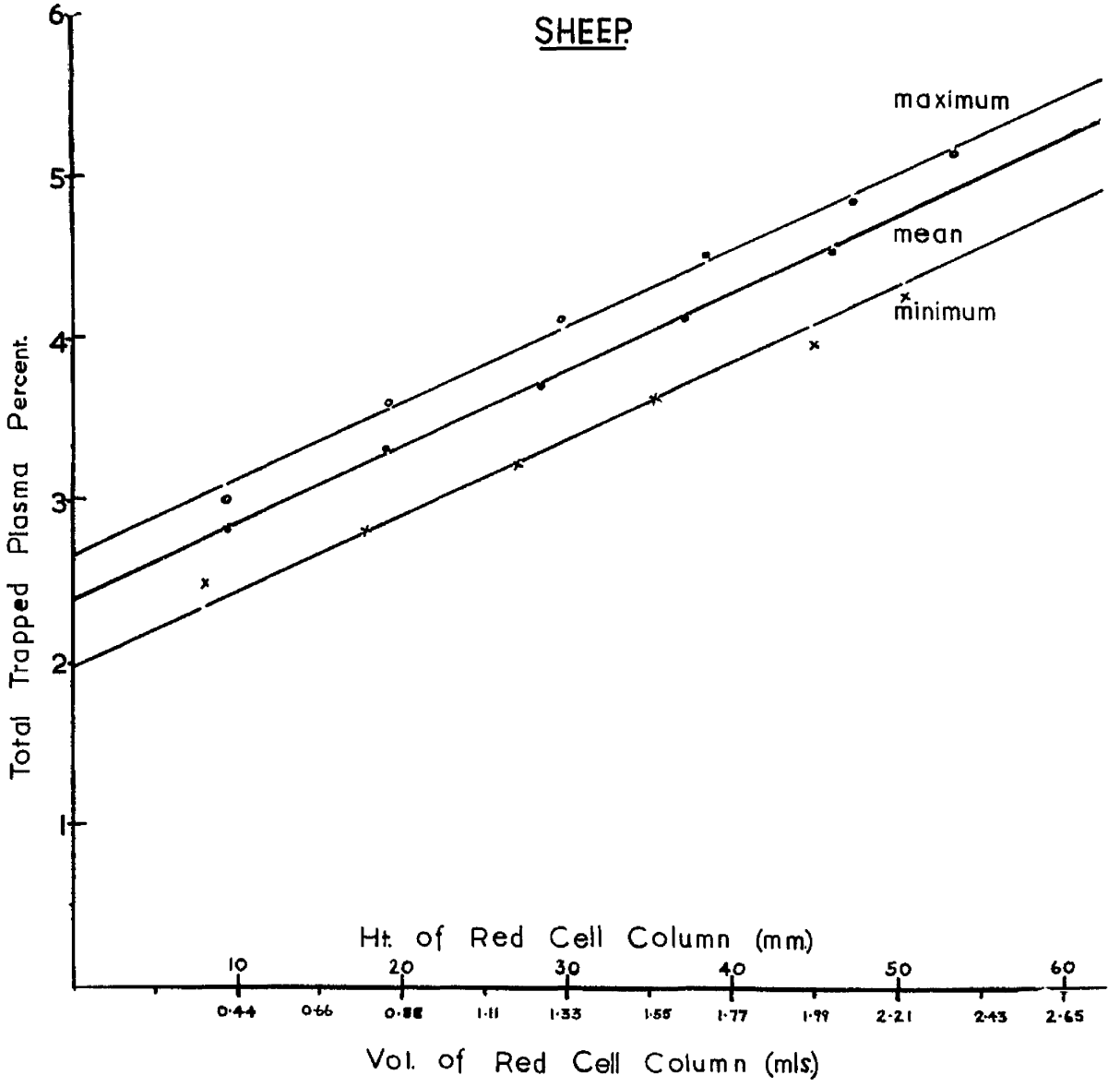


TABLE 23

THE PERCENTAGE OF TRAPPED PLASMA IN THE RED CELL COLUMN OF THE SHEEP AFTER CENTRIFUGATION AT 1500 *g* AND 25°C FOR 2½ HOURS

Sheep No.	Ht	Percentage Trapped Plasma	
		From Mean Graph	From Individual Graph
1	22.4	3.4	3.7
2	29.3	3.8	3.4
3	32.5	3.9	3.9
4	23.8	3.5	3.5
5	32.7	3.9	3.9
6	35.2	4.0	3.8
7	23.1	3.5	3.3
8	31.3	3.8	4.0
9	36.7	4.1	4.2
10	44.0	4.5	4.6
11	41.4	4.3	4.5
12	43.9	4.4	4.5
13	40.2	4.3	4.6
	Mean	4.0 ± 0.4	4.0 ± 0.5
	Range	3.4 - 4.5	3.3 - 4.6

trapped plasma estimated by reading off individual graphs has a narrower range than similarly estimated figures on bovine blood.

(d) The Estimation of Trapped Plasma in the Blood of the Pig After 1 Hour's Centrifugation

The study of the distribution of trapped plasma in the red cell column of the blood of the pig was carried out as for the bovine and sheep. Blood was taken from 21 animals and duplicate Wintrobe haematocrit tubes filled from each. Duplicate enriched samples were then prepared and centrifuged in polythene tubes. An indication of the range of figures obtained is given in Table 24 and Figure 13. A mean graph and individual graphs were drawn and the percentage of trapped plasma for each animal read off according to the haematocrit of its normal blood. The results are shown in Table 25. It can be seen that estimations based on the mean graph have a good correlation with those based on individual graphs.

(e) The Estimation of Trapped Plasma in the Blood of the Dog After 1 Hour's Centrifugation

Trapped plasma in the red cell column of canine blood was examined in duplicate samples from 13 dogs as previously described for cow, sheep and pig. The findings are summarised on Tables 26 and 27, and Figure 14.

TABLE 24

DISTRIBUTION OF TRAPPED PLASMA IN THE RED CELL COLUMN OF THE ENRICHED BLOOD OF THE PIG AFTER CENTRIFUGATION AT 1500 g AND 25°C FOR 1 HOUR

	Tube Section No.	Volume (mls)	% Trapped Plasma
Average of 21 pigs	1	0.44	2.7
	1 + 2	0.85	3.1
	1 + 2 + 3	1.24	3.5
	1 + 2 + 3 + 4	1.58	3.8
	1 + 2 + 3 + 4 + 5	2.00	4.1
	1 + 2 + 3 + 4 + 5 + 6	2.38	4.3
Maximum (Pig 15A)	1	0.49	2.4
	1 + 2	0.89	3.6
	1 + 2 + 3	1.28	4.0
	1 + 2 + 3 + 4	1.65	4.4
	1 + 2 + 3 + 4 + 5	2.06	4.8
	1 + 2 + 3 + 4 + 5 + 6	2.42	5.1
Minimum (Pig 12A)	1	0.42	2.4
	1 + 2	0.85	2.8
	1 + 2 + 3	1.22	3.1
	1 + 2 + 3 + 4	1.62	3.4
	1 + 2 + 3 + 4 + 5	2.00	3.6
	1 + 2 + 3 + 4 + 5 + 6	2.39	3.8

FIGURE 13

The proportion of trapped plasma in relation to the height of the red cell column.

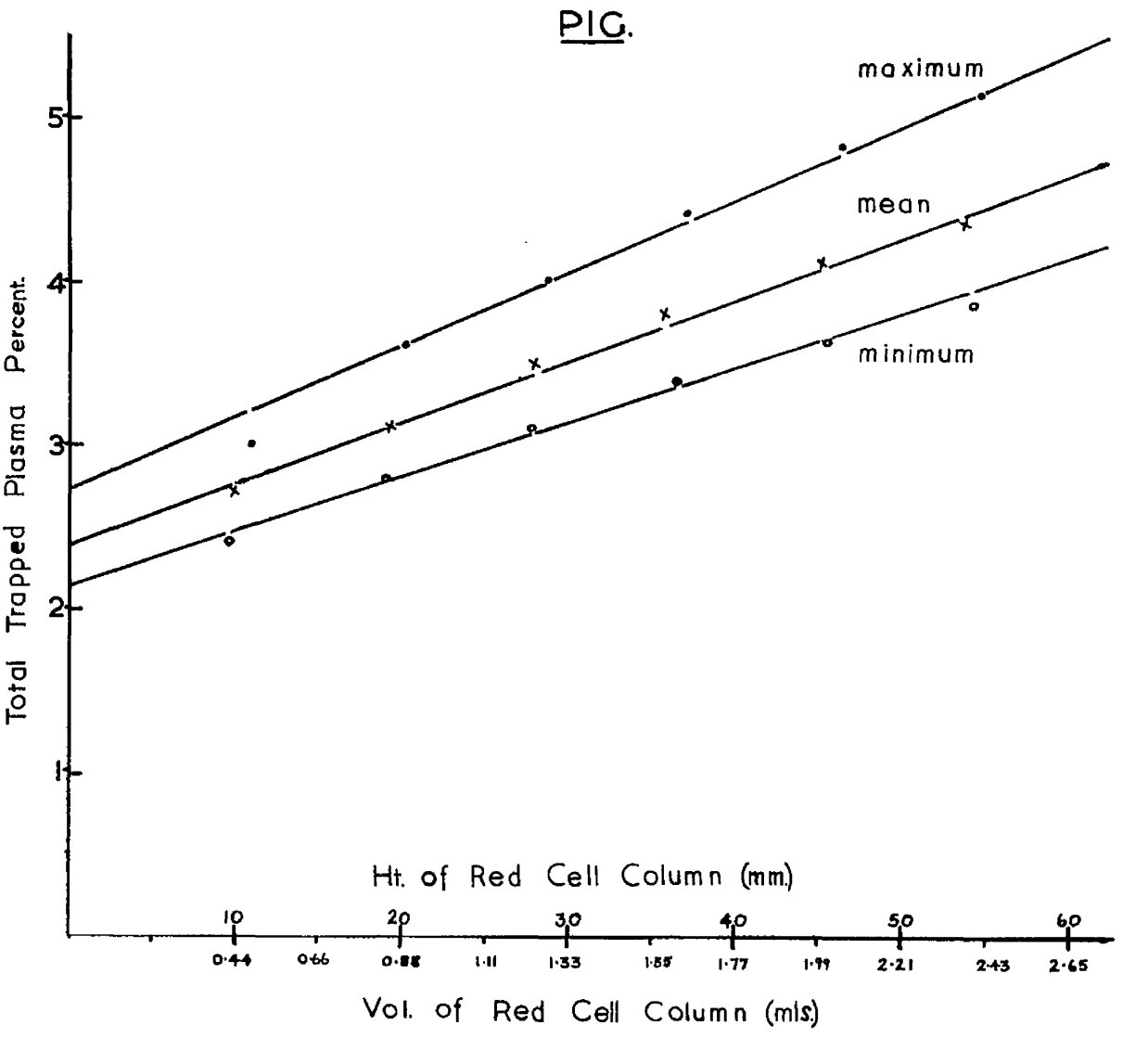


TABLE 25

THE PERCENTAGE OF TRAPPED PLASMA IN THE RED CELL COLUMN OF THE PIG AFTER CENTRIFUGATION AT 1500 g AND 25°C for 1 HOUR

Pig No.	Ht	Percentage Trapped Plasma	
		From Mean Graph	From Individual Graph
1	46.4	4.0	4.3
2	43.3	3.9	4.1
3	44.2	3.9	3.9
4	43.9	3.9	4.5
5	45.8	4.0	3.9
6	44.5	4.0	4.1
7	42.6	3.9	3.9
8	47.8	4.1	4.0
9	44.3	4.0	3.8
10	48.6	4.1	3.9
11	45.9	4.0	4.1
12	46.6	4.0	3.7
13	46.5	4.0	3.9
14	45.6	4.0	3.7
15	44.9	4.0	4.7
16	51.0	4.2	4.2
17	47.0	4.1	4.2
18	43.0	3.9	4.1
19	40.8	3.8	4.3
20	46.5	4.0	4.4
21	44.7	4.0	4.0
		Mean 4.0 ± 0.1	Mean 4.1 ± 0.2
		Range 3.8 - 4.2	Range 3.7 - 4.7

TABLE 26

DISTRIBUTION OF TRAPPED PLASMA IN THE RED CELL COLUMN OF THE ENRICHED BLOOD OF THE DOG AFTER CENTRIFUGATION AT 1500 g AND 25°C FOR 1 HOUR

	Tube Section No.	Volume (mls)	% Trapped Plasma
Average of 13 dogs	1	0.44	3.0
	1 + 2	0.86	3.3
	1 + 2 + 3	1.26	3.6
	1 + 2 + 3 + 4	1.67	3.8
	1 + 2 + 3 + 4 + 5	2.06	4.0
	1 + 2 + 3 + 4 + 5 + 6	2.48	4.2
Maximum	1	0.41	3.3
	1 + 2	0.88	3.7
	1 + 2 + 3	1.29	3.9
	1 + 2 + 3 + 4	1.66	4.2
	1 + 2 + 3 + 4 + 5	1.99	4.4
	1 + 2 + 3 + 4 + 5 + 6	2.38	4.6
Minimum	1	0.40	2.8
	1 + 2	0.80	3.0
	1 + 2 + 3	1.20	3.2
	1 + 2 + 3 + 4	1.62	3.5
	1 + 2 + 3 + 4 + 5	1.99	3.7
	1 + 2 + 3 + 4 + 5 + 6	2.42	3.8

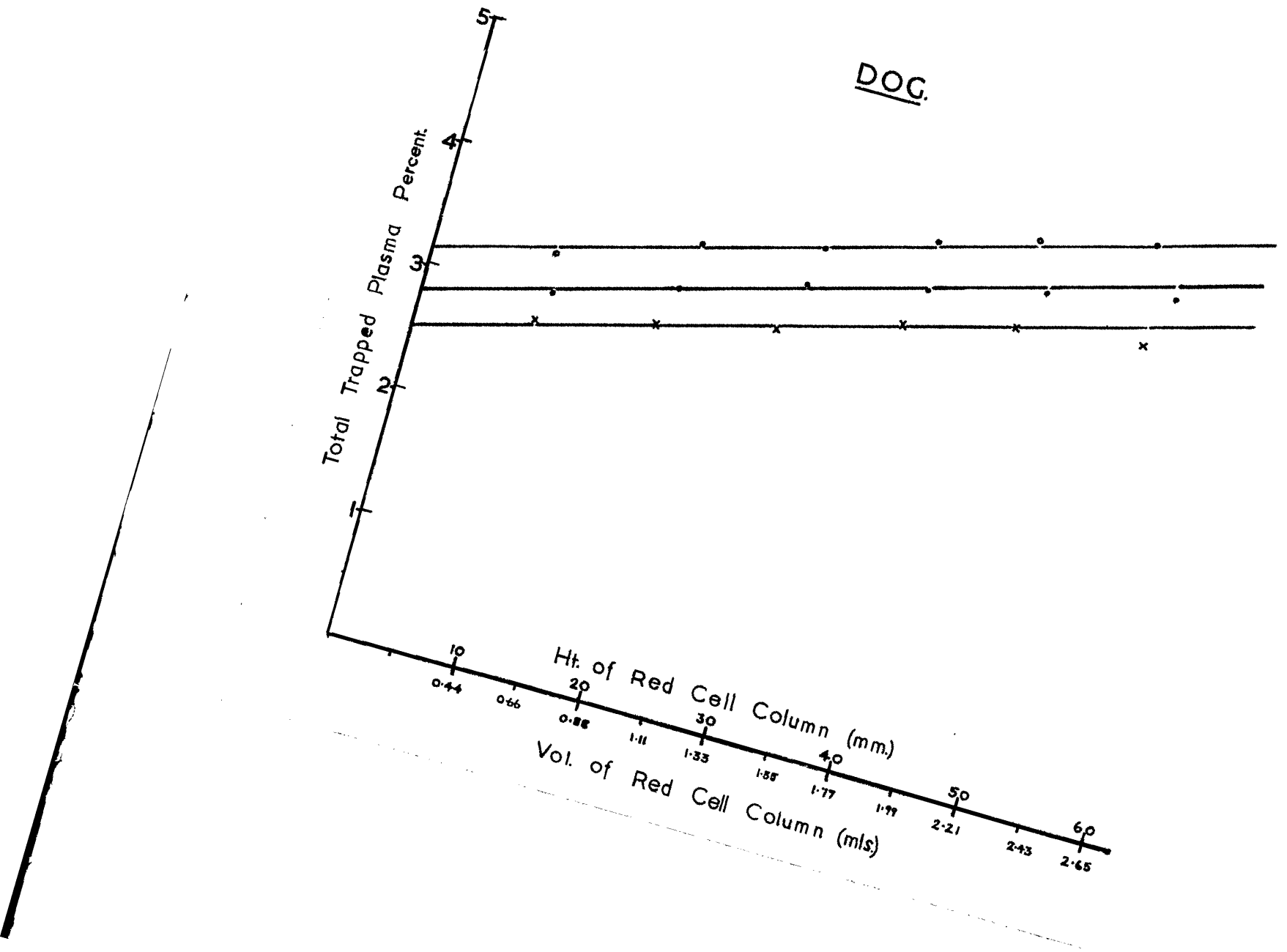
TABLE 27

THE PERCENTAGE OF TRAPPED PLASMA IN THE RED CELL COLUMN OF THE DOG AFTER CENTRIFUGATION AT 1500 g AND 25°C FOR 1 HOUR

Dog No.	Ht	Percentage Trapped Plasma	
		From Mean Graph	From Individual Graph
1	49.2	4.0	4.0
2	50.8	4.1	3.7
3	50.2	4.0	3.9
4	41.3	3.8	3.7
5	39.5	3.8	3.5
6	52.5	4.1	4.5
7	4.69	4.0	4.2
8	55.8	4.2	4.2
9	57.7	4.2	4.1
10	53.1	4.1	4.4
11	48.4	4.0	3.1
12	50.8	4.1	4.0
13	50.7	4.1	4.0
		Mean 4.0 ± 0.1	Mean 4.0 ± 0.3
		Range 3.8 - 4.2	Range 3.5 - 4.5

The proportion of trapped plasma in relation to the height of the red cell column.

FIGURE 14



(f) The Estimation of Trapped Plasma in the Blood of the Horse After 1 Hour's Centrifugation

Heparinised blood samples were obtained from 10 horses. Wintrobe haematocrit tubes were filled then red-cell enriched samples were prepared for centrifugation in polythene tubes. Homologous ^{131}I-labelled albumin was added to those samples which were mixed, centrifuged, frozen, cut and examined as previously described for other species. Individual and mean graphs were drawn and the results are shown in Table 28, Figure 15 and Table 29. It can be seen that estimations of trapped plasma made by reading the haematocrit against the mean graph are fairly close to estimations made by reading off individual graphs.

The distribution of trapped plasma in the haematocrits of the different species is illustrated in Figure 16.

TABLE 28

DISTRIBUTION OF TRAPPED PLASMA IN THE RED CELL COLUMN OF THE ENRICHED BLOOD OF THE HORSE AFTER CENTRIFUGATION AT 1500 g AND 25°C FOR 1 HOUR

	Tube Section No.	Volume (mls)	% Trapped Plasma
Average of 10 horses	1	0.44	1.8
	1 + 2	0.87	2.3
	1 + 2 + 3	1.28	2.5
	1 + 2 + 3 + 4	1.08	3.0
	1 + 2 + 3 + 4 + 5	2.06	3.0
	1 + 2 + 3 + 4 + 5 + 6	2.43	3.2
Maximum (No. 7)	1	0.44	1.9
	1 + 2	0.86	2.4
	1 + 2 + 3	1.27	2.8
	1 + 2 + 3 + 4	1.48	3.1
	1 + 2 + 3 + 4 + 5	2.04	3.3
	1 + 2 + 3 + 4 + 5 + 6	2.47	3.6
Minimum (No. 5)	1	0.48	1.7
	1 + 2	0.90	2.0
	1 + 2 + 3	1.32	2.3
	1 + 2 + 3 + 4	1.71	2.5
	1 + 2 + 3 + 4 + 5	2.11	2.7
	1 + 2 + 3 + 4 + 5 + 6	2.53	2.9

FIGURE 15

The proportion of trapped plasma in relation to the height of the red cell column.

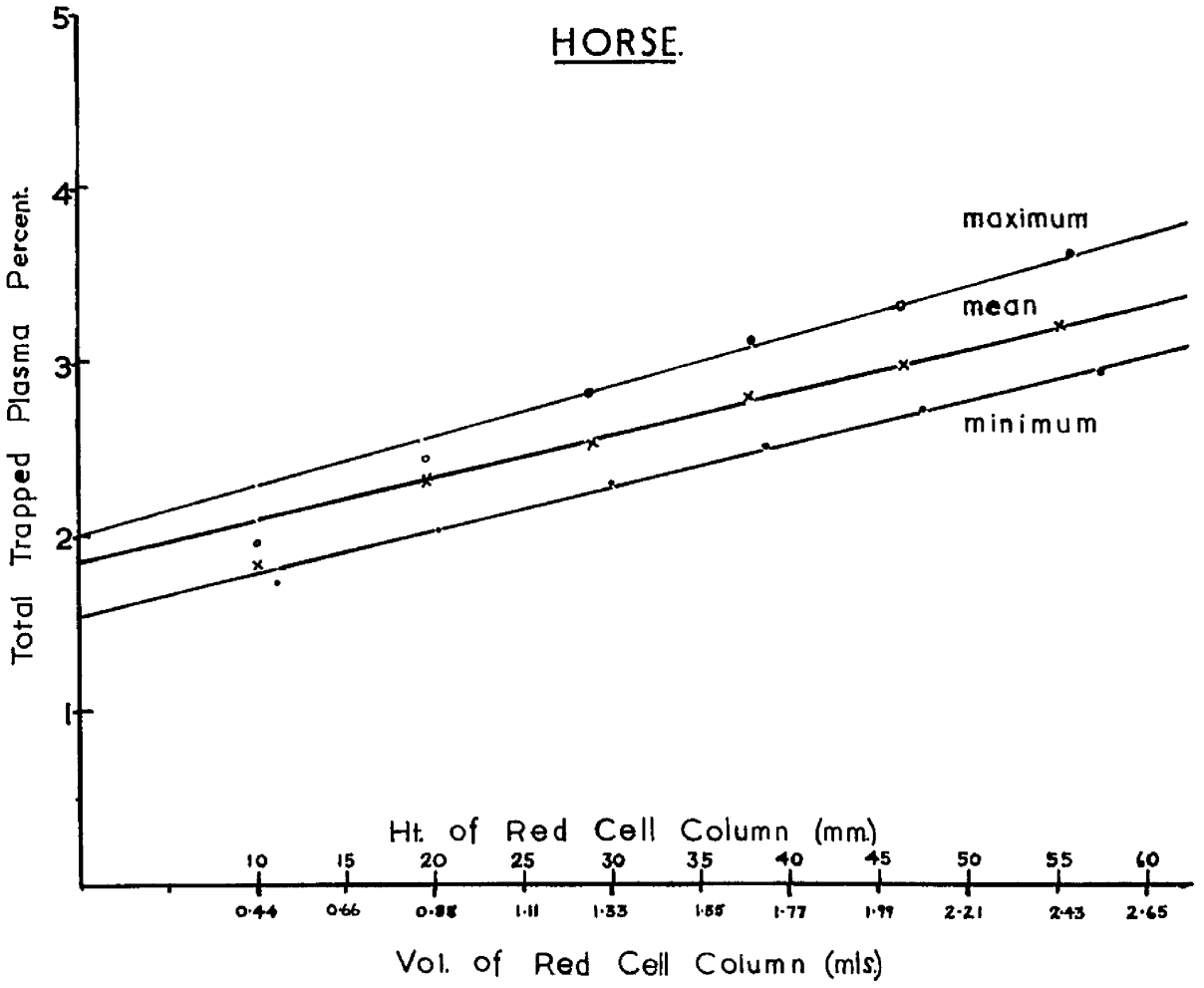


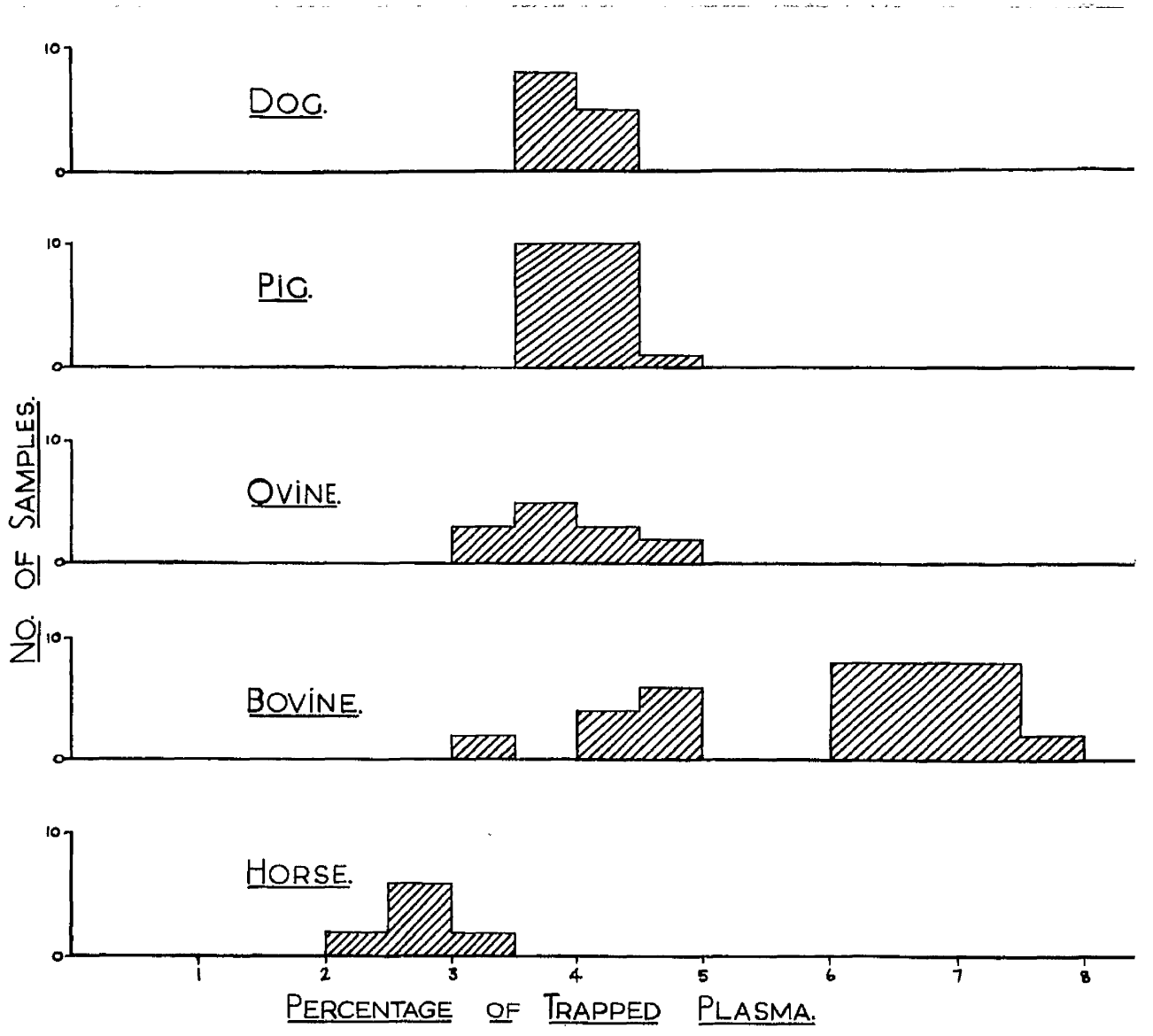
TABLE 29

THE PERCENTAGE OF TRAPPED PLASMA IN THE RED CELL COLUMN OF THE BLOOD OF THE HORSE AFTER CENTRIFUGATION AT 1500 *g* AND 25°C FOR 1 HOUR

Horse No.	Ht	Percentage Trapped Plasma	
		From Mean Graph	From Individual Graph
1	36.5	2.7	2.5
2	37.1	2.7	2.7
3	35.4	2.7	2.7
4	34.9	2.7	2.6
5	37.2	2.7	2.4
6	40.5	2.8	2.7
7	37.7	2.7	3.1
8	50.2	3.0	3.3
9	36.0	2.7	2.8
10	30.1	2.6	2.7
		Mean 2.7 ± 0.1	Mean 2.8 ± 0.3
		Range 2.6 - 3.0	Range 2.4 - 3.3

FIGURE 16

Percentage of trapped plasma in the haematocrit of different species.



DISCUSSION

In the preliminary experiment (A), estimation of the proportion of "trapped" plasma in the haematocrits of rabbit, dog, horse and cow by the direct method showed that the amount in bovine blood was obviously in a different category and much larger than in the other bloods.

It was decided to study the effect of increasing the time of centrifugation on haematocrit determinations of bovine, sheep, dog, rabbit, pig and horse bloods. Because it was thought that prolonged centrifugation might cause over-heating in the ordinary centrifuge a refrigerator centrifuge was used and set at 15°C. The results showed that dog, rabbit, pig and horse blood had cells which packed fairly well after one hour, and bovine and sheep samples required 2-3 hours spinning time. Hodgetts (1959), Kennedy and Millikan (1938) and Chien, Dellenback, Usami and Gregersen (1965) had recorded that sheep cells did not pack as readily as those of some other species on centrifugation.

Estimations of intercellular plasma were made on bovine blood after centrifugation for 2 hours at 1500 g and 15°C. As haematocrits had reduced with increased time of spinning it was expected that the trapped plasma would also be less than that found in the preliminary experiment (A). However little difference was found and an experiment was carried out to check the validity of the method of estimation of trapped plasma by

the direct method. It appeared that the method was valid.

It was then considered that the only real difference between centrifugation conditions during the first 30 minutes in the preliminary experiment (A) and in the later estimation of bovine trapped plasma at 15°C, was that of temperature.

The effect of the temperature of the centrifuge on haematocrit determinations of bloods of various species was then studied and increase in temperature was found to be associated with reduction in the height of the haematocrit. The effect of the temperature of the centrifuge was then studied with regard to its effect on the percentage of plasma trapped in the blood of the pig. Increase in the temperature of the centrifuge was found to be associated with a lowering of the amount of trapped plasma.

This effect of the centrifuge temperature had not been recorded previously in the literature, and it explained the apparent anomaly that after 2 hours centrifugation the amount of trapped plasma in some bloods centrifuged at 15°C was no less than that found when centrifugation at the same RCF had been carried out for only 30 minutes in a centrifuge without temperature control, which ran at a higher temperature than 15°C.

All later experiments were carried out with the centrifuge at 25°C, and with an RCF of 1500 g.

A modified analytical method was devised which proved effective in the study of the distribution of trapped plasma at different levels of the packed red cell column.

It was desired to study the distribution of trapped plasma in red cell columns which were at the top of or beyond the normal range, so bloods were prepared with an artificially increased content of red cells by removal of some of the plasma. Experiments were carried out which showed that the increased amount of red cells did not change the proportion of trapped plasma in the lower sections of the column.

Accurate determinations of trapped plasma in the bloods of different species were then performed. For each species mean and individual graphs were prepared by plotting the proportion of trapped plasma against the height of the red cell column. Hlad and Holmes (1953), on theoretical considerations, stated that the distribution of trapped plasma in the haematocrit could not be shown by a straight line. They pointed out that under reasonable conditions of centrifugation the amount of trapped plasma in the bottom of the tube would probably be very small whereas at the top of the red cell column or in the buffy coat the proportion might well be over 50%. In the experiments at present being discussed the buffy coat was excluded and in practice the distribution of trapped plasma was found to be linear. Hodgetts (1959) also found this distribution to be linear.

Trapped plasma was estimated by reading off mean and individual graphs for each species, reading the proportion of trapped plasma against the height of the haematocrit. There was fairly good agreement between estimations made from mean graphs and from individual graphs although in the case of the bovine the readings from the mean graph were subject to a degree of error greater than that found in results in other species.

The finding of 6% trapped plasma in the haematocrit of cow blood agrees with the results of Reynolds (1953a) but as the conditions of centrifugation she used were 1260 g for 30 minutes she might well have found a much greater percentage than recorded. The findings in the sheep were of a trapped plasma somewhat higher than that recorded by Schambye (1952b) and Hodgetts (1959). The former used an indirect method of estimation and while the latter used a direct method she obtained the red cells for intercellular plasma estimation by first removing the supernatant plasma by frequent pipetting. Although this was done carefully there was some danger that the cells were a little disturbed and some of the saline used for washing entered the trapped plasma and reduced its radioactivity per unit volume, which would make it appear that there was less plasma present that there was in fact. Hodgetts however used an RCF of 2756 g and it is possible that the samples did in fact contain less trapped plasma than was found in the experiments

being discussed at present. The trapped plasma recorded in sheep blood was less than that recorded by Chien et al (1965) when they used the same RCF, but for a shorter time. When they used an RCF of 15000 g in capillary haematocrit tubes they reduced the proportion of trapped plasma, but still found it higher than in non-ruminant species.

SUMMARY

1. Species differences in the intercellular plasma of the haematocrit were described for the first time. It was shown that under conditions of centrifugation suitable for human blood, the red cell columns of bovine, sheep and pig bloods contained anomalously large amounts of trapped plasma.

2. While more appropriate centrifuge conditions for bovine blood were being investigated an important temperature effect on trapped plasma was observed. This had not been described previously.

3. A direct method for measurement of trapped plasma was developed which took into account the variation in trapped plasma along the red cell column. Graphs have been prepared from the results on distribution of trapped plasma in the red cell column, from which trapped plasma can be estimated if the haematocrit is determined.

PART 2

BLOOD VOLUME MEASUREMENTS ON DOMESTICATED ANIMALS

INTRODUCTION

The estimation of blood volume by the dilution technique is based on the injection into the circulation of a measured amount of some test substance associated with either the red cells or the plasma, and the determination of its "dilution". A great deal of the literature is concerned with refinements of methods of estimation of dye dilution in the attempt to attain a high degree of accuracy, but these will not be dealt with in detail.

The Blue Dye T-1824 (Evans' Blue)

After injection of this dye there may be some difficulty in assessing its dilution in a plasma sample because of lipaemia or haemolysis. Such difficulties may be overcome by extraction of the dye from the sample as described by Campbell, Frohman and Reeve (1958). Allen, Ochoa, Roth and Gregersen (1953) have shown that the spectral absorption curve of T-1824 is not the same in all species of animals.

T-1824 was used by Cronin (1954) and Courtice (1943) to estimate plasma volume in a small number of horses and it was considered a reasonably satisfactory method, even though the plasma of equine blood is naturally of a rather deep yellow or light brown colour. Stahl and Dale (1958) found that in repeat estimations on the cow the disappearance curve of the dye was anomalous, and there was an anomalous rise in the T-1824 plasma

volume. Reynolds (1953) commented that the disappearance rate of the dye from the circulation of the cow was greater than that in man or the dog. She found that the mean mixing time of the dye with the general circulation was 7.7 minutes, with a range of 5-10 minutes. Lipaemia was not found to be a hazard in bovine animals. Schambye (1952b) noted that the time-concentration levels of the dye in the plasma of the sheep showed a three phase curve. When repeat estimations were made different methods of correcting for dye already present resulted in fairly wide differences in the results obtained. Courtice (1943) found that this dye disappeared from the circulating plasma of the goat more readily than from the plasma of the dog. Klement, Ayer and Rogers (1955) estimated that complete mixing in the blood of the goat occurred in 5 minutes after injection. Loss from the plasma in the first 30 minutes varied from 6 to 28% and the authors commented that this was higher than reported for other species. They pointed out that as the loss rate did not appear to be uniform, errors in plasma volume determinations might have been larger than expected.

^{131}I-Albumin

Gibson, Seligman, Peacock, Aub, Fine and Evans (1946) compared the use of ^{131}I-albumin with T-1824 and in general the results were similar. Some commercial preparations contained excessive free ^{131}I (Crane and Adams, 1954) necessitating purification by precipitation. Methods of labelling and purification

described previously (Francis, Halligan and Wormall, 1951) overcome this difficulty. The physical half-life of the isotope is 8.07 days.

Radioactivity determinations can be carried out much more simply than dye determinations and alteration of samples either by haemolysis or lipaemia introduces no errors. ^{131}I-albumin may become adsorbed on glassware (Reeve and Franks, 1956), and as the adsorption is progressive an error is introduced if there is undue delay between filling of the injection syringe and delivery. Adsorption may be reduced by the use of carrier iodide in glassware or by detergent (Sear, Allen and Gregersen, 1953). Bovine albumin may induce an immune reaction in other species, however there is no reason why homologous albumin should not always be employed.

^{131}I-albumin was used by Hansard, Butler, Comar and Hobbs (1953) to measure plasma volume in the burro. About 200 µc was injected into each animal. Samples were withdrawn at 15 and 30 minutes post injection for radioactivity determinations. Sear, Allen and Gregersen (1953) reviewed the literature on simultaneous determinations of plasma volume in the dog with ^{131}I-albumin and T-1824 and carried out similar experiments on 13 dogs using ^{131}I-human albumin. Determinations agreed on the average to within 0.5%. The loss rates during the first hour after injection were found to be the same, but over a longer period albumin was retained

longer than T-1824. Hansard et al (1953) carried out plasma determinations on cow, sheep and pig, but were dissatisfied with the results obtained. It appears that workers have been rather slow to adopt isotopic methods for the determination of plasma volumes in farm animals.

^{32}P Cells

The labelling of red cells with ^{32}P and their use for measurement of the circulating red cell volume had been shown to be practicable by Hevesy, Koster, Sørensen, Warburg and Zerahn (1944). The radioactive phosphate ions penetrate the red cells and tend to become fixed as organic phosphorus compounds.

In labelling burro cells for the practical determination of blood volume, Hansard et al (1953) added approximately 150 μc ^{32}P to 15 ml of whole blood from the animal on which the determination was to be performed, and incubated the mixture at 37.5°C for 4 hours. After repeated washing of the red cells and reconstitution of the volume with saline, 10 ml was injected intravenously and a sample taken 10-15 minutes later. It was considered that mixing was probably complete in three minutes. Persistence curves of radioactivity in the circulating blood were not drawn for each burro, but several samples were taken from some animals and a graph plotted with extrapolation back to zero time. When the radioactivity of a 10-15 minute sample was ascertained, the theoretical radioactivity at the time of injection was calculated by reference to the graph.

They considered that their methods were reasonably consistent and practicable as they performed four blood volume estimations on each of three burros at intervals of one week. The mean blood volume estimate for each animal was 62, 61 and 62 ml/Kg and the S.D. 2.0 in each case. They pointed out that the radioactivity of a second or further injection had to be of a degree well above the residual activity of the previous injection or injections. The amounts of ^{32}P injected into cows were similar to those used in the burro. Bush, Jensen, Cartwright and Wintrobe (1955) incubated about 50 µc ^{32}P with 6 ml of homologous blood for one hour when estimating blood volume in the pig. Blood samples were taken 10-15 minutes post injection for radioactive assay. During the estimation the pigs were anaesthetised with pentobarbital.

^{51}Cr and Combined Methods

Sterling and Gray (1950) showed that red cells readily took up ^{51}Cr and this isotope will be discussed further in Part II Section (B). True blood volume can only be determined when simultaneous measurement of the red cell volume and plasma volume is carried out. Stahl and Dale (1958) used ^{51}Cr-labelled red cells in combination with T-1824 for blood volume estimation in calves. Hodgetts (1961) used the same labels in sheep. Schambye (1952b) used T-1824 and ^{32}P cells simultaneously in twelve sheep. T-1824 was used in conjunction with ^{51}Cr-labelled red cells by Klement, Ayer and Rogers (1955) in estimations on the goat.

Calculation of Blood Volume

Some aspects of calculation of blood volume have been dealt with in the general introduction. If only plasma volume is determined directly, blood volume is calculated from that and the haematocrit. If only the red cells are labelled blood volume may be estimated by assay of a whole blood sample, but the red cell volume and the plasma volume can only be calculated by means of the haematocrit. The total blood volumes calculated in the above manner require to be corrected for the "F cells" factor which relates body haematocrit to venous haematocrit.

The greatest errors in blood volume calculation arise from failure to make an accurate estimation of the amount of material injected and failure to make the correct allowance for trapped plasma in the observed haematocrit. The correction for trapped plasma has been dealt with in Part I. Estimation of the amount of material injected depends on careful weighing or volumetric assay. A suitable apparatus for injection will be described later.

Extrapolation:

Immediately after injection into the circulation the test substance is not mixed homogeneously, therefore a sample taken soon after injection might be too dilute or too concentrated and blood volumes calculated from such samples would be incorrect.

If the injected material escapes too readily from the circulation blood samples will show a reduced concentration so the calculated blood volume will be larger than the true figure. If serial samples are taken after injection and the concentration of the test substance plotted on semi-log paper a disappearance curve related to the loss rate may be shown. The curve on the graph can be extrapolated back to the time of injection whereby the theoretical initial concentration of test substance is indicated. The validity of this method depends on the assumption that the loss rate is correctly estimated on the slope of the disappearance curve (Gregersen and Rawson, 1943). This is probably true, as by using this method of calculation similar results are obtained using test substances with differing loss rates, and high and low concentrations of the same dye give comparable results.

It is of value to estimate plasma proteins and haematocrits on all samples in an attempt to detect any volume change by comparison with the findings on a pre-injection sample. Any such shift can then be allowed for in the interpretation of the test substance time-concentration curve (Gregersen and Rawson, 1959). Extrapolation also overcomes the difficulty of determining the 'mixing time' of the test substance with the circulating blood, which may vary between species and individuals and in some pathological states.

Red cell storage depots:

As the circulation is not equal in all tissues, the injected substance may not always reach some "backwaters" or "depots" where blood movement is sluggish, or it may not be thoroughly mixed in a depot such as the spleen. Gregersen (1953) and Reeve, Gregersen, Allen and Sear (1953) carried out experiments in which it was estimated that the spleen of the dog under nembutal anaesthesia contained approximately one quarter of the total red cells. Hodgetts (1961) found that mixing of test substance after injection into the sheep could be delayed for 30-40 minutes. Some sheep given adrenalin at that time showed a marked decrease in cell radioactivity per unit of whole blood. The T-1824 curves did not alter at the same time so the fall must have been due to unmixed unlabelled cells extruded from the spleen.

Anderson and Rogers (1957) found the haematocrit very variable in goats. When adrenalin was administered it was found to raise the jugular haematocrit by 15 to 51 per cent. The time-concentration curve was anomalous in some normal goats, but a straight line resulted when levels were graphed from determinations in splenectomised animals. They stated that it was generally accepted that mixing in the blood of dog and man was complete within three minutes. They cited Barcroft (1923-4) as explaining increases in haematocrit after adrenalin in terms of spleen

contraction, and commented that since then there had been much controversy over his theory. Bush et al (1955) noted that the haematocrits of pig blood were higher before anaesthesia and attributed this to enlargement of the spleen during the influence of pentobarbital.

In the literature on blood volume determinations on farm animals there have been variations in the methods of calculation. An allowance for trapped plasma and extrapolation may or may not have been taken into consideration. Stahl and Dale (1958) calculated blood volume in the same 17 calves in five different ways with five different results as shown below.

	Blood Volume ml/Kg
1. From T-1824 plasma volume and the haematocrit	105.6
2. Estimation of whole blood ^{51}Cr without extrapolation	78.2
3. Estimation of whole blood ^{51}Cr with extrapolation	69.3
4. From ^{51}Cr packed red cell volume and the haematocrit without extrapolation	82.5
5. From ^{51}Cr packed red cell volume and the haematocrit with extrapolation	74.0

It is to be expected that blood volume derived from plasma volume will be over-estimated if "F cells" is not taken into account, but not to the extent shown above. With fairly rapid loss of a test substance from the circulation, whether or not

extrapolation is used will have a marked effect on the calculated blood volume. In the Table above the result by method 2 should have been identical with that by method 4, and the result in 3 should have been identical with that in 5, as the same sample of blood was being assayed each time.

In one case the assay was made on a sample of whole blood, say 1 ml. In the other case the sample was first spun and packed cells obtained for assay. Had the packed cells been obtained from one ml of blood, the radioactivity determined should have been identical to that obtained from the whole blood sample, in which the radioactivity is present only in the red cells. The difference shown in results which should be identical can be explained by failure to make the correct allowance for trapped plasma in the haematocrit and the sample of packed red cells, or some other fault in measuring technique.

Figures from the literature on blood volume determinations on farm animals are shown in the following tables - Table 30 - horse and burro, Table 31 - cow, Table 32 - sheep, Table 33 - goat, Table 34 - pig and Table 35 - dog. Included therein are some results which were published after the greater part of the work described in the present thesis was completed.

When this work was started, the decision to employ red cell-labelling rather than plasma-labelling methods was made almost arbitrarily. It was thought that the cells were the more solid and stable component of whole blood.

TABLE 30 - HORSE AND BURRO

BLOOD VOLUME DETERMINATIONS. FIGURES FROM THE LITERATURE

Author		No.	Age	Method	Ht	Plasma Volume mls/Kg	Red Cell Volume mls/Kg	Blood Volume mls/Kg	Remarks
Courtice	(1943)	2		T-1824 Plasma		51	21	72	Trapped Plasma ignored
Hansard, Butler, Comar & Hobbs	(1953)	(BURRO)							
		2	1 day	^{131}I Plasma	52			190±9	Estimations
		4	1-3 mths	^{32}P Cells	38			95±2	based on whole
		6	1-2 yrs	^{131}I Plasma	34			71±6	blood sample
		2	2 yrs	^{32}P Cells	35			67	Trapped plasma 5%
		7	3 yrs	^{131}I Plasma	36			64±4	
		2	3 yrs	^{32}P Cells	36			65	
		9	4 yrs	^{131}I Plasma	37			66±4	
		13	4 yrs	^{32}P Cells	35			62±1	
		5	4 yrs	^{32}P Cells	37			79±2	
Cronin	(1954)	1		T-1824 Plasma	36.9	52.4	28.7	81.1	Trapped Plasma 4%
Julian, Lawrence, Berlin & Hyde	(1956)	6		^{32}P Cells		61.9	47.1	109.6	Racehorses
Julian, Lawrence, Berlin & Hyde	(1956)	4		^{32}P Cells		43.2	28.5	71.7	Percheron breed quoted by Julian et al

TABLE 31 - COW

BLOOD VOLUME DETERMINATIONS. FIGURES FROM THE LITERATURE

Author		No.	Age	Method	Ht	Plasma Volume mls/Kg	Blood Volume mls/Kg	Remarks
Turner & Herman	(1931)	24		Vital Red		38.0	64.0	Not pregnant) Trapped plasma
		41		Vital Red		49.0	81.0	Pregnant) ignored
Miller	(1932)	19		Vital Red			59.7	Trapped plasma ignored
Reynolds	(1953)	10		T-1824			57.4±2.1	Trapped Plasma 6%
Hansard, Butler, Comar & Hobbs	(1953)	2	2-6 days	^{32}P	39		120	
		1	3 weeks	^{32}P	30		85	Based on estimation of
		3	2-3 mths	^{32}P	45		62±3	whole blood
		5	6-8 mths	^{32}P	39		58±3	
		3	14-15 mths	^{32}P	38		57±5	
		4	8-12 yrs	^{32}P	41		57±4	
Dale, Burge & Brodie	(1957)	3		T-1824	40.9	36.6	62±5.1	Dry Jerseys T40-70°F
		3		T-1824	31.6	47.1	68.8±7.0	Lactating Jerseys T40-70°F
		3		T-1824	35.3	54.5	84.4±9.8	Lactating H. Friesians T40-70°F
Stahl & Dale	(1958)	17		T-1824			105.6±2.6	Simultaneous measurement
		17		^{51}Cr Cells			74±1.8	on young calves
Howes, Hentges & Feaster	(1963)	6	2 yrs	^{131}I		28±0.8	56±2.8	Brahman breed) Trapped
		6	2 yrs	^{131}I		28±0.8	49±3.0	) Plasma
		6	4 yrs	^{131}I		39±1.6	84±3.8	Brahman breed) Ignored
		6	4 yrs	^{131}I		31±1.1	58±2.0	Brahman breed)

TABLE 32 - SHEEP

BLOOD VOLUME DETERMINATIONS. FIGURES FROM THE LITERATURE

Author	No.	Age	Method	Plasma Volume mls/Kg	Red Cell Volume mls/Kg	Blood Volume mls/Kg	Remarks
Barcroft, Kennedy & Mason (1939)	3	Adult	T-1824	ca.43	ca.17	ca.60	Splenectomised Non-pregnant
Gotsev (1939)	4	1 day	Congo red			169	
Schambye (1952b)	6	Adult	T-1824 & ^{32}P simultaneously			58	Trapped plasma 5% Blood volume from cell and plasma volume
Hansard, Butler, Comar & Hobbs (1953)	5	1 month	^{32}P			80±2	Estimated on whole blood
	4	6 months	^{32}P			63±1	" " " "
	4	1 year	^{32}P			55±3	" " " "
	4	3 years	^{32}P			58±3	" " " "
	3	7 years	^{32}P			80±4	" " " "
Hodgetts (1961)	5	Adult	T-1824 & ^{51}Cr simultaneously	46.7	19.7	66.4	Corrected for trapped plasma. Blood volume from cell volume plus plasma volume

TABLE 33 - GOAT

BLOOD VOLUME DETERMINATIONS. FIGURES FROM THE LITERATURE

Author	No.	Method	Plasma Volume ml/Kg	Red Cell Volume ml/Kg	Blood Volume ml/Kg	Remarks
Courtice (1943)	32	T-1824			70	Trapped plasma ignored
Walker & Dziemian (1950)	50				90.2	
Klement, Ayer & Rogers (1955)	20	T-1824 & ^{51}Cr simultaneously	55.9	14.7	70.5	Trapped plasma 19%
	5	^{51}Cr alone		13.4	62.8	Trapped plasma 19%
	6	T-1824 alone	56.7		80.1	Trapped Plasma 19%

TABLE 34 - PIG

BLOOD VOLUME DETERMINATIONS. FIGURES FROM THE LITERATURE

Author	No.	Age	Wt. Kgs.	Method	Ht	Plasma Volume mls/Kg	Red Cell Volume mls/Kg	Blood Volume mls/Kg	Remarks
Hansard, Butler, Comar & Hobbs (1951)	3	2 weeks		^{32}P	36			74±3	Blood volume based on estimations of whole blood
	4	4 weeks		^{32}P	41			67±1	
	8	3-4 mths		^{32}P	38			63±1	
	3	4-5 mths		^{32}P	37			59±3	
	3	7-8 mths		^{32}P	40			54±5	
	4	2 years		^{32}P	38			46±2	
	3	3 years		^{32}P	48			35±3	
Bush, Jensen, Cartwright & Wintrobe (1955)	31		10	^{32}P		62.5	32.5	95.0	
			30	^{32}P		48.3	30.0	78.3	
			50	^{32}P		41.9	27.5	69.4	
			70	^{32}P		38.9	24.6	63.5	Trapped plasma ignored
			90	^{32}P		37.2	22.5	59.7	
			110	^{32}P		35.4	21.1	56.5	

TABLE 35 - DOG

BLOOD VOLUME DETERMINATIONS. FIGURES FROM THE LITERATURE

Authors	No.	Method	Blood Volume - mls/Kg
Hooper, Smith, Belt & Whipple (1920)	22	Brilliant vital red	101.3
McQuarrie & Davis (1920)	25	Acacia - Refractometer	97.6
Dawson, Evans & Whipple (1920)	67	Various dyes	92 - 93
Gibson, Keeley & Pijoan (1938)	50	Dye	84.0 - 97.3
Bonnycastle & Cleghorn (1942)	106	T-1824	79
Courtice (1943)	29	T-1824	60 - 107.5
Clark & Woodley (1959)	39	T-1824	87.2
	9	Rose bengal	91.9
	17	^{51}Cr Cells	70.7
	41	T-1824 & ^{51}Cr Cells	81.0

(A) BLOOD VOLUME MEASUREMENTS ON DOMESTIC ANIMALS WITH ^{32}P-LABELLED RED CELLS

METHODS AND MATERIALS

In the initial experiments when whole blood was withdrawn the anticoagulant used was oxalate, but later it was considered that heparin was a more physiologically normal substance and in much the greater part of the work, the latter was used.

^{32}P was obtained as inorganic phosphate from the Radiochemical Centre, Amersham, Buckinghamshire. In labelling the red blood corpuscles of various species for blood volume determinations, about 25-250 μc of the isotope in isotonic solution, which contained a little phosphate, was added to about 5 ml of the withdrawn blood. As will be described later, in labelling the blood of some species it was found advisable to remove some of the plasma before addition of ^{32}P.

The bottle containing the cells being labelled was rotated frequently for 1 minute to ensure effective mixing, and placed in an incubator at 37°C for a period of 1-2 hours. During this time the bottle was rotated for a short period every 20 minutes or so to facilitate mixing, then centrifuged for a short time to separate cells and plasma, and the latter removed by pipette and discarded. The cells were washed by addition of an amount of ice-cold normal saline at least 5-10 times their own volume, gentle but thorough mixing, centrifugation, and removal of the supernatant fluid. This procedure was repeated a further two to three times to eliminate any extraneous radioactive material.

From time to time the supernatant fluid from the last washing was checked for radioactivity and this was found to be negligible.

It was found that there was occasionally some tendency for cells to become haemolysed during washing, and this was reduced in later experiments by retaining plasma from the original blood sample and adding a little of this to the saline before each wash. After the last wash the cells were made up to their original volume with saline or original plasma for injection.

Injection of Labelled Cells

In the calculation of blood volume based on measurement of red cell volume, a great deal of the accuracy depends on the measurement of the relatively small volume of labelled cells injected into the circulation.

In initial experiments the syringe was filled to the requisite mark, a needle inserted into a vein, the syringe attached and the contents injected. The syringe was then detached and the needle left in place in the vein. Another syringe, filled with saline, was used to flush residual material in the needle into the circulation. During the process of changing syringes, there was a danger of loss of radioactive material from the needle by seepage before the "flushing" syringe could be attached.

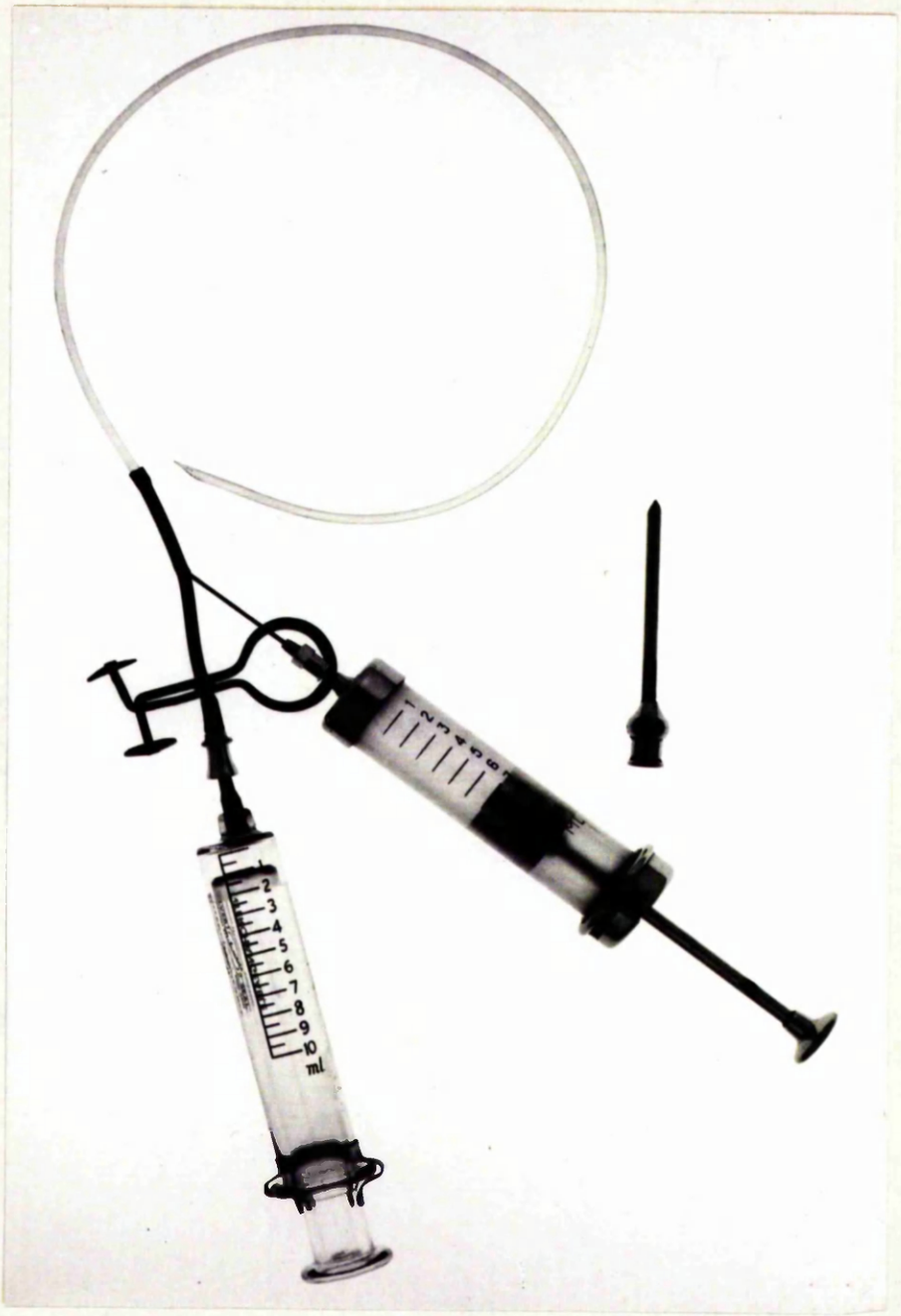
This difficulty was overcome by the following method, which was applicable in all species with the exception of the pig. A large bore needle or McGregor introducing needle was inserted intravenously and through it was threaded several inches of polythene

catheter, some of which was retained externally. The needle was extracted from the skin, slipped over the tubing and removed after which part of the tubing was fixed to the animal's skin by adhesive tape. The catheter was filled with heparinised saline to prevent clotting and to it was attached a piece of rubber tubing approximately 2 inches long, which had a clamp near its free end which terminated in a "Record" syringe mount (Figure 17). The mount and rubber tube were also filled with heparinised saline and the clamp closed. Labelled cells were drawn into a syringe to the requisite mark through a 22 gauge 15 mm hypodermic needle, which was then inserted into the lumen of the rubber tube, the injection made into that site and the needle withdrawn. A 10 or 20 ml syringe containing heparinised saline was attached to the proximal "Record" mount, the clamp loosened and all the material previously injected into the tubing was flushed into the vein. The volume of saline used for this purpose was not large enough to alter the recipient's blood volume significantly.

A source of error that remained was that it was not possible to flush into the circulation radioactive material remaining on the lining of the syringe that had contained the labelled cells, without encountering the problem of involving material lying in the nozzle of the syringe. This difficulty could only be overcome by first calibrating the syringe to allow for the volume held in the nozzle.

FIGURE 17

Apparatus for injection.



Collection and Treatment of Samples after Injection

A vein or veins different from that used for injection was used for withdrawals. Whole blood samples were collected into heparinised bottles at intervals after injection either by venepuncture, or through an indwelling intravenous catheter which was filled with heparinised saline between successive samples. It was usually possible to obtain samples without causing engorgement of the vein during the withdrawal of blood.

Usually 1.0 or 2.0 ml of each sample was pipetted into a graduated centrifuge tube containing a little 0.1N NaOH, made up to 10.0 ml with distilled water and shaken before counting in the M6 liquid counter. To conserve ^{32}P and increase the speed of counting of samples from the large farm animals, a successful attempt was made to count 9.0 ml of whole blood at one time. 1.0 ml of 50% aqueous "Teepol" was added to lyse the cells and excessive frothing during the shaking process was controlled by the addition of a few drops of capryl alcohol. A little sludge tended to settle at the bottom of the centrifuge tube, and a considerable amount of shaking was required to disperse it. In the later experiments it was found more satisfactory to count 10.0 ml of whole blood to which a knife point of saponin and a few drops of capryl alcohol had been added before shaking.

Haematocrit determinations were made on each sample by filling two Wintrobe haematocrit tubes and centrifuging at 1500g and 25°C for 2 hours in the case of bovine animals, 2½ hours for sheep blood samples and 1 hour in the case of the bloods of other

species (See Part 1 Experimental Section (B)).

Preparation of a "Standard"

Some of the original material injected, labelled red cells re-suspended in original plasma or saline, was retained in order that its radioactivity might be determined. A sample, usually 1.0 ml, was carefully measured by pipette and made up to 1 litre with distilled water containing a little carrier phosphate, some of which was used to rinse the pipette six times. 1.0 ml of this "standard" solution was made up to 10 ml with distilled water for counting. If the original activity was known or found to be higher than usual the standard was prepared with a greater dilution than the 1:1000 indicated above. In later experiments the standard was prepared by suitably diluting the same volume of labelled cells in suspension as had been injected into the experimental animal. The volume was measured in the same syringe as had been used for injection.

Counting of Samples and "Standard" and Corrections to Observed Count Rates

Counting of ^{32}P-labelled cells was carried out on lysed samples in the M6 liquid Geiger-Muller tube, with correction for background activity as described under "Materials and Methods" in Part 1. In addition the following corrections to observed count rates were applied.

(a) Correction for Radioactive Decay: ^{32}P decays with a half-life of 14.3 days. If counting of samples which have to be related to each other is spread over a sufficient time, differences due to decay become significant. Such differences were resolved by repeatedly counting the "standard" and relating sample counts to standard counts carried out at times close enough for no significant decay to have occurred. For example, if over a period of 24 hours the standard was found to have decayed by 7%, any sample counted at the later time could be compared to an earlier one by allowing for this figure, as standard and sample decay at the same rate.

(b) Correction for Variations in Sensitivity of the Tube and Equipment

Such variations were seldom found to occur to an extent which necessitated correction, but on occasion repeated counts of the standard indicated that correction should be made. This was done by noting the ratio between the counts of standard under both conditions and using this factor to adjust the counts accordingly.

(c) Correction for Self Absorption

When a 9.0 or 10.0 ml sample of blood was counted, the increase in specific gravity of the sample relative to one which consisted of 1.0 ml of blood and 9.0 ml of water, resulted in the

absorption within the sample itself of a proportion of the impulses. An experiment in counting equal amounts of isotope in watery solution and in whole blood showed absorption to be of the order of 5% and the counts of whole blood were corrected by that factor. In later experiments it was found more convenient to ignore this factor, but make up the 'standard' sample for counting in retained whole blood instead of water, so that standard and unknown sample were of similar densities.

(d) Correction for Resolving Time

The resolving times of the tubes and mechanical register used were considered to be in a range for which, in this type of experiment, no correction was necessary.

EXPERIMENTAL

During the first attempts at blood volume determinations on horses and cattle such low activities were obtained after carrying out the conventional labelling procedure that preparations were useless for the determination of blood volume, but they indicated that the uptake of ^{32}P varied markedly in the different species and an experiment was devised to study these differences.

(a) The Relative Rates of Labelling with ^{32}P of the Red Cells of Different Species

Whole blood was withdrawn into heparinised bottles from three animals of each of the following species - horse, cow, sheep, pig and dog. From each sample two haematocrit determinations were made and the mean value noted. A known volume of each sample was then spun in a centrifuge tube to separate cells and plasma and by subtraction of plasma each sample was adjusted to a haematocrit of approximately 50 per cent. This was done so that equal volumes of cells would be exposed to equal volumes of radioactive plasma. To 5 ml of each sample of blood was added 1 ml of a solution of ^{32}P containing approximately 10 μc of radioactivity. Each sample was mixed thoroughly, 1 ml removed for the preparation of a "standard" solution, and the remainder placed in an incubator at 37°C. The contents were mixed gently from time to time and 1 ml samples removed at intervals of 30, 60 and 120 minutes.

On removal after a period of incubation the cells of each sample were washed as described previously, then dissolved by addition of a little 0.1N NaOH solution. Each sample was finally made up to 10 ml with water for radioactivity determinations in the M6 liquid counter. The samples removed immediately before incubation for preparation of a "standard" were diluted to 1 litre with water and 10 ml of the mixture was counted. After adjustment for dilution this count represented the total available ^{32}P per ml of mixture. The radioactivity determined in samples removed after incubation indicated the amount which had entered the red cells and this was expressed as a percentage of that originally available. As examples, figures for one cow and one dog are shown in Table 36. The mean findings for each species are shown in Figure 18 and individual graphs for pigs and dogs are shown in Figure 19.

Summary

The uptake of ^{32}P by the red cells of pig, horse, sheep and cow was shown to be very much less than the uptake by the red cells of the dog.

It was decided to determine if the rate of labelling of horse red cells could be increased by reducing the amount of plasma to which ^{32}P was added.

TABLE 36

RELATIVE RATES OF UPTAKE OF ^{32}P BY R.B.CS. OF COW AND DOG

Subject	Sample	Corrected Counts/ ml/min.	Uptake %
Cow	Before incubation	41823	
No.	30 minutes	330	0.8
1	60 minutes	684	1.6
	90 minutes	1119	2.7
	120 minutes	1504	3.6
Dog	Before incubation	41720	
No.	30 minutes	3098	7.4
1	60 minutes	6284	15.1
	90 minutes	9536	22.7
	120 minutes	11815	28.3

FIGURE 18

The uptake of ^{32}P by the r.b.cs. of different species.

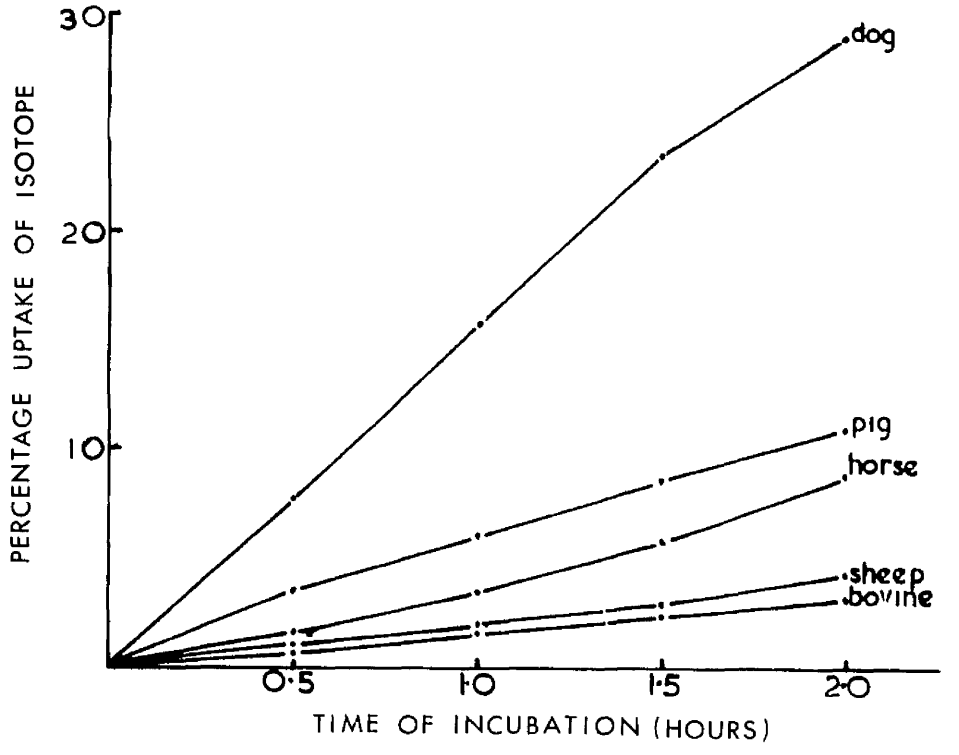
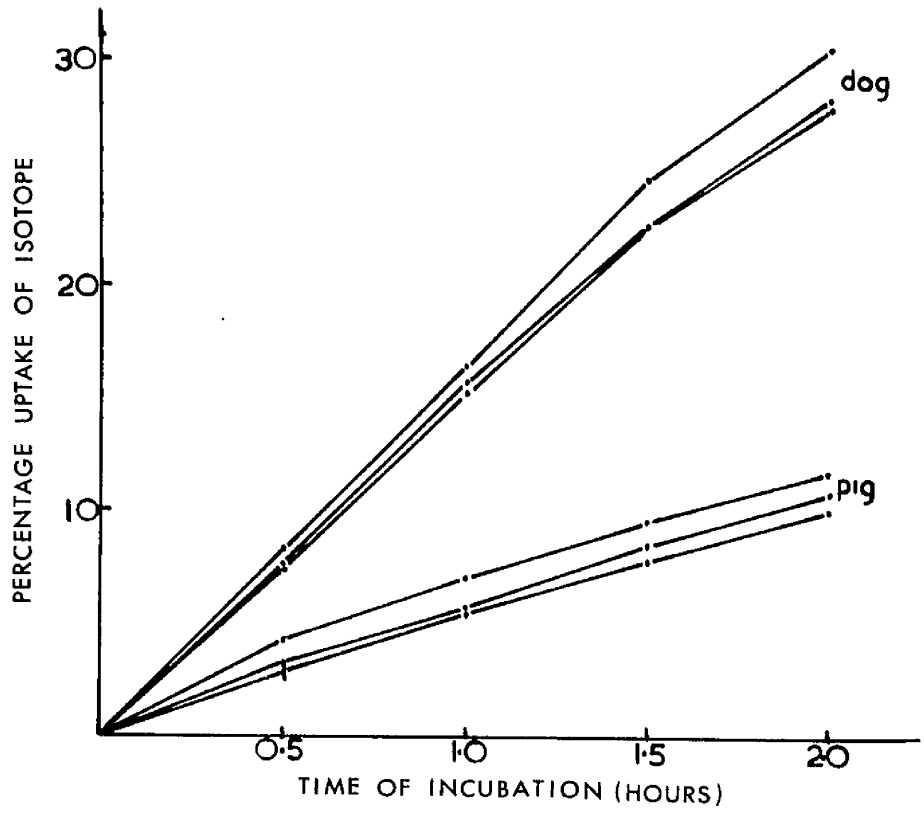


FIGURE 19

The uptake of ^{32}P by the r.b.cs. of individual dog and pigs



(b) The Rate of Labelling with ^{32}P of Horse Red Cells in Whole Blood, in Blood with Reduced Plasma, and Washed Free of Plasma

Nine similar 10 ml portions of a single sample of heparinised horse blood were each placed in a centrifuge tube. Three tubes were retained intact and three were centrifuged to separate cells and plasma and most of the latter was removed and discarded. The remaining three tubes were similarly spun and the plasma removed, then the cells were washed three times with normal saline, the supernatant fluid being discarded each time. To the contents of all tubes was then added 0.5 ml of a solution of ^{32}P containing 0.27 μc, and the preparations mixed and placed in an incubator at 37°C. One tube from each group of three was removed after one hour, one after two hours and the remainder at three hours. Immediately after removal, the contents were spun down, the supernatant removed and the cells washed three times with ice-cold normal saline. Special care was taken that no cells were removed during the process and they were then made up to 10 ml with normal saline. From those tubes which had been incubated for only one hour 5 ml of the mixed contents was taken and made up to 10 ml with distilled water before counting in the M6 liquid counter. From those tubes which had been incubated for a longer period 1 ml of the mixed contents was taken, made up to 10 ml with distilled water and counted similarly. The results are shown in Table 37 and Figure 20.

TABLE 37

RATE OF LABELLING WITH ^{32}P OF HORSE R.B.CS. WHEN IN WHOLE BLOOD, IN BLOOD WITH REDUCED PLASMA AND WHEN WASHED FREE OF PLASMA

Red Cells	Time of Incubation		
	1 Hour	2 Hours	3 Hours
	Corrected Counts/ml/min.		
Whole blood	51	135	377
Reduced Plasma	229	890	1282
Washed free of plasma	673	1275	2008

Summary

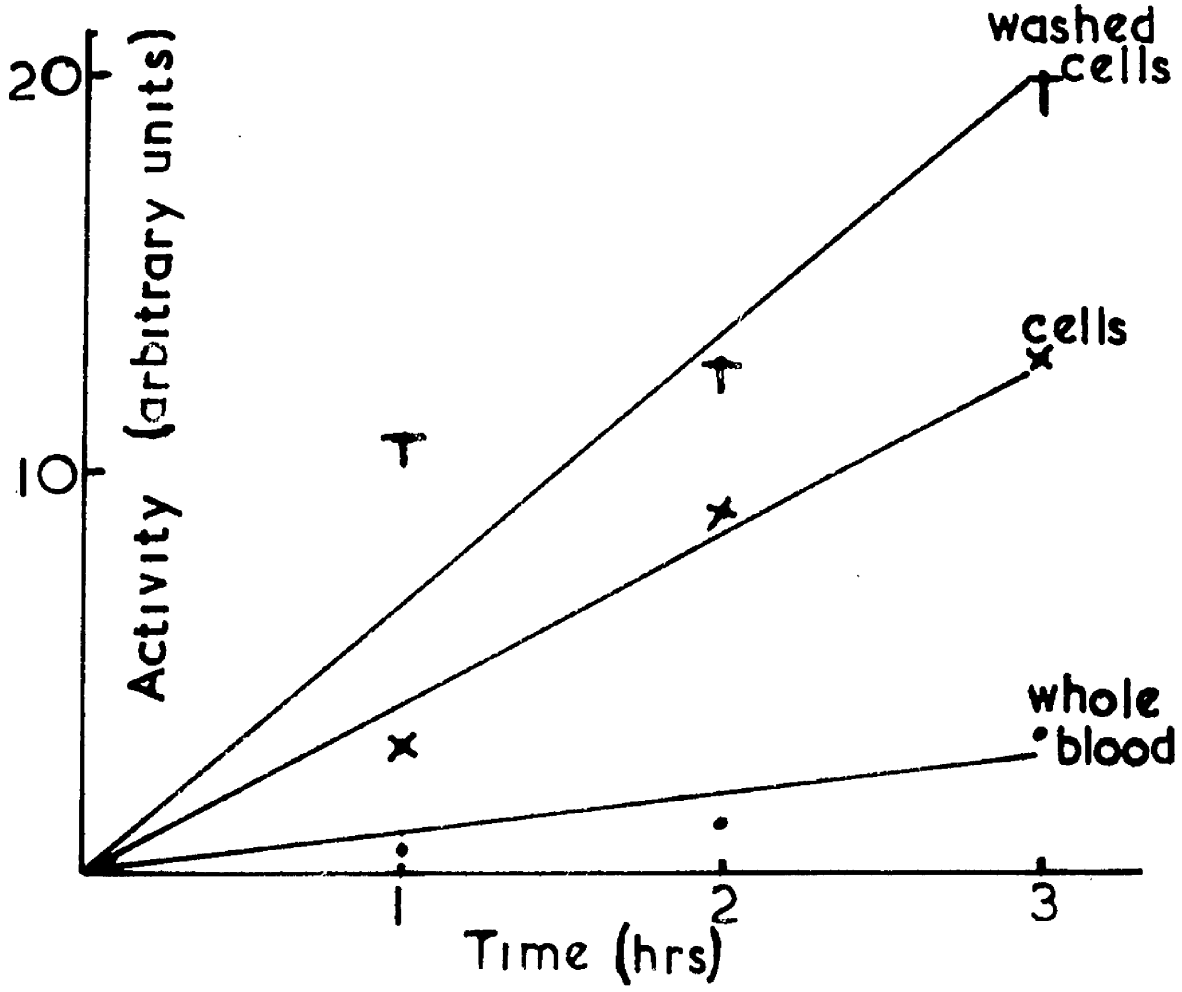
The uptake of ^{32}P by horse red cells was shown to be markedly increased by removal of plasma from the cells before addition of radioactive material.

(c) The Persistence of ^{32}P-labelled Red Cells in the Circulation of Different Species and Blood Volume Measurements

In the preparation of ^{32}P-labelled cells for injection into animals, advantage was taken of the findings shown in Table 37 and Figure 19 to conserve time in labelling or the amount of ^{32}P used. In the case of the comparatively large horse and cow, whose cells label relatively slowly and whose blood volumes are so large that

FIGURE 20

Rate of labelling with ^{32}P of horse r.b.cs. in whole blood, in blood with reduced plasma ("cells"), and when washed free of plasma.



injected material becomes greatly diluted, it became standard practice to remove plasma before adding ^{32}P for labelling purposes. When the initial experiments were being performed the cost of ^{32}P was high enough for economy to be of importance and adaptation of the above method of labelling was helpful in this respect.

A few minutes after injection of labelled red cells the first blood samples were collected and samples were taken at intervals for 60 minutes in most cases. Determinations of radioactivity with corrections of the observed count rate were made as described previously. Haematocrits of all samples were determined, corrected for trapped plasma, and the mean calculated. In determination of radioactivity of serial samples from one animal if the haematocrit of a sample differed from the mean a proportionate adjustment was made to the corrected observed count rate/minute of the sample. The results so obtained were equivalent to determinations on serial samples with identical volumes of red cells.

In order that the results in different animals and different species might be compared with each other more readily, the figure which was determined for each sample was adjusted so that the first determinations made in each animal were related to a common figure. From the adjusted figures points were plotted on semi-logarithmic paper and graphs were drawn which showed the persistence of the labelled cells in the circulation. The results on

ten horses (twelve estimations) are shown in Table 38 and Figure 21; on four cows in Table 39 and Figure 22; on four pigs in Table 40 and Figure 23 and on three dogs in Table 41 and Figure 24.

Calculation of Blood Volume: Estimations were carried out on 17 horses, five cows, four pigs and 11 dogs, and some were repeated once or twice. The graphed persistence curves of ^{32}P cells in the circulation were somewhat anomalous and made extrapolation difficult. Blood volume was usually calculated from the mean of two samples taken between five and ten minutes post injection, according to the formula

$$Bv_c = \frac{\text{Total counts per minute injected}}{\text{Counts/ml/minute of whole blood withdrawn}} \quad \text{ml}$$

An example of counts obtained on one horse is shown in Table 42. No correction for haematocrit is shown as the 8, 11 and 14 minute samples had the same haematocrit as the mean of all samples. The calculation of the estimated blood volume was as follows -

$$\text{Total counts/min. injected} = \text{Counts/ml/min. of standard} \times \text{Dilution of standard} \times \text{No. ml Injected}$$

$$= 3012 \times 1000 \times 10$$

$$\text{Counts/ml/min Sample} = \frac{4389}{10}$$

(Average of 8, 11, 14 min. samples)

$$\text{Therefore } Bv_c = \frac{3012 \times 1000 \times 10 \times 10}{4389} \text{ ml}$$

$$= 68.6 \text{ litres}$$

Weight of horse = 660 Kg

Therefore Bv_c/Kg = 103.9 ml

Further results on horses are shown in Table 43. Results on the cow are shown in Table 44; on the pig in Table 45 and on the dog in Table 46.

TABLE 38

PERSISTENCE OF ^{32}P-LABELLED CELLS IN THE CIRCULATION OF THE HORSE

Time After Injection (mins.)	Horse No. 6		7		8		9	
	Corrected Counts/ml/min.							
	Actual	Adjusted*	Actual	Adjusted*	Actual	Adjusted*	Actual	Adjusted*
2			6051	756			1660	756
3	1263	756			1706	756		
5			5900	737			1489	678
6.5	1138	681						
7			5728	715	1513	670		
8							1434	653
10	1057	632	5068	633	1373	608		
11							1340	609
14							1264	575
15	997	597	4430	553	1184	524		
19							1266	576
20	914	547	4323	539	1132	501		
25	871	521	4295	536	1023	453	1194	544
30	835	499	4206	525	966	428		
35							1136	517
40	795	476	4230	528	890	394		
45							1092	497
50	774	463	4110	513	822	364		
55							1007	458
60	730	437	4108	513	816	361		

* Adjusted to common starting figure

TABLE 38 contd.

PERSISTENCE OF ^{32}P-LABELLED CELLS IN THE CIRCULATION OF THE HORSE

Time After Injection (mins.)	Horse Number 9 (repeat)		9 (repeat)		10		11	
	Corrected counts/ml/min.							
	Actual	Adjusted*	Actual	Adjusted*	Actual	Adjusted*	Actual	Adjusted*
2	3734	756			1163	756	4488	756
3			4785	756				
5	3586	726	4714	744	1119	727	4125	696
8	3362	680	4542	698	1071	696	4000	673
11	3246	657	4430	679	1051	683	3983	671
14	3103	628	4194	641			3860	649
15					995	647		
19	3050	617	3990	630			3727	628
20					954	619		
25	2879	583	3978	628	918	596	3666	617
35	2855	578	4000	631	916	595	3415	575
45	2798	566	3754	593	882	573	3396	571
55	2802	567	3550	561	871	566	3394	571

* Adjusted to common starting figure

TABLE 38 contd.

PERSISTENCE OF ^{32}P-LABELLED CELLS IN THE CIRCULATION OF THE HORSE

Time After Injection (mins.)	Horse Number 12		13		14		16	
	Corrected Counts/ml/min.							
	Actual	Adjusted*	Actual	Adjusted*	Actual	Adjusted*	Actual	Adjusted*
2	5456	756						
2.5			7556	756				
3.5							952	756
5	4695	650	7243	724			884	701
6					4007	724		
7							812	645
8	4298	595	7298	730				
10							705	560
11	3987	552	7040	704				
12					3734	675		
14	3701	513	7057	706				
18					3538	639		
19	3501	485						
20			6978	698			674	535
25	3350	464						
27			6911	691				
30					3297	596	670	532
35	3352	464						
37			6774	677				
40							661	525
45	3313	459	6768	677	3112	563		
55	3311	459	6958	696	3142	568		
60							619	491

* Adjusted to common starting figure

FIGURE 21.

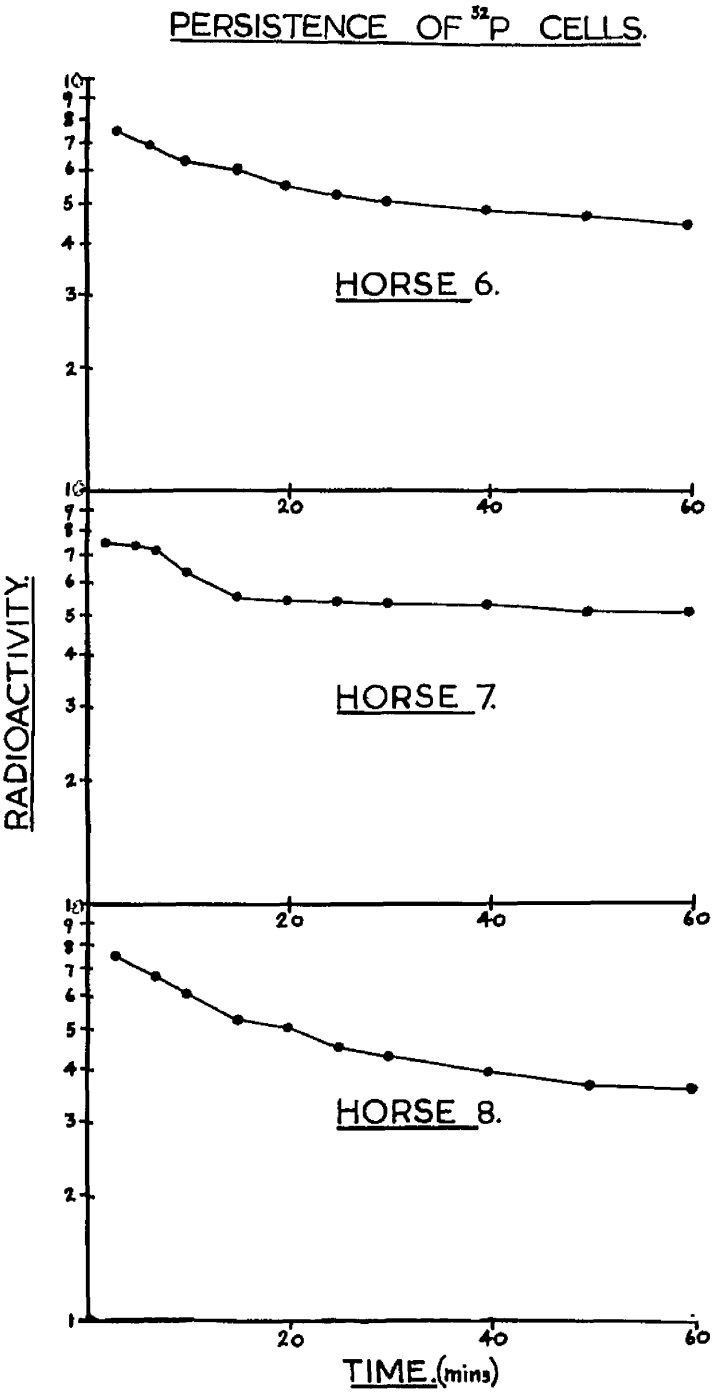

PERSISTENCE OF ^{32}P CELLS.
RADIOACTIVITY.
HORSE 6.
HORSE 7.
HORSE 8.
20
40
60
TIME (mins)

FIGURE 21 contd.

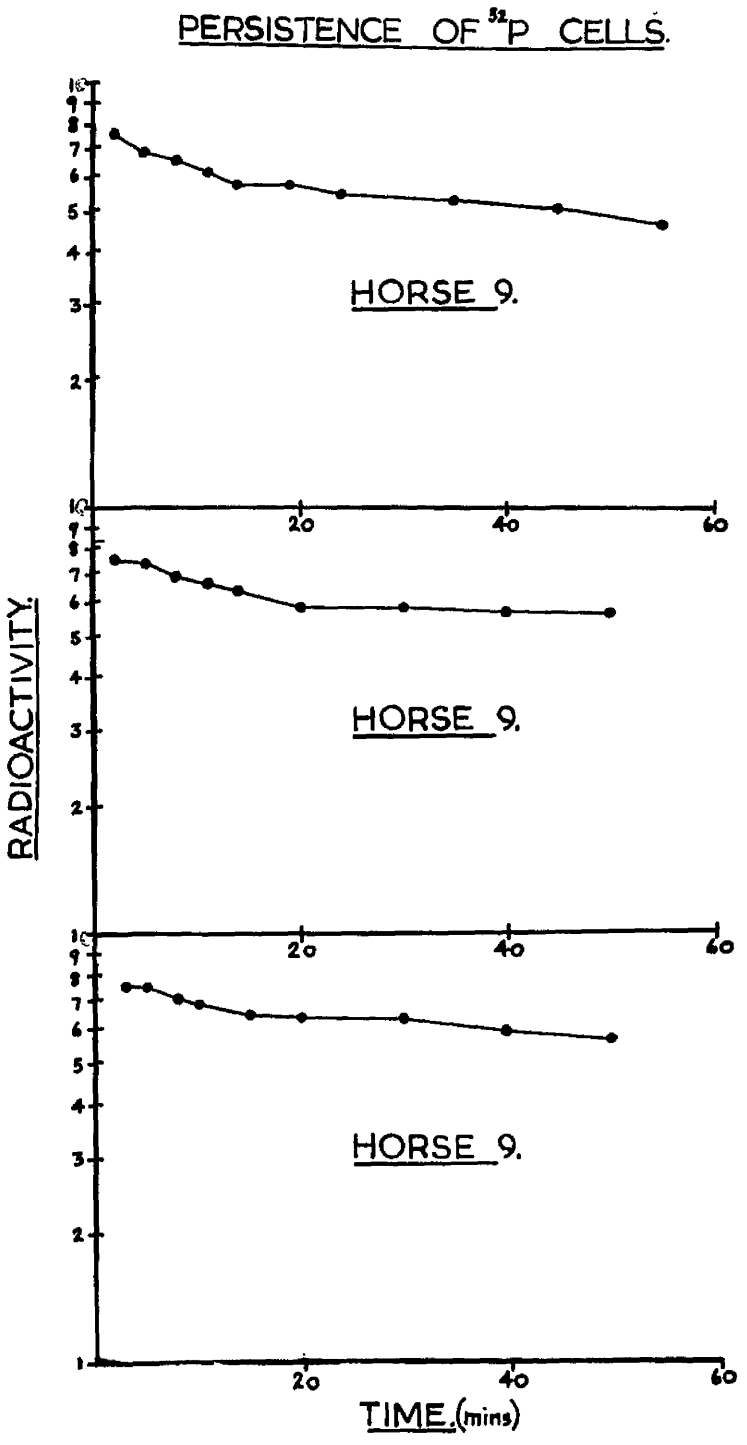


FIGURE 21 Contd.

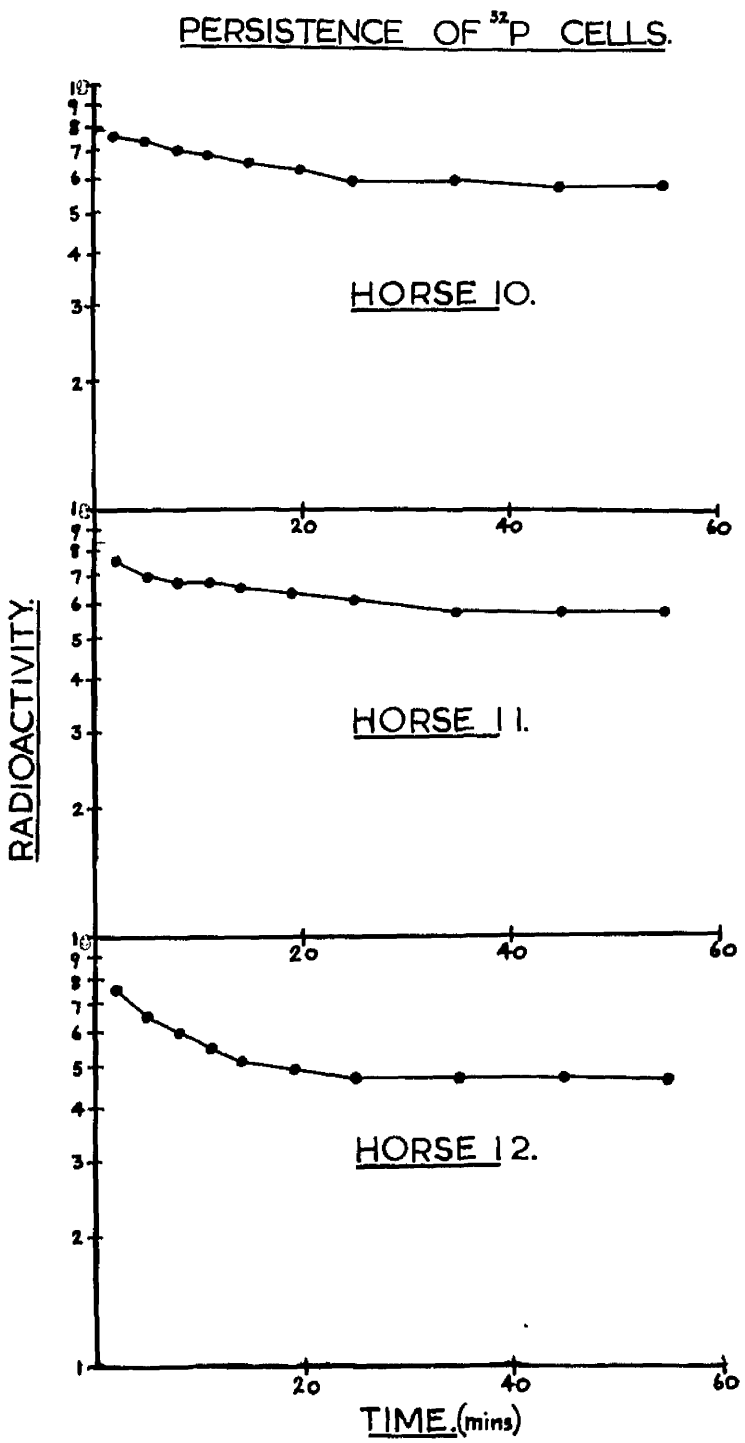


FIGURE 21 Contd.

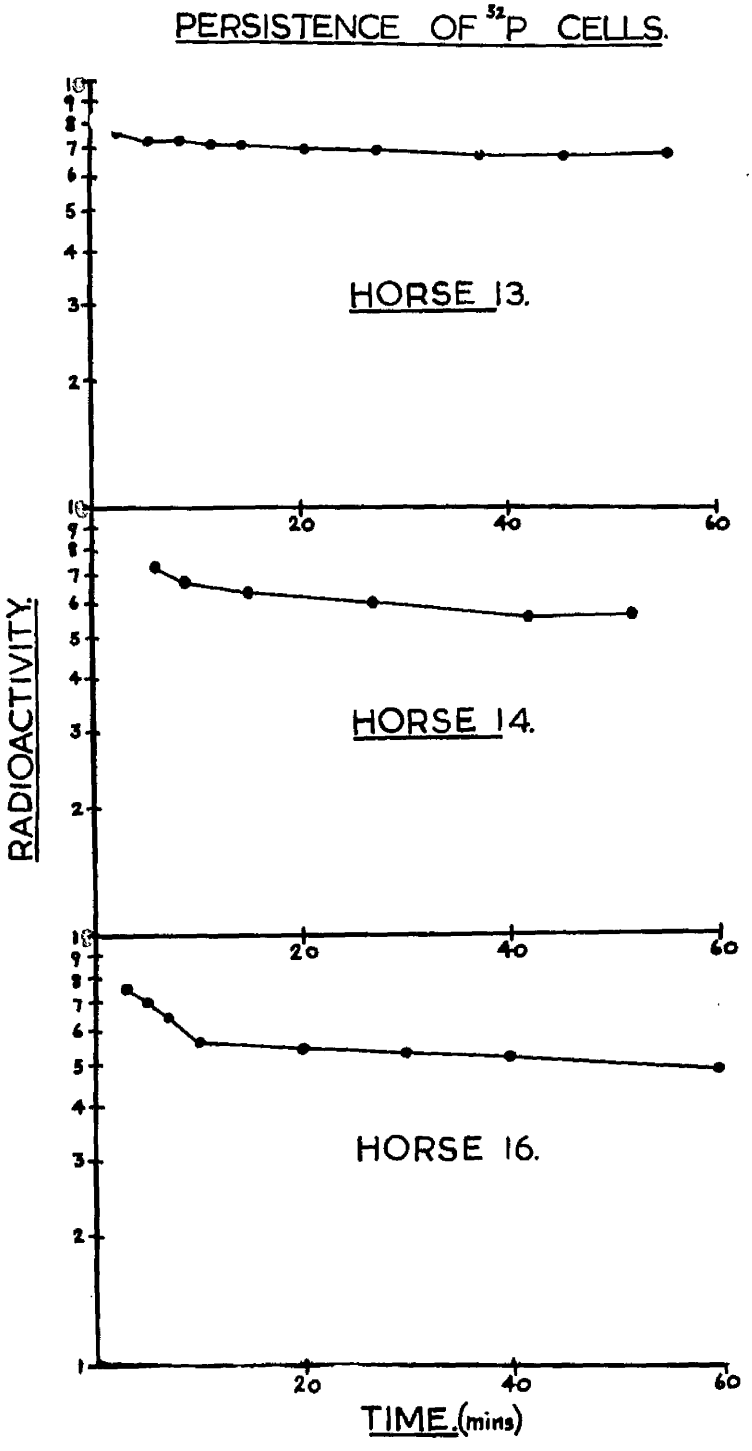


TABLE 39

PERSISTENCE OF ^{32}P-LABELLED CELLS IN THE CIRCULATION OF THE COW

Time After Injection (mins.)	Cow Number 1		2		5		6	
	Corrected Counts/ml/min.							
	Actual	Adjusted*	Actual	Adjusted*	Actual	Adjusted*	Actual	Adjusted*
2	393	756	444	756	511	756	730	756
5	370	711	426	728	491	727		
10	371	714	437	743	520	770	779	807
20	327	629	417	709	520	769	771	799
40	337	647	395	672	502	742	735	761
60	301	580	392	667	491	727	723	749

* Adjusted to common starting figure

FIGURE 22

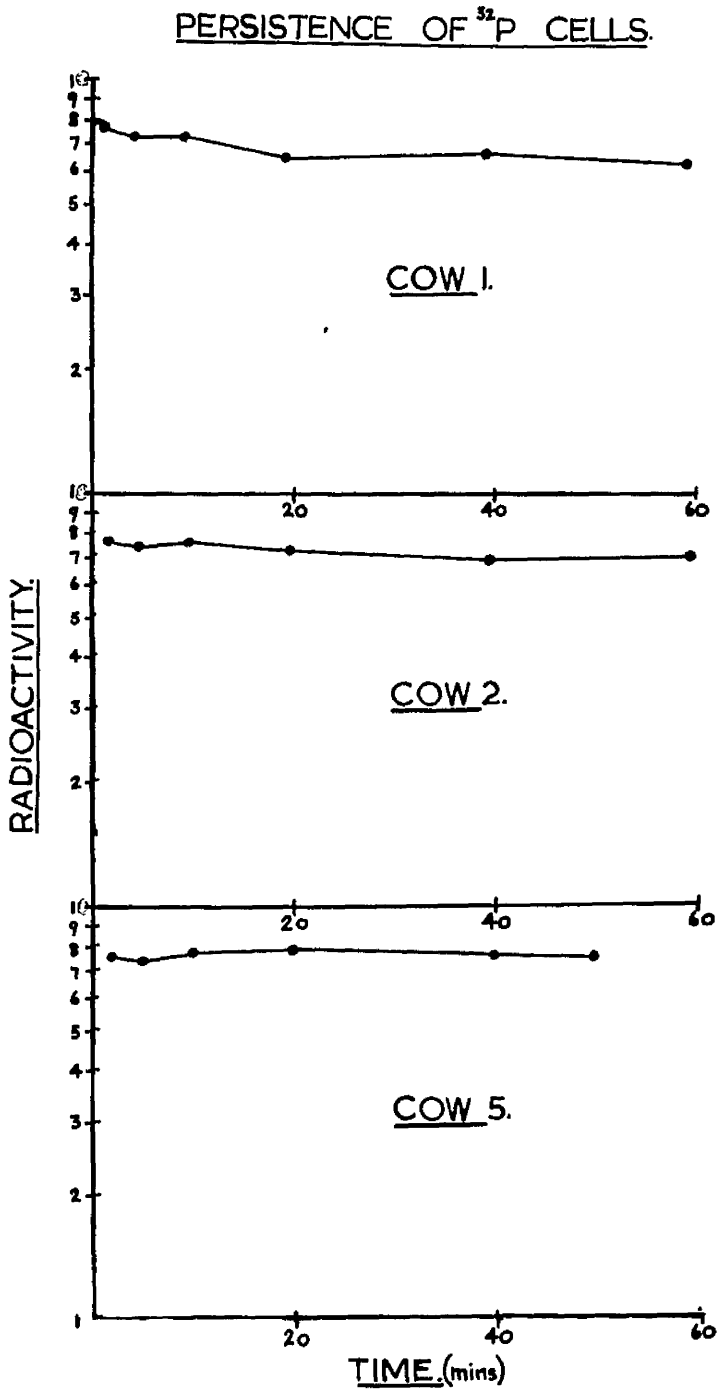

PERSISTENCE OF ^{32}P CELLS.
RADIOACTIVITY.
COW 1.
COW 2.
COW 5.
20
40
60
TIME.(mins)

FIGURE 22 contd.

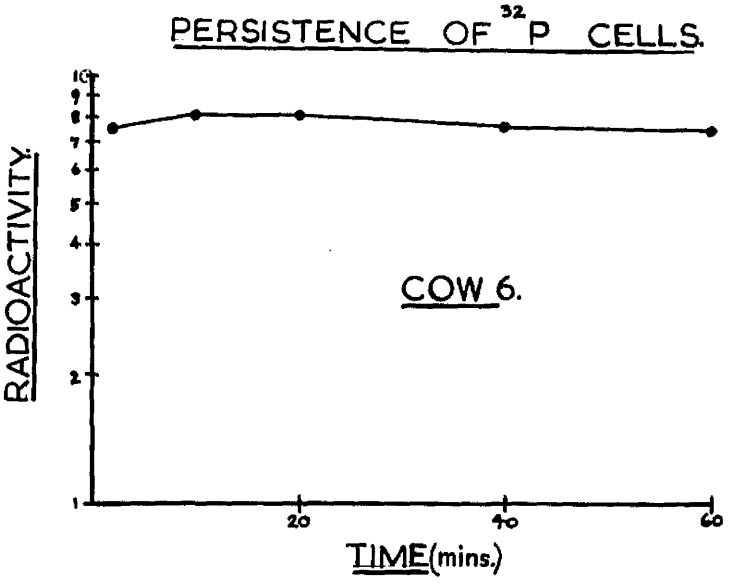


TABLE 40

PERSISTENCE OF ^{32}P-LABELLED CELLS IN THE CIRCULATION OF THE PIG

Time After Injection (mins.)	Pig Number 1		2		3		4	
	Corrected Counts/ml/min.							
	Actual	Adjusted*	Actual	Adjusted*	Actual	Adjusted*	Actual	Adjusted*
3	9409	756	3650	756	5463	756		
5			3490	723	5228	724		
6	9047	727						
10			3400	704			2771	756
11	9094	731						
15					5045	698	2668	728
18	8419	676						
20			3262	676				
25	8567	688			4828	668	2727	744
40	7820	628	3003	622	4993	691	2686	733
50	7674	633	3110	644	4902	678	2347	640

* Adjusted to common starting figure

FIGURE 23

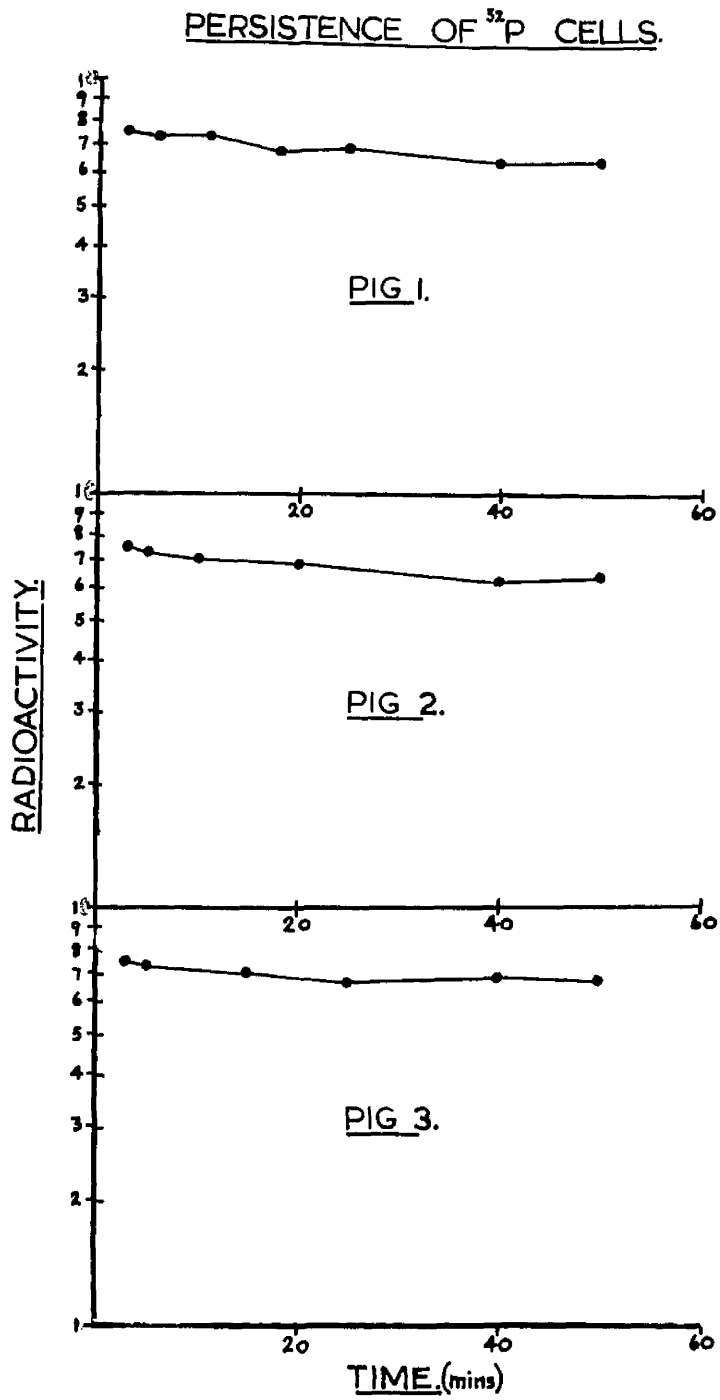
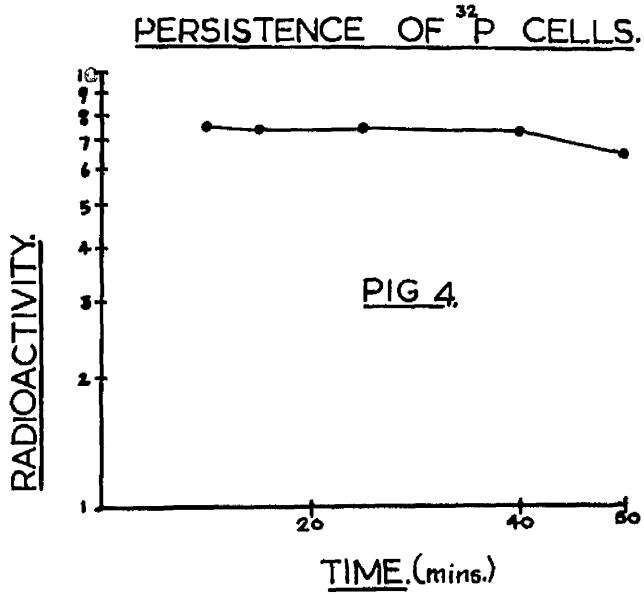

PERSISTENCE OF ^{32}P CELLS.
PIG 1.
PIG 2.
PIG 3.
RADIOACTIVITY.
TIME.(mins)
20
40
60

FIGURE 23 contd.



PIG 4.

TABLE 41

PERSISTENCE OF ^{32}P-LABELLED CELLS IN THE CIRCULATION OF THE DOG

Time After Injection (mins.)	Dog Number 3		4		5	
	Corrected Counts/ml/min.					
	Actual	Adjusted*	Actual	Adjusted*	Actual	Adjusted*
3			670	756	522	756
4	541	756				
6	540	755				
7					563	815
8			631	712		
10					521	754
11	515	719	617	696		
20	490	684	565	637	492	712
30	506	707				
60			572	645	353	512
90	392	547			258	373
120	419	586			110	159

* Adjusted to common starting figure

FIGURE 24

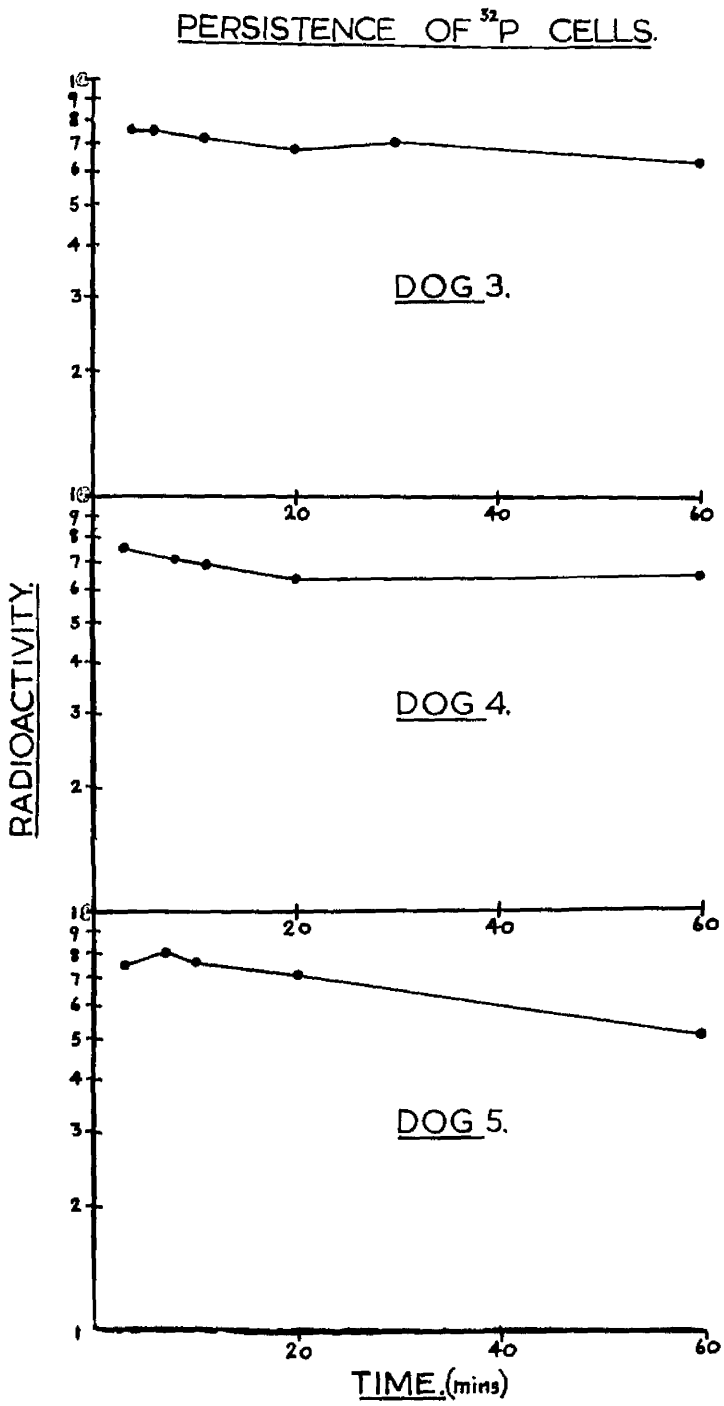

PERSISTENCE OF ^{32}P CELLS.
RADIOACTIVITY.
DOG 3.
DOG 4.
DOG 5.
20
40
60
TIME (mins)

TABLE 42

EXAMPLE OF COUNTS OBTAINED IN BLOOD VOLUME DETERMINATION ON A HORSE BY MEANS OF ^{32}P-LABELLED R.B.CS.

(Horse Number 9)

Sample	No. mins. Counted	Total Counts	Counts /min.	Average Counts/ min.	Corrected for Background
Background (Natural)	16	208	13.0	13.4	
	10	137	13.7		
	12	162	13.5		
Background (Natural and Residual Activity in 10.0 ml blood)*	3	92	30.7	40.0	
	5	104	36.8		
	10	444	44.4		
Standard 1.0 ml of 1/1000 in 10.0 ml whole blood	1	3069	3069	3052	3012
	1	3087	3087		
	1	3136	3136		
	1	2940	2940		
	1	3011	3011		
	1	2955	2955		
	1	3090	3090		
	1	3130	3130		
	1	3044	3044		
	1	3052	3052		
14 min after injection	1	4178	4178	4234	4194
	1	4272	4272		
	1	4256	4256		
	1	4229	4229		
11 min.				4470	4430
8 min.				4592	4542
Average of 14, 11 and 8 minute samples					4389

* Similar experiment carried out on same horse 6 weeks previously

TABLE 43

ESTIMATED BLOOD VOLUMES BASED ON ^{32}P-LABELLED R.B.CS. IN THE HORSE

Horse No.	Ml Injected	Dilution Standard	Corr. Counts /ml/min. Standard	Corr. Counts /ml/min. Sample	Blood Volume Litres	Wt. Kgs.	Blood Vol./Kg. ml
1	5	1-1000	3134	236.6	66.3	724	91.5
2	5	"	2628	231.4	56.8	775	73.4
3	5	"	1536	135.6	56.7	762	74.4
4	5	"	799	62.8	63.7	711	89.5
5	4.5	"	1391	952.5	65.7	800	82.1
6	5	"	1253	117.4	53.3		
7	5	"	6116	1093.6	28.0		
8	4.5	"	1565	152.6	46.2		
9	10	1- 500	2023	134.6	75.2	665	112.8
9 repeat	10	1-1000	2301	323.7	71.8	660	108.9
9 repeat	10	"	3052	438.9	68.6	660	103.9
10	10	1- 500	5306	1039.0	25.5	274	93.3
11	10	1-1000	2600	394.8	65.9	641	102.7
12	9.5	1-1000	2305	404.2	54.2	602	90.0
13	10	1- 500	1244	1426.4	4.4	77	56.5*
14	10	"	2070	376.0	27.5	318	86.5
15	5	1-1000	1363	118.1	57.7	623	92.5
16	5	"	1749	185.0	47.3	559	84.6
17	5	"	749	56.7	66.1		

Mean = 89.5

S.D. = ±14.9

* Small pony-emaciated

TABLE 44

ESTIMATED BLOOD VOLUMES BASED ON ^{32}P-LABELLED R.B.CS. IN THE COW

Cow No.	Ml Injected	Dilution Standard	Corr. Counts /ml/min. Standard	Corr. Counts /ml/min.	Blood Volume Litres
1	5	1-1000	658.9	371.1	8.88
1a	5	"	747.0	500.3	7.43
2	5	"	954.0	436.5	10.93
2a	5	"	869.0	478.0	9.09
2b	5	1- 500	899.7	251.1	8.96
3	5	1-1000	726.0	356.8	10.17
3a	5	1- 500	1287.2	346.7	9.28
4	5	"	840.4	213.5	9.84
5	10	1-1000	265.6	77.7	34.18*

* Adult. Others were 3-4 months of age

TABLE 45

ESTIMATED BLOOD VOLUMES BASED ON ^{32}P-LABELLED R.B.CS. IN THE PIG

Pig No.	Ml Injected	Dilution Standard	Corr. Counts /ml/min. Standard	Corr. Counts /ml/min. Sample	Blood Volume ml	Weight Kgs.	Blood Vol./Kg. ml
1	1	1-2500	6425	9093.5	1774	21.4	82.9
2	1	1-1000	3743	3400.0	1101	15.2	72.4
3	1	1-1000	4831	5228.0	924	15.4	60.0
4	1	1-1000	4465	2771.3	1611	32.3	49.9

Mean = 66.3

S.D. = ±14.4

TABLE 46

ESTIMATED BLOOD VOLUMES BASED ON ^{32}P-LABELLED R.B.CS. IN THE DOG

Dog No.	Ml Injected	Dilution Standard	Corr. Counts /ml/min. Standard	Corr. Counts /ml/min. Sample	Blood Volume ml	Weight Kgs.	Blood Vol./Kg. ml
1	1	1-100	2992	267.9	1,120	10.7	104.7
2	1	1- 500	3259	569.7	2,860	35.2	81.3
3	1	1- 250	2986	541.2	1,379	10.9	126.5*
4	1	1- 250	2488	650.7	956	10.9	87.7
5	1	1- 250	1748	541.8	807	8.2	98.4
6	1	1- 250	1238	318.4	972	10.3	94.4
7	1	1- 250	1422	1078.0	660	6.7	98.5
8	1	1- 250	7075	627.5	2,819	31.8	88.6
9	1	1- 250	3823	1022.5	935	12.8	73.0
10	1	1- 100	1358	188.7	721	10.9	66.2
11	1	1- 250	2127	224.4	2.370	21.1	112.4

* Pregnant

Mean = 93.8

S.D. = ±17.3

DISCUSSION

The red cells of ruminants were found to take up less ^{32}P than those of other species, confirming the findings of Hansard et al (1953) and Schambye (1952a). The former authors found that the uptake of ^{32}P by pig red cells was a little greater than that of the cells of the burro, which however had a greater uptake than that of cow or sheep red cells. Schambye found that the uptake of ^{32}P by the red cells of man and the rabbit was markedly more than that by the cells of the horse, which in turn had a greater uptake than that of the cells of the ruminants - cattle, sheep and goat.

The uptake of ^{32}P by the red cells of various species, after 2 hours incubation at 37°C, was found to be approximately half of that recorded by Hansard et al (1953). These authors found that the proportion of ^{32}P added in vitro to burro red cells which was taken up by the cells was 6 per cent in 30 minutes, 10 per cent in one hour and 16 per cent in 2 hours. The uptake of ^{32}P by bovine red cells was recorded as 3 per cent in 30 minutes, 6 per cent in one hour, and 10 per cent in 2 hours. The uptake by sheep red cells was 3 per cent in 30 minutes, 5 per cent in one hour and 8 per cent in 2 hours. Experiments on pig blood showed that of the ^{32}P available 7 per cent was taken up in 30 minutes, 12 per dent in one hour and 17 per cent in 2 hours.

After injection of labelled cells damaged cells may be removed from the circulation quickly or the label may become detached from the cells, enter the plasma and from there leave

the circulating blood. Hansard et al (1953) measured the loss of ^{32}P from the circulating blood of various species, with the following results. In the burro it was found that the proportional loss of ^{32}P from the circulating blood was 10 per cent in 15 minutes post-injection, 23 per cent in 30 minutes and 50 per cent in 1 hour. In the cow the loss of radioactivity was 10 per cent in 15 minutes, 20 per cent in 30 minutes and 40 per cent in one hour. After injection of sheep the loss was 12 per cent in 15 minutes, 25 per cent in 30 minutes and 48 per cent in one hour. In the pig the loss of injected radioactivity was 6 per cent in 15 minutes, 14 per cent in 30 minutes and 20 per cent in one hour. Schambye (1952b) found the in vitro loss of ^{32}P from sheep red cells to be 5 per cent in 5-30 minutes.

O'Brien, Howie and Crosby (1957) considered that the phosphorus turnover in goat erythrocytes was so rapid that the radioactive tag was lost within minutes after injection.

In the present studies the average percentage loss of ^{32}P-labelled red cells in one hour after injection was found to be markedly less in each species than that recorded by Hansard et al (1953), but in the horse it still amounted to 31 per cent. It was thought that this figure might be excessive and associated with cell damage during labelling and injection procedures.

The figures for blood volume per kilogram body weight obtained in the horse were found to be equal to the higher range of those quoted in the meagre literature on this species (Table 30),

but were higher than those determined in the burro by Hansard et al (1953). Results obtained in bovine animals cannot be compared with those of other workers as it was not possible to obtain an accurate record of body weight. Wide variation was found in the determinations of blood volume on four pigs, but the results were within the range described in the literature (Table 34). Results in the dog were found to be in the higher zone of the range recorded in the literature (Table 35). Further comparison of the results are made in the discussion following Part 2 Section (B).

SUMMARY

1. The uptake of ^{32}P by red blood cells was determined in the blood of the horse, cow, sheep, pig and dog. Marked species differences were found and uptake by the cells of the cow, sheep and horse was poor.

2. It was found that the uptake of ^{32}P by horse red cells was markedly increased by removing plasma prior to the addition of the isotope.

3. Suitable methods were evolved for the injection of an accurately measured amount of ^{32}P-labelled red cells, the collection of serial blood samples, and the counting of large (9-10 ml) samples of whole blood.

4. The persistence of ^{32}P-labelled red cells in the circulation was determined in horses, cows, pigs and dogs.

5. Blood volume estimations based on ^{32}P-labelled red cells were carried out on seventeen horses, five cattle, four pigs and eleven dogs.

(B) BLOOD VOLUME MEASUREMENTS ON DOMESTIC ANIMALS USING RED CELLS LABELLED WITH ^{32}P AND WITH ^{51}Cr

INTRODUCTION

As the results obtained on measuring the persistence of ^{32}P-labelled red cells were equivocal and as ^{51}Cr had become available it was decided to carry out experiments using both isotopes for the labelling of red cells and the calculation of circulating red cell volume and blood volume.

^{51}Cr

Sterling and Gray (1950) showed that red cells readily took up ^{51}Cr and the percentage uptake was in inverse ratio to the amount of Cr added to the cells. When anionic chromium ($Na_2{}^{51}CrO_4$) was injected into the circulation of a dog the radioactivity was found to be predominantly in the red cells and plasma activity rapidly declined. For in vitro tagging 7-26 μc ^{51}Cr was mixed with blood for 1 hour at room temperature. 80-90 per cent of the radioactive material entered the red cells in two hours, and remained firmly bound to the cell even when excess non-radioactive chromate was added to the suspension. When labelled cells were fractionated the radioactivity was shown to be 97 per cent associated with stroma-free haemoglobin, particularly the globin fraction. The uptake into cells in saline was appreciably greater than when the cells were in whole blood. After entering the red cell $Na_2{}^{51}CrO_4$ is probably reduced to the cationic trivalent state before binding with haemoglobin. Very little of the radioactivity left the red cells during the first 24 hours

after labelling.

^{51}Cr emits gamma rays and soft x-rays with a half-life of 26.5 days. The amounts commonly used for labelling red cells do not injure the cells and are below the safe dose for man. Labelled cells can be stored in saline for 24 hours, but long storage is associated with some loss of ^{51}Cr from the cells and early sequestration after injection (Gregersen and Rawson, 1959).

Cationic trivalent chromium ($^{51}CrCl_3$) was shown by Gray and Sterling (1950) to label plasma in vivo and in vitro. A firm chromium - protein bond was shown with bovine albumin. However there are some disadvantages in labelling albumin with ^{51}Cr and the method is not commonly used.

METHODS AND MATERIALS

Because of the difference in the type of radiation emitted by ^{32}P and ^{51}Cr it seemed likely that a determination of each in the same sample could be effected by using an M6 liquid counter (sensitive to β rays largely) and a scintillation counter (largely sensitive to electromagnetic radiation).

^{51}Cr was obtained from the Radiochemical Centre, Amersham, in the form of $Na_2\ ^{51}Cr\ O_4$ in isotonic solution. For blood volume estimations the amount of ^{51}Cr added to red cells varied from 100 c to 850 c and the amount of ^{32}P varied from 150 c to 1600 c. Plasma was removed from the cells of slow-labelling species before addition of the isotopes. The injection of labelled cells and withdrawal of samples was carried out as described in Section A.

For estimation of ^{32}P radioactivity, samples were treated as described in Section A and the same corrections were applied. It was considered that counts in the M6 liquid counter were not significantly altered by the amount of ^{51}Cr present and could be ascribed solely to ^{32}P. For determination of ^{51}Cr radioactivity, samples were assayed in an annular ring well-type scintillation counter (E.K. Cole Ltd.). The same corrections were applied as described previously, and in addition a correction for the proportion of radioactivity recorded which was due to ^{32}P, as described below.

EXPERIMENTAL

(a) The Determination of ^{32}P and ^{51}Cr Activities in the Same Sample

Approximately 200 μc of ^{32}P was added to distilled water in a 2 litre flask containing a trace of carrier phosphate. Approximately 500 μc of ^{51}Cr was added to a similar flask containing a trace of carrier chromate, the flasks made up to their marks with distilled water and the contents of each thoroughly mixed. The solutions were termed P and Cr Primary Standards. Samples of 0.1 ml and 1.0 ml were taken from the flask containing ^{32}P. The former was made up to 10.0 ml with distilled water and counted in the M6 liquid counter; the latter was made up to 5.0 ml and counted in the scintillation counter. Samples of 0.1 ml and 10.0 ml were taken from the flask containing ^{51}Cr. The former was made up to 5.0 ml and counted in the scintillation counter; the latter was counted directly in the M6 liquid counter. The count-rates were corrected for background and the results are shown in Table 47.

It can be seen that the proportion of counts of ^{32}P in the M6 which is recorded by the scintillation counter is $\frac{1485}{31540}$ which is equal to 4.71%. The proportion of counts of ^{51}Cr in the scintillation counter which is recorded by the M6 is $\frac{38.8}{30920}$ = 0.125%. As the latter figure is well within the range of experimental error in the estimation of red cell volume it can be

TABLE 47

THE DETERMINATION OF ^{32}P AND ^{51}Cr ACTIVITIES IN THE SAME SAMPLE

1.0 ml Primary Standard	Counts/100 secs. in M6 Counter	Counts/100 secs. in Scintillation Counter
^{32}P	31,540	1,485
^{51}Cr	38.8	30,920

ignored in experiments involving the use of red cells labelled with both ^{32}P and ^{51}Cr, unless the radioactivity due to ^{51}Cr is very grossly in excess of that due to ^{32}P.

The proportion of ^{32}P-M6 counts recorded by the scintillation counter has been stated to be 4.71%. In the practical use of the M6 and scintillation counters for the estimation of radioactivity of double-labelled red cells, the former requires a sample made up to 10.0 ml and the latter one of 5.0 ml. It is more suitable to count 10.0 ml first in the M6 then take 5.0 ml of this for estimation in the scintillation counter. Using this method it follows that the proportion of ^{32}P-M6 counts recorded by the scintillation counter will be $\frac{4.71}{2}$ = 2.36%.

Summary

In the practical estimation of blood volume with cells labelled with both ^{32}P and ^{51}Cr the proportion of ^{51}Cr-scintillation counter counts which is recorded by the M6 is negligible. The proportion of ^{32}P-M6 counts recorded by the scintillation counter is 2.36%.

(b) The Simultaneous Labelling of Red Cells of Different Species with ^{32}P and ^{51}Cr

About 12 ml of blood was withdrawn into heparinised bottles from one individual of each of the following species - horse, cow, sheep, pig, dog and rabbit. From each sample two haematocrit determinations were made and the mean value noted. A known volume of each sample was then spun in a centrifuge tube to separate cells and plasma, and by subtraction of plasma each sample was adjusted to a haematocrit of approximately 50%. The samples were transferred to 10.0 ml bottles and 50 μc of ^{32}P and 20 μc of ^{51}Cr were added to each. Samples were then well mixed, placed in a water bath at 37.5°C, and gently agitated every five minutes. 50 c of ^{32}P and 20 μc of ^{51}Cr were added to a 250 ml flask containing a little carrier phosphate and carrier chromate, and made up to the mark with distilled water to serve as a standard.

From each incubating blood sample 0.5 ml was withdrawn 15, 30, 45, 60 and 90 minutes after the addition of the isotopes. The cells were washed three times in ice-cold plasma-saline, by repeated mixing, centrifugation and discarding of plasma and

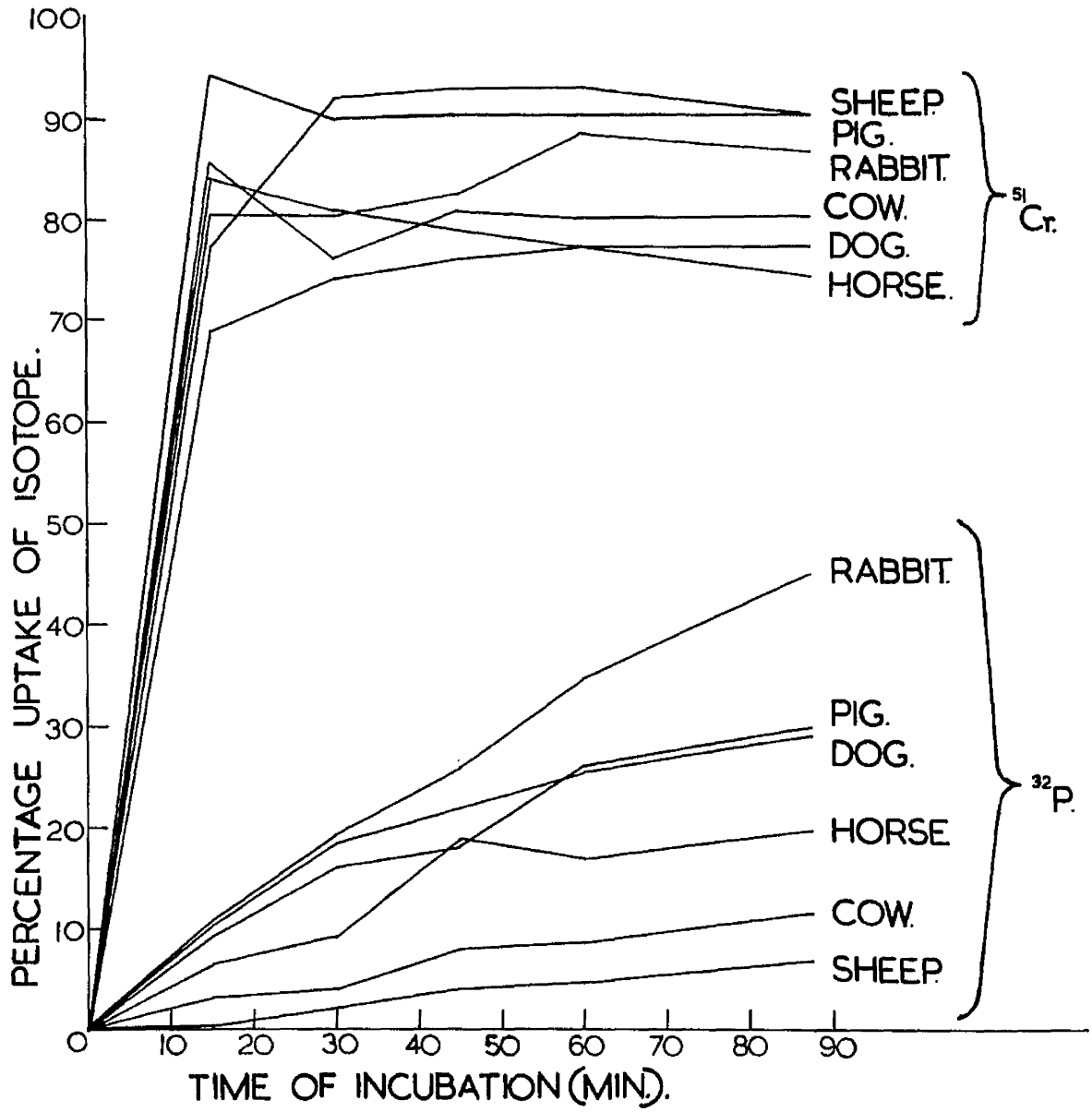
supernatant fluid. The cells were then lysed by the addition of a little 0.1 N NaOH, and after suitable dilution with distilled water 10.0 ml samples were counted in the M6 liquid counter. 5.0 ml of the mixture assayed in the liquid counter was counted in the scintillation counter. The count rates were corrected for background, and those observed in the scintillation counter were also corrected for the contribution due to ^{32}P. The radioactivity of the samples was expressed as a percentage of that originally available to the cells, as determined by means of the standard solution, and the results are shown in Figure 25.

Summary

The red cells of different species were labelled simultaneously with ^{32}P and ^{51}Cr. The species differences in rates of labelling with ^{32}P were again noted. The red cells of all the species labelled rapidly and well with ^{51}Cr.

FIGURE 25

The simultaneous labelling of red cells of different species with ^{32}P and ^{51}Cr.



(c) The Persistence of ^{32}P and ^{51}Cr-Labelled Cells in the Blood of Different Species and Blood Volume Measurements

(1) The Dog

Approximately 12.0 ml of blood were withdrawn into a 30 ml heparinised bottle. A sample of 4.0 to 5.0 ml was removed, the cells spun down and the plasma added to a 250 ml flask of ice-cold normal saline. Approximately 500 μc of ^{32}P and 200 μc of ^{51}Cr solutions were added to the blood remaining in the 30 ml bottle, which was gently but thoroughly shaken, and placed in a water bath at 37.5°C for 60-70 minutes. During incubation the mixture was gently agitated at approximately 15 minute intervals to ensure complete mixing. Following incubation the bottle was filled with plasma-saline and the cells washed by repeated inversion, spinning down and discarding of the supernatant fluid. The washing procedure was carried out four times then without delay the cells were made up to a volume of 7.0-8.0 ml with plasma-saline.

A measured quantity, usually 5.0 ml, was injected into the left radial vein within a few minutes of its preparation. 2.5 ml samples were obtained from the right radial vein, usually at 2, 5, 10, 15, 20, 30, 45 and 60 minutes after injection. Difficulties were encountered in the use of an indwelling polythene catheter for sampling. It was simple to introduce a

catheter into the vein through a McGregor introducing needle, but it tended to leak at the junction with the mount, or it became displaced too readily and no foolproof method of fixation was devised. In some cases it was found preferable to perform repeated venepuncture with a 20 gauge $\frac{5}{8}$" needle on a syringe. When haemorrhage was carefully controlled by pressure repeated withdrawals were made quite satisfactorily.

From each sample two Wintrobe haematocrit tubes were filled and centrifuged at 1500g for one hour at 25°C. The mean reading was noted and corrected by a factor of 4% for trapped plasma.

Preparation and Counting of Standard: 1.0 ml of the suspension which was used for injection was carefully measured and added to a one or two litre flask containing distilled water with a trace of carrier phosphate and chromate. The contents of the flask were made up to the mark with distilled water and mixed thoroughly. A measured quantity, usually 1.0 ml, was withdrawn, made up to 10.0 ml in a graduated centrifuge tube, and counted in the M6 liquid counter. From the 10.0 ml sample 5.0 ml was taken for counting in the scintillation counter. The standards were counted before, in the course of and after the counting of blood samples.

Preparation and Counting of Samples: Usually 1.0 ml of the sample was added to distilled water containing a few drops of 0.1 N NaOH in a graduated centrifuge tube, and made up to the 10.0 ml mark with water. This volume was counted in the M6

counter then half of it (5.0 ml) transferred for counting in the scintillation counter.

Corrections: Count-rates of standard and samples were corrected for background. Count-rates recorded in the scintillation counter were mainly those of the ^{51}Cr content of the sample, but were corrected by subtraction of the counts due to ^{32}P. The correction figure for a specific sample was obtained by multiplying the ^{32}P-M6 count-rates by the figure of 2.36 per cent, as derived earlier. No correction for self-absorption was required, and correction for decay of ^{32}P was applied on only one occasion, when the counting of some samples in the M6 counter had to be delayed.

Corrected counts were first expressed per 100 secs. per 1.0 ml of sample or standard. As a convention, ^{51}Cr counts were so expressed though in fact 1.0 ml of sample had been made up to 10.0 ml, counted for ^{32}P content in the M6 counter, then 5.0 ml of this taken for estimation of the ^{51}Cr content, the equivalent of only 0.5 ml of the sample. In order to avoid unnecessary doubling of corrected count-rates registered in the scintillation counter it was decided to express the rates as per 1.0 ml of sample, prepared for counting in the above manner.

The count-rate/ml of packed cells was calculated from the measured activity for whole blood and the appropriate haematocrit. Reference to count-rates/ml of packed cells for the study of persistence, in effect ruled out differences between samples which were due solely to changes in haematocrit. The corrected count-rate of 1.0 ml of packed cells of each sample was adjusted for each

dog to relate it to a common figure for the earliest sample withdrawn from each dog. The results are shown in Table 48 and Figure 26.

Calculation: Calculation of cell volume was based on the formula -

$$CV = \frac{\text{Total counts/100 secs. injected}}{\text{Counts/100 secs/ml packed cells in sample}} \text{ ml}$$

As the persistence curves of ^{32}P and ^{51}Cr usually showed some irregularity, extra-polation was not carried out, but calculation of cell volume was based usually on the sample taken ten minutes after injection.

When cell volume had been determined, blood volume was calculated according to the formula:

$$Bv_c = CV \times \frac{100}{\text{corrected peripheral haematocrit}}$$

As an example the calculations for dog No. 1 are shown below.

Dog No. 1 (^{32}P)

$$CV = \frac{\text{Counts/100 secs/ml standard} \times \text{dilution standard} \times \text{No. ml injected}}{\text{Counts/100 secs/ml packed cells in sample}}$$

$$= \frac{2160 \times 2000 \times 1}{4861} \text{ ml}$$

$$= 889 \text{ ml}$$

Body Weight = 21.8 Kg $\therefore$ Cell Volume = 40.8 ml/Kg

Corrected peripheral Ht = 51.8

$$\therefore Bv_c = \frac{889 \times 100}{51.8} \text{ ml}$$

$$= 1,716 \text{ ml}$$

$$= 78.7 \text{ ml/Kg}$$

Dog No. 1 (^{51}Cr)

$$CV = \frac{3583 \times 2000 \times 1}{7394} \text{ ml}$$

$$= 969 \text{ ml}$$

$$= 44.5 \text{ ml/Kg}$$

$$Bv_c = \frac{969 \times 100}{51.8} \text{ ml}$$

$$= 1,871 \text{ ml}$$

$$85.8 \text{ ml/Kg}$$

This and other results on the dog are shown in Tables 49 and 50.

TABLE 48

PERSISTENCE OF ^{32}P AND ^{51}Cr LABELLED CELLS IN THE CIRCULATION OF THE DOG

Time After Injection (mins.)	Dog No. 1		Dog No. 3		Dog No. 4	
	Corrected Counts/ml Packed Cells/100 secs.					
	^{32}P	^{51}Cr	^{32}P	^{51}Cr	^{32}P	^{51}Cr
2					7500*	7500*
5	7500*	7500*	7500*	7500*	7299	7595
10	5743	6202	7114	7123	7149	7160
15	5415	6131	7057	6854	6955	7050
20	5447	5849	7175	6797	6694	6875
30	5215	6036	7172	7097	6691	7044
45	5219	6507	7006	6929	6410	6838
60	5561	5988	7057	6848	6277	6723

Time After Injection (mins.)	Dog No. 5		Dog No. 6	
	Corrected Counts/ml Packed Cells/100 secs.			
	^{32}P	^{51}Cr	^{32}P	^{51}Cr
2	7500*	7500*	7500*	7500*
5	7960	7220	7610	7768
10	7849	6738	7847	7717
15	8054	6883	7247	7308
20	7868	7223	8365	8008
30	8043	7082	7529	7790
45	7379	6808	7515	7595
60	7262	6847	6877	7254

* Adjusted to common starting figure

FIGURE 26

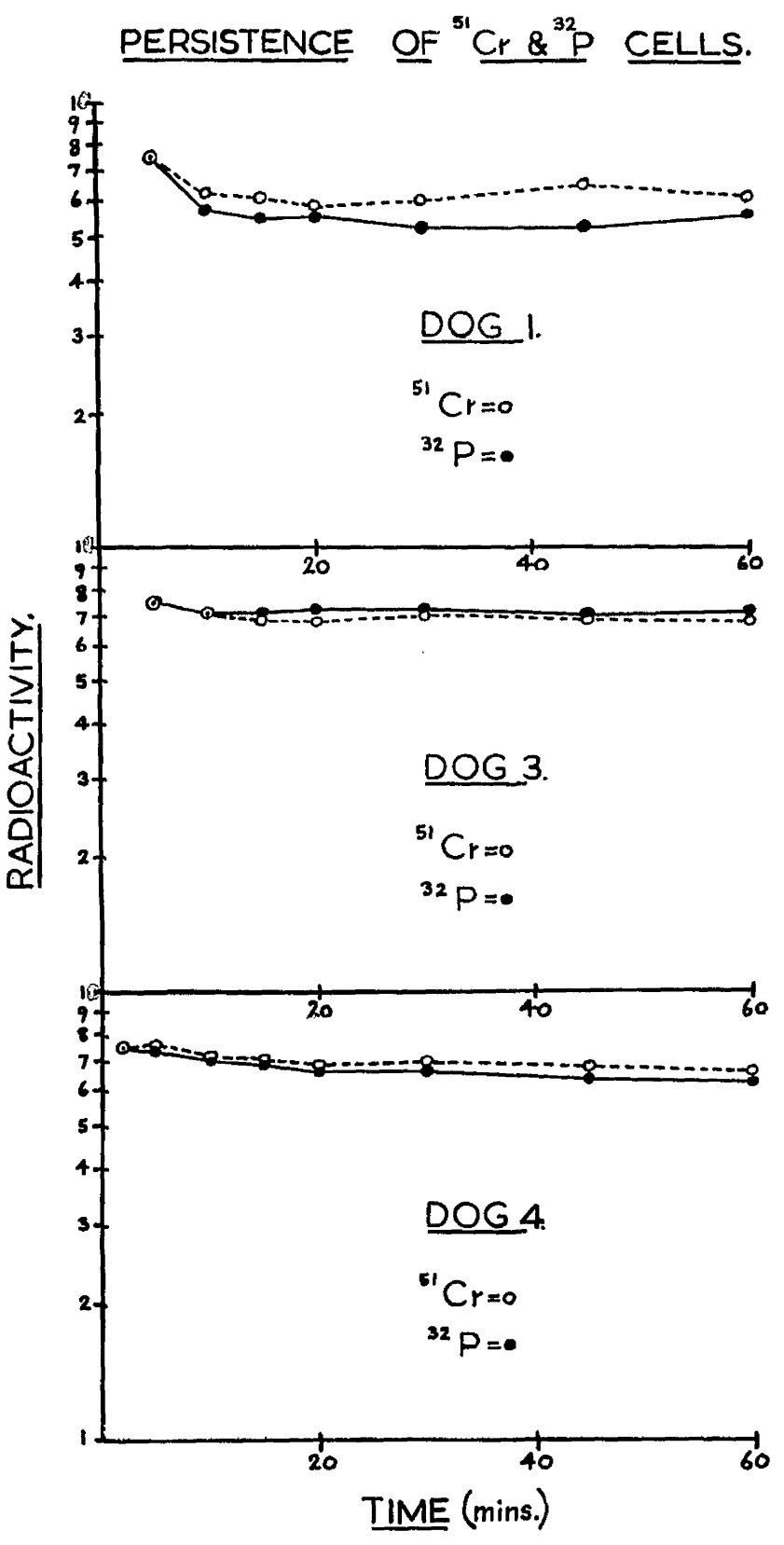

PERSISTENCE OF ^{51}Cr & ^{32}P CELLS.
RADIOACTIVITY.
DOG 1.
^{51}Cr = o
^{32}P = •
DOG 3.
^{51}Cr = o
^{32}P = •
DOG 4.
^{51}Cr = o
^{32}P = •
20
40
60
TIME (mins.)

FIGURE 26 contd.

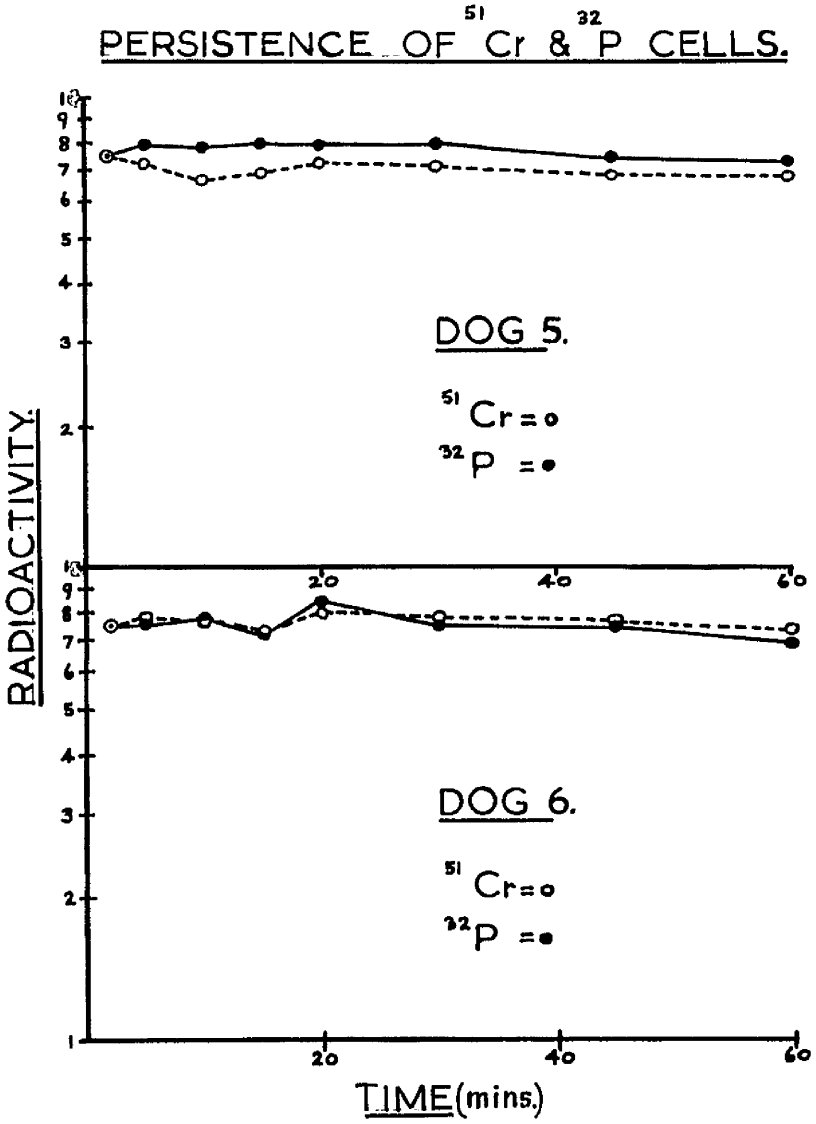


TABLE 49

CELL VOLUME ESTIMATIONS ON DOGS, BASED ON CELLS LABELLED WITH ^{32}P AND ^{51}Cr

Dog No.	No. ml Injected	Dilution Standard		Corr. Counts /ml/100s Standard	Corr. Counts /ml/100s Sample	Cell Volume Litres	Wt. Kg.	Cell Vol/Kg ml
1*	1	1-2000	^{32}P	2,160	4.861	0.889	21.8	40.8
			^{51}Cr	3,583	7,394	0.969		44.4
3*	5	1-1000	^{32}P	3,476	7,826	2.221	32.9	67.5
			^{51}Cr	2,111	7.710	1.369		41.6
4*	4	1-1000	^{32}P	4,815	11,042	2.180	29.9	72.9
			^{51}Cr	2,982	7,068	2.110		70.6
5	5	1-1000	^{32}P	3,926	25,519	0.769	18.6	41.3
			^{51}Cr	426	2,576	0.827		44.5
6	5	1-1000	^{32}P	4,491	40,196	0.559	11.8	47.4
			^{51}Cr	3,271	35,728	0.458		38.8

* Greyhounds

^{32}P Mean 53.9 $\pm$15.2

^{51}Cr Mean 47.9 $\pm$12.9

TABLE 50

BLOOD VOLUME ESTIMATIONS ON DOGS BASED ON CELLS LABELLED WITH ^{32}P AND ^{51}Cr

Dog No.	Cell Vol.	Litres	True Peripheral Ht	Blood Volume Litres	Blood Volume ml/Kg.
1*	^{32}P	0.889	51.8	1.716	78.7
	^{51}Cr	0.962		1.871	85.8
2*	^{32}P	x	x	3.117	110.1
	^{51}Cr	x	x	2.418	85.4
3*	^{32}P	2.221	52.8	4.206	127.8
	^{51}Cr	1.369		2.593	78.8
4*	^{32}P	2.180	55.2	3.949	135.2
	^{51}Cr	2.110		3.822	127.8
5	^{32}P	0.769	42.2	1.882	98.0
	^{51}Cr	0.827		1.960	105.4
6	^{32}P	0.559	46.6	1.201	101.8
	^{51}Cr	0.458		0.984	83.4

* Greyhounds
x Ht omitted

^{32}P Mean 108.6 ±20.6

^{51}Cr Mean 94.4 ±18.7

(2) The Sheep

The subjects were six adult Blackface ewes which were suckling two-month-old lambs. The techniques used were similar to those described previously for the dog. About 15.0 ml of blood was withdrawn, and 3.0 ml retained for the preparation of plasma-saline for cell-washing. Before labelling, the heparinised blood was spun and the plasma removed and retained. 200-270 μc of ^{32}P and 300-500 c of ^{51}Cr were then added and the cells incubated for 65-70 minutes. After four washes in plasma-saline the cells were reconstituted with original plasma for injection. Injection was made into the right radial vein and blood samples were collected from the right jugular vein. Haematocrits of each sample were determined in Wintrobe tubes spun at 1500g and 25°C for 2½ hours. A correction for trapped plasma of 4% was applied.

The determination of ^{51}Cr and ^{32}P in the blood samples and the subsequent calculations were as described for the dog. The results on persistence of ^{32}P and ^{51}Cr in circulating red cells are shown in Table 51 and Figure 27. The results of estimation of red cell volume (CV) and blood volume based on labelled cells (Bv_c) are shown in Tables 52 and 53.

TABLE 51

PERSISTENCE OF ^{32}P AND ^{51}Cr LABELLED CELLS IN THE CIRCULATION OF THE SHEEP

Time After Injection (mins.)	Sheep No. 1		2		3	
	Corrected Counts/ml Packed Cells/100 Secs.					
	^{32}P	^{51}Cr	^{32}P	^{51}Cr	^{32}P	^{51}Cr
2	7500*	7500*	7500*	7500*	7500*	7500*
5	7040	6768	7531	8049	7690	7051
10	6766	6501	7088	7029	7497	7400
15	6202	6341	6712	7037	7139	7246
20	6232	6354	7054	7114	7063	7424
30	6011	5910	6656	6937	7531	7375
45	5553	5769	-	-	6912	7141
60	5313	5992	5917	6421	7293	7404
	Sheep No. 4		5		6	
2	7500*	7500*	7500*	7500*	7500*	7500*
5	7591	7348	7457	7309	7399	8059
10	7804	7679	7063	7167	5987	6991
15	7513	7346	7247	6930	6972	7147
20	7677	7835	6832	6893	6939	6920
30	7023	7058	6703	7630	6868	6829
45	7554	7513	6751	6976	6647	6720
60	7260	7383	6872	6783	6480	6563

* Adjusted to same starting figure

FIGURE 27

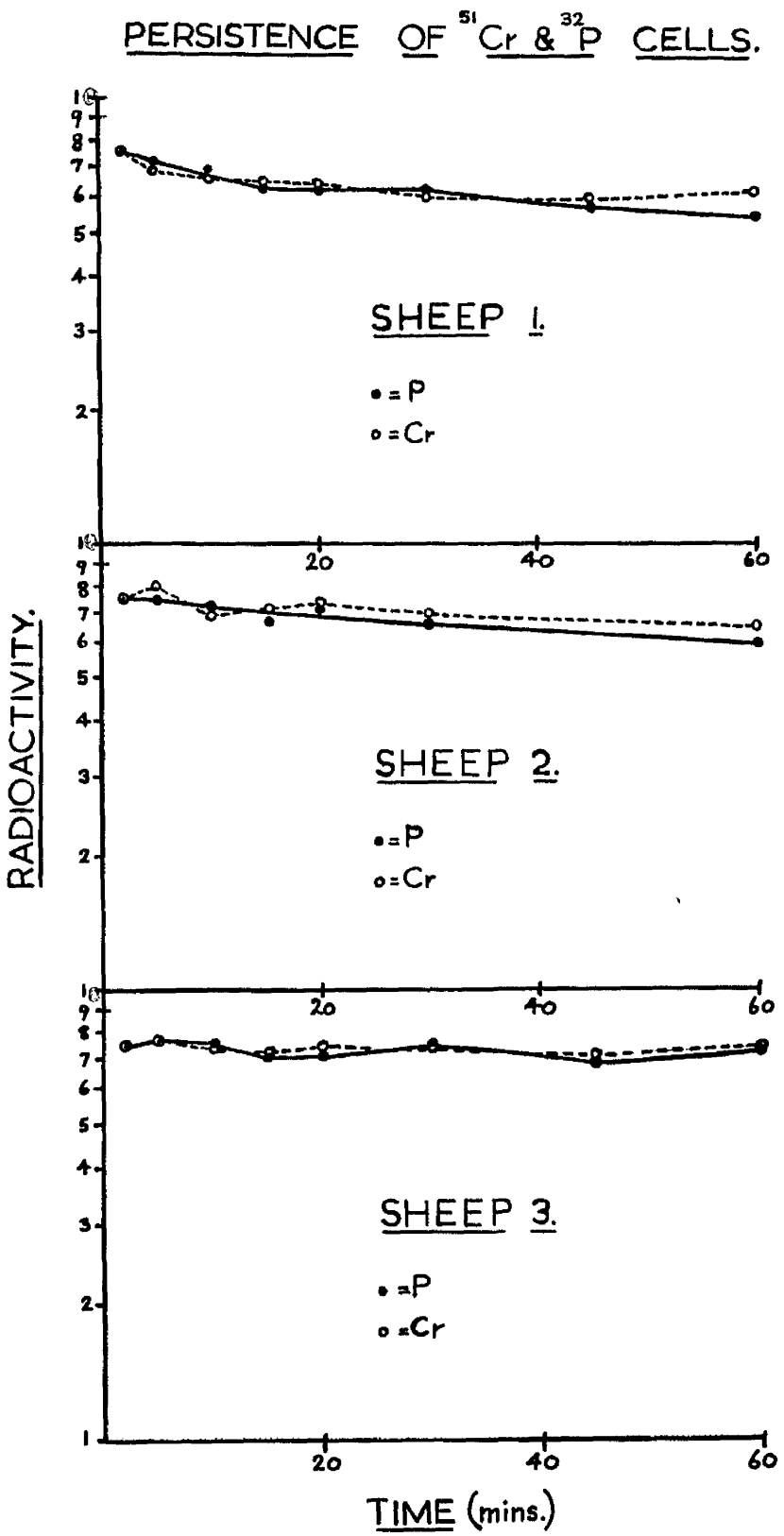

PERSISTENCE OF 51Cr & 32P CELLS.
RADIOACTIVITY.
SHEEP 1.
• = P
o = Cr
SHEEP 2.
• = P
o = Cr
SHEEP 3.
• = P
o = Cr
20
40
60
TIME (mins.)

FIGURE 27 contd.

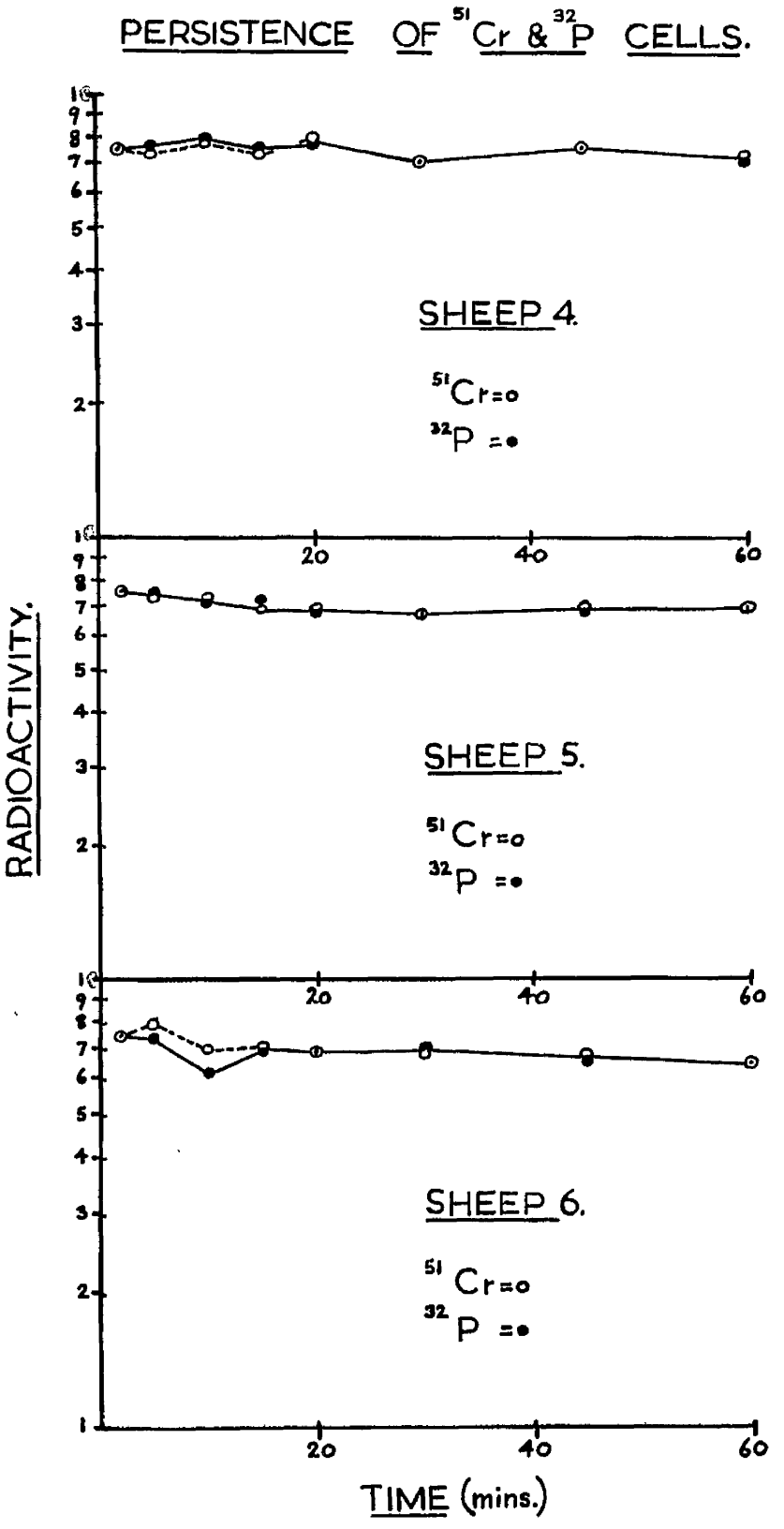


TABLE 52

RED CELL VOLUME ESTIMATIONS ON SHEEP, BASED ON CELLS LABELLED WITH ^{32}P AND ^{51}Cr

Sheep No.	No. ml Injected	Dilution Standard 1-		Corr. Counts /ml/100s Standard	Corr. Counts /ml/100s Sample	Cell Volume Litres	Wt. Kgs.	Cell Vol/Kg.
1	1.0	1,000	^{32}P	1,133	6,463	1.753	45.4	38.6
			^{51}Cr	286.7	1,979	1,449		31.9
2	1.0	1,000	^{32}P	1,103	9,659	1,142	41.3	27.7
			^{51}Cr	1,417	11,776	1.203		29.1
3	1.0	1,000	^{32}P	781	9,148	0.854	32.2	26.5
			^{51}Cr	2,754	36,566	0.739		23.0
4	1.0	1,000	^{32}P	971	12.377	0.785	36.7	21.4
			^{51}Cr	1,637	26,120	0.627		17.1
5	1.0	1,000	^{32}P	948	7,334	1.293	52.2	24.8
			^{51}Cr	798	7,978	1.000		19.2
6	1.0	1,000	^{32}P	900	5,211	1.727	63.5	27.2
			^{51}Cr	1,241	9,381	1.323		20.8

^{32}P Mean - 27.7
^{32}P S.D. - $\pm$ 5.8

^{51}Cr Mean - 23.5
^{51}Cr S.D. - $\pm$ 5.8

BLOOD VOLUME ESTIMATIONS ON SHEEP BASED ON CELLS LABELLED WITH ^{32}P AND ^{51}Cr

BLOOD VOLUME ESTIMATIONS ON SHEEP BASED ON CELLS
LABELLED WITH ^{32}P AND ^{51}Cr

Sheep No.		Cell Vol. Litres	True Peripheral Ht	Blood Volume Litres	Blood Volume ml/Kg.
1	^{32}P	1.753	37.4	4.862	103.1
	^{51}Cr	1.449		3.870	85.2
2	^{32}P	1.142	26.4	4.326	104.8
	^{51}Cr	1.203		4.557	110.3
3	^{32}P	0.854	31.7	2.696	83.7
	^{51}Cr	0.739		2.332	72.4
4	^{32}P	0.785	26.9	2.921	79.6
	^{51}Cr	0.627		2.332	63.6
5	^{32}P	1.293	26.7	4.989	95.6
	^{51}Cr	1.000		3.858	73.9
6	^{32}P	1.727	33.6	5.140	80.9
	^{51}Cr	1.323		3.938	62.0

^{32}P Mean - 91.3 $\pm$11.3

^{51}Cr Mean - 77.9 $\pm$17.9

(3) The Bovine Animal

The subjects were five 2-month-old calves (one male Jersey and two male and two female Ayrshires), and one adult Ayrshire dairy cow. When red cells were labelled, most of the plasma was removed before addition of the isotopes. 750 μc of ^{32}P and 300-400 μc of ^{51}Cr were added to the calves' red cells, but in the case of the cow 1600 μc of ^{32}P and 600 μc of ^{51}Cr were used. After incubation and washing, the cells were not reconstituted to their original volume with plasma, but were injected in a more concentrated form, with a haematocrit of approximately 75 per cent.

The animals were injected into the left jugular vein and withdrawals were made from the corresponding vein on the right side. In the case of the larger individuals, samples of 8.0 ml of whole blood were counted in the M6 liquid counter after being made up to 10.0 ml with water, a knife tip of saponin and a few drops of capryl alcohol. In such cases the diluted standard was made up for counting with a similar concentration of original blood, to eliminate the necessity of making a self-absorption correction to the M6 counts because of the increased density of the sample. Haematocrit tubes were spun at 1500g and 25°C for 2 hours and corrected for a trapped plasma of 6 per cent. The determination of ^{51}Cr and ^{32}P in the blood samples and the

subsequent calculations were as described for the dog. The results are shown in Figure 28 and Tables 54 and 55.

FIGURE 28

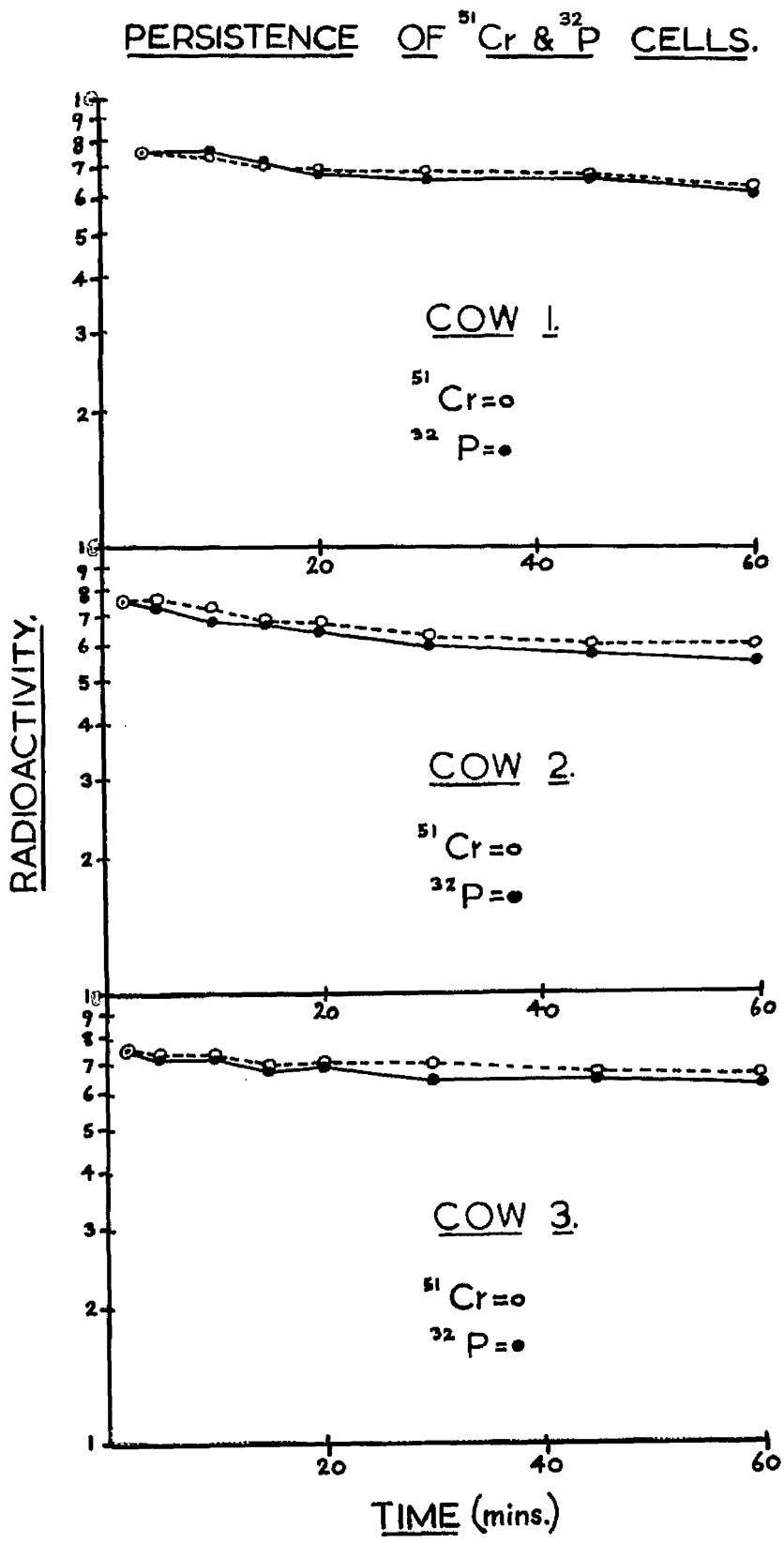

PERSISTENCE OF ^{51}Cr & ^{32}P CELLS.
RADIOACTIVITY.
COW 1.
^{51}Cr=o
^{32}P=●
COW 2.
^{51}Cr=o
^{32}P=●
COW 3.
^{51}Cr=o
^{32}P=●
20
40
60
TIME (mins.)

FIGURE 28 contd.

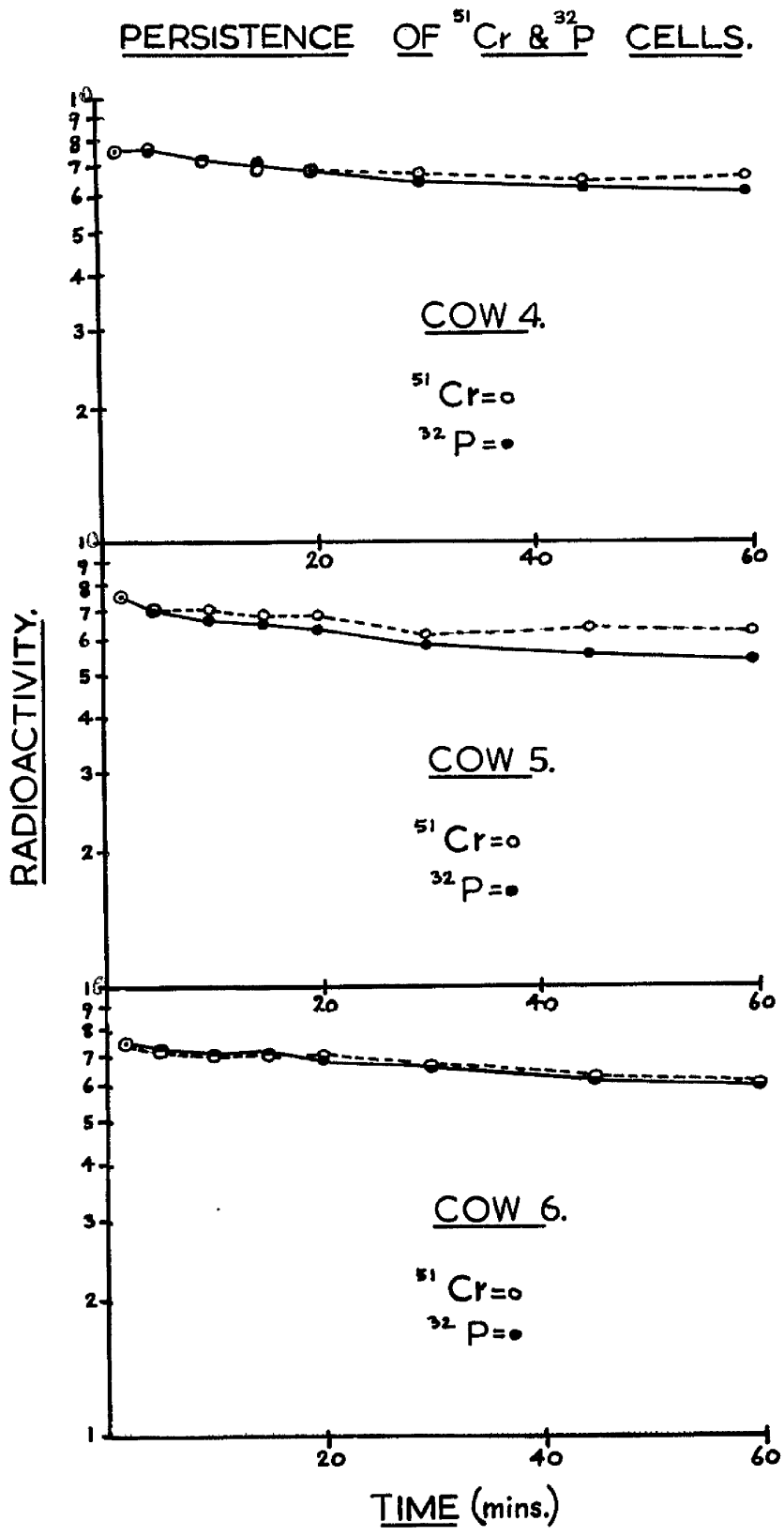


TABLE 54

RED CELL VOLUME ESTIMATIONS ON BOVINES, BASED ON CELLS LABELLED WITH ^{32}P AND ^{51}Cr

Bovine No.	No. ml Injected	Dilution Standard l.		Corr. Counts /ml/100s Standard	Corr. Counts /ml/100s Sample	Cell Vol. Litres	Wt. Kg	Cell Vol/Kg ml
1	7.5	1,000	^{32}P	2,287	14,725	1.165	60.3	19.3
			^{51}Cr	5,414	40,347	1.006		16.7
2	10.0	1,000	^{32}P	2,693	17,676	1.524	57.6	26.5
			^{51}Cr	1,569	14,038	1.118		19.4
3	8.5	1,000	^{32}P	3,957	21,695	1.550	78.9	19.7
			^{51}Cr	1,939	13,351	1.234		15.6
4	10.0	1,000	^{32}P	2,499	8,255	3.027	97.1	31.2
			^{51}Cr	1,251	5,500	2.274		23.4
5	10.0	1,000	^{32}P	2,712	14,364	1.888	95.3	19.8
			^{51}Cr	1,465	9,471	1.547		16.2
6	10.0	2,000	^{32}P	2,687	5,477	9.812	469.0	20.9
			^{51}Cr	1,976	4,335	9.116		19.4

^{32}P Mean - 22.9
^{32}P S.D. - $\pm$4.9

^{51}Cr Mean - 18.5
^{51}Cr S.D. - $\pm$2.9

TABLE 55

BLOOD VOLUME ESTIMATIONS ON BOVINES BASED ON CELLS LABELLED WITH ^{32}P AND ^{51}Cr

Bovine No.		Cell Vol. Litres	True Peripheral Ht	Blood Volume Litres	Blood Volume ml/Kg
1	^{32}P	1.165	29.1	4.003	66.4
	^{51}Cr	1.006		3.457	57.3
2	^{32}P	1.524	34.3	4.443	77.1
	^{51}Cr	1.118		3.259	56.6
3	^{32}P	1.550	28.2	5.496	69.7
	^{51}Cr	1.234		4.376	55.5
4	^{32}P	3.027	31.0	9.765	100.6
	^{51}Cr	2.274		7.335	75.5
5	^{32}P	1.888	27.2	6.941	72.8
	^{51}Cr	1.547		5.688	59.7
6	^{32}P	9.812	31.0	31.652	67.5
	^{51}Cr	9.116		29.456	62.7

^{32}P Mean - 75.7 $\pm$13.3

^{51}Cr Mean - 61.2 $\pm$7.5

(4) The Equine Animal

The subjects were two 3-month-old male Shetland ponies, two 3-month-old female donkeys, one aged Hunter gelding and one aged Clydesdale gelding. The techniques of labelling, injection, withdrawal of samples and treatment of samples were as described for the bovine animal. Haematocrit determinations of each sample were made after centrifugation at 1500g and 25°C for one hour. A correction factor of 2.7 per cent for trapped plasma was applied. The determination of ^{51}Cr and ^{32}P in the blood samples and the subsequent calculations were as described for the dog. The results are shown in Figure 29 and Tables 56 and 57.

It can be seen that red cell volumes based on ^{51}Cr-labelled cells are in general smaller than those based on ^{32}P-labelled cells. The relationship of the two sets of results is indicated in Table 58.

An examination of the persistence curves indicates that the discrepancy in ^{32}P and ^{51}Cr cell volume results is not due simply to increased rate of loss of ^{32}P-labelled cells between two and fifteen minutes post injection. It appears that there may be a loss of ^{32}P from the injected suspension of cells and the circulation within two minutes of injection. It was thought that even after four washes in plasma-saline the suspending liquid might contain an excessive amount of ^{32}P which would be rapidly removed from the circulation.

FIGURE 29

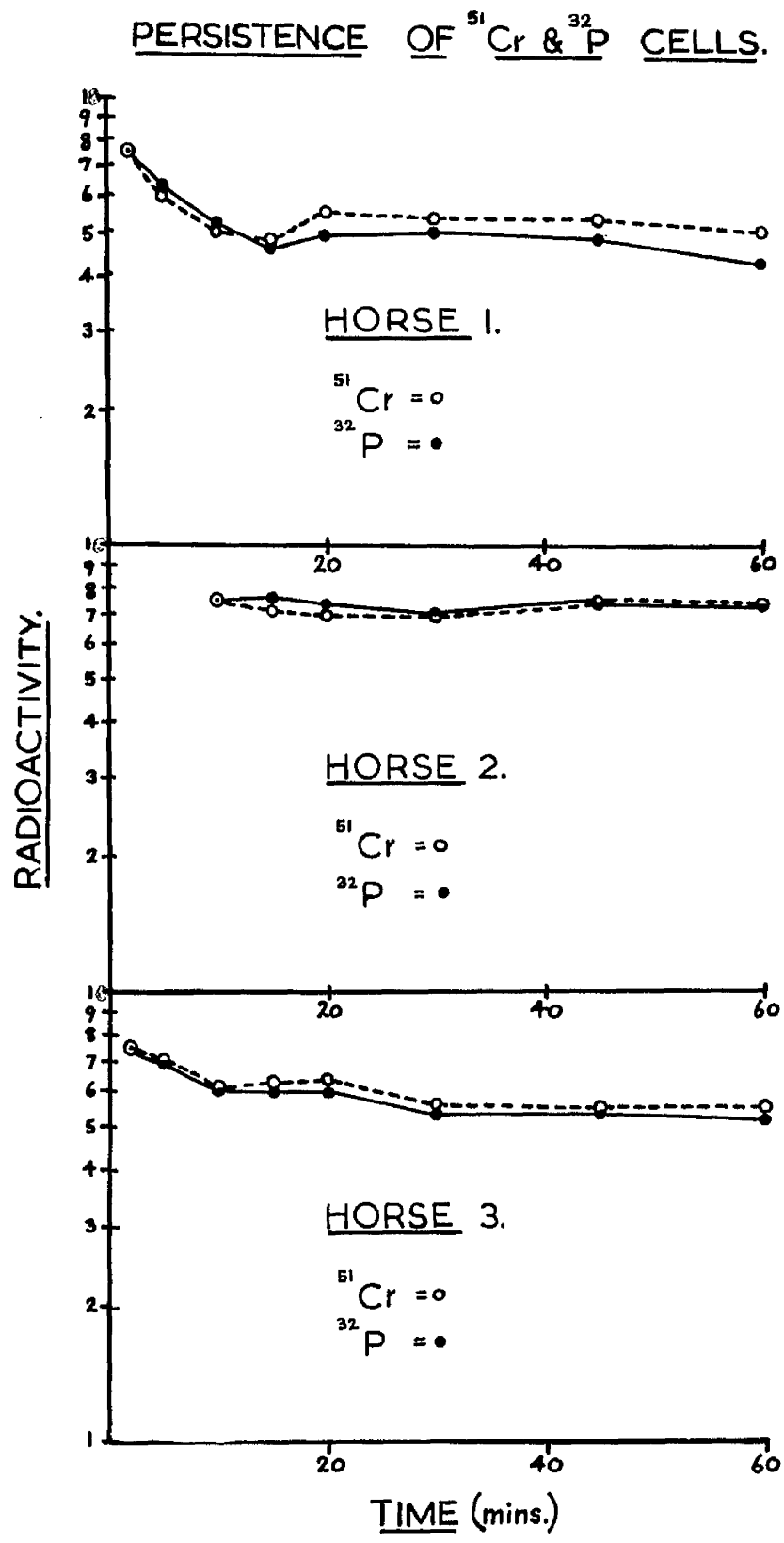

PERSISTENCE OF 51Cr & 32P CELLS.
RADIOACTIVITY.
HORSE 1.
51Cr = o
32P = •
HORSE 2.
51Cr = o
32P = •
HORSE 3.
51Cr = o
32P = •
20
40
60
TIME (mins.)

FIGURE 29 contd.

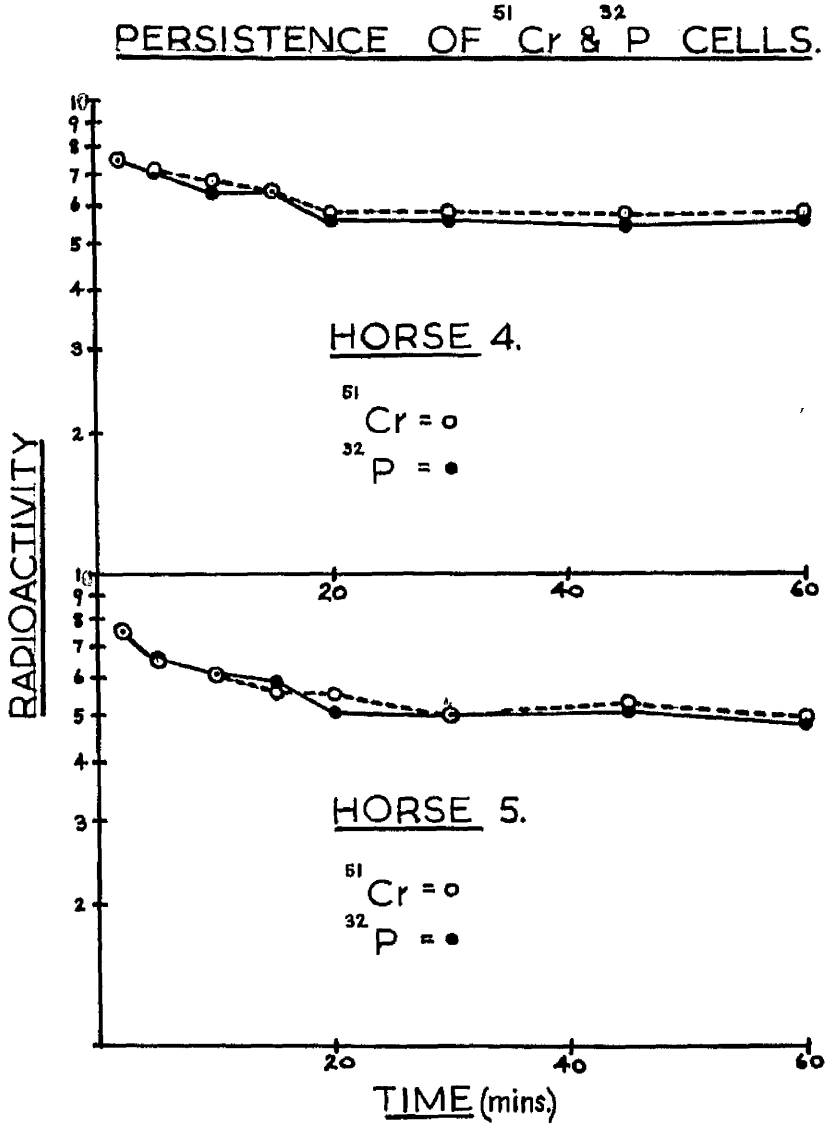


TABLE 56

RED CELL VOLUME ESTIMATIONS ON EQUINES, BASED ON CELLS LABELLED WITH ^{32}P AND ^{51}Cr

Equine No.	No. ml Injected	Dilution Standard 1-		Corr. Counts /ml/100s Standard	Corr. Counts /ml/100s Sample	Cell Volume Litres	Wt. Kgs.	Cell Vol/Kg ml
1	10.0	1,000	^{32}P	4,614	58,949	0.783	46.3	16.9
			^{51}Cr	3,408	43,445	0.784		16.9
2	7.5	1,000	^{32}P	6,902	21,670	2.389	86.0	27.8
			^{51}Cr	1,654	5,625	2.205		25.6
3 Donkey	10.0	1,000	^{32}P	2,770	14,997	1.847	54.4	34.0
			^{51}Cr	702	4,142	1.695		31.2
4 Donkey	10.0	1,000	^{32}P	2,667	14,977	1.781	51.7	34.4
			^{51}Cr	802	5,178	1.549		30.0
5	10.0	1,000	^{32}P	8,445	3,939	21.439	618.7	34.6
			^{51}Cr	2,708	1,252	21.629		35.0
6	10.0	1,000	^{32}P	11,450	5,100	22.451	607.8	34.9
			^{51}Cr	3,733	1,726	21.628		35.6

^{32}P Mean - 30.4 $\pm$7.2

^{51}Cr Mean - 29.1 $\pm$7.0

TABLE 57

BLOOD VOLUME ESTIMATIONS ON EQUINES BASED ON CELLS LABELLED WITH ^{32}P AND ^{51}Cr

Equine No.		Cell Vol. Litres	True Peripheral Ht	Blood Volume Litres	Blood Volume ml/Kg.
1	^{32}P	0.783	23.8	3.290	71.1
	^{51}Cr	0.784		3.295	71.1
2	^{32}P	2.389	35.5	6.730	78.3
	^{51}Cr	2.205		6.211	72.2
3	^{32}P	1.847	37.9	4.873	89.6
	^{51}Cr	1.695		4.472	82.2
4	^{32}P	1.781	39.9	4.464	86.3
	^{51}Cr	1.549		3.882	75.1
5	^{32}P	21.439	34.5	62.142	100.4
	^{51}Cr	21.629		62.692	101.3
6	^{32}P	22.451	37.9	59.237	97.5
	^{51}Cr	21.628		57.066	93.9

^{32}P Mean - 87.2 $\pm$11.2

^{51}Cr Mean - 82.6 $\pm$12.5

TABLE 58

RED CELL VOLUME BASED ON ^{51}Cr-LABELLED CELLS EXPRESSED AS A PERCENTAGE OF RED CELL VOLUME BASED ON ^{32}P-LABELLED CELLS

No.	Dog %	Sheep %	Bovine %	Equine %
1	109.0	82.7	86.4	100.0
2		105.3	73.4	92.3
3	61.6	86.5	79.6	91.8
4	96.8	79.9	75.1	87.0
5	107.5	77.3	81.9	101.2
6	81.9	76.6	92.9	96.3
AVERAGE	91.4	84.7	81.6	94.8

Supernatant liquid from the fourth wash of the labelled red cells of horse No. 6 was retained for estimation of ^{32}P radioactivity. The fluid in which the cells are finally suspended for injection, when it is first prepared, can be assumed to have less radioactivity than the liquid from the fourth wash. Assuming however, that the suspending fluid of the injected material contained as much radioactivity as that in the supernatant from the fourth wash, the following relationships were determined -

Corrected haematocrit of injected suspension = 73.9

Corrected counts/100 secs./1.0 ml/fourth wash supernatant = 370,500

Percentage of suspending fluid in 1.0 ml cell suspension = 26.1

$\therefore$ Corrected counts/100 secs. of suspending fluid in 1.0 ml suspension = 96,700

Total count/100 secs/1.0 ml injected suspension = 11,450 x 1,000

Percentage of total counts injected due to suspending fluid $= \frac{96,700 \times 100}{11,450 \times 1000}$

$= 0.84$

This result confirms that the fluid in which the injected cells were suspended contained little free ^{32}P a few minutes before injection. It does not obviate the possibility that some ^{32}P was loosely attached to the surface of the red cells, or that it became extruded from the cells to a significant degree in the few minutes between the last wash and collection of the first sample.

DISCUSSION

The amount of ^{51}Cr used to label the red cells of calves was 6-10 times less than that used by Stahl and Dale (1958) who incubated 50 μc ^{51}Cr per kilogram body weight in a whole blood sample of the calf under investigation for one hour at room temperature. Hodgetts (1961) injected about 33-150 μc ^{51}Cr into each sheep.

The uptake of ^{51}Cr by sheep red cells was found to be more than 90% in ten minutes and this differs from the findings of Hodgetts (1961). She recorded that the uptake of ^{51}Cr by sheep red cells in acid-citrate-dextrose at 12-14°C was 80-96 per cent in 30 minutes, but this anticoagulant was sometimes associated with haemolysis, and when heparin was used the uptake of radioactivity was slowed to 30 per cent in 30 minutes and 75 per cent in one hour. Klement, Ayer and Rogers (1957) found the percentage uptake of ^{51}Cr by goat red cells to be in inverse proportion to the original concentration of $Na_2{}^{51}CrO_4$ in the blood and it was of the order of 50-85 per cent in one hour. High concentrations of chromate were found to haemolyse cells readily and some haemolysis was nearly always seen by those workers.

Stahl and Dale (1958) found that ^{51}Cr-tagged bovine red cells did not lose radioactivity readily when suspended in saline, but that washing in homologous plasma was associated with significant

loss. After injection of labelled cells into calves, up to 20 per cent loss of radioactivity from the circulation was found two hours after injection. Hodgetts (1961) found the loss of ^{51}Cr radioactivity from the circulation of the sheep to be 7 per cent in one hour and 20 per cent in four hours post injection if labelling had taken 30 minutes, regardless of whether the anti-coagulant was heparin or acid-citrate-dextrose. If labelling had taken one hour, the loss in one hour post injection was 11.5 per cent.

Anderson and Rogers (1957) found the time-concentration curve of ^{51}Cr-tagged cells in the circulation of the goat to be anomalous. 20 goats were injected and 11 of these lost 1-9 per cent of the original activity in 30 minutes, whereas nine animals lost 15-25 per cent. Klement, Ayer and Rogers (1957) found that the loss from the circulation of the goat during the first 30 minutes post injection was generally uniform and logarithmic and about 10 per cent per hour, but occasionally it was much more. In some cases a second logarithmic phase appeared 45-60 minutes post injection. Chien and Gregersen (1962), writing mainly about determinations in man and the dog state that the loss of chromium label is negligible during the course of an experiment while in contrast ^{32}P labelled cells lost activity at a rate of about 6 per cent during the first hour.

In the experiments described in Part 2 Section (B) the loss of ^{51}Cr from the circulation of the dog was approximately 7 per

cent in the first hour after injection, more than reported by Chien and Gregersen (1962). The loss of chromium from the circulation of the sheep was approximately 10% in one hour, a little more than reported by Hodgetts (1961). The loss rate of ^{51}Cr and ^{32}P from the circulation of the horse was approximately 30 per cent in one hour, more than in any of the other species.

The uptake of ^{32}P by the red cells of different species illustrated in Figure 25 is in general similar to that found previously (Figure 18). In the more recent experiments however the uptake of ^{32}P by the cells of species other than the dog was greater than determined previously, though the uptake by sheep and bovine blood remained relatively low.

The persistence curves of ^{51}Cr and ^{32}P-labelled cells usually showed significant fluctuations and no satisfactory explanation for this was found. They were based on estimations of radioactivity in equal volumes of packed red cells which were determined after correction for trapped plasma. Fluctuations in the curve were therefore not due simply to changes in the haematocrits of different samples, but they were of such a nature that it was not practicable to use extrapolation for the calculation of cell volumes.

The relatively high blood volume/Kg results in greyhounds agree with the findings of Courtice (1943) who reported that greyhounds had relatively greater blood volumes than other breeds

of dog, due to increased red cell volume. Kjellberg, Rudhe and Sjostrand (1949) reported that blood volume is increased by increase in muscle volume, as in a trained athlete. Julian, Lawrence, Berlin and Hyde(1956) used ^{32}P-labelled cells in six race-horses and four Percherons. The blood volume/Kg body-weight was much higher in the race-horses.

Pregnancy is known to be associated with an increase in blood volume (Berlin, Goetsch, Hyde and Parsons, 1953; Verel, Bury and Hope, 1956). Barcroft, Kennedy and Mason (1939) determined increase in blood volume, essentially a plasma-volume increase, as pregnancy developed in sheep, compared with non-pregnant controls. The blood volume increase was of the order of 20-25 per cent. Reynolds (1953b) determined plasma and blood volumes in 20 pregnant and 7 recently calved cows. It was found that the increase in plasma and blood volume with pregnancy was maintained during lactation. The only pregnant animal investigated in the experiments at present being reported did have a noteably high blood volume per kilogramme body weight (Dog No. 3. Table 46. Bv = 126.5 ml/Kg).

Dale et al (1957) compared blood volumes of three lactating Jersey cows and three which were dry. The lactating animals had plasma volumes about 30 per cent greater. Hansard et al (1953) did not find increase in blood volume in four lactating Hereford cows, but reported that five lactating burros had blood volumes about 25% larger than non-lactating mature animals. The only

lactating animals investigated in the current experiments were six sheep. The blood volumes/Kg determined in these were in the higher range of those reported in the literature.

Blood volume has been found to be increased at an increased altitude (Lawrence, 1955), and under warm conditions (Yoshimura, 1958). Dale et al (1957) performed estimates of blood volume and plasma volume in cattle at temperatures with a marked diurnal variation. Serum and blood volume estimations were increased by 10-18% at the higher ranges of temperature.

Hansard et al (1953) found significantly higher blood volume/Kg measurements in the young of all species than in older animals. Gotsev (1939) found significantly higher values in lambs than in adult sheep. In the experiments currently reported, several young bovines and equines were used. The results in these are in line with the findings of Hansard.

The results of blood volume determinations using ^{51}Cr and ^{32}P-labelled cells, expressed in ml/Kg are in line with those determined in previous experiments, though the ^{32}P-cell volume figures are somewhat higher. This is explained by the high proportion of greyhounds in the most recent experiments. The lower readings obtained in ^{51}Cr experiments are associated in part with the slower loss of this isotope from the circulation. As ^{51}Cr labels red cells more effectively and more rapidly than ^{32}P it appears to be the isotope of choice for this type of experiment.

In the estimation of blood volume by means of labelled cells there appear to be two main disadvantages. Every animal must have its blood individually labelled, and the persistence of labelled red cells in the circulation after injection appears to be irregular. The results obtained with labelled red cells in these experiments do not appear to be accurate enough to enable changes in circulating blood volume which occur over a short period of time to be accurately assessed.

Blood volume might be better assessed by the use of labelled plasma protein. In this respect ^{131}I has advantages over Evans' blue and as this isotopic method has been little investigated in animals it seems worthy of further study.

SUMMARY

1. ^{32}P and ^{51}Cr radioactivity was determined in the same sample by counting in different counters.

2. The red cells of different species were labelled simultaneously with ^{32}P and ^{51}Cr. The uptake of ^{32}P was found to be similar to that determined previously while the uptake of ^{51}Cr was found to be of a much higher degree than that of ^{32}P, and much more rapid.

3. The persistence of ^{32}P and ^{51}Cr-labelled cells in the circulation of various species was studied by the estimation of radioactivity in 1 ml of packed red cells, derived from a haematocrit for which the correct allowance for trapped plasma had been made. ^{51}Cr was lost less rapidly than ^{32}P.

4. Determinations were made on 6 dogs, 6 sheep, 6 bovine animals and 6 equine animals. Blood volume measurements were determined and when expressed as ml/Kg the results based on ^{51}Cr labelled cells were smaller than those based on ^{32}P-labelled cells. The results based on ^{51}Cr-labelled cells are probably the more accurate and this isotope is to be preferred for labelling purposes.

5. The results of the experiments are discussed in relation to the results determined in Part 2, Section (A) and the literature.

GENERAL SUMMARY

Part 1

1. After centrifugation of blood under conditions acceptable for measurements on the blood of man and the dog, the amount of plasma trapped in the red cell column of cows' blood was found to be much greater than that trapped in the blood of rabbit, dog or horse.

2. Haematocrit determinations were carried out on bovine, sheep, dog, rabbit, pig and horse bloods under varying conditions of centrifugation. It was found that before reaching stabilisation, bovine and sheep samples required to be centrifuged for a much longer period than the samples of other species. Estimations of intercellular plasma were made on bovine blood after centrifugation for 2 hours at 1500 g in a refrigerator centrifuge. Despite the extended period of centrifugation the amount of intercellular plasma was still very high.

3. The effect of the temperature of the centrifuge on haematocrit determinations and the percentage of trapped plasma was studied. Increase in temperature of the centrifuge was found to be associated with a reduction in the height of the haematocrit and the amount of trapped plasma. A modified analytical method was devised which proved effective in the study of the total and distribution of trapped plasma at different levels of the packed red cell column.

4. It was demonstrated that increasing the proportion of red cells in a blood sample did not change the proportion of trapped plasma in the lower sections of the haematocrit column.

5. A substantial number of accurate determinations of trapped plasma in the bloods of different species were performed. For each species graphs were constructed from which it was possible, when the height of the haematocrit of an individual of the species was determined, to read off the amount of plasma trapped under standard conditions of centrifugation.

Part 2 (A)

6. Marked species differences were found in the uptake of ^{32}P by the red cells of different species. Uptake by the cells of cow, sheep and horse was poor.

7. It was found that the uptake of ^{32}P by horse red cells was markedly increased by removing plasma before adding the isotope.

8. The persistence of ^{32}P-labelled red cells in the circulation, and blood volumes were determined in horses, cattle, pigs and dogs.

Part 2 (B)

9. It was found practicable to determine ^{32}P and ^{51}Cr activities in the same sample by using different counters.

10. The uptake of ^{32}P and ^{51}Cr by red cells was studied in different species after simultaneous labelling.

11. The persistence of ^{32}P and ^{51}Cr-labelled cells in the circulation was studied. Blood volume estimations were made, based on ^{32}P-labelled cells and on ^{51}Cr-labelled cells and the results compared.

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