THE BLOOD VOLUME IN MAN AND ANIMALS

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ABSTRACT OF THE DISSERTATION

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In general two methods have been used for the determination of blood volume in man and animals. These are, the CO method which measures red cell volume directly, and the dye method which measures plasma volume directly. Total blood volume in each case is calculated from the cell to plasma ratio given by the central venous or arterial haematocrit. Both methods have been subject to criticism; the CO method on the grounds of technical difficulties in CO in blood analyses and the fact that it may include an unknown quantity of muscle myoglobin in addition to circulating haemoglobin; the dye method because injected dye substances disappear from the circulation and degree of dilution in the plasmas is calculated by extrapolation of the disappearance curve. In addition, the assumption that the central venous haematocrit is truly representative of the ratio of the body's total red cell volume to plasma volume has been questioned. If 'body' haematocrit and the central venous haematocrit are identical, simultaneous determinations of total blood volume by the CO method and the dye method should give equal values.

In this work both methods have been re-investigated in humans and rabbits. Simultaneous estimations of total blood volume by CO and the blue dye T-1824 have been carried out. The

CO method has been tested in humans during muscular exercise and in rabbits by the addition and removal of known quantities of red cells. The effect of variations in the fluid content of the diet of rabbits, and the rate of replacement of circulating fluid volume following haemorrhage in unanaesthetized and nembutalized animals, have been studied.

The CO Method.

CO was administered by having the subject breathe from a closed respiratory circuit consisting of a Krogh spirometer, inspiratory and expiratory valves and a soda-lime container for absorption of CO_2, with O_2 admitted at intervals. In the human experiments approximately 90 c.c. of CO were admitted to the inspiratory side of the circuit. The rate of uptake and disappearance of CO in the blood was investigated by taking blood samples at intervals for one hour with the subject at rest, and continuing to breathe from the closed circuit. CO in blood analyses were done simply and accurately using the syringe-capillary apparatus described by Scholander and Roughton.

These experiments showed that maximum absorption had occurred 10 minutes after administration of CO and that there was a slow fall in CO concentration in the blood thereafter which amounted to 0.13 c.c. per 100 c.c. in one hour when the original concentration

had been 2.00 c.c. per 100 c.c. This fall in CO concentration was observed in the rabbit and cat, as well as the human. It was not due to haemodilution and could not be explained by leakage from the breathing system or taking up of CO in physical solution by the body fluids. It was believed to be due to combination of CO with some haemoglobin-like substance outside the circulation, notably muscle myoglobin.

On theoretical grounds, the total amount of CO which disappeared slowly from the circulation over the course of one hour was just sufficient to satisfy equilibrium conditions with muscle myoglobin considering the various factors of CO and O_2 tensions involved. In that case, calculation of blood volume from the 10 minute CO level would not be incorrect due to the inclusion of myoglobin.

CO curves were studied on human subjects performing moderate muscular exercise on the electric-brake bicycle ergometer. It was shown that with this degree of exercise (420 Kgm. metres per min.) the pulmonary ventilation, cardiac output and O_2 consumption were increased to three times the resting value. With this degree of increase in blood flow to active muscles, the curves showed no greater disappearance of CO than at rest. Unless there had been a very rapid uptake of CO by myoglobin during muscular exercise

before the first blood sample was taken, the total myoglobin effect could not have been greater than that observed at rest. That this rapid uptake did not occur was shown by comparing the effect of a repeat dose of CO at rest and during exercise on the same group of subjects.

The amount of CO left unabsorbed when equilibrium had been reached between the CO tension in the air of the closed circuit and the blood was determined. In human subjects this fraction was 1.6% and in rabbits 3.3% of the volume of CO administered. In the calculation of blood volume from the CO concentration in the blood at 10 minutes the appropriate correction factor was applied. The 10 minute value was used because it allowed time for absorption and mixing of CO without including a significant disappearance effect.

The Dye Method.

A known amount of the blue tetrazo dye T-1824 in aqueous solution was injected intravenously. The plasma dye concentration 10, 20 and 30 minutes after the injection was determined in a photo-electric colorimeter. These values were plotted linearly in the form of a time-concentration curve; the theoretical dye concentration at zero time was obtained by extrapolation of this curve. For human work the dye was extracted from the plasma

into clear butyl alcohol by the method of Harington, Pochin and Squire to avoid the errors in colorimetry arising from lipemia and plasma pigments. Plasma volume was calculated from dye dilution; total blood volume from plasma volume and the venous haematocrit. Studies of the effect of anticoagulants on the haematocrit value revealed that potassium oxalate alone or a mixture of potassium and ammonium oxalate caused shrinkage of the red cells with resultant errors in cell and plasma volume calculations. Heparin was used to avoid these errors.

In rabbits the disappearance curve of T-1824 was studied after single and repeat dye injections. It was shown that there was no alteration in the shape of this curve after the repeat injection. Dye dilution calculated by the extrapolation procedure described gave plasma volume values which showed agreement between the first and second injections.

CO and Dye Methods Simultaneously.

The two methods were used simultaneously to determine total blood volume in 20 human and 15 rabbit experiments. In both species the results agreed to within ± 5%. These findings were in support of those of Root, Roughton and Gregersen who maintain that there is no significant difference between central venous and 'body' haematocrit; they are opposed to those of Whipple et

et al. and Gibson et al. who report that 'body' haematocrit is lower than central venous haematocrit.

Average figures from human subjects demonstrated a total blood volume of 74.7 c.c. per Kgm. of body weight; a cell volume of 33.0 c.c. per Kgm.; and a plasma volume of 41.7 c.c. per Kgm. Average figures from the rabbits gave a total blood volume of 57.7 c.c. per Kgm. of body weight; a cell volume of 18.0 c.c. per Kgm.; and a plasma volume of 39.8 c.c. per Kgm. Moderate muscular exercise in humans was not attended by any increase in circulating red cell volume or rise in haematocrit.

Although the two methods gave numerically equivalent values it was considered possible that each could have included systematic sources of error in the same direction so that neither measured absolute blood volume accurately. To clarify this point the CO method was tested in rabbits by the removal and addition of known volumes of red cells to the circulation. The estimated reduction or increase in red cell volume agreed closely with the volume of cells actually removed or added.

Emphasis has been placed in this work on the CO method for determining blood volume in man and animals. Use of standard closed-circuit breathing systems and the syringe-capillary method of CO analysis have made the procedure simple and accurate. It

has been found that the CO method offers certain advantages over the dye method. Only two small blood samples are required. Haemolysis in these is of no importance. The method can be repeated several times in one day with the same degree of accuracy. In human subjects repeated determinations by the dye method sometimes result in discoloration of the skin. CO, being more 'physiological' in its behaviour, is breathed off in several hours. When the apparatus is set up a blood volume determination can be carried out in 10-15 minutes. These considerations recommend the CO method for use in clinical or laboratory investigations where it is desired to perform repeated determinations of the blood volume in a short period of time.

Haemorrhage Experiments in the Rabbit and Cat.

The rate of replacement of fluid to the circulation following haemorrhage of 30% of the blood volume was studied in unanaesthetized rabbits. It was demonstrated that 2 hours after bleeding the circulating fluid volume had returned to its original level; in 24 hours it was in many cases greater than the original value. This overcompensation was still present at 72 hours, but at 14 days when regeneration of red cell volume was almost complete the total volume had returned to normal. This rapid restoration of fluid to the circulation in rabbits stands in contrast to

observations in other species such as the dog and human where 72 hours may be required.

The effect of nembutal (sodium pentobarbital) anaesthesia on the rate of haemodilution following haemorrhage was investigated in rabbits and cats. In rabbits, fluid was replaced but at a slower rate than in an unanaesthetized group; in cats there was no restoration of fluid during the period of anaesthesia. This observation is of practical importance because studies of experimental shock in animals are often carried out under nembutal and it is worthwhile to know the effect this anaesthetic exerts on replacement of circulating fluid volume.

In the haemorrhage experiments on rabbits and cats the red cell volume was measured before and after the removal of known amounts of blood. These figures, together with others in the literature for humans and dogs, show that haemorrhage is not followed by the addition of red cells to the circulation from such storage depots as the spleen, bone marrow and liver sinusoids. Other experiments on muscular exercise in humans supply additional evidence that the concept of reserve stores of red cells may be open to question.

Fluid Content of the Diet and Blood Volume.

In rabbits it was shown that alteration in the fluid content of

the diet resulted in changes in plasma volume and haematocrit. On a diet consisting chiefly of cabbage the blood volume of a series of animals was 10% higher than the blood volume of the same group on a dry diet of oats, bran and water. The rise in blood volume was due to a greater plasma fraction; the red cell volume remained constant. This rise in plasma volume, accompanied by a corresponding rise in the fluid stores of the body would aid the compensatory mechanisms for restoration of fluid volume in shock and blood loss.

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PREFACE

I would like to acknowledge my indebtedness to my supervisor, Dr. F.C. Courtice, in whose laboratory this work was carried out. His help, encouragement and example were invaluable.

I am also indebted to Mr. P.J. Phipps and Mr. T.J. Meadows for their technical assistance and willingness to act as subjects in many of the human experiments.

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INTRODUCTION

General

The measurement of the total volume of circulating blood is a valuable procedure in studying the physiology of man and experimental animals. It provides information not supplied by the analysis of unit volumes of blood. Haemoglobin content and oxygen capacity are commonly expressed in terms of unit volumes; yet these values give no indication of the body's total oxygen capacity without simultaneous knowledge of the total blood volume. In investigations on acclimatization to the conditions at high altitudes it is important to ascertain the rate and degree of increase in the body's total oxygen capacity. Likewise in the abnormal conditions of anaemia, polycythaemia, erythroblastosis foetalis, studies of the changes in total oxygen capacity resulting from treatment provide useful guidance in therapy.

In metabolic studies the total amounts of protein, sugar, urea, sodium, calcium present in the circulating blood can be calculated only with the knowledge of their respective concentrations and the total blood volume. Recent developments relating to water balance have made it possible to estimate the

volume of intracellular fluid and extracellular fluid. Measurements of plasma volume changes as fluid moves to and from these compartments are important in a variety of clinical conditions such as salt depletion, dehydration, adrenal cortical insufficiency.

The hydrodynamics of the circulation can be more accurately studied if the investigator knows the total quantity of blood involved. The changes with posture, muscular exercise and environmental temperature show interesting physiological adaptations in the volume of blood. The pathological states of shock, heart failure, hypertension may be further clarified by blood volume studies.

The importance of the problem has not gone unrecognized in the history of physiological investigation. Indeed, the literature pertaining to methods of blood volume estimation has paralleled in time the development of the literature of modern experimental physiology. In 1854 Welcker described his direct method for the estimation of blood volume. Yet almost a century later there is major disagreement on the accuracy of modern methods of cell and plasma volume determinations.

Erlanger (1921) has provided a comprehensive review of earlier work. In the classical direct method of Welcker the

blood is actually collected by bleeding, washing out the vascular bed and extracting the small fraction remaining from ground-up tissues. The blood volume is then calculated from the degree of dilution of the blood, washings, and extractions by comparison with the haemoglobin concentration of an original undiluted normal sample of blood. The Welcker method, with modifications, was used by many early workers without any universal agreement in results. One of the chief criticisms of the method was that it included in the blood volume, the muscle myoglobin. A more important disadvantage of the method was that it involved sacrifice of the experimental animal and could not be used for humans, except in the unique circumstances described by Bischoff who determined the blood volume of two executed criminals. It was of value for comparison with results obtained by other methods and was so used in rabbits by Boycott and Douglas (1909) and in dogs by Smith, Arnold, and Whipple (1921).

Indirect methods for ascertaining the blood volume have been divided by Salvesen (1919) into concentration and dilution methods, and foreign substance methods. In the dilution method as used by Valentin (1838), Cohnstein and Zuntz (1888), and Sherrington and Copeman (1893), a known volume of distilled water or isotonic saline is injected into the circulation and

from the change in concentration of one of the blood constituents, the original blood volume is calculated. In the concentration method of Tarchanoff (1880) the increase in haemoglobin concentration resulting from a steam bath and the measured loss of body weight, are used to calculate the blood volume. Both these methods are based on principles which later work has shown to be invalid or of limited accuracy.

The foreign substance method is based on the principle that a known volume of some easily detectable and measureable substance introduced into the circulation will be diluted by the blood and its concentration when completely mixed in the circulation will provide a figure from which the blood volume can be calculated. To satisfy ideal criteria this substance should be non-toxic; it should be capable of accurate measurement; and it should not escape from the circulation in the time required for complete mixing.

Many substances have been used for this purpose including: dextrin by Aberhalden and Schmid (1910); tetanus antitoxin by von Behring (1912); foreign serum by Schurer (1911); gum acacia by Meek and Gasser (1918); colloidal gelatin solution by McQuarrie and Davis (1920). Although no substance has yet been found to satisfy the ideal criteria completely, two have proved

more satisfactory than all others and have occupied the most prominent positions in the literature on blood volume. These are carbon monoxide, introduced by Grehant and Quinquaud in 1882, and dye, introduced by Keith, Rowntree and Geraghty in 1915. More recently radioactive substances have been used; radioactive iron incorporated in the haemoglobin molecule and radioactive phosphorus incorporated in the red cells (Hahn, Ross, Bale, Balfour and Whipple, 1942) (Gibson, Peacock, Seligman and Sack, 1946) (Meneely, Wells and Hahn, 1947).

The carbon monoxide method.

This method utilizes the capacity of haemoglobin for forming with carbon monoxide a relatively stable compound, carboxyhaemoglobin. Because of the great affinity of haemoglobin for carbon monoxide, which in different species varies from 100 to 300 times the affinity for oxygen, this gas when inhaled is rapidly absorbed by the blood. If the amount of CO introduced into the circulation is known, and the amount of CO present in 100 c.c. of blood is estimated by some analytical procedure, then the blood volume in c.c. is given by the simple calculation:

$$\frac{\text{Volume CO introduced in c.c.}}{\text{CO in blood in c.c. per 100 c.c.}} \times 100$$

The CO method, as other foreign substance methods, gives a true

estimate of the blood volume only if the ideal criteria mentioned above are satisfied. Its accuracy is directly limited by the accuracy of the CO in blood analysis.

Gréhant and Quinquaud first used the method in dogs. Haldane and Lorrain-Smith (1900) described a method for the estimation of blood volume in humans using CO. The subject breathed from a closed circuit consisting of a rubber bag and soda-lime container for absorption of the CO_2. A known volume of CO was introduced into the circuit and was rebreathed for several minutes, O_2 being added as required. The CO content of the blood was estimated by Haldane's carmine titration method. Most subsequent workers have used some modification of this original closed circuit apparatus described by Haldane and Lorrain-Smith.

Douglas (1906) applied the method to rabbits, used the carmine titration procedure for HbCO analysis, and improved the technical accuracy by measuring the amount of unabsorbed CO remaining in the circuit at the time the blood sample was taken. Further work in rabbits was reported by Boycott and Douglas (1909), Boycott (1911) and in man by Douglas (1910).

Oerum (1906) and Zuntz and Plesch (1908) used the CO method, describing improved gasometric techniques for the estimation of

CO in blood. In the method of Zuntz and Plesch the CO is liberated from the blood by the action of ferricyanide, burnt to CO_2, absorbed with KOH and the change in pressure noted as in Barcroft's method for blood gas analysis.

Van Slyke and Salvesen (1919) described an improved method for CO in blood analysis using the Van Slyke volumetric apparatus and Salvesen (1919) estimated the blood volume of rabbits using Douglas's (1906) circuit and of humans using Haldane & Lorrain-Smith's (1900) circuit. Refinements in the analytical procedure involving the use of the Van Slyke manometric apparatus were described by Van Slyke and Neill (1924), and the use of Winkler's ammoniacal cuprous chloride solution for absorption of CO by Van Slyke and Robscheit-Robbins (1927), and Sendroy and Liu (1930).

Using these methods of analysis for CO and a closed circuit respiratory apparatus Arnold, Carrier, Smith and Whipple (1921) estimated the blood volume of dogs, and Smith, Belt, Arnold and Carrier (1925) studied blood volume changes at high altitude in humans. Chang and Harrop (1928) re-investigated the CO method in man and Waterfield (1931) used their technique to study blood volume alterations with changes in posture. The method has been used further in man by Asmussen (1941), Asmussen and Consolazio (1941), Hopper, Tabor and Winkler (1944) and Hopper, Winkler and

Elkington (1944).

Bazett, Sunderman, Maxfield and Scott (1940) in their CO method, estimate the HbCO percentage in blood using laked red blood cells and a photo-electric device with appropriate light filter. In 1942, 1943, 1945, Scholander and Roughton described an accurate microgasometric method for estimating the carbon monoxide content in small samples of blood. Using this method of blood carbon monoxide analysis, Root, Roughton, and Gregersen (1946) determined the blood volume by the CO method, but for the first time they abandoned the closed circuit apparatus for absorption of the carbon monoxide and employed an open circuit.

The dye method.

Keith, Rowntree and Geraghty (1915) introduced the use of brilliant vital red dye for plasma and blood volume determination in man. They injected a known amount of the dye in solution in distilled water into an arm vein, allowed 3 to 6 minutes for complete mixing, removed a sample of blood and estimated the concentration of dye in the plasma in an ordinary colorimeter by comparison against a standard dye in plasma solution of known concentration. The plasma volume could then be calculated by the degree of dilution of the injected dye. The total blood volume was calculated by determining the cell to plasma ratio (haematocrit).

In the 33 years which have elapsed since this original publication many improvements in technical procedures and theoretical interpretations of results have accrued from numerous investigations on the dye method of blood volume estimation. Only the most important of these will be mentioned here. Dawson, Evans and Whipple (1920) described the behaviour of a large series of dyes injected into the circulating blood. They discovered that a blue tetrazo dye, T-1824 (Evans Blue) exhibited optimal properties for use in blood volume determinations. It disappeared slowly from the circulation, was non-toxic, and was suitable for colorimetric estimation. Gregersen, Gibson and Stead (1935) investigated the spectral absorption curve of T-1824 and found that it gave maximal light absorption at a wave length of 620-630 millimicrons. This fact allowed correction to be made for the presence of slight haemolysis in plasma samples by the insertion of light filters which would eliminate light of the wave length absorbed by oxyhaemoglobin (540 to 578 millimicrons). The principle was used by Gibson and Evans (1937), Gibson and Evelyn (1938), Kennedy and Millikan (1938) and Morris (1944). Gregersen and Gibson (1937) showed that salt or protein, present in dye solutions, altered their colour intensity and these authors pointed out the inaccuracy of using dye in water

solutions for comparison against dye in plasma solutions.

Gibson and Gregersen (1935) investigated the toxicity of T-1824 in young male rats and found that 100 times the dose used in blood volume determinations did not cause any slowing in growth. They reported that the dye was present in reticulo-endothelial cells and was apparently slowly excreted by the liver and biliary tract. No dye was found to have passed through the kidney glomerulus. This fact was later explained by the discovery that T-1824 is firmly combined with plasma albumin - demonstrated by the dialysis and electrophoretic studies of Gregersen and Rawson (1943) and through the use of the ultra-centrifuge by Ogston (quoted by Courtice 1943).

These properties of T-1824, its non-toxicity, its slow disappearance from the circulation and its ease in optical measurement encouraged blood volume studies by the dye method. Improvements in colorimetric technique were described. The use of the monochromatic visual spectrophotometer by Gregersen et al. (1935) in which only a narrow wave length band near 620 millimicrons is allowed to pass through the dyed plasma, increased the precision of measurement. Kennedy and Millikan (1938) estimated T-1824 concentration, using a photo-electric colorimeter. Harington, Pochin and Squire described a method for the

quantitative extraction of T-1824 from plasma samples into clear butyl alcohol. In this procedure plasma pigments, cloudiness due to lipemia and 1% haemolysis were removed, rendering the colorimetric analysis by a simple photocell even more precise. An alternative extraction method using alcoholic phosphotungstic acid precipitation was described by Crooke and Morris (1942) and later a chromatographic method by Morris (1944).

In the original method as described by Keith, Rowntree and Geraghty it was assumed that complete mixing of the dye in the circulation had occurred in 4 minutes. The plasma volume was calculated from the dye concentration at that time. Further studies by Dawson et al. (1920) of the behaviour of a large number of dyes injected into the circulation showed that all of these substances, including T-1824, disappeared slowly from the circulation. This rate of disappearance varied with the dye used and with the species of experimental animal used. It became common practice in blood volume studies to plot time-concentration curves of dye disappearance. At varying times after injection these curves assumed a linear disappearance rate. The theoretical dye concentration at the moment of injection was determined by extrapolating the linear part of the curve back to 0 minutes and reading the dye concentration on the

ordinate scale.

From their data on the disappearance rate of T-1824 in humans, Gibson and Evans described an initial rapidly falling phase of the curve occupying the first 10 minutes after injection, followed by a linear disappearance curve. They ascribed the initial rapid fall to mixing of the dye with all parts of the circulation. In calculating the theoretical dye concentration at the moment of injection they extrapolated the linear disappearance curve back to 0 min. from the 10 minute value. A similar practice has been followed by Kennedy and Millikan, Gibson and Evelyn, Asmussen. Gregersen and Rawson (1943) also conclude that mixing is complete in 10 minutes but believe dye concentration is plotted more accurately semi-logarithmically against time. Other workers, Sunderman and Austin (1936), Mather, Bowler, Crooke and Morris (1947), Bazett, Sunderman, Maxfield and Scott (1940) allow 30 minutes for mixing.

Use of CO and dye methods simultaneously.

Inhaled carbon monoxide is carried by the haemoglobin of the red blood corpuscles. The CO method, therefore, measures directly only the total red cell volume. It has been shown by Gregersen and Schiro (1938) and Courtice (1943) that T-1824 does not diffuse into red blood cells. The dye is therefore distributed in the

plasma and this method measures only plasma volume directly. Calculation of the total blood volume in each case is based on the cell to plasma ratio measured by the haematocrit and on the assumption that the haematocrit value is the same throughout the circulation.

If the technical procedures and theoretical assumptions involved in these two methods are valid, blood volume estimations carried out simultaneously in one subject by both methods should give similar values. This critical test has been applied by several investigators with varying results.

Smith, Arnold and Whipple (1921) using dogs reported that the average results in 14 experiments gave a value of 103.9 c.c. blood per Kilogram body weight by the dye method and 86.9 c.c. per Kilogram by the CO method.

McKintosh (1929) in infants found that the CO method gave a value of 71 c.c./Kgm and the dye method (brilliant vital red) 93 c.c./Kgm.

Bazett et al. in man reported their results as percent of "true" blood volume. "True" blood volume was the sum of plasma volume by the dye method and cell volume by the CO method. The dye method gave a value of 103.2% and the CO method 96.2% of the "true" blood volume.

Asmussen found in 15 experiments on humans that the CO method estimated the blood volume as much as 40% higher than the dye method. These results are in direct disagreement with those of Smith et al., Bazett et al., and McKintosh mentioned above.

Hopper, Tabor and Winkler using man and dogs, reported that although the averages of their series by CO and dye were equal, there were large discrepancies in individual experiments. They concluded that neither method measured absolute blood volume.

Root, Roughton and Gregersen (1946) using an elaborate open circuit method for administering CO in man and dogs, obtained results which agreed with the dye method to within ± 10%.

Purpose of this work.

The variability of the results obtained has been explained in different ways. Smith et al. who found a higher blood volume by the dye method maintained that the haematocrit value was much lower in the small blood vessels and that the true blood volume could only be obtained by estimating the red cell volume by the CO method and the plasma volume by the dye method and adding the results. Asmussen, however, who found that the blood volume by the CO method was the greater, maintained that the discrepancy was due to an uptake of CO by muscle myoglobin. From the litera-

ture, therefore, it is evident that no general agreement exists regarding the accuracy of blood volume measurements.

In recent years the dye method of estimating blood volume using T-1824 has enjoyed a virtual monopoly in this field. The chemical and optical properties of T-1824, its rate of disappearance from the plasma, its degree of combination with plasma proteins, have been the subject of numerous scientific papers. It has displaced CO for use in blood volume studies. It was felt nevertheless that the CO method offered advantages over the dye method worthy of consideration. Blood volume estimations can be made regardless of the fat content or haemolysis of samples of blood. It is unnecessary for the subject to fast prior to the experiment. Estimations can be repeated daily because the amount of CO used in a determination is breathed off in 24 hours. In the dye method daily estimations soon result in discoloration of the subject's skin and correction for residual dye in plasma complicates the colorimetric technique.

The development of a simple, accurate micro-gasometric method for the determination of CO in blood by Scholander and Roughton (1942) (1943) made it feasible to re-investigate the CO method. Further work on the behaviour of CO in the body by Roughton et al. (Roughton and Darling (1944), (Forbes, Sargent

and Roughton, 1945), (Tobias, Lawrence, Roughton and Root, 1945), (Roughton and Root, 1945) provided information of value in interpreting results. Despite the considerable volume of literature on the CO method, very little work had been done on the uptake and disappearance curves of CO in blood using a closed circuit respiratory apparatus. These data are as important with reference to mixing time and disappearance from the circulation as corresponding data in the dye method.

It was therefore decided to make further investigations of the carbon monoxide method using Scholander and Roughton's improved technique for estimating blood CO, and to compare the results obtained with those given by the dye method. Humans and rabits have been used.

PROCEDURE

I Humans

General method for administration of CO and taking of blood samples.

The apparatus for administering CO to humans is shown in Fig. 1. It is a closed breathing circuit consisting of a 5 litre Krogh spirometer, a soda-lime container and Douglas valves. The subject is seated in front of the apparatus with a noseclip in

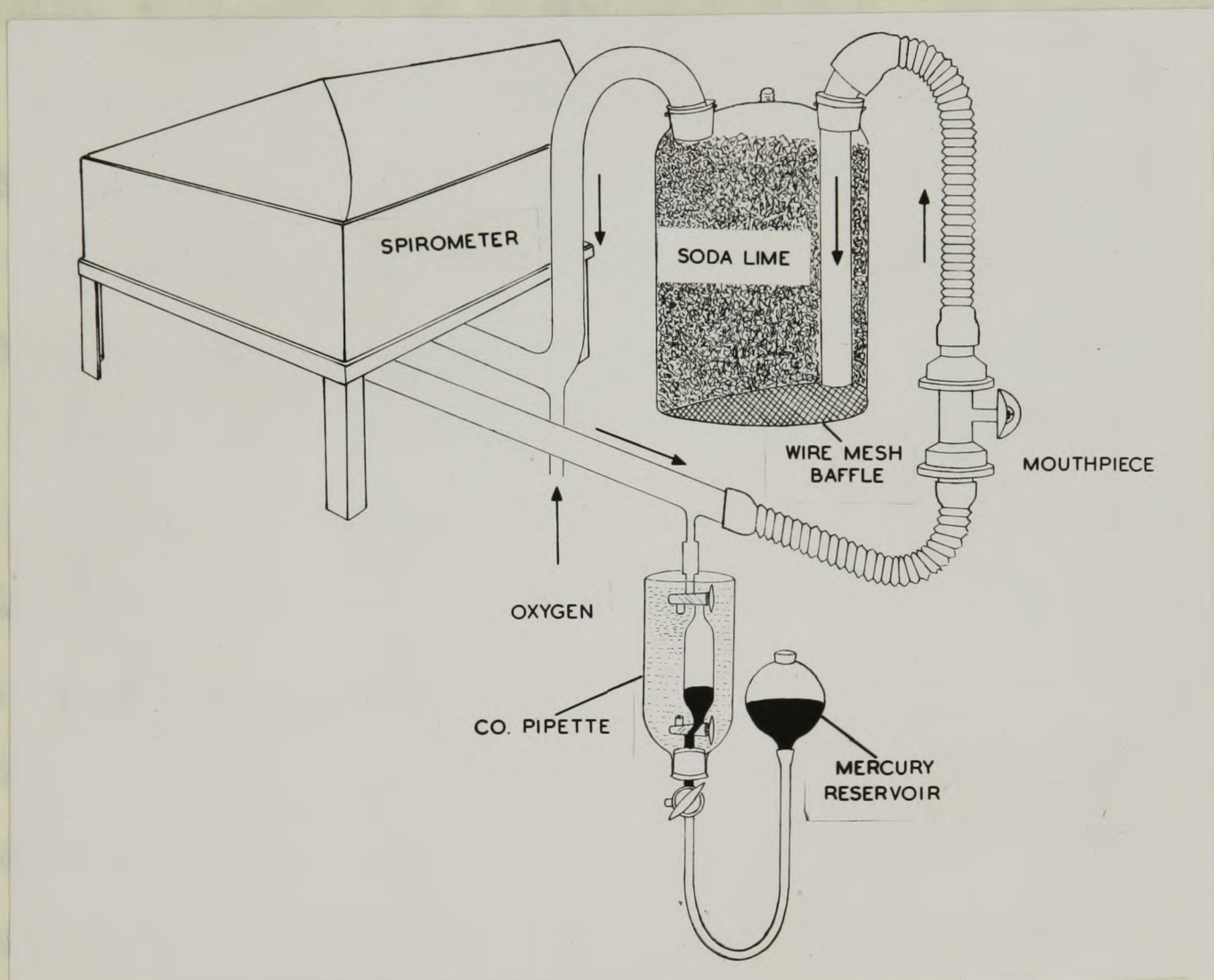


Figure 1. Apparatus for administering CO to humans.

in place and breathes through the mouthpiece of the valve unit. Air is inspired from the spirometer through large bore glass and flexible corrugated rubber tubing, and is expired through the soda-lime container back to the spirometer. The glass portion of the expiratory tubing carries the air through a wire mesh screen in the bottom of the soda lime container. In this unit the air does circulate and in this respect is different from the Haldane-Lorrain-Smith apparatus used by many investigators where the air is simply breathed back and forth into a rubber bag. It was felt that this movement of the CO containing air would facilitate rapid uptake of CO by the blood. Except for the two short pieces of rubber tubing on either side of the valve unit which allow the subject to move about in the chair, the apparatus consists entirely of glass and metal parts. There is some evidence (Roughton 1946) that CO is absorbed by rubber and although the amount lost in this manner might be negligible, the elimination of non-essential rubber parts from the apparatus was thought to be advisable.

At the beginning of each experiment the apparatus was washed out thoroughly with air and the spirometer emptied. Oxygen was then introduced from a cylinder through an inlet in the glass part of the expiratory tubing until the spirometer was

half-filled. As the subject breathed through the circuit and the CO_2 was absorbed, O_2 was admitted at intervals to maintain the spirometer at the same level. This procedure resulted in an air mixture in the circuit containing approximately 40% O_2. Because of the competition between O_2 and CO for combination with haemoglobin, this 40% O_2 caused the equilibrium tension of CO to be slightly higher than would be the case with 20% O_2 as in room air. This small increase in the amount of CO remaining unabsorbed was accounted for in the determinations of CO content of the circuit at the end of an experiment. In some trials where the apparatus was filled with air at the start and O_2 admitted at intervals to maintain a concentration of 20%, the subject several times experienced symptoms of anoxia because of the difficulty in judging the level of the spirometer exactly. It was therefore found safer, more convenient and no less accurate to breath 40% O_2.

Any accumulation of CO_2 in the circuit while the subject was rebreathing could have caused undesired changes in the circulatory system through vasodilation and hyperpnea. To test the efficiency of the soda-lime absorber, analyses of CO_2 content of the air were made at various times after commencement of rebreathing. The results are given below.

Subject	10 min.	30 min.	60 min.
R.W.G.	0	0	-
T.J.M.	-	0	-
R.W.G.	-	-	0.08
R.W.G.	-	-	0.75
F.C.C.	-	-	0.01

The soda lime was renewed after 2 hours use at rest.

The CO was introduced directly into the inspiratory tubing from a gas sampling tube encased in a water jacket. The CO was prepared by the addition of sulphuric acid to boiling formic acid and was collected in a 10 litre aspirator bottle over alkali (Peters and Van Slyke, 1932). The first litre of gas evolved from this reaction was discarded in order to remove the air present in the reagent container and delivery tube. The gas was stored under slight positive pressure in order that any leak at the stopper or rubber tubing should be outward. In this manner CO was obtained with a purity of 95 to 99%. It was analyzed before each experiment even if these were on successive days, using the Scholander-Roughton syringe capillary apparatus. (See under Detailed Methods for a description of this apparatus.) A bubble of the gas to be used in the experiment was collected over 30% saline and drawn into the graduated capillary tube of the apparatus. Its length was measured to the nearest 0.1 of a

division. The cup was then filled with Winkler's cuprous chloride solution and the CO absorbed. The length of the very small residual bubble was again measured to the nearest 0.1 of a division at the same temperature. From these figures the percentage of CO in the original bubble was calculated. The analysis was done in duplicate. Sample figures appear below.

Original Bubble length	After Winkler	CO%	Mean CO%
(32.2	0.4	98.8	98.9
(29.8	0.3	99.0	
(31.0	1.1	96.5	96.3
(32.2	1.3	96.1	
(28.7	0.7	97.6	
(30.6	0.8	97.4	97.4
(29.2	0.8	97.3	

It was found convenient to deliver the CO from a gas sampling tube connected to a mercury reservoir. The same tube was used in all the human experiments. Its volume from tap to tap was 102.2 c.c. The CO was delivered into this apparatus from the storage bottle and after allowing a few moments for the equilibration of its temperature with that of the water bath surrounding the sampling tube, it was adjusted to atmospheric pressure and the taps were closed. The temperature of the water bath and the existing barometric pressure were recorded and the volume of pure

CO at S.T.P. was calculated. Applications of these corrections reduced the amount of pure CO admitted to about 90.0 c.c. In a subject of average size this is sufficient to raise the CO content of the blood by about 2.0 volumes per cent, a concentration measured most conveniently by the apparatus used in the analysis. At this low level of CO in blood the subject experiences no discomfort whatsoever and the decrease in O_2 carrying capacity of the blood is not sufficient to produce any alterations in normal physiological processes. Even when the dose of CO is repeated and a blood concentration of around 4.0 volumes % is reached there are no objectionable symptoms. The delivery of the CO directly into the inspired air as quickly as possible (administration was usually finished in 1 to 1½ minutes) ensured the rapid uptake of CO by the blood. Data to be presented later will show that this was 98% completed within the first 5 minutes of rebreathing.

Blood samples were drawn from an ante-cubital vein using a record syringe. The barrel of the syringe was lightly greased with vaseline to prevent sticking and make it more airtight for use with the small bore needle. Several minutes before a sample was taken the subject immersed his arm in a warm water (44° C.)

bath to ensure adequate blood flow and promote mixing of the sample received. Using this procedure the blood samples are essentially arterial in character. Minimal compression for the purpose of distending the vein was employed as this may cause a change in the normal cell volume ratio.

Although 0.2 c.c. of blood will suffice for the CO in blood analysis, 2 or 3 c.c. were usually taken to allow for duplicate estimations and wastage. The blood was transferred immediately from the syringe to a small glass cup containing a trace of dry powdered heparin, the cup was stoppered, and rotated to dissolve the heparin.

For anaerobic storage the method described by Scholander and Roughton was used. The blood was transferred to 1 or 2 c.c. all glass syringes, a drop of mercury was sucked in to aid in mixing, any air was expelled from the syringe, and it was sealed by attaching a short length of capillary bore pressure tubing to the nozzle and inserting a cylindrical glass plug into the open end. These storage syringes were kept in the dark in an ice bath. Analyses could be made any time within 24 hours.

Uptake and disappearance of CO.

It has already been pointed out that the dilution of a known amount of a foreign substance introduced into the circulating

blood will give an accurate figure for total blood volume only if that substance is completely mixed with, and has not disappeared from, the blood at the time the determination is made. It was therefore important in using the CO method to find out (1) how much time was required for absorption of the gas from the respiratory circuit and (2) whether any CO disappeared from the circulating blood after absorption was complete. With this information it would be possible to allow sufficient time for absorption and mixing. If there were any significant degree of disappearance of CO which might have occurred during the absorption-mixing period, the necessary allowance could be made in the calculation. To study these factors human subjects breathed through the closed circuit for an hour after administration of the usual 90 c.c. of CO and the blood CO content was determined at intervals.

The apparatus and CO were prepared in the manner described. The subject refrained from smoking for 12 hours preceding the experiment but ate a normal breakfast. Most experiments were conducted about 10 A.M. at normal room temperature. The subject sat reclining in a deck chair with the noseclip in place and the valves strapped to his face. He breathed from the circuit for a period of 5 minutes before the CO was admitted, to

establish equilibrium between the air in the system and the small amount of CO which might be present in his blood. A 'blank' sample of blood was taken. The CO was admitted and a stopwatch started. Blood samples were drawn at 5,10,15,30,45 and 60 minutes. This procedure was followed in 11 experiments on resting subjects.

The results, which will be presented in full detail later, showed a gradual but definite disappearance of CO from the blood during the 60 minute period of rebreathing. This could possibly have been due to slow diffusion of CO into the tissues and combination with muscle myoglobin. During muscular exercise the blood flow to the muscles is increased. Muscular exercise would be expected to accelerate the disappearance phenomenon if it were in fact due to slow combination of CO with myoglobin. The disappearance curve of CO in blood was studied in 4 subjects during moderate muscular exercise on the Krogh bicycle ergometer.

In each experiment the subject got in a 'steady' state of muscular exercise by pedalling the bicycle for 10 minutes with a load of 1.0 kgm. on the pan and the metronome set at 120 per minute. The mechanical work performed was 420 Kgm-Metres per minute. At the end of this period breathing was commenced through the same closed circuit used in the experiments at rest. It

was found necessary to insert two soda-lime containers in series to absorb the extra CO_2 given off. After 5 minutes rebreathing to equilibrate the CO in blood and air, a 'blank' blood sample was drawn. The same 90 c.c. volume of CO was admitted to the circuit, the stopwatch started and blood samples drawn at 5, 10, 15, and 30 minutes. At the conclusion of the experiment the noseclip and mouthpiece were removed but the subject continued the exercise for a further 5 minutes. Then, to determine more accurately the degree of exercise which had been performed, the respiratory exchange was estimated by the bag method of Haldane and Douglas.

During all these experiments simultaneous estimations of blood volume were carried out by the dye method (T-1824) to be described below.

Measurement of CO remaining in closed circuit

All of the CO admitted to the circuit will not be absorbed. After a certain time an equilibrium will be reached between the CO tension in the alveolar air and that in the blood. From this point onwards the amount of CO absorbed from the inspired air will be equal to the amount of CO breathed off in the expired air. The CO tension of the air in the closed circuit will remain constant. Because of the high affinity of haemoglobin for CO this equilibrium point is reached when

this equilibrium point is reached when there is a very low tension of CO in the alveolar air. This equilibrium is governed by the equation $\frac{HbCO}{HbO_2} = K \frac{pCO}{pO_2}$ where K in man equals 200-300.

Although the tension and therefore the percentage of CO in the air of the circuit should be very low it is present in a large volume comprised of the spirometer, soda-lime container, connecting tubes and lungs. It might total 1 or 2% of the amount of CO administered and unless corrected for, would give a falsely high value for the blood volume.

In several experiments on 4 subjects the CO percentage of the air in the circuit was estimated at intervals after administration of CO. The experiments were conducted in the same manner as those for studying the uptake and disappearance of CO in the blood. At 5, 10, and 30 minutes 500 c.c. samples of air were taken from the inspiratory side of the circuit for CO in air analysis. In two experiments the dose of CO was repeated at 30 minutes and another analysis was made after a further 10 minutes of rebreathing.

In addition to indicating the correction which must be applied for unabsorbed CO in the blood volume calculations, these experiments provide complementary information to those on CO

blood levels in determining when absorption of CO is complete. As a test of accuracy the value of K for humans in vivo can be compared with the results of in vitro experiments by other workers.

CO repeated at rest and during exercise

To throw further light on the CO disappearance curve it was decided to repeat the administration of CO after rebreathing for 30 minutes into the spirometer unit. This procedure would demonstrate the effect of higher blood CO concentration on the curve. The fact that the dissociation curve of muscle myoglobin is a rectangular hyperbola suggested that even at the low tensions of CO used in these experiments the myoglobin might be saturated with CO by the first CO administration and when a second was given with the myoglobin 'blocked', the rise in blood CO content would be greater than with the first, yielding a lower figure for the blood volume. Failing this, the procedure would show the accuracy with which blood volume estimations by the CO method could be repeated at rest and would serve as a control experiment for studying changes induced in the blood volume over a short period of time.

Four young male subjects were studied; F.C.C., R.W.G., P.J.P., T.J.M. The procedure for the first half of the

experiment was identical with that for uptake and disappearance of CO at rest. After the 30 min. blood sample had been taken the subject removed the noseclip and mouthpiece and continued to sit in the deck chair breathing room air for 15 minutes. Breathing from the circuit was begun again and continued for the 5 minute equilibration period after which the second 'blank' blood sample was taken, the same volume of CO admitted, and blood samples as usual at 5, 10, 15 and 30 minutes. Only a small amount of CO was expired during the 15 minute resting interval but this loss was accounted for by taking the second 'blank' sample.

An almost identical series of experiments was conducted on these same four subjects except that the second dose of CO was given during the performance of moderate muscular exercise. The resting curves were done in the usual manner with the subject sitting at rest on the seat of the bicycle ergometer. When the first 30 min. sample had been taken and the noseclip and mouthpiece removed, the subject began to pedal the bicycle performing 420 Kgm. Metres of work per minute. After 10 min. for the development of a 'steady' state he breathed from the closed system for a further 5 minutes. Blood samples were taken at 0 min. and at 5, 10, 15, and 30 min. after administration of CO with the same degree of exercise continued. At the conclusion

of the experiment respiratory exchange by the bag method was estimated.

Cardiac output and pulmonary ventilation at rest and during moderate exercise.

In the preceding sections experiments have been described in which the effect of moderate muscular exercise on the uptake and disappearance of CO from the blood was studied. During exercise the cardiac output and pulmonary ventilation are increased several times. These factors would accelerate absorption and mixing of CO in the blood as well as demonstrating any significant disappearance due to myoglobin. It was decided to obtain quantitative information on this subject by estimating pulmonary ventilation and cardiac output at rest and during moderate muscular exercise on the bicycle ergometer.

Three subjects: F.C.C., R.W.G., and T.J.M. were used in the experiments. Cardiac output was determined by Grollman's acetylene method in the manner described by Douglas and Priestly (1937). Protocol and calculations of a typical experiment are given below. All analyses were done in duplicate. Values given are the averages of the duplicates.

Subject: T.J.M. Bar. 767 Date 9/12/47

9.45 A.M. - sat at rest on bicycle ergometer

10.00 " - respiratory exchange at rest. Pulse 73

10.25 " - C_2H_2 6 + 3 in 19"

10.35 " - began moderate exercise on ergometer

1.0 Kgm. on pan. Metronome at 120 per min

Mechanical work 420 Kgm-Metres per min.

10.45 " - respiratory exchange during exercise. Pulse 90

10.50 " - C_2H_2 5 + 3 in 17"

Resp. exchange at rest

42.40 litres in 6' 18" @ 11.9° C and 767 mm. Hg.
= 6,418 c.c. per min. @ S.T.P.

Analysis:- 4.12% CO_2
15.88% O_2
80.00% N_2

CO_2 given off: 263 cc/min
O_2 absorbed: 341 cc/min
R.Q. .....: 0.77

Resp. exchange during exercise

47.10 litres in 1' 55" @ 11.7° C and 767 mm. Hg.
= 2,343 c.c. per min @ S.T.P.

Analysis: 4.42% CO_2
15.73% O_2
79.85% N_2

CO_2 given off:	1029 c.c. per min.	
O_2 absorbed :	1272 c.c. per min.	
R.Q. :	0.81	

Acetylene analysis

Rest	I	5.28% CO_2
		15.25% C_2H_2
		11.92% O_2
		67.55% N_2
	II	6.01% CO_2
		13.28% C_2H_2
		10.48% O_2
		70.23% N_2
Exercise	I	6.40% CO_2
		14.66% C_2H_2
		9.42% O_2
		69.52% N_2
	II	7.35% CO_2
		12.22% C_2H_2
		7.36% O_2
		73.07% N_2

Applying the formula cardiac output in litres per min.

$$= \frac{(O_2 \text{ consumption}) \times \left[\frac{N_2\ II}{N_2\ I}(C_2H_2)_I - (C_2H_2)_{II}\right] \times 760}{\left[\frac{N_2\ II}{N_2\ I}(O_2)_I - (O_2)_{II}\right] \times 740 \times (C_2H_2)\ \text{pres.}}$$

Cardiac output at rest = 4.61 litres per min.
" " during exercise = 16.95 litres per min.

All data obtained in the experiment are summarized in the table below.

	Pulm. vent. l./min.	CO_2 cc/min.	O_2 cc/min.	R.Q.	Pulse rate	Stroke volume c.c.	Cardiac output l./min
Rest	6.42	263	341	0.77	73	63	4.61
Exercise	23.4	1029	1272	0.81	90	188	16.95

General method for estimation of blood volume by T-1824

The principles used in the dye method of blood volume estimation in this work are those of Gibson and Evans and Kennedy and Millikan with some modifications for the colorimetric determination of dye concentration. A known amount of T-1824 was injected into an arm vein and blood samples were drawn at 10, 20 and 30 min. thereafter. Plasma dye concentration was plotted linearly against time; the theoretical dye concentration at 0 min. being obtained by extrapolating this curve. Total blood volume was calculated from plasma volume and the venous haematocrit.

The dye used in this work was obtained from Messrs. Eastman Kodak Co. U.S.A. For injection it was prepared in solution in distilled water at a concentration of 2.5 mgs. per c.c. After thorough mixing the dye solution was filtered and transferred in 10 c.c. lots to chemically clean test tubes which were stoppered with cotton wool and autoclaved. The final sterile test tubes

containing dye solution were stored in a closed tin in the refrigerator until used. To check against evaporation through the cotton wool stopper, the dye concentration in these tubes was frequently tested in the photo-electric colorimeter against a freshly prepared standard solution of the same lot of dye.

In the human experiments 5.0 c.c. of dye solution (containing a total of 12.5 mgs. of T-1824) were injected for each determination. A 5.0 c.c. record syringe was used. To test the calibration of the syringe it was filled with water, a small needle attached, air bubbles expelled, and the plunger adjusted to the 5.0 c.c. mark. In this way the barrel of the syringe, the dead space and the needle were filled with fluid. The water was 'injected' into a weighing cup by forcing the plunger home at the same rate as dye injection into the vein was made (45 secs.) This left the dead space of the syringe and the needle still filled with water. Dye injections were made in the same way. The plunger was forced home but no attempt was made to wash the dye out of the needle and dead space. The water delivered from 50 c.c. mark was weighed with these results:

(1) 4.9905 gs.

(2) 4.9968 "

No correction for the volume of fluid delivered was necessary.

The same syringe and same size needle were used in all experiments.

The general behaviour of the subject was the same as for the CO experiments described above. The subject ate his usual breakfast. The estimations were done at about 10 A.M. with the subject sitting at rest in a deck chair. A 10 c.c. sample of blood containing undyed plasma was taken to use as a control in the colorimetric determination. The dye was injected into an antecubital vein and the stopwatch started when the injection was half completed. Care was taken not to cause any extravasation of dye at the site of injection for this would introduce an error in the amount delivered to the circulation. 10 c.c. blood samples were removed from the opposite arm vein at 10, 20, 30 min. with the precautions for mixing and stasis described under the CO method. The blood was immediately transferred to small beakers containing a trace of dry heparin and agitated gently to dissolve the heparin and prevent coagulation. 2.0 c.c. of the blood were then placed in a stoppered glass tube for haemoglobin and haematocrit determination. The remaining 8.0 c.c. were transferred to 15 c.c. pyrex centrifuge tubes and centrifuged for 20 min. at 2000 r.p.m., to separate cells and plasma. The supernatant plasma was carefully pipetted off and 3.0 c.c. amounts were taken with a volumetric pipette for extraction of the dye

by the butyl alcohol method of Harington, Pochin and Squire.

If the blood samples were taken quickly into greased syringes and handled gently in the various containers there was no haemolysis. However in the early work it was found that the variations in opalescence and lipemia of plasma samples, even in the short period of 30 minutes required for a determination, introduced great errors in colorimetry. The alcoholic phosphotungstic acid method of extraction of T-1824 described by Crooke and Morris (1942) was tried but gave unsatisfactory results. The butyl alcohol method of Harington et al. was tried and found to be very satisfactory. It required the overnight digestion of the plasma proteins but resulted in complete extraction of the dye free from plasma pigments, lipemia and 1% haemolysis into clear butyl alcohol. Extracts of undyed plasma were indistinguishable with the naked eye from pure butyl alcohol.

Estimation of dye in plasma using the photo-electric colorimeter.

A diagram of the photo-electric colorimeter used is presented in Fig. 2. The light source is powered by a 6 volt storage battery which provides a more constant current than could be obtained from the mains. Coarse and fine variable resistances are placed in series to allow adjustment of the intensity of the light source. The lens system directs a beam of parallel light through

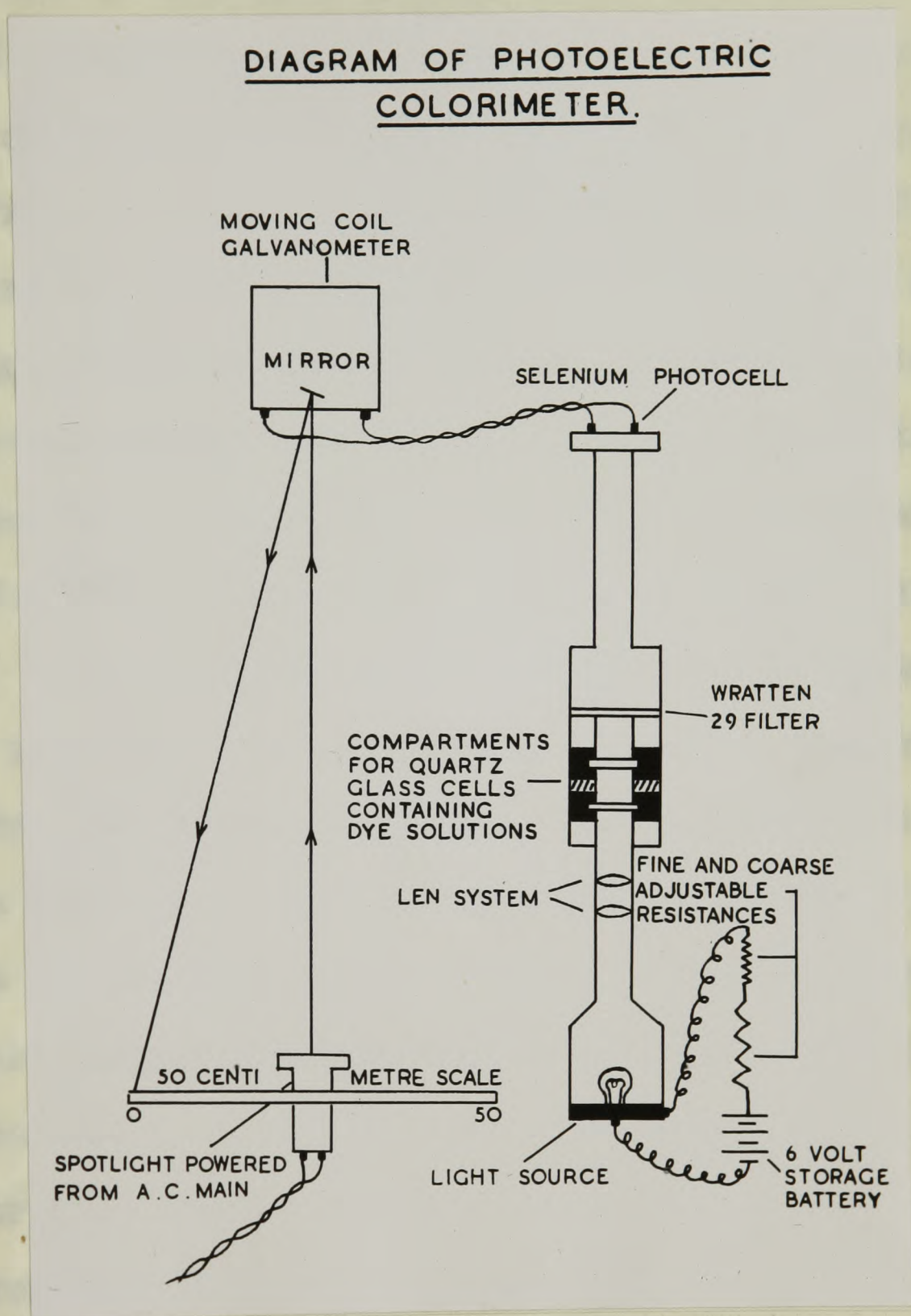


Figure 2. Diagram of photo-electric colorimeter.

the glass cell containing the dyed solution and through the Wratten filter to a selenium photo-cell. The lens system, cells, filter and photocell are in a closed metal casing to prevent the admission of light from the room. The photo-cell is some distance from the light source to eliminate heating effects.

Current output of the photo-cell is recorded by a sensitive mirror type moving coil galvanometer. The mirror reflects a linear vertical shadow from the spotlight on a 50 cm. transparent scale placed at a distance of 1 metre from the galvanometer. All the recording parts of the apparatus are firmly fixed in position. With no light passing through the colorimeter system, the scale is adjusted so that in the resting position of the galvanometer the reading is 50 cm. At full scale deflection the reading is 0 cm. The Wratten filter eliminates light up to a wavelength of 600 millimicrons. Since T-1824 gives maximum spectral absorption at 620 millimicrons, and the extraction process leaves only this substance present in butyl alcohol, light absorption must be due almost exclusively to the dye. In making a reading the spotlight is switched on and the resting position of the galvanometer at 50 cm. is checked. The butyl alcohol extract of the undyed plasma sample (taken just before dye injection in each experiment) is placed in a polished glass

cell having a depth of 10 mm. and a volume of approximately 3.0 c.c. The light source is switched on, the cell is placed in position between the light source and the photo-cell, and the resistances adjusted so that the galvanometer reads 0. The 10 mm. cell is removed and replaced by another cell containing dyed water. The galvanometer reading with this new cell in position is recorded as the 'blank' setting. It is usually of the order of 0.5 to 1.0 cm. The undyed plasma sample and the 'blank' are interchanged in the colorimeter several times in order to be sure that the "blank" setting corresponds to full scale deflection with the undyed plasma sample in position. This precaution is taken to allow for any changes which might occur in the intensity of the light source while the estimations are being carried out. The "blank" cell is kept on hand and its setting checked between each reading on an unknown solution. Should the light source vary, this can be corrected by suitable adjustment of the resistances.

The 10 mm. cell is emptied completely with a fine pipette and filled with the dyed solution. It is placed again in the colorimeter and the galvanometer reading recorded. In this manner the galvanometer moves from its position at full scale deflection (0 cm.) towards the resting position (50 cm.) as more light is absorbed by stronger dye solutions. Because the

same 10 mm. cell is used for the undyed control sample and for all other dyed samples, there is no error due to different light absorption by the glass of different cells. Setting the galvanometer at full scale deflection with the undyed plasma samples in position means that light absorption by the dyed samples is due to dye only. If any plasma pigments remain after the butyl alcohol extraction, they must be present in identical amount in all samples.

Dye concentration from galvanometer reading was obtained from a calibration curve. This curve was plotted by making up dye in human plasma solutions of known concentration, extracting the dye by the butyl alcohol method and recording the galvanometer readings in the manner just described. This calibration curve was repeated several times during the course of the experiments using different lots of human plasma. The calibration curve is shown in Fig. 3. Dye concentration is expressed in mgs./litre of plasma, Galvanometer reading in centimetres.

The results of three calibration curves done at different times are shown below.

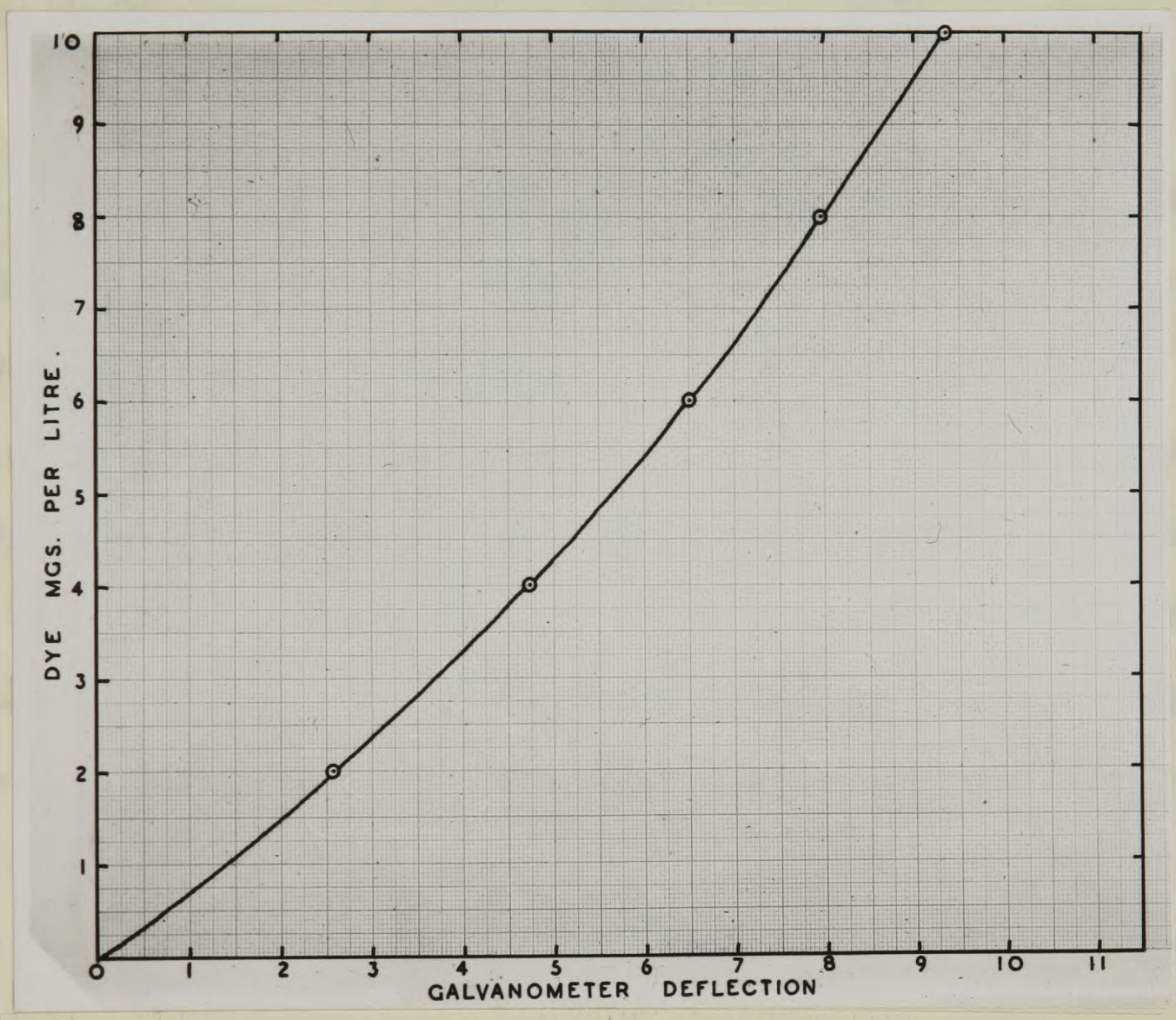


Figure 3. Calibration curve of T-1824 in human plasma extracted with butyl alcohol.

Dye concentration mgs. per litre	Galvanometer deflection in cm. 16.3.47	28.3.47	11.6.47
0	0	0	0
2	2.60	2.50	2.55
4	4.70	4.50	4.75
6	6.45	6.40	6.55
8	7.90	7.85	8.05
10	9.20	9.05	9.50

It will be seen that in the range of dye concentrations used for the human experiments (4 to 8 mgs./litre) these results agree to within 3%. (Excepting the value for 4 mgs./litre on 28.3.47 where the error is 5%.) This 3% discrepancy could represent the sum of all the possible errors in the procedures used for a calibration curve, i.e. weighing of dye, preparation of dilutions with volumetric apparatus, extraction into butyl alcohol, setting of 'blank', and actual reading of the galvanometer. When readings were made on a single sample several times in one day or after an interval of 2 or 3 days the values always agreed to within 1% (0.05 cm. on scale). Duplicate readings were done routinely on every sample.

When plasma volume estimations by the dye method were repeated, dye from the previous injection was present in the 0 min. sample. In this case undyed plasma from another individual was used for the control. That this substitution is valid may be inferred from the fact that the calibration curves shown above

were done on plasma from three different individuals. The dye concentration present in the repeat 0 min. sample was estimated and subtracted from the values after injection.

The Haematocrit

Total blood volume is calculated from plasma volume by determining the cell to plasma ratio in the samples of blood used in the dye determinations. The cell volume expressed as a percentage of the total volume of blood in a sample has come to be known as the 'haematocrit' although this term originally referred to an instrument used to measure this ratio.

Many methods have been described for determination of the haematocrit. Because none of the commonly used tubes were available, a simple and accurate procedure was devised. Pyrex tubing having an external diameter of 6 mm. and an internal diameter of 1.5 mm. was tested for uniformity of bore. A pellet of mercury was sucked into the tubing. It was mounted on the stage of a travelling microscope in which the ocular-objective unit could be moved horizontally. The cross-hairs of the eye-piece were adjusted to the outer convex end of the mercury pellet, the ocular-objective unit was moved until the cross hairs were opposite the inner convex end of the mercury pellet, and the distance travelled was measured on a vernier scale to the nearest 0.01 mm. The mercury pellet was moved along the tubing and

its length measured again. Variations in the length of the pellet indicated uneven bore and these portions were discarded. The tubing was cut in lengths of 10 cm.

Blood for analysis was thoroughly mixed and sucked up to almost fill the tube. The bottom end of the tube was then pushed firmly into a ½ inch thick slab of plasticene which had been pressed against a table top so as to present a flat smooth upper surface. When the haematocrit tube was removed from the plasticene it contained a firm, even plug in the lower end about 5 mm. in thickness and presenting a cleanly demarcated upper surface. This plug was not forced out during centrifugation. Tubes prepared in this way could be used many times and were easily cleaned by pushing out the plasticene with copper wire, scrubbing the inner surface with a pipe-cleaner and drying with acetone.

Four such tubes fitted neatly into the ordinary sized metal centrifuge tube container without requiring additional support. They were spun at 3000 r.p.m. for 1 hour. The length of the cell column including the buffy coat due to white cells, and the total length of cell plus plasma column were read against a millimeter scale. The cell to plasma ratio was rapidly calculated by means of a slide rule.

To test the accuracy of the method, simultaneous haematocrit

determinations were made on the same sample of blood using different tubes. The final value would include errors due to non-uniformity of bore, leakage around the plasticene plug and inaccuracy in measuring the lengths of the columns. Results of three experiments are given below.

	Hct.	Deviation from Mean
Sample I	40.6	- .1
	41.0	+ .3
	40.8	+ .1
	40.9	+ .2
	40.7	0
	40.9	+ .2
	40.6	- .1
	40.8	+ .2
	40.2	- .5
	40.3	- .4
Mean	40.7	
Sample II	46.5	+ .1
	46.3	- .1
	46.6	+ .2
	46.6	+ .2
	46.2	- .2
	46.4	0
	46.2	- .2
	46.4	0
Mean	46.4	
Sample III	43.0	+ .2
	42.3	- .5
	42.7	- .1
	42.9	+ .1
	42.7	- .1
	42.9	+ .1
	42.6	- .2
	42.8	0
	43.2	+ .4
Mean	42.8	

In the early part of the work, the effect of the anticoagulant used on the haematocrit value was not appreciated. In these early experiments roughly 0.2% dry powdered potassium oxalate was placed in the container for the blood. Different samples taken from humans or rabbits within a short space of time showed wide variations in haematocrit. This was due to the fact that this concentration of oxalate resulted in a hypertonic solution in the plasma surrounding the red cells. Fluid passed from the cells into the plasma and reduced them in size. Very slight difference in the amount of oxalate used caused significant changes in the haematocrit.

To test the magnitude of this error haematocrit values using heparin as the anticoagulant were compared with values using 0.1%, 0.2%, and 0.5% potassium oxalate using the same samples of blood. Heparin, with its large polysaccharide molecule, has little effect on tonicity. The required amount of oxalate for these concentrations in 5.0 c.c. of blood was weighed out and placed in beakers. A barely visible trace of dry powdered heparin was sufficient. Haematocrits were done in duplicate. The results of 4 experiments are shown below.

Blood sample	Heparin	0.1% K Oxalate	0.2% Oxalate	0.5% Oxalate
R.W.G.	49.9	47.9	46.2	41.8
F.C.C.	43.0	41.7	40.3	36.8
P.J.P.	43.6	42.3	41.1	37.4
R.W.G.	47.8	45.9	44.2	40.3
Mean	46.1	44.5	43.0	39.1
% of Heparin value	100%	96.5%	93.3%	84.8%

Similar results have been reported by Hooper, Smith, Belt and Whipple (1920). Because of the shrinkage of red cells caused by potassium oxalate, a 2/3 mixture of potassium and ammonium oxalate has been recommended (Wintrobe and Landsberg 1935). The K ion cannot pass through the red cell membrane and exerts its tonicity on the surrounding plasma; the NH_4 ion is able to enter the red cell and counteracts the effect of K ion outside. Crooke and Morris (1942) have used approximately 30 mgs. of this mixture in 3.0 c.c. of blood (1.0%) in their T-1824 method for plasma volume determination.

To test the accuracy of this mixture of K and NH_4 oxalate the following experiment was performed. Four test tubes were prepared containing heparin, 31 mgs. of the mixture, 35 mgs. of the mixture and 31 mgs. K oxalate respectively, as the anticoagulants. 3.0 c.c. of freshly drawn blood were placed in the tubes. These were inverted 20 times for mixing. The haematocrit

values were read after 10 min. and 1 hour of centrifuging at 3000 r.p.m. Results are given below.

Anti-coagulant	10 min.	1 hour
Heparin	49.1	48.2
31 mgs. mixture	46.3	45.1
35 mgs. mixture	45.0	43.6
31 mgs. K oxalate	40.8	31.4

Results expressed as % of 'Heparin' Hct.

	10 min.	1 hour
31 mgs. mixture	94.3%	93.6%
35 mgs. mixture	91.7%	90.5%
31 mgs. K oxalate	83.1%	77.6%

In the concentrations used this mixture caused a 6 to 10% reduction in the haematocrit. That the shrinkage of the red cells occurs quickly is shown by comparison of the figures at 10 min. and 1 hour. Even where the difference in the quantity used was 4 mgs. there were significant effects on cell size. If the red cells are reduced in size by 6%, the fluid derived from them must dilute the plasma, yielding too high a figure for plasma volume. Results of the above experiment calculated in terms of 'Heparin' plasma volume are given below.

31 mgs. mixture = 106% of 'Heparin' plasma vol.
35 " " = 109% " " " "
31 " K.oxalate = 121% " " " "

Although the mixture of potassium and ammonium oxalate gives

more satisfactory results than pure potassium oxalate, it introduces uncertainties in the haematocrit value and dye concentration in plasma. In this work heparin has been used.

Simultaneous estimation of blood volume by CO and dye methods.

The general methods used to determine blood volume by CO and T-1824 have been described. These two methods were used simultaneously in 20 experiments on 12 different subjects. To illustrate the detailed procedure and sample calculation of results a typical experiment will be described.

Subject: R.W.G. Wt. 82.2 Kgm. Had not smoked for 12 hours. At rest in deck chair. Bar. 746.

CO admitted: 102 c.c. @ 17.2° C and 746 mm. Kg.
Purity of CO = 97.9%
= 90.6 c.c. CO pure @ S.T.P.

Dye: 5.0 c.c. of T-1824 solution containing 2.5 mgs. per c.c.
= 125mgs. T-1824

Blood samples taken at 0, 5, 10, 15, 30, 45, 60 min.

CO in blood analysis

Sample	CO in vols. % at S.T.P.
0 mins.	0.53
5 "	2.05
10 "	1.99
15 "	1.99
30 "	1.99
45 "	1.90
60 "	1.88

Dye and haematocrit

Sample	Hct.	Hb	R.B.C's	Galvanometer Deflection	Dye con. in plasma mgs./litre
0 min.	45.9	109	5,135,000	0	0
5 "	-	-	-	4.30	3.60
10 "	45.7	-	-	4.10	3.40
15 "	45.0	-	-	4.05	3.35
30 "	44.9	108	5,070,000	4.00	3.30
45 "	45.6	-	-	4.05	3.35
60 "	45.0	108.5	5,015,000	3.95	3.25
Mean	45.3				

The haematocrit is multiplied by the factor 0.96 to correct for plasma trapped between the packed cells (Gregersen and Schiro, 1938). Corrected haematocrit = 43.5.

Calculation

CO method: CO admitted to circuit = 90.6 c.c. @ S.T.P.

From other experiments CO remaining in circuit at equilibrium = 1.6% of CO admitted.

CO absorbed by subject = $90.6 \times \frac{98.4}{100} = 89.1$ c.c.

CO content of blood at 10 min. = 1.99 c.c. per 100 c.c.

" " " " " 0 min. = 0.53 c.c. per 100 c.c.

rise in CO content due to CO absorbed = 1.46 c.c. per 100 c.c.

Total blood volume = $\frac{89.1}{1.46} \times 100 = 6110$ c.c.

Red Cell volume = $6110 \times \frac{43.5}{100}$(hct.) = 2660 c.c.

Plasma volume = 3450 c.c.

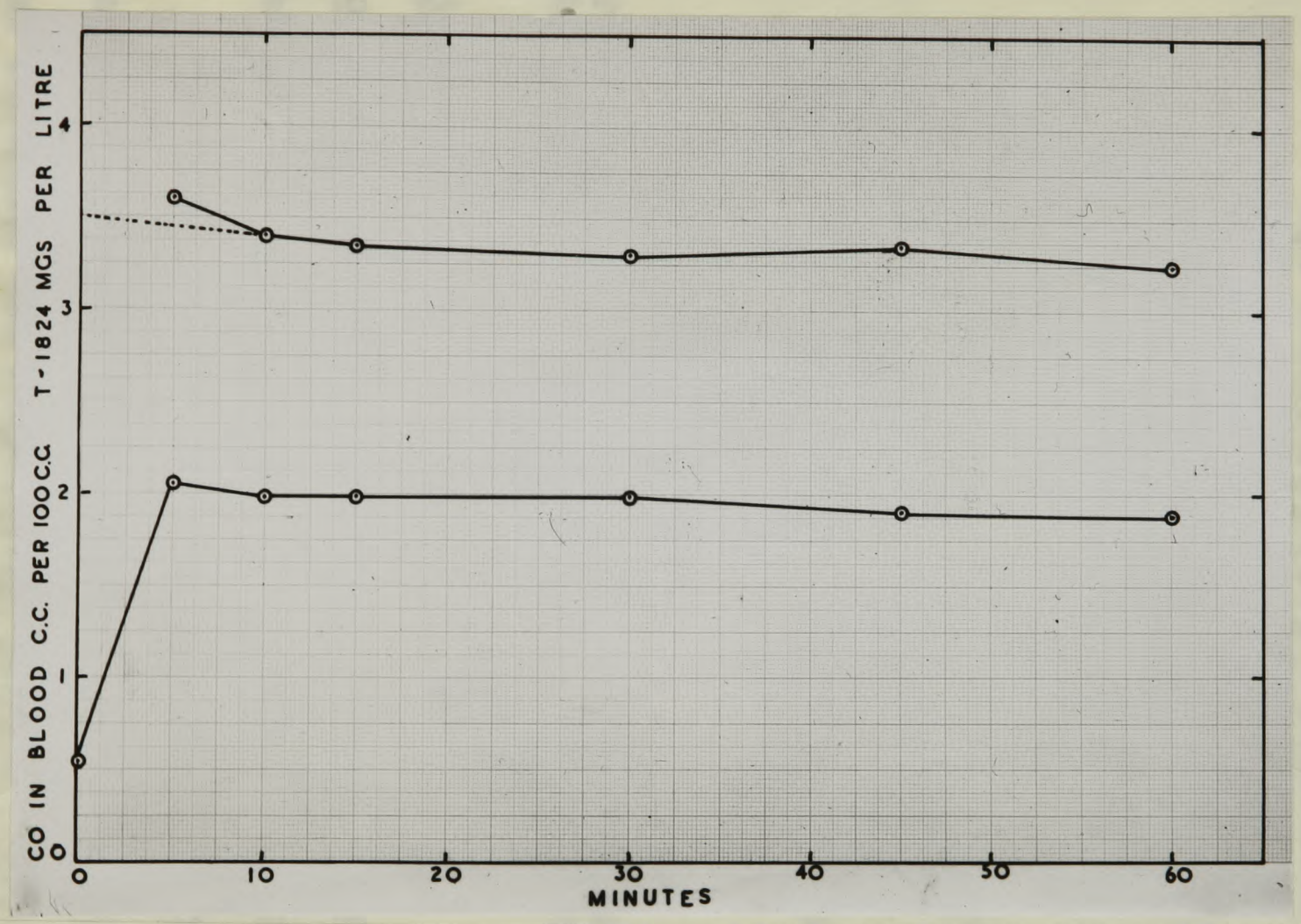


Figure 4. CO concentration in blood and T-1824 concentration in plasma of human subject at intervals for one hour after the administration of CO to the closed circuit and the intravenous injection of the dye.

Dye method: Fig. 4 shows the dye disappearance curve plotted for 1 hour and the method of extrapolation from the 10 min. value to 0 min. This curve also shows the initial rapidly falling phase of the dye curve in the first 10 min. due to mixing.

Dye concentration at 0 min. = 3.5 mgs. per litre.
(the extrapolated value)

Total amount of dye injected = 12.5 mgs.

$$\text{Plasma volume} = \frac{12.5}{3.5} \times 1000 = 3570 \text{ c.c.}$$

From the haematocrit value of 43.5% this volume of plasma represents 56.5% of the total blood volume.

$$\text{i.e. Total Blood volume} = \frac{3570}{56.5} \times 100 = 6320 \text{ c.c.}$$

Red Cell volume = 2750 c.c.

The reasons for using the CO content of the blood at 10 min. in the calculation will be explained under Discussion.

II Rabbits

Method of administering CO and taking blood samples

The apparatus for administering CO to rabbits is illustrated in Fig. 5. The principle is the same as that used in the human experiments. It consists of a 200 c.c. Krogh spirometer, glass soda-lime container and respiratory valves. The valves were prepared by cutting holes of ½ inch diameter in sections of a rubber stopper. Diaphragms of thin rubber were cemented to the

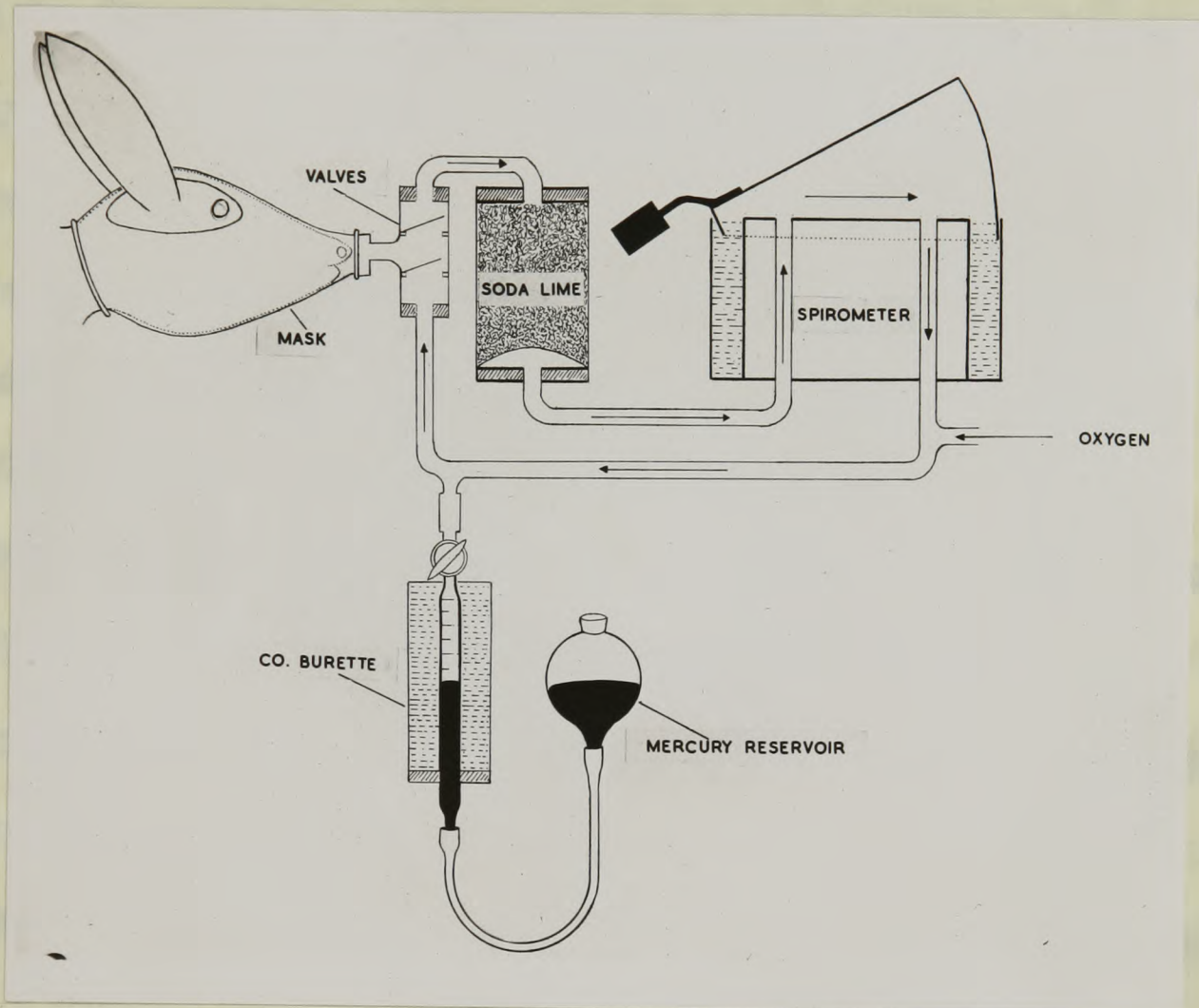


Figure 5. Apparatus for administering CO to rabbits.

upper surface of these sections so that about 2/3 of the valve leaf was free to move. These were mounted as shown in an upright glass cylinder having a side-arm leading from between the two valves to the rabbit mask. Air is inspired from the spirometer and expired through soda lime back to spirometer. This circuit appeared to cause no resistance to breathing. Unanaesthetized animals sat quietly at rest breathing at a normal rate and when excited by the taking of a blood sample easily increased the rate without apparent discomfort.

The mask was made of thin black rubber and was so shaped that it fitted tightly to all parts of the animal's face and was held in position by straps which extended back to the angle of the mandible and the occiput leaving an aperture for the ears and eyes. The anterior end of the mask was cemented tightly around a rubber stopper perforated by a glass tube which could be connected to the side-arm of the valve unit. The inner surface of the mask was lined by a thin layer of compressible rubber which stopped about ½" short of the nostrils. With the mask in position the elasticity of the black rubber compressed this rubber lining tightly against all irregularities in the animal's face and left a small free space surrounding the nostrils.

O_2 was admitted to the spirometer from a cylinder. At the

commencement of an experiment the spirometer was half-filled with O_2 and as it was used up more O_2 was added from the cylinder at intervals to maintain a constant volume in the circuit.

The CO was admitted into the inspiratory side of the circuit from a gas burette immersed in a water jacket and connected to a mercury levelling bulb. The burette was calibrated in 0.05 c.c. divisions and the volume of gas delivered could be read to 0.01 c.c. The CO was prepared, stored, analyzed for purity and its volume corrected to S.T.P. as in the human experiments. In the rabbit experiments a volume of approximately 3.0 c.c. was admitted for a blood volume determination. This raised the CO content of the blood to about 2.00 c.c. per 100 c.c. in an animal of average size.

Blood samples were taken from the marginal vein of the ear. Adequate blood flow was encouraged by warming the ear with an electric light for several minutes prior to the venipuncture; samples so obtained were essentially arterial in character. For the CO in blood analysis 1.5 c.c. samples were collected in small glass containers and transferred immediately to the glass syringes for anaerobic storage. Potassium oxalate crystals or dry heparin were used as anti-coagulants. In samples used for haematocrit determination, heparin was used exclusively.

In the experiments on unanaesthetized rabbits the animal was placed in a wooden box with its head outside. The box had an aperture in the front just large enough to fit the neck and prevent the head being withdrawn.

Uptake and disappearance of CO

As in the human experiments it was considered necessary to determine the rate at which the CO was absorbed from the breathing circuit and the rate at which it disappeared from the circulating blood during a 1 hour rebreathing period. In the earlier experiments this was done on anaesthetized animals with a slightly different circuit than that described in the preceding section. A 100 c.c. cylindrical glass 'mixing chamber' was interposed between the spirometer and the inspiratory and expiratory glass tubing. The CO was delivered into this mixing chamber.

The animals were anaesthetized with intravenous nembutal (1 grain per 5 lbs. body weight) and the mask was applied. O_2 was run into the spirometer and rebreathing commenced. A 'blank' sample of blood for initial CO level was taken. The measured amount of CO was introduced into the circuit as rapidly as possible (10-15 seconds) and the stopwatch started. Samples of blood were withdrawn at 3, 6, 9, 15, 30, 45 and 60 minutes. The results of experiments on 7 rabbits showed that absorption of CO

was complete by 9 minutes. It appeared that the inclusion of a mixing chamber served no useful purpose. This was eliminated and the CO was admitted directly to the inspiratory tubing. These two changes speeded up the rate of absorption of CO. Uptake and disappearance curves were done on unanaesthetized rabbits with this modified circuit.

Measurement of CO remaining in circuit at equilibrium

It has already been pointed out in the methods for humans that measurement of CO remaining unabsorbed is necessary for accurate determination of blood volume. This factor is of even greater importance in rabbits because of the lower affinity of rabbit haemoglobin for CO. With a lower K value and higher oxygen tensions the residual CO at equilibrium given by the equation: $p\,CO = \frac{HbCO \times pO_2}{HbO_2 \times K}$ would be expected to comprise a greater percentage of the CO admitted to the circuit. Experiments were conducted to determine the correction required.

The procedure was the same as that for uptake and disappearance curves except that 80-100 c.c. samples of the air in the circuit were taken into gas sampling tubes at 10 minutes and 30 minutes after administration of CO. Percentage of O_2 and CO in these samples was estimated; O_2 by the Haldane gas analysis apparatus and CO by the technique described under Analytical

Methods.

Blood volume by CO method repeated.

As a test of the accuracy of the CO method in rabbits the blood volume estimation was repeated on the same animal within a short space of time. In several experiments the CO was administered and blood levels followed for a period of 30 minutes. Then, while the animal continued to breathe from the closed circuit, a second dose of CO was admitted and the increase in blood CO content was estimated. In this way two determinations of blood volume were made within a period of 30 minutes under identical conditions.

In another series of experiments, the blood volume was determined by the CO method after an interval of 3-4 hours. The first determination was carried out in the usual way with rebreathing continued for 15 minutes. The animal was then removed from the apparatus and allowed to breathe room air for several hours. No food or water were given during this period. This lapse of time allowed the animal to breathe off most of the CO which had been absorbed during the first determination. The blood volume estimation was done a second time. The amount of CO remaining in the animal's blood was measured in the 'blank' sample prior to administration of the second dose of CO and this value was subtracted from that obtained after absorption. The

haematocrit was repeated during the second determination in order to reveal any changes which might have occurred in the cell to plasma ratio during the interval.

Dye method in rabbits.

For injection into rabbits the T-1824 was dissolved in distilled water to make a concentration of 1.20 mgs. dye per 2.0 c.c. of solution (60 mgs. %). This stock solution of dye was stored in the dark in a tightly stoppered volumetric flask. Its strength was checked several times in the colorimeter against a freshly prepared solution of the same concentration. The dye injections were made with a 2.0 c.c. record syringe. The volume of fluid delivered by this syringe with needle attached was tested by weighing in the same manner as for the syringe used in human experiments. Results of this calibration in terms of weight of water delivered and actual amount of dye delivered with a solution of 60 mgs. % are given below.

Wt. water delivered		Amt. dye delivered
1.9640 gs.		1.178 mgs.
1.9790 "		1.187 "
1.9637 "		1.178 "
1.9754 "		1.185 "
1.9668 "		1.180 "
	Mean	1.1816 "

Injections were always made using the same syringe and needle. The mean value 1.1816 mgs. was used in the calculations. The

dye was injected into the marginal vein of the rabbit's ear.

Blood samples (1.0 to 1.5 c.c.) were taken from the opposite ear before and at intervals after the injection using heparin as anticoagulant. These samples were centrifuged for 20 minutes at 2500 r.p.m. and the plasma pipetted off. Dye concentration in plasma was estimated in the photo-electric colorimeter using the same procedure as described for the human experiments, except that a cell of 2.0 mm. depth and 0.5 c.c. capacity was employed and the dye was not extracted with butyl alcohol. The dye concentration in plasma was of the order of 12-15 mgs. per litre in the rabbit experiments (compared with 3-4 in humans) so that the smaller cell gave adequate galvanometer deflections and allowed smaller blood samples to be used. Undyed rabbit plasma was found to be very clear under all conditions and its pigment content low. Plasma samples from different animals gave identical readings in the colorimeter. For this reason it was not necessary to extract the dye with butyl alcohol. Dye concentration was read from a calibration curve in mgs. per litre. The calibration curve was repeated several times during the course of the experiments.

Study of dye disappearance curve.

It has already been mentioned that because T-1824 disappears

slowly from the circulation, the theoretical dye concentration at the time of injection is obtained by extrapolation of the disappearance curve back to 0 mins. These curves have been studied in man and dogs by various investigators. They commonly show an initial rapid fall in dye concentration occupying the first 10 minutes which is attributed to mixing, followed by a linear disappearance 'slope' up to 1 hour. Recently Cruikshank and Whitfield (1944) have questioned the validity of this interpretation. They have pointed out that T-1824 is taken up by the reticulo-endothelial system and believe the early rapid fall in dye concentration is due to 'gobbling' of dye by this tissue. To support this claim they have 'blocked' the reticulo-endothelial system by an injection of T-1824 or india-ink and have found in the cat that a second injection of dye does not show the early rapid fall. They have suggested that the blood volume estimated from a single dye injection gives falsely high values.

In order to study this phenomenon in rabbits, disappearance curves of T-1824 were followed in anaesthetized animals for 60 minutes and then a repeat dye injection was made and the dye concentration followed for a further interval. Time-concentration curves were plotted; plasma volumes calculated from the original and repeat dye injection were compared. 2.0 c.c. of dye solution

were injected in the usual manner; 1.5 c.c. blood samples were taken at 0, 2, 4, 6, 9, 15, 30, 45, 60 minutes; the dye injection was repeated at 60 minutes; further samples were taken at 62,64, 66, 69, 75, 90 minutes.

The removal of ten 1.5 c.c. samples of blood from a rabbit with a blood volume of approximately 150 c.c. would be expected to alter the original haematocrit value. Haemodilution should occur to compensate for the blood loss and this haemodilution would make the observed dye disappearance curve steeper. Therefore in these experiments, haematocrit and haemoglobin values were determined on the samples of blood taken at 0, 15, 30, 45 and 60 minutes.

Simultaneous determination of blood volume by CO and dye methods. To compare the results by CO and T-1824 methods, simultaneous determinations were made on 17 animals. With the rabbit breathing through the closed circuit the dye was injected into an ear vein at the same time as the CO was admitted. Blood samples were taken at 0, 9, 15, and 30 minutes. CO in blood analyses were done on the 0, 9, and 15 min. blood samples. Dye concentration in plasma was estimated using the 0, 9, 15 and 30 min. samples. To illustrate the method of calculation of results, a typical experiment will be described. The analytical results

willare plotted in Fig. 6.

Rabbit: male; natural; Wt. 2.50 Kgms.

CO: 3.40 c.c. measured in gas burette @ 17.0° C and 753 mm. Hg. with a purity of 97.0%

= 3.02 c.c. pure CO @ S.T.P. admitted to circuit.

T-1824: 1.1816 mgs. injected into ear vein.

Analyses.

Sample	Dye mgs./litre	CO c.c./ 100 c.c.	Haematocrit%
0 min.	0	0	40.3) 40.9) 40.6
9 "	12.8	2.07	-
15 "	12.3	2.02	-
30 "	11.4	-	-

Observed Hct. = 40.6%
True Hct. = 40.6 x 0.96 = 39.0

Calculation

CO method: CO admitted to circuit = 3.02 c.c. pure @ S.T.P.

From other data CO unabsorbed = 3.3% of CO admitted.

$$\text{CO absorbed} = 3.02 \times \frac{96.7}{100} = 2.92 \text{ c.c.}$$

CO content of blood at 9 mins. = 2.07 c.c. per 100 c.c.

$$\text{Total Blood volume} = \frac{2.92}{2.07} \times 100 = 141 \text{ c.c.}$$

$$\text{Red Cell volume} = 141 \times \frac{39.0}{100}(\text{Hct.}) = 55 \text{ c.c.}$$

Plasma " = ... 86 c.c.

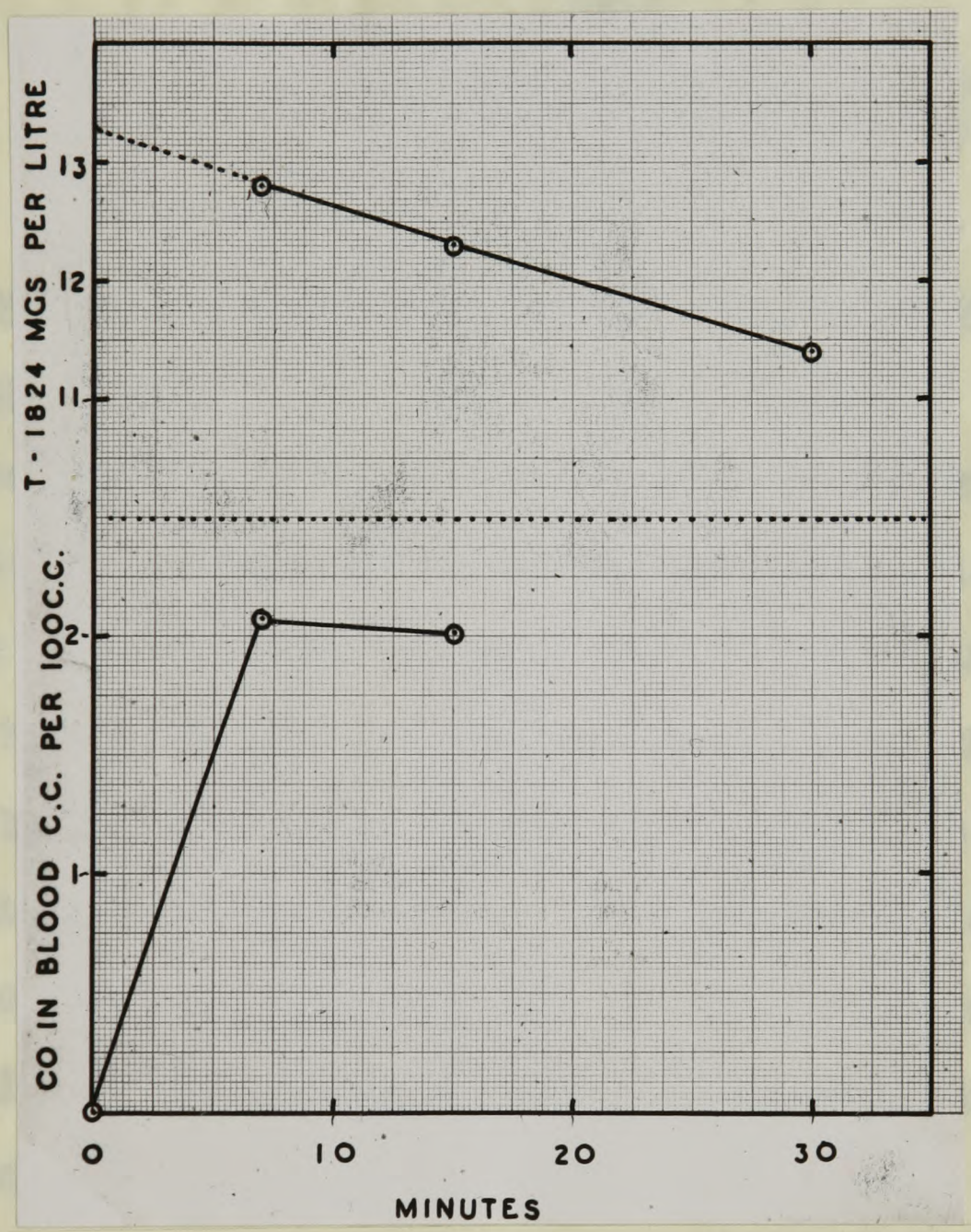


Figure 6. CO concentration in blood and T-1824 concentration in plasma of rabbit at intervals after injection of dye and administration of CO at 0 min. Dotted part of dye curve shows method of extrapolation from 10 min. to theoretical concentration at the moment of injection.

Dye method: Fig. 6. shows the method of extrapolation of the dye disappearance curve to 0 mins. Theoretical dye concentration at 0 mins. = 13.4 mgs. per litre.

$$\text{Plasma volume} = \frac{1.1816}{13.4} \times 1000 = 88 \text{ c.c.}$$

$$\text{Total blood volume} = \frac{88}{100 - 39.0(\text{Hct.})} \times 100 = 145 \text{ c.c.}$$

Red cell volume = ... 57 c.c.

Test of accuracy of CO method in rabbits by the removal of a known amount of blood.

In the procedure described thus far the CO and dye methods have been studied and compared. Because both measure blood volume indirectly it is possible that although they give similar values there may be systematic errors in each which cause the blood volume to be estimated higher or lower than the true value. It was felt that a more direct test of the accuracy of either method would be to measure the reduction in blood volume resulting from the removal of a known amount of blood from the circulation. This was carried out in rabbits using the CO method. The CO method was chosen because it could be used repeatedly in the same animal several times in one day.

Unanaesthetized rabbits were used in the experiments. The blood volume was estimated in the usual way at about 10 A.M. Approximately 40 c.c. of blood were removed by bleeding from the

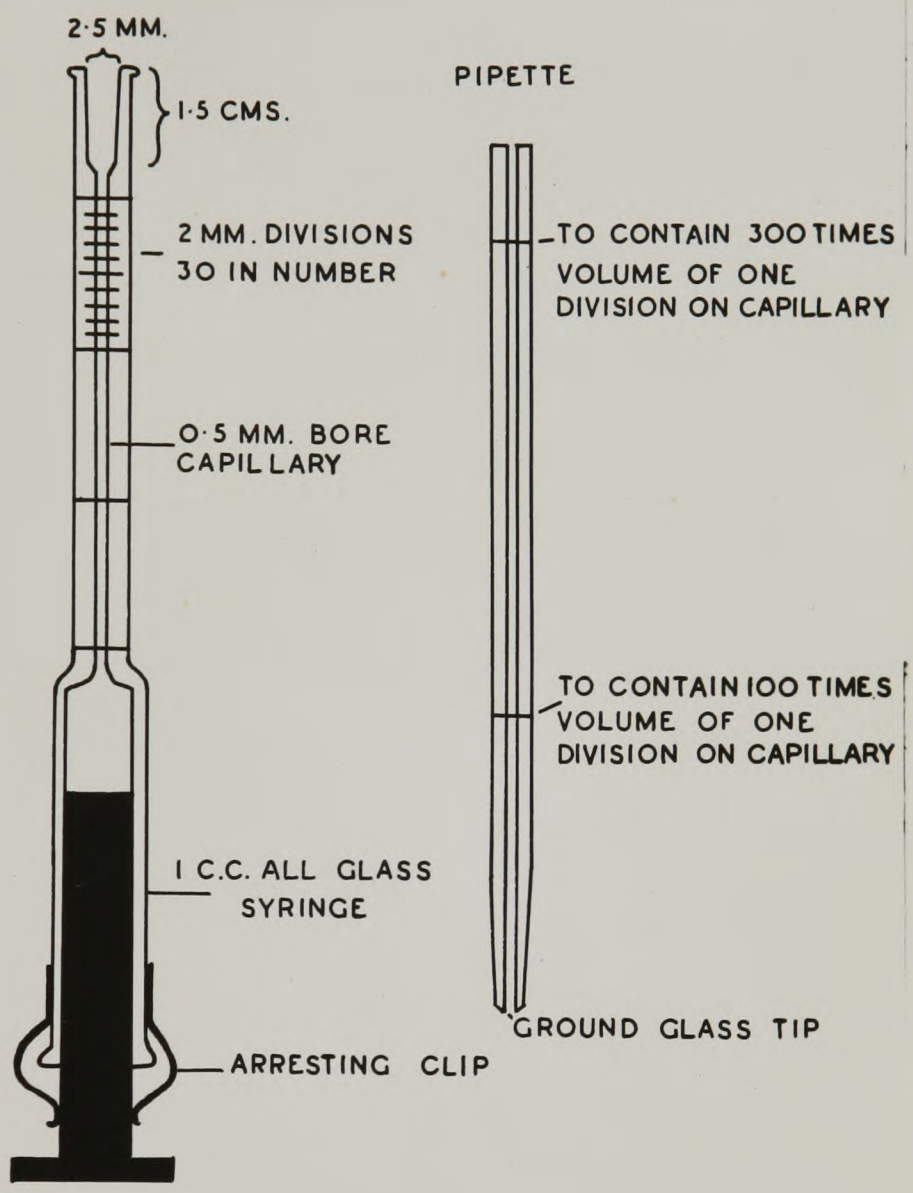
marginal vein of the ear. This blood was collected in a small flask; coagulation was prevented by dry heparin; the volume removed was measured; a small sample was saved for haematocrit determination; and the remainder was stored in the refrigerator. The rabbit was kept in the laboratory without food or water and a second blood volume determination was done 4 hours later. Unaesthetized rabbits restore their circulating fluid volume very rapidly after haemorrhage. It was possible therefore to compare only changes in the total red cell volume. Haematocrits were done in duplicate on the mixed sample of blood which had been removed and on the circulating blood during each blood volume determination.

In another series of experiments, blood which had been removed from one rabbit was injected slowly into another rabbit. Red cell volume was estimated before and after addition of this known amount of red cells to the circulation.

DETAILED ANALYTICAL METHODS

Estimation of CO in blood.

The CO content of the blood was estimated by the method of Scholander and Roughton (1943). The syringe capillary apparatus used in this method is shown diagrammatically in Fig. 7. It consists of a pyrex 1.0 c.c. syringe with an 0.5 mm. bore capillary



THE SCHOLANDER - ROUGHTON SYRINGE

CAPILLARY APPARATUS.

Figure 7. Diagram of the Scholander-Roughton syringe capillary apparatus.

tube fused to the nozzle end. The free end of the capillary tube is expanded to form a cup 1.5 cm. in length and 2.5 mm. in diameter, having a sloping ground glass floor. The shaft of the capillary tube is marked off in 30 or more equal divisions of approximately 2 mm. length. The pipette has a ground glass tip made to form a joint with the floor of the cup. The pipette is calibrated to contain 100 and 300 times the volume of 1 division of the capillary tube.

The apparatus is simple and easy to use. It depends for its accuracy on the precision of calibration of the capillary and pipette. The apparatus used in these experiments was made in the laboratory.

A section of pyrex glass capillary tubing with a bore of approximately 0.5 mm. was selected and cleaned with water and acetone. A pellet of mercury was introduced into the bore and the tubing was mounted on the stage of a travelling microscope. The length of the pellet of mercury in all parts of the tubing was measured to verify the uniformity of the bore. The tube was then covered by a thin film of paraffin wax and mounted in the chucks of a machine lathe. A needle point was fixed in position in the tool holder of the lathe and brought into apposition with the wax coated capillary tube. The needle point was maintained

under slight tension against the tubing by means of a wire spring. When the lathe was rotated by hand with the needle in position, a clean narrow line was etched in the wax covering the capillary tubing. The needle point was withdrawn and the carriage of the lathe bearing the capillary tubing was moved forward 2 mm. The needle point was moved into cutting position and another line was etched in the wax. This process was repeated until 30 or more lines 2 mm. apart had been marked on the shaft of the glass tubing. It was then carefully removed from the lathe and wrapped in blotting paper saturated with hydro-fluoric acid. The acid etched clean narrow lines in the glass where it had been exposed by the needle point. When the acid and wax were cleaned off, the tubing bore 30 easily readable and permanent division lines.

The next problem was to determine the volume of one divison of the capillary tube and calibrate the pipette to contain 100 and 300 times this volume. This was done by weighing mercury. The capillary tube was weighed empty; mercury was sucked in until it occupied exactly 30 divisions; the tube was weighed again. Sample results:

Capillary tube empty: (1) 9.1980 gs.
(2) 9.1980 "

Capillary tube plus 30 divisions Hg. @ 20° C	(1)	9.4046 gs.
	(2)	9.4045 "
Wt. 30 divisions of Hg. @ 20° C =		0.2066 gs.
Wt. 1 divison of Hg. @ 20° C =		$\frac{0.2066}{30}$ gs.
Wt. 100 divisions of Hg. @ 20° C =		$\frac{0.2066}{30} \times 100 = \underline{0.6881 \text{ gs.}}$
and Wt. 300 divisions of Hg. @ 20° C =		$\frac{0.2066}{30} \times 300 = \underline{2.0660 \text{ gs.}}$

These amounts of mercury were carefully weighed out in a clean crucible and were sucked up into the pipette. With the pipette held vertically mercury was allowed to fill the bore from the tip. A line was etched in the glass at the top of the mercury column corresponding to 100 and 300 divisions. The marking of the pipette was always done at the same temperature as the original weighing of mercury in order that no volume changes would result. With calibration complete, the capillary tubing was fused to the syringe and the cup was blown in the free end.

At the start of an analysis the dead space of the syringe and capillary were filled with hydro-sulphite borate solution. Blood was drawn up to the 100 mark on the pipette and transferred to

the syringe. A cupful of ferricyanide and potassium bicarbonate solution was added, followed by an equal volume of acetate buffer solution. The index finger was held over the mouth of the cup to prevent the escape of CO_2 evolved from the reaction between the potassium bicarbonate and acetate buffer. The whole apparatus was shaken horizontally for 5 minutes. During this time approximately 0.75 c.c. of CO_2 were evolved. Adjustment to atmospheric pressure was made by gently withdrawing the plunger of the syringe. CO was liberated from haemoglobin by the action of ferricyanide and was present in the gas phase. The CO_2 was then absorbed by alkaline pyrogallol admitted through the cup and a bubble of gas containing N_2 and CO remained in the barrel of the syringe. This bubble was screwed into the capillary and its length V1 measured after immersion of the apparatus in a water bath of known temperature. With the aid of a magnifying glass the bubble length could be read to the nearest 0.1 of a division. The cup was cleaned out and filled with Winkler's cuprous chloride solution. The bubble of N_2 and CO was forced down into the cup by inverting the apparatus and gently screwing down the plunger of the syringe. The Winkler's solution absorbed the CO and the length of the residual bubble of nitrogen V2, was read after equilibration of its temperature. The value V1-V2

gave the volume in divisions of CO which had been present in the original 100 divisions of blood used in the analysis. Thus, the answer was obtained in terms of c.c. of CO per 100 c.c. of blood and was corrected to S.T.P. by noting the temperature of the water bath with which the bubble was equilibrated and the existing barometric pressure. The accuracy of the method was increased by using 3 times the volume of blood (i.e. 300 division mark on the pipette) and dividing the difference between V1 and V2 by 3. The method of tabulating analytical results is shown below.

Sample	V1	V2	CO	Temp.	Bar	f	Corrected CO c.c. per 100 c.c.	Mean CO c.c. per 100 c.c.
15 mins.	19.7	12.4	2.43	22.3	156	8952	2.18)	2.20
	19.6	12.2	2.47	22.4	"	8948	2.21)	
30 mins.	19.6	12.2	2.47	22.4	"	8948	2.21)	2.20
	20.0	12.7	2.43	22.3	"	8952	2.18)	
36 mins.	25.9	12.4	4.50	22.2	"	8957	4.03)	4.04
	25.9	12.4	4.50	22.0	"	8966	4.04)	

In order to determine the accuracy with which the CO content of blood was determined by this apparatus, samples of blood with known CO concentrations were analyzed. These samples were prepared by mixing CO saturated blood with blood containing little or no CO. The O_2 capacity of the sample blood was first deter-

mined on the Haldane blood gas analysis apparatus. Several c.c. of this blood were placed in a 400 c.c. tonometer containing nitrogen. 25-50 c.c. of pure CO from the reservoir were added to the tonometer and the pressure was brought to atmospheric by momentarily releasing the clamp on the outlet tube of the tonometer. This percentage of CO in nitrogen was sufficient to saturate the haemoglobin 100% with CO after 2 hours rotation. The value obtained for O_2 capacity of the blood was also the value for CO content of the treated blood. Careful dilutions with Ostwald pipettes were made and the resulting samples stored anaerobically in an ice bath.

Example:

A sample of ox blood containing no CO was found to have an O_2 capacity of 16.85 c.c. per 100 c.c. 1.0 c.c. of this blood saturated with CO (and containing therefore 16.85 c.c. CO per 100 c.c. blood) was mixed with 7.0 c.c. of untreated blood.

The CO content of the mixture was $\frac{1}{8}$ x 16.85 = 2.11 c.c. per 100 c.c. Duplicate analyses with the syringe capillary apparatus gave the values . . . . . 2.06) 2.09 c.c. per
2.12) 100 c.c.

All the tests by the method of mixtures using rabbit, ox, sheep, and human blood are summarized in Table 1 below.

Table 1. Accuracy of CO in blood determinations by the method of mixtures. CO content expressed as c.c. per 100 c.c. blood.

xperi-ent	Dilution: CO sa-turated Blood	Dilution: Untreat-ed Blood	CO content of untreat-ed blood	CO content of blood saturated with CO	O_2 capa-city (Haldane)	Observed CO con-tent	Mean of observed values	Expected CO con-tent	Error in c.c. per 100 c.c.
.11.46 abbit	1 part	8 parts	0	-	13.8	1.67 1.60 1.58	1.62	1.53	+ 0.09
	1 "	6 "	0	-	13.8	2.07 2.01 1.95	2.01	1.97	+ 0.04
8.11.46 Rabbit	1 "	8 "	-	-	14.9	1.61 1.68 1.58	1.62	1.67	- 0.05
	1 "	6 "	-	-	14.9	2.16 2.10 2.06	2.11	2.13	- 0.02
12.11.46 Sheep	1 "	8 "	-	18.1) 18.0) 18.05	17.95) 18.05) 18.0	2.01 1.97	1.99	2.00	- 0.01
13.11.46 Sheep	1 "	5 "	-	18.05	18.0	3.10 3.07	3.09	3.00	+ 0.09
	1 "	8 "	-	18.05	18.0	2.00 2.03	2.02	2.00	+ 0.02

Table 1 cont.

Experiment	Dilution: CO saturated Blood	Dilution: Untreated Blood	CO content of untreated blood	CO content of blood saturated with CO	O_2 capacity (Haldane)	Observed CO content	Mean of observed values	Expected CO content	Error in c.c. per 100 c.c.
5.11.46	1 part	10 parts	-	19.3) 18.9)19.0 18.8)	-	1.74 1.79	1.77	1.73	+ 0.04
	1 "	20 "	-	"	-	0.95 0.95	0.95	0.09	+ 0.05
25.11.46 Human	1 "	8 "	1.20) 1.20)1.20	19.5) 19.7)19.6 19.7)	19.7	3.26 3.24	3.25	3.24	+ 0.01
	1 "	5 "	"	"	"	4.37 4.40	4.39	4.40	- 0.01
14.4.47 Human	1 "	11 "	0.56) 0.62)0.59	20.6	-	2.43 2.33 2.49	2.42	2.41	+ 0.01
29.8.47 Human	1 "	7 "	0.18) 0.15)0.17	18.8) 18.2)18.7 19.0)	-	2.54 2.50	2.52	2.49	+ 0.03
	1 "	7 "	"	"	-	2.47 2.51 2.47	2.48	2.49	- 0.01
7.4.47 Ox	1 "	7 "	0	-	16.85	2.06 2.12	2.09	2.11	- 0.02

Inspection of the deviations of observed from expected CO content of blood indicates that over the range of CO values studied the spread of these deviations is independent of the CO content of the blood. The mean deviation of observed from expected CO content is 0.012 ± 0.009, i.e. the deviation is trivial and not significantly different from zero. The standard errors of the means of duplicate and triplicate determinations are found to be ± 0.037 and ± 0.030 c.c. per 100 c.c. of blood respectively. Since these estimates are based on 36 individual estimations, they imply that there is less than one chance in 20 of the mean of duplicate determinations deviating by more than 0.075 c.c. per 100 c.c. or the mean of triplicates deviating by more than 0.06 c.c. per 100 c.c. from the true CO content of the blood.

In several of the experiments appearing in Table 1 the CO content of CO saturated blood was measured by the syringe capillary apparatus and compared with the O_2 capacity determined by the Haldane blood gas analysis apparatus. It will be noted that the figures agree to within 0.05 vols. %.

Estimation of CO in air.

The method used for the determination of CO in air was a modification of several other methods described in the literature. (Sendroy and Fitzsimons 1944) (Roughton 1945) Blood was used

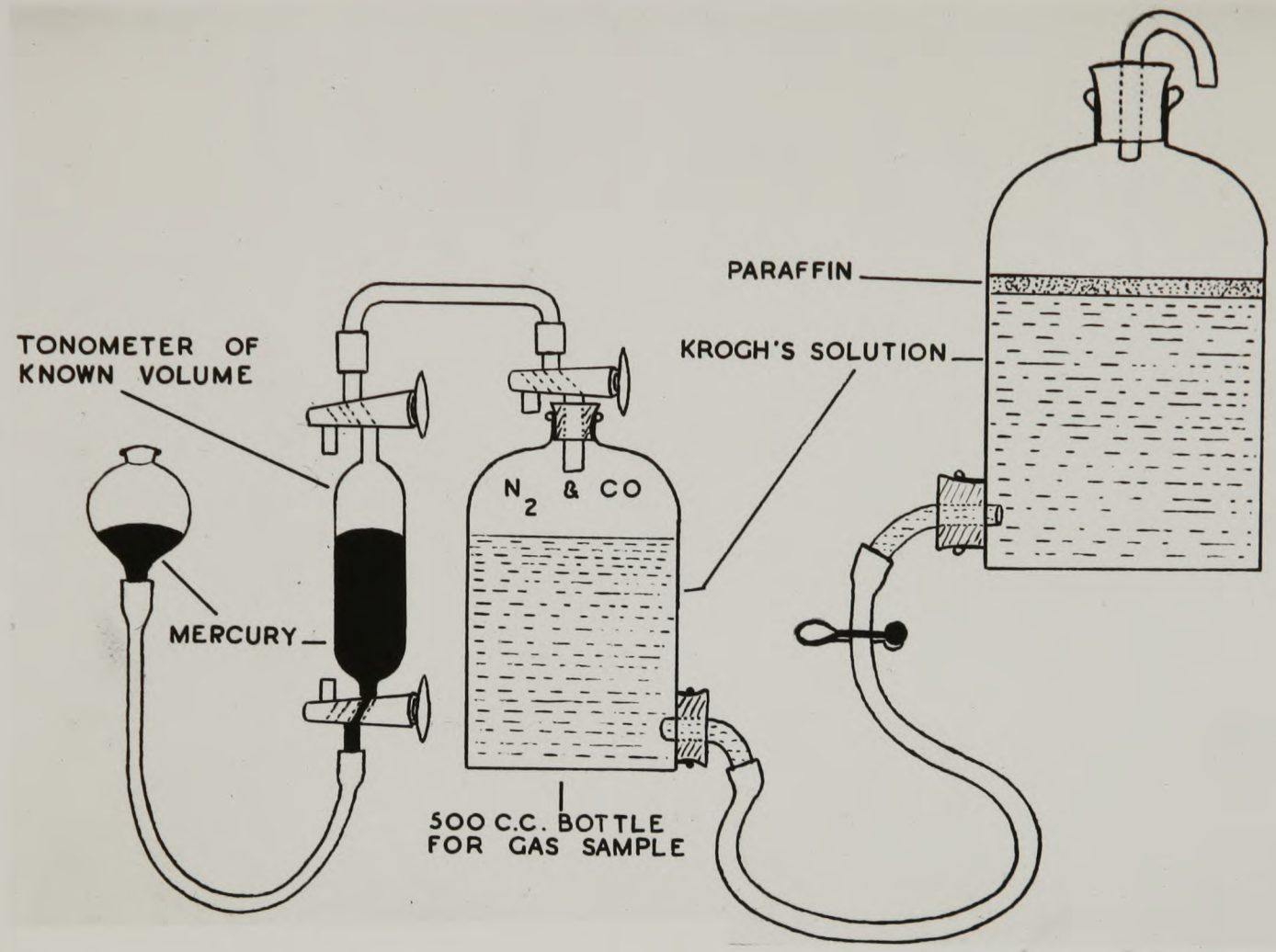


Figure 8. Apparatus used for de-oxygenation of air sample and transference to a tonometer of known volume in the method for CO in air analysis.

as the active reagent to absorb CO from a known quantity of de-oxygenated air. The amount of CO absorbed by the reagent blood was measured by the syringe capillary apparatus. The O_2 percentage of the original air before de-oxygenation was determined by the Haldane gas analysis apparatus. With these data the CO percentage of the original air could be calculated.

Part of the apparatus used is shown in Fig. 8. A sample of air for analysis was collected over saline in a 500 c.c. aspirator bottle fitted with rubber stoppers and a 3 way tap. A sample of the same air was collected in a gas sampling tube over mercury for determination of CO_2 and O_2 content on the Haldane apparatus. The air was allowed to displace all the saline from the 500 c.c. bottle; the upper tap was closed; and a clamp was applied to the rubber tubing leading to the saline reservoir. The saline reservoir was replaced by another aspirator bottle containing Krogh's solution (100 c.c. N.NaOH; 20 gs. sodium hydrosulphite; 2 gms. B anthraquinone sulphonate) covered by a layer of liquid paraffin. Care was taken to fill the rubber tubing with Krogh's solution before connecting it to the 500 c.c. air bottle, in order that no foreign gas would be introduced. The Krogh's solution was allowed to run into the 500 c.c. bottle under slight positive pressure. The bottle was

shaken vigorously to facilitate absorption of O_2. Fifteen minutes were allowed for this absorption which left N_2 and CO remaining in the 500 c.c. bottle. The N_2 and CO were then transferred to a tonometer of known volume (80-100 c.c.), using a glass connecting bridge with a mercury seal. The gas in the tonometer was brought to atmospheric pressure by adjusting the level of the mercury bulb and bottle of Krogh's solution, and the taps were closed.

Several c.c. of freshly drawn human blood containing little or no CO were placed in a 400 c.c. tonometer filled with nitrogen. This tonometer was rotated for 2 hours to reduce the reagent blood. A sample of the reduced blood was stored in an all glass syringe for 'blank' CO analysis; the remainder was kept in a small tightly stoppered bottle. Exactly 1.0 or 2.0 c.c. of this blood were introduced into the tonometer containing the test gas by means of record syringe with a rubber tube fitted to the nozzle. The tonometer was rotated in the dark for 2 hours at 15° C. During this period the CO in the air entered into combination with the haemoglobin. The blood was then completely drained from the tonometer and stored anaerobically for CO analysis.

The increase in the CO content of the blood after equilibration showed how much CO had been absorbed from the tonometer.

It was assumed that the CO present was absorbed completely. This amount of CO had originally been present in a larger volume of air before the O_2 absorption by Krogh's solution. With the volume of the tonometer and the O_2 percentage of the original air known, the volume of original air involved was easily calculated.

Example: Volume of tonometer used: 88.0 c.c.

Analyses of original air for
O_2 and CO_2 percentage 0% CO_2
48.2% O_2

1.0 c.c. of blood introduced into tonometer.

CO in blood analysis by syringe capillary apparatus:

'Blank' CO content: 0.34 c.c. per 100 c.c.
CO content after equilibration:
3.98 c.c. per 100 c.c.

Increase in CO content of blood due to absorption of CO from tonometer = 3.98 - 0.34
= 3.64 c.c. per 100 c.c. blood
= 0.0364 c.c. per 1.0 c.c. blood.

i.e. 0.0364 c.c. CO was present in the 88 c.c. of gas in the tonometer.

O_2% of original gas was 48.2%

88.0 c.c. comprised 51.8% of original gas.

Volume of original gas = $\frac{88.0}{51.8} \times 100 = 170$ c.c.

CO content of original air = $\frac{0.0364}{170} \times 100 = \underline{0.0214}$ c.c. CO per 100 c.c. of original air.

In order to determine the accuracy of this method, analyses were done on air with a known CO content. These CO in air mixtures were prepared quite simply using the apparatus shown in Fig. 9. The volume of the aspirator bottle was calibrated by weighing the water delivered from the tap to the clamp. The bottle was filled with 30% saline. A Douglas bag was filled with an air mixture containing 50% O_2 and 50% N_2 and was connected by a glass T-piece to the upper tubing of the aspirator bottle. A gas burette containing CO of known purity was connected with the side arm of the T-piece. The air mixture from the Douglas bag was allowed to displace the saline from the aspirator bottle. As it passed through the T-piece the CO from the gas burette was slowly admitted to this moving stream of air. When the aspirator bottle was just filled by the gas mixture the tap and the clamp were closed. The volume of CO delivered was read on the burette to the nearest 0.01 c.c. and corrected for purity, temperature and barometric pressure to standard conditions.

Example: 2.48 c.c. CO from gas burette at 18.5° & 757 mm. Hg. with a purity of 99.5% = 2.28 c.c. CO pure at S.T.P.

These 2.28 c.c. of CO were present in 10,893 c.c. of air

Expected CO content = $\frac{2.28}{10,983} \times 100 = \underline{0.0207}$ c.c. per 100 c.c. of original air.

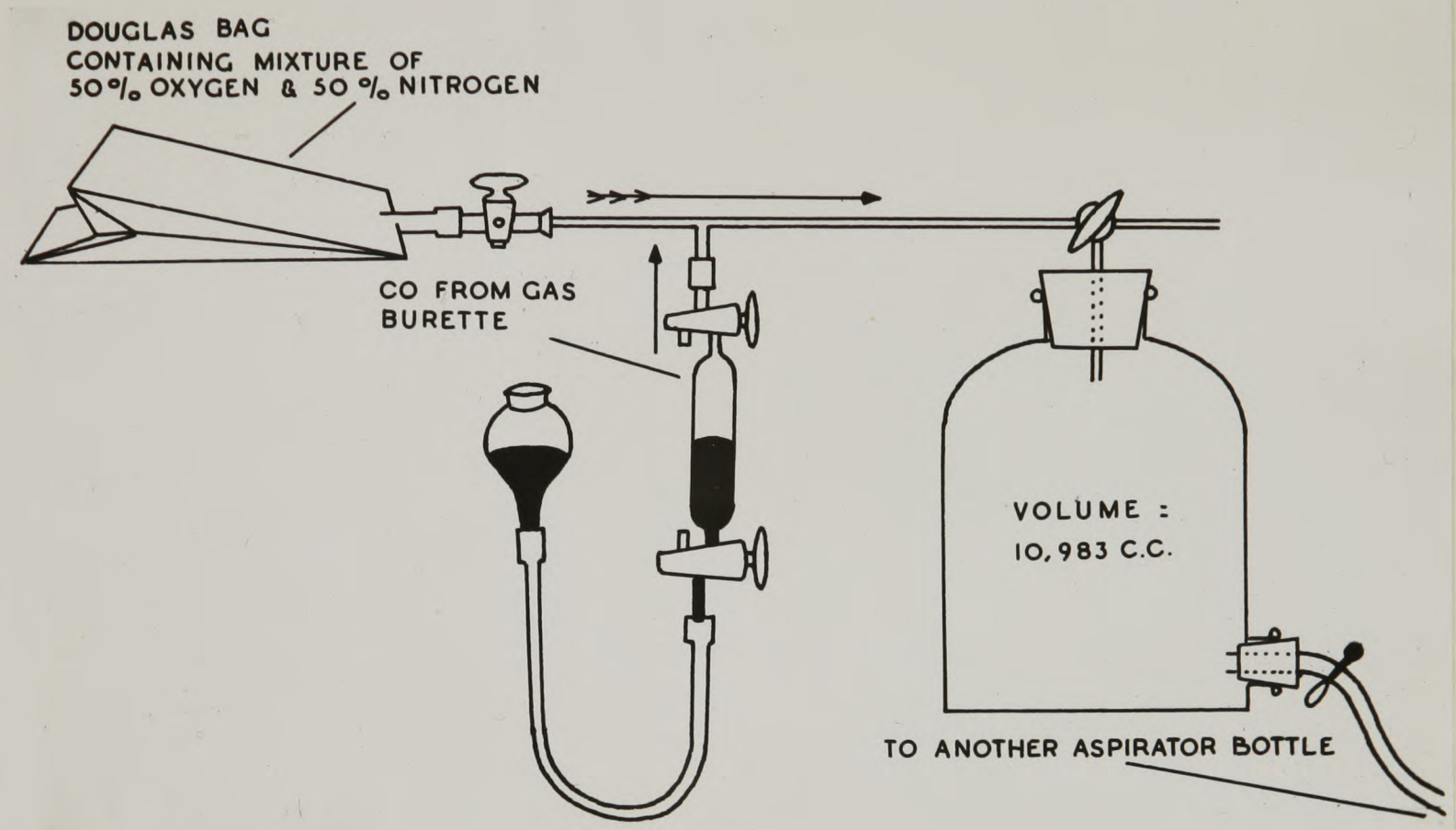


Figure 9. Apparatus used for preparing CO in air mixture of known concentration.

Samples of this air mixture from the aspirator bottle were analyzed for CO content by the method just described. Results of two experiments are given below.

Experiment		Values obtained by analysis	Expected Values
(1)		0.0200%	
		0.0199"	0.0207%
		0.0206"	
		0.0211"	
	Mean	0.0204"	
(2)		0.0193%	
		0.0196"	0.0205%
		0.0200"	
		0.0210"	
	Mean	0.0200"	

These results were very satisfactory. Something less than complete recovery was expected because of failure to account for the very small fraction of CO left unabsorbed in the tonometer at equilibrium. This fraction was reduced to a minimum by excluding O_2 from the tonometer and reducing the reagent blood before equilibration. A discrepancy of 5% in this analysis would have little effect on the final blood volume determination because the amount of residual CO in the closed circuit which this technique was designed to measure was only of the order of 1 to 2% of the CO admitted.

RESULTS

I Humans: Uptake and disappearance of CO at rest.

In 11 experiments the CO content of the blood was determined at intervals for a period of 1 hour after the administration of approximately 90 c.c. of pure CO to the closed circuit. The results appear below in Table 2. Mean values are plotted on the graph in Fig. 10.

Table 2: Human CO curves at rest followed for 60 minutes. CO concentration in blood in c.c. per 100 c.c.

Subject	Date	Pure CO admitted to circuit at S.T.P.		0'	5'	10'	15'	30'	45'	60'
F.C.C.	27.2.47	90.5	c.c.	0.20	2.12	2.05	1.99	1.98	1.93	1.87
R.W.G.	28.2.47	90.0	"	0.57	2.17	2.14	2.02	1.91	1.93	1.90
R.W.G.	7.3.47	92.7	"	0.57	2.13	2.12	2.07	1.98	1.98	2.02
F.C.C.	17.3.47	90.8	"	0.22	2.04	2.05	1.99	1.93	1.82	1.83
R.W.G.	18.3.47	90.6	"	0.53	2.05	1.99	1.99	1.99	1.90	1.88
T.J.M.	19.3.47	90.2	"	0.28	1.75	1.75	1.75	1.75	1.69	1.67
W.J.S.	3.4.47	89.9	"	0.47	1.92	2.09	2.00	2.00	2.03	2.03
F.C.C.	16.4.47	87.3	"	0.24	2.13	2.08	2.05	1.99	1.92	1.90
M.V.B.	7.5.47	84.9	"	0.17	1.85	1.91	1.89	1.85	1.79	1.80
G.R.	8.5.47	84.3	"	0.14	2.11	2.08	2.03	1.93	1.96	1.95
W.M.J.	9.5.47	84.7	"	0.12	1.74	1.79	1.74	1.67	1.70	1.67
	Mean	88.7	"	0.32	2.00	2.00	1.96	1.91	1.88	1.87
	Difference			1.68	0	0.04	0.05	0.03	0.01	

In these experiments the subjects had not smoked for 12 hours prior to the taking of the initial blood sample for 'blank' CO content. Several of the subjects, M.V.B., G.R., W.M.J. were

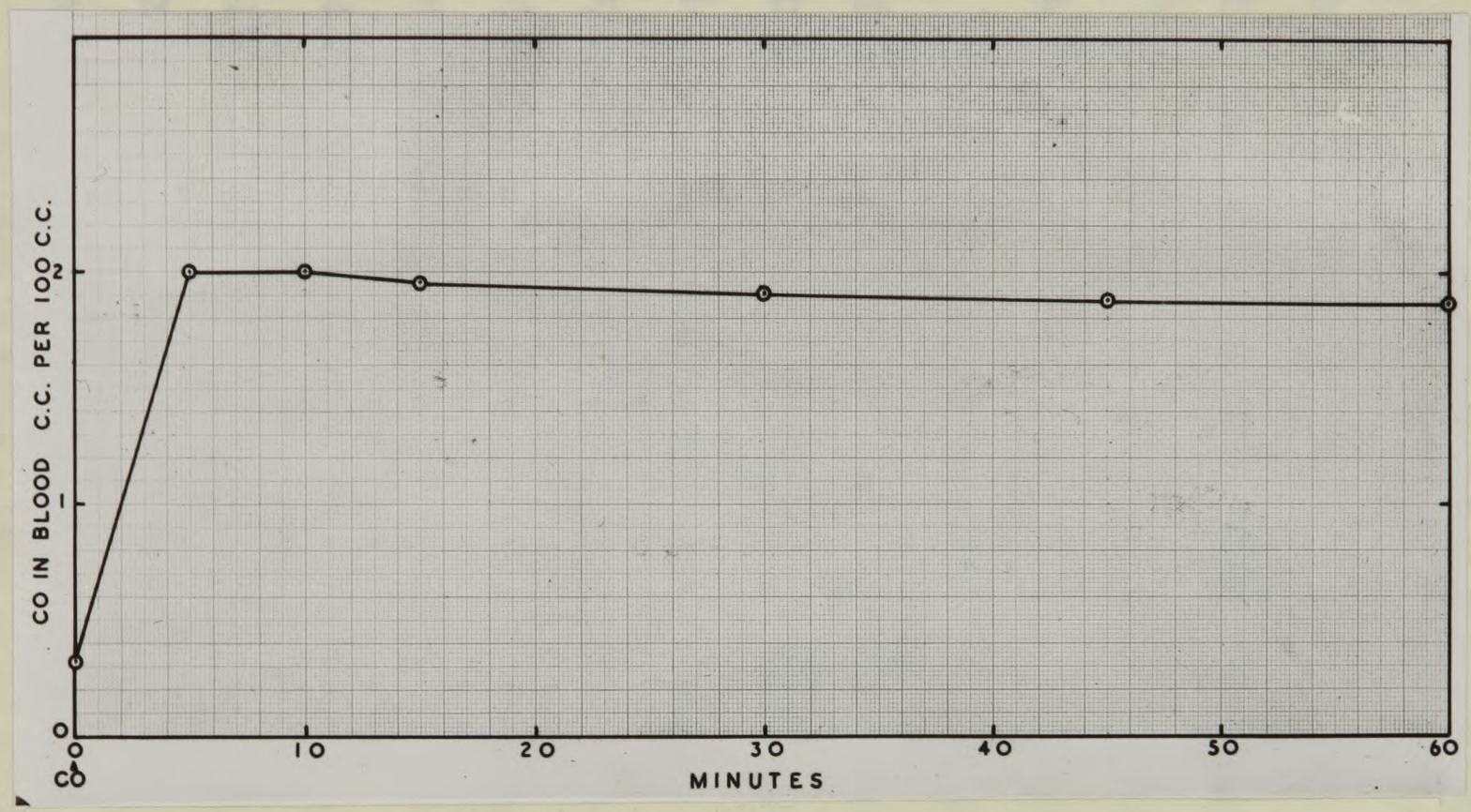


Figure 10. CO uptake and disappearance curve in human subjects breathing from the closed circuit for one hour at rest. Average values from 11 experiments. The 'blank' CO content of the blood before administration of CO and the slow disappearance over the hour period are illustrated.

non-smokers. Despite these facts, the blank CO content of the blood comprised a significant percentage of the final CO level after absorption of the 88.7 c.c. If this factor had been disregarded as was the case in some of the earlier work on blood volume by the CO method, appreciable error would have been introduced in the calculation. Using the average values from Table 2 the blood volume would have been estimated 15% too low. For this reason initial CO blood levels were done in every case. The subject breathed from the closed circuit for 5 minutes to establish equilibrium between the blood and the air in the circuit, before the initial sample was drawn. Individual smokers such as F.C.C. and R.W.G. showed very constant initial CO levels on different days after 12 hours abstention. These two subjects also showed very different responses to smoking 5 cigarettes in 1 hour (Courtice and Simmonds 1948). The initial CO content of non-smokers' blood may be attributed to small percentages of CO in the atmosphere resulting from exhaust fumes, coal gas fires and smoke from industrial plants.

Examination of individual curves in these experiments showed in most cases maximum CO levels at 5 minutes with no significant change at 10 min. It also showed a slow but definite fall in CO levels from 10 min. to 60 min. Mean values showed a fall from

2.00 vols.% at 10 minutes to 1.87 vols.% at 60 min. In this total fall of 0.13 vols.%, a major part, 0.09 vols.%, occurred from 10 to 30 min. In the last 30 min. the rate of disappearance of CO decreased and the curve became almost flat from 45 min. to 60 min.

Mean values from these experiments also showed an initial CO concentration of 0.32 vols.% and a concentration of 2.00 vols.% after absorption of 88.7 c.c. of the gas. The increase was 1.68 vols.% and of this amount 0.13 vols.% disappeared from the circulation in 50 min. That is, 7.7% of the CO absorbed into the blood disappeared.

The actual volume of CO absorbed was slightly less than the 88.7 c.c. admitted to the circuit. Measurement of CO remaining in the circuit at 10 minutes showed that 1.4 c.c. was not absorbed. Therefore the volume of CO which disappeared from the circulating blood in this series of experiments was 7.7% of 87.3 c.c. = 6.72 c.c. This figure will be used later in discussing the part played by myoglobin in blood volume determinations.

The CO disappearance curve in human subjects was studied for 30 minutes in a larger series of experiments. These data are shown in Table 3. The 25 curves include those already given in Table 2 plus others taken from routine blood volume

determinations performed in the same manner.

Table 3. Human CO curves at rest followed for 30 minutes. CO concentration in blood in c.c. per 100 c.c.

	0'	5'	10'	15'	30'
	0.20	2.12	2.05	1.99	1.97
	0.22	2.04	2.05	1.99	1.93
	0.24	2.13	2.08	2.05	1.99
	0.17	2.08	2.11	2.08	2.06
	0.57	2.17	2.14	2.02	1.91
	0.57	2.13	2.12	2.07	1.98
	0.53	2.05	1.99	1.99	1.99
	0.80	2.45	2.36	2.33	2.32
	0.65	2.30	2.25	2.19	2.20
	0.28	1.75	1.75	1.75	1.75
	0.17	1.85	1.91	1.89	1.85
	0.14	2.11	2.08	2.03	1.93
	0.12	1.74	1.79	1.74	1.67
	0.27	1.97	2.03	2.03	2.00
	0.34	2.32	2.29	2.22	2.16
	1.01	2.57	2.55	2.51	2.48
	0.17	1.79	1.74	1.67	1.66
	0.17	1.82	1.81	1.82	1.76
	0.65	2.21	2.23	2.12	2.00
	0.47	1.92	2.09	2.00	2.00
	1.96	3.87	3.89	3.89	3.84
	2.17	3.81	3.75	3.73	3.70
	2.09	3.87	3.90	3.85	3.83
	1.81	3.60	3.55	3.55	3.58
	1.55	3.12	3.08	3.03	3.03
Mean	0.69	2.39	2.38	2.34	2.30
Difference		1.70	0.01	0.04	0.04

Average values from this larger series showed a fall in CO of 0.08 vols.% from 10 min. to 30 min. This was 4.7% of the CO absorbed.

The interpretation of this fall in CO content as due to a disappearance of CO from the circulating blood would be incorrect

if haemodilution had occurred during the course of the experiments. A reduction in the haematocrit of later samples would leave less haemoglobin in those samples to carry COHb and the determined CO percentage would reflect degree of dilution rather than disappearance of CO. Gibson et al. (1937) have in fact observed this phenomenon in man while taking serial blood samples for the determination of dye disappearance curves. In their experiments larger blood samples were drawn over a longer period of time. To make sure that no haemodilution occurred during the experiments tabulated in Table 3, haematocrits were done on the 0, 10, 15, and 30 min. samples. The results on 20 experiments shown in Table 4 indicate that haemodilution was not a factor in lowering the blood CO content.

Table 4. Haematocrit values on blood samples used to study CO disappearance curves.

0'	10'	15'	30'
45.5	45.5	45.7	45.2
46.5	47.1	47.6	47.1
41.9	41.8	41.6	41.7
47.2	46.8	47.0	48.0
44.7	45.0	44.2	45.1
40.1	39.7	39.9	40.2
40.7	40.5	40.5	39.7
44.2	43.3	42.5	42.6
42.6	42.2	42.7	43.3
38.9	39.9	38.9	39.1
40.2	39.6	40.4	40.9
47.1	47.3	47.2	48.0
42.4	42.6	43.8	43.5
42.1	41.3	41.9	41.4
43.7	43.5	44.1	44.7
40.4	41.5	41.9	41.7
42.1	42.4	41.7	41.7

Table 4 continued.

	0'	10'	15'	30'
	44.1	44.3	44.8	44.8
	40.7	41.3	40.8	41.9
	41.3	40.7	39.7	40.6
Mean	42.8	42.8	42.8	43.1

In 4 subjects the effect of moderate muscular exercise on the CO disappearance was studied. In these experiments the exercise was begun 10 minutes prior to the administration of CO so that the subject was in a steady state. The data for volume of CO admitted, mechanical work performed, O_2 consumption, mean haematocrit and CO content of blood at intervals up to 30 minutes are recorded in Table 5.

Table 5. Human CO curves during moderate exercise.

Subject	Date	c.c. CO @ S.T.P.	O_2 consumption c.c. per min.	Work Kgm.M /min.	CO in blood c.c. per 100 c.c. 0'	5'	10'	15'	20'	30'
F.C.C.	3.6.47	89.3	1072	420	0.15	1.97	1.98	1.91	1.91	1.95
R.W.G.	5.6.47	89.9	1333	420	0.54	2.16	2.10	2.05	2.05	2.08
W.J.S.	13.6.47	91.6	1604	630	0.56	2.60	2.63	2.58	2.63	2.59
H.	24.6.47	92.2	1439	525	0.05	1.48	1.54	1.49	1.49	1.53
Mean CO levels					0.33	2.05	2.06	2.01	2.02	2.04
Mean Hct. of the 4 subjects					43.6	-	43.7	-	43.9	43.8

These results demonstrate that although the O_2 consumption by the tissues was increased to 4 or 5 times the normal resting value there was no significant disappearance of CO from the circulating blood. If the muscle myoglobin does take up an appreciable quantity of CO and if the gradual disappearance of the CO from the blood observed at rest is due to this slow diffusion, it might be expected that the increased blood supply to the muscles during exercise would augment the rate and degree of this process. The figures in Table 5 show that the CO curves are very similar to those at rest. The maximum concentration was reached between 5 min. and 10 min; there was a fall of 0.05 vols.% at 15 min; and no further fall thereafter. The figures for 20 min. and 30 min. were slightly higher than those for 15 min. but considering the small series, these were within the error of the analytical method. The haematocrit values remained constant throughout the experiment so that no concentration or dilution effects were included.

These observations do <u>not</u> exclude the possibility that a considerable amount of CO diffused into the muscle myoglobin within the first 5 minutes before a blood sample was taken.

<u>Measurement of CO remaining in closed circuit</u>

As described under Procedure, air samples were taken from the

spirometer at 5, 10 and 30 min. after admitting CO to the closed circuit and at 10 min. after a repeat dose of the same volume of CO. These samples were analyzed for O_2 on the Haldane gas analysis apparatus and for CO by the method described. The results on 4 subjects are summarized in Table 6.

Table 6. CO content of air in lung-spirometer system.

	After single 90 c.c. dose CO. Time in minutes CO & O_2%						After repeated 90 c.c. dose CO. Time in minutes CO & O_2%	
	5'		10'		30'		10'	
Subject	CO	O_2	CO	O_2	CO	O_2	CO	O_2
T.J.M.	-	-	-	-	0.0127	35.8	-	-
R.W.G.	-	-	0.0214 0.0224	48.2	0.0216 0.0217	44.6	-	-
F.C.C.	-	-	0.0210 0.0227	53.0	0.0210 0.0206	50.8	0.0470 0.0511	54.6
P.J.P.	0.0394 0.0413	44.1	0.0219 0.0217	44.0	0.0217 0.0221	49.1	-	-
T.J.M.	-	-	0.0134 0.0133	39.4	0.0136 0.0125	49.4	0.0279 0.0275	40.1
R.W.G.	0.0425 0.0430	51.5	0.0248 0.0242	49.6	-	-	0.0476 0.0468	49.5

In the 4 experiments where the residual CO was measured at 10 min. and again at 30 min. the values from individual cases showed that no further absorption occurred after 10 minutes. The average 10 min. CO percentage was 0.0197; the 30 min.

percentage 0.0196. This meant that equilibrium between the CO in the air of the closed circuit and the blood had been reached after 10 minutes of rebreathing.

For the subjects F.C.C., R.W.G. and P.J.P. the percentage of CO at equilibrium was very constant and of the order of 0.0220%. In the case of T.J.M. lower values were obtained on two occasions. To obtain a figure for the correction factor for residual unabsorbed CO, the mean of all the 10 min. values, 0.0207% was taken. The volume of the lung-spirometer system was calculated as follows:

Spirometer at ½ full level	2500 c.c.
Soda-lime container and tubes	2000 c.c.
Air in lungs	2500 c.c.
Total	7000 c.c.

The total amount of unabsorbed CO at equilibrium would be:

$$\frac{0.0207}{100} \times 7000 = 1.45 \text{ c.c.}$$

Since the volume administered was approximately 90 c.c. pure CO @ S.T.P. 1.45 c.c. would comprise $\frac{1.45}{90} \times 100 = 1.6\%$ of CO administered. In all the blood volume estimations the conditions affecting this equilibrium value, i.e. O_2% in circuit (40-50) and CO concentration in blood (2.00 vols.%) were kept constant. A correction factor of 0.984 was therefore applied in all calculations where the CO method was used.

In two experiments on P.J.P. and R.W.G. the residual CO was measured at 5 min. and again at 10 min. after commencement of rebreathing. The mean values were 0.0416% at 5 min. and 0.0232% at 10 min. Using again the figure 7000 c.c. for volume of lung-spirometer system the actual amounts of CO left unabsorbed were respectively 2.9 and 1.8 c.c. That is, 5 minutes of rebreathing were sufficient for absorption of 96.8% of the admitted CO and equilibrium was achieved at 10 min. with 98.2% absorption. Thus, using the circuit described in this work, with administration of the CO directly into the inspiratory side within 1 min. 15 sec., 98.6% of the CO destined for absorption was taken up in the first 5 minutes. From these figures it may be inferred that the equilibrium level was probably reached in 6-7 minutes. Complementary evidence was supplied by the uptake and disappearance curves of CO in blood. Mean values from the 25 curves (Table 3) showed that the blood CO concentration remained essentially unchanged between 5 min. and 10 min. It is probable that the additional 1.4% of CO absorbed after 5 min. disappeared from or was mixed with the circulating blood so that the 10 min. level remained constant.

In 3 experiments on the subjects, F.C.C., T.J.M. and R.W.G. the CO percentage in the lung-spirometer system was estimated

at equilibrium after a single 90 c.c. dose of CO and again 10 minutes after this same dose of CO was repeated with the subject continuing to breath from the spirometer. In each of the three experiments (Table 6) the residual CO after the second administration was approximately double that after the first. The mean values were 0.0195% and 0.0413%. At the first equilibrium tension roughly 1.4 c.c. of CO remained in the lung-spirometer system and at the second 2.8 c.c. Assuming that 90.0 c.c. CO had been administered each time, the volume absorbed from the first dose would be 90.0 - 1.4 = 88.6 c.c.; and the volume absorbed from the second (90.0 + 1.4) - 2.8 = 88.6 c.c. Thus, in the experiments where the blood volume estimation by the CO method was repeated no error was introduced by having the residual CO at a higher tension. The volume of CO remaining at the original equilibrium cancelled this factor. Care was taken to have the subject breathe from the closed circuit for 5 minutes to re-establish this equilibrium tension before the initial blood sample was drawn.

These data are presented in schematic form in Fig. 11 to illustrate the rise in blood CO concentration and co-incident fall in CO content of air in the circuit when the same dose of CO was administered to a subject and then repeated

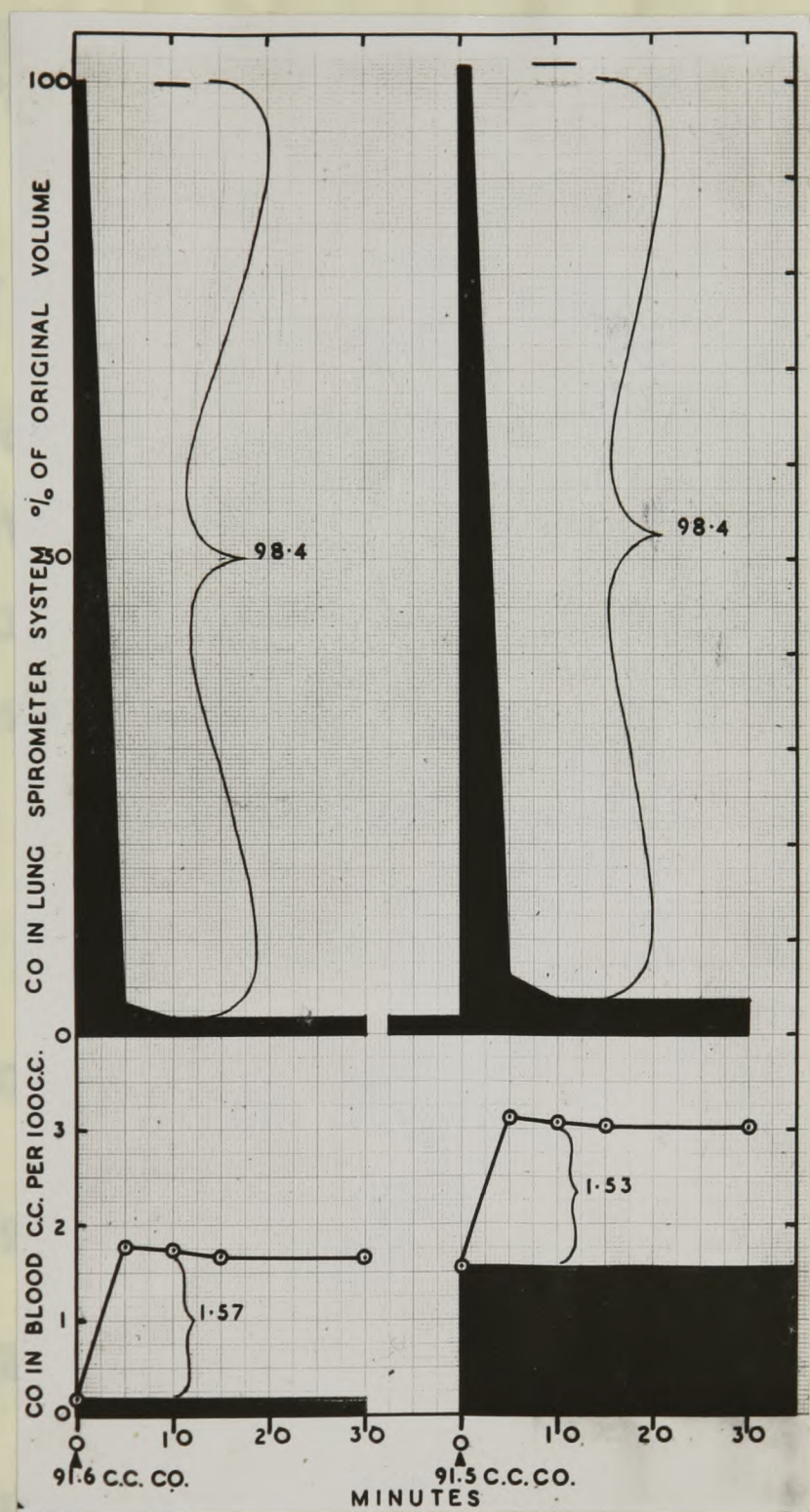


Figure 11. Rise in CO concentration in the blood and concurrent fall in CO content of lung-spirometer system after single and repeat dose of the same volume of CO in human subjects.

The value for K, the equilibrium constant of the reaction $\frac{HbCO}{HbO_2} = K \frac{pCO}{pO_2}$ was determined from the results of the preceding experiments. By changing the equation

$$K = \frac{HbCO}{HbO_2} \times \frac{pO_2}{pCO}$$

pO_2 and pCO were calculated from the average results in Table 6 on all subjects at 10 minutes. With a barometer reading of 760 mm. Hg. and allowance for CO_2 and aqueous vapour pressure the conditions obtaining in the lung alveoli would have been:

$$pO_2 = \frac{39.9}{100} \times 713 = 284 \text{ mm. Hg.}$$

$$pCO = \frac{0.0207}{100} \times 713 = 0.148 \text{ mm. Hg.}$$

HbCO = 10% (2.00 vols.% CO in a total O_2 capacity of 20.0 vols.%)

HbO_2 = 85% (Assuming Hb 95% saturated and deducting 10% for COHb measured)

$$K = \frac{10 \times 284}{85 \times 0.148} = 226.$$

In vitro experiments by other investigators have yielded values for K in human blood at 37° ranging from 210-300 (Killick 1940). The figure 226 obtained here represents only average results on a series of subjects but it indicates that the physico-chemical laws governing the partition of haemoglobin between O_2 and CO

are being met under the conditions of the experiment.

CO repeated at rest and exercise

On the 4 subjects F.C.C., R.W.G., P.J.P. and T.J.M. the CO curves were followed for 30 minutes after a single administration of CO and for another 30 minutes after the CO had been repeated. Haematocrits were done on each blood sample. In the exercise experiments the respiratory exchange was determined. The results are presented in Table 7.

Table 7. Human CO curves repeated at rest and during moderate muscular exercise. Time in minutes. CO in c.c. per 100 c.c.

	I Rest						II Rest					
Subject	0'	5'	10'	15'	30'	Hct.	0'	5'	10'	15'	30'	Hct.
F.C.C.	0.17	2.08	2.11	2.08	2.06	40.0	1.96	3.87	3.89	3.89	3.84	40.2
P.J.P.	0.27	1.97	2.03	2.03	2.00	40.8	1.81	3.60	3.55	3.55	3.58	39.3
R.W.G.	0.80	2.45	2.36	2.33	2.32	43.3	2.17	3.81	3.75	3.73	3.70	42.7
T.J.M.	0.17	1.79	1.74	1.67	1.66	40.3	1.55	3.12	3.08	3.03	3.03	40.4
Mean	0.35	2.07	2.06	2.03	2.01	41.1	1.87	3.60	3.57	3.55	3.54	40.7

	I Rest						II Exercise						O_2 c.c./min	Work Kgm.M./min
F.C.C.	0.34	2.32	2.29	2.22	2.16	41.4	1.86	3.85	3.87	3.85	3.81	41.2	1144	420
P.J.P.	0.65	2.21	2.23	2.12	2.00	41.8	1.93	3.57	3.54	3.48	3.48	42.0	1167	420
R.W.G.	1.01	2.57	2.55	2.51	2.48	44.0	2.47	4.09	4.03	3.99	3.97	44.5	1126	420
T.J.M.	0.17	1.82	1.81	1.82	1.76	43.1	1.60	3.29	3.22	3.23	3.26	43.1	1119	420
Mean	0.54	2.23	2.22	2.17	2.10	42.6	1.97	3.70	3.67	3.64	3.63	42.7		

The average values for the blood CO concentrations are plotted on the graphs in Figure 12.

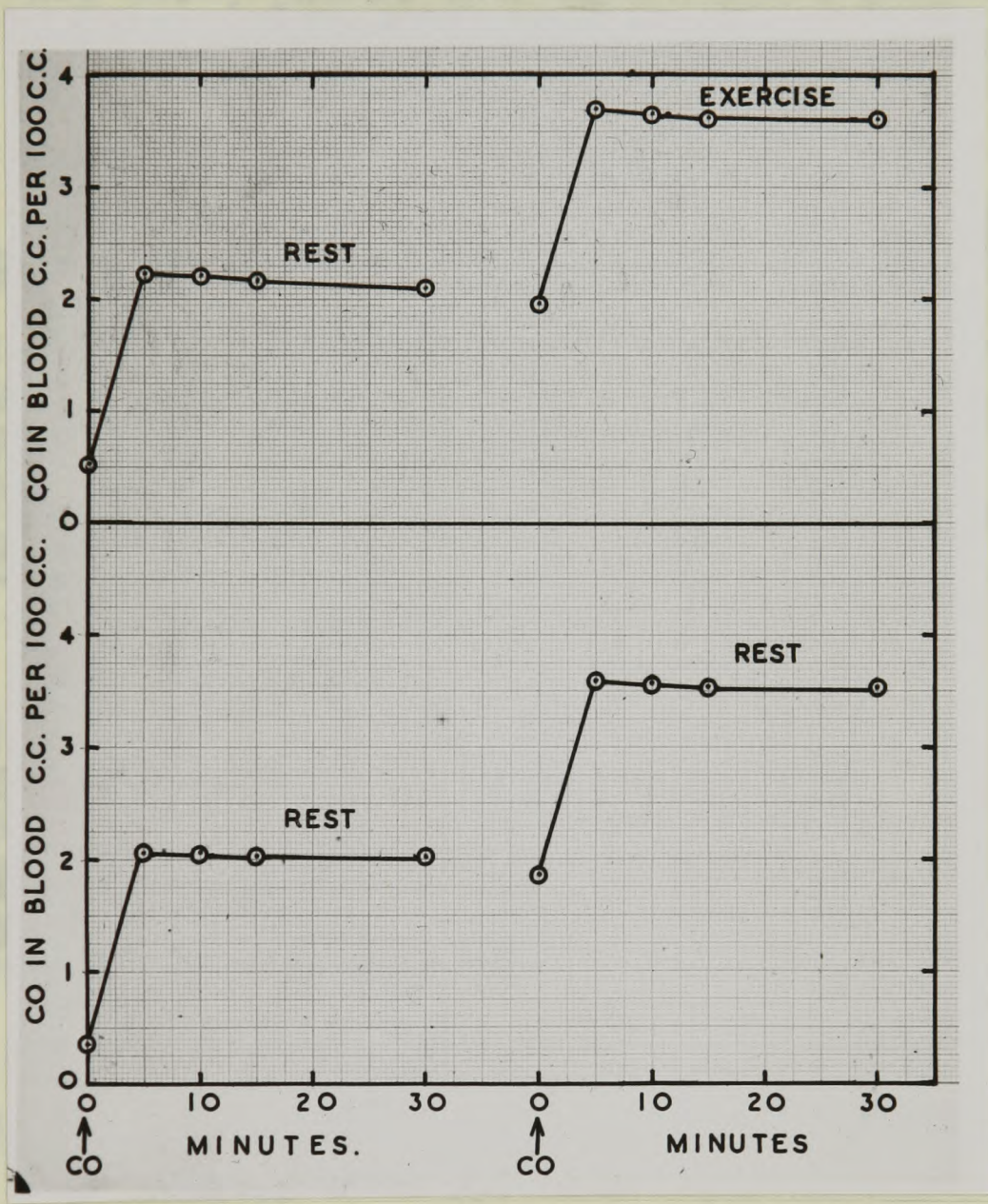


Figure 12. CO curves in human subjects followed for 30 minutes after a single and repeat dose of the same volume of CO. Bottom: subjects seated at rest throughout experiment. Top: subjects performing moderate exercise on bicycle ergometer during repeat administration.

The blood volume was calculated in each of the experiments tabulated above. The results appear in Table 8.

Table 8. Red cell, plasma and total blood volume in 4 human subjects by the CO method repeated at rest and during moderate muscular exercise.

	I Rest			II Rest			
Subject	Total	Cell	Plasma	Total	Cell	Plasma	Total II / Total I
F.C.C.	4710	1880	2830	4740	1910	2830	100.6
P.J.P.	4990	2040	2950	5080	2000	3080	101.8
R.W.G.	5690	2460	3230	5660	2420	3240	99.5
T.J.M.	5750	2320	3430	5880	2380	3500	102.3
Mean	5290	2180	3110	5340	2180	3160	101.0
	I Rest			II Exercise			
F.C.C.	4610	1910	2700	4550	1870	2680	98.7
P.J.P.	5330	2230	3100	5230	2200	3030	98.1
R.W.G.	5850	2570	3280	5670	2520	3150	96.9
T.J.M.	5520	2380	3140	5510	2370	3140	99.8
Mean	5330	2270	3060	5240	2240	3000	98.3

One purpose of these experiments was to determine the effect of higher blood CO concentrations on the disappearance curve. When a second dose of CO was given the concentration approached 4.0 vols.%, or 20% COHb. This was nearer the levels used in earlier investigations on the CO method. It might be expected that with the blood CO content and CO tension in the air and tissue fluids doubled the disappearance rate would be doubled. This would be true if the CO escaped from the closed-circuit -blood-body compartment into the external air by some leakage

in the breathing system, or if it were excreted by the skin according to some physical law. In both these cases the pressure gradient to the exterior would be doubled and the quantity of CO disappearing in 30 minutes should be doubled. In the experiments where the CO was repeated at rest the average values at the lower tension showed a disappearance of 0.06 vols.% in 25 minutes and at the higher tension also 0.06 vols.% in 25 minutes. These observations suggest that leakage of CO from the breathing circuit or excretion through the skin were not responsible for the disappearance of CO from the blood.

Returning again to myoglobin, it was proposed in the discussion of methods that since the dissociation curve of myoglobin with O_2 or CO is a rectangular hyperbola, this substance might be saturated with CO by the first administration, even at the low tensions used. The second dose of CO would then be distributed only in the blood haemoglobin, the blood CO concentration would be higher, and the blood volume calculated lower. The results in Table 8 demonstrated that the blood volume estimated from the second CO at rest did not vary by more than 1% from the original estimation. The question of degree of myoglobin saturation will receive further treatment under Discussion.

The results from these repeat determinations at rest estab-

lished that the CO method gives two almost identical values for blood volume in humans within a period of 30 minutes, and further, that these values are independent of the blood CO concentration used used. These experiments were used as a control for repeat determinations during muscular exercise.

The results of the four individual experiments where the CO was repeated during exercise on the bicycle ergometer are shown in Table 7. The mean values are plotted in Fig. 12. The curves during exercise revealed no greater disappearance of CO for a period of 30 minutes than corresponding curves at rest. They verified in this respect the results obtained where exercise was performed during a single CO administration (Table 5).

It was pointed out that failure of exercise to increase the rate of disappearance of CO from the circulating blood did not exclude the possibility that a considerable quantity of CO had diffused into the muscle myoglobin before the 5 minute blood sample was taken. There was every reason to expect that muscular exercise would promote this diffusion. With the degree of exercise performed in these experiments (420 Kgm.Meters/min.; 1100 c.c. O_2 consumption per min.) the cardiac output was increased to 3 times the resting value (see below). The blood supply to the active muscles must therefore have been increased by an even

greater amount. The subjects achieved a steady state of exercise before the CO was admitted so that the arterioles and capillaries in the active muscles must have been dilated at the time the blood CO concentration began to rise. Removal of some of the CO by the myoglobin in this 5 minute period would have left less CO distributed in the haemoglobin of the circulating blood. The blood volume would therefore have been calculated higher than the control value at rest.

The figures in Table 8 for blood volume repeated during exercise show that this was not the case. The values indicate, in fact, a slightly lower blood volume during the exercise period. From these experiments it may be concluded that muscular exercise does not have any great effect on the quantity of CO which disappears from the circulating blood. Any disappearance of CO in the first 5 minutes must occur in almost equal amounts at rest and exercise.

Haematocrit values listed in Table 7 show no significant change between rest and exercise. The red cell volume remained constant indicating that contraction of the spleen or liberation of red cells from extra-circulatory depots such as the liver and bone marrow, were not stimulated by this degree of exercise.

Although there was no significant difference in the quantity

of CO removed from the circulation during the first 5 minutes at rest and exercise, there was a difference in the pattern of the slow disappearance from 10 min. to 30 min. This was best illustrated by tabulating average values from the 25 experiments at rest and the 8 experiments during exercise.

	Time in minutes. CO in c.c. per 100 c.c.				
	0'	5'	10'	15'	30'
Rest (25 expts.)	0.69	2.39	2.38	2.34	2.30
		.01	.04	.04	
Exercise (8 expts.)	1.15	2.88	2.87	2.83	2.83
		.01	.04	0	

These showed that during exercise the CO concentration in the blood reached a constant level between 15 min. and 30 min., while at rest it continued to show a slight fall past 30 min. The degree of exercise performed increased the cardiac output and pulmonary ventilation to about 3 times the resting value. These factors would have speeded up the absorption, mixing and disappearance of CO, so that the slow fall in CO concentrations in the resting state would be expected to take place earlier during exercise. These observations were not easily reconciled with those just made that exercise does not affect the amount of CO which disappears from the circulating blood in the first few

minutes. However the discrepancy between these two sets of observations is very near the limit of accuracy of the analytical method. The experiments where the CO was repeated during exercise established that the quantity of CO which disappeared from the circulation in the first few minutes was certainly not of the order of 15%, a figure which has been suggested as the probable error of the CO method due to inclusion of myoglobin.

Respiratory and circulatory effects of moderate exercise

The respiratory exchange and cardiac output were determined on 3 subjects at rest and then at moderate exercise (420 Kgm. Meters per min.) on the bicycle ergometer. The results are set forth in Table 9.

Table 9. Respiratory and circulatory effects of moderate exercise.

Subject	State	Pulm. vent. ls./min. @ S.T.P.	CO_2 c.c./min.	O_2 c.c./min.	R.Q.	Pulse rate	Stroke Vol. c.c.	Cardiac output ls. per min.
F.C.C.	Rest	5.43	207	261	0.79	74	57	4.2
	Exercise	17.81	917	1127	0.81	104	117	12.2
R.W.G.	Rest	6.92	281	350	0.80	68	91	6.2
	Exercise	21.10	1186	1376	0.86	106	177	18.8
T.J.M.	Rest	6.42	263	341	0.77	73	63	4.61
	Exercise	23.4	1029	1272	0.81	90	188	16.95

These figures demonstrated that with the degree of exercise performed on the bicycle ergometer the pulmonary ventilation and cardiac output were increased to greater than 3 times the resting value. It has already been shown that the CO admitted to the circuit was 98% absorbed in the first 5 minutes with the subject at rest. During exercise the volume of CO laden air entering the lungs per minute was trebled; the volume of blood passing through the lungs per minute was trebled. It is a reasonable assumption that during exercise the CO must have been almost completely absorbed in 2 minutes. With the blood supply to the active muscles increased to a similar degree, disappearance of CO into myoglobin with exercise, if it occurred at all, would have taken place by the time the 5 minute blood sample was drawn.

Simultaneous determination of blood volume by CO and dye methods in humans.

The data from 16 experiments at rest and 4 experiments during muscular exercise in which the blood volume was estimated simultaneously by CO and T-1824 are presented in Table 10. It will be seen that there is very close agreement between results of the two methods. Of these 20 comparisons, 16 show agreement within 5% and the remaining 4 within 10%. This degree of discrepancy was to be expected from the possible errors in the

Table 10. Blood volume on humans. Summary of data by CO and dye methods simultaneously.

Subject	Date	Age	Weight Kgms.	Haemoglobin % (Haldane)	Haematocrit observed x 0.96	CO Method: Plasma Volume c.c.	CO Method: Plasma volume c.c./Kgm.	CO Method: Cell volume	CO Method: Cell volume c.c./Kgm.	CO Method: Total Blood Volume c.c.	CO Method: Total Blood Volume c.c./Kgm.	Dye Method: Plasma Volume	Dye Method: Plasma Volume c.c./Kgm.	Dye Method: Cell Volume c.c.	Dye Method: Cell Volume c.c./Kgm.	Dye Method: Total Blood Volume c.c.	Dye Method: Total Blood Volume c.c./Kgm.	CO Blood Volume / Dye Blood Volume
Rest.																		
F.C.C.	17.3.47	36	66.1	109	40.6	2900	43.9	1980	30.0	4880	73.9	2740	41.5	1870	28.3	4610	69.8	1.06
F.C.C.	16.4.47	36	66.6	103	40.9	2760	41.4	1910	28.7	4670	70.1	2650	39.8	1830	27.5	4480	67.3	1.04
R.W.G.	18.3.47	24	82.2	109	43.4	3460	42.1	2650	32.2	6110	74.3	3570	43.4	2750	33.5	6320	76.9	0.97
R.W.G.	21.4.47	24	80.7	108	44.0	3070	38.0	2400	29.7	5470	67.7	3150	39.0	2480	30.7	5630	69.7	0.97
R.W.G.	21.4.47	24	80.7	108	44.0	3330	41.3	2620	32.5	5950	73.8	3290	40.8	2590	32.1	5880	72.9	1.01
T.J.M.	19.3.47	30	86.8	100	43.4	3420	39.4	2620	30.2	6040	69.6	3290	37.9	2520	29.0	5810	66.9	1.04
M.V.B.	7.5.47	20	64.8	91	40.5	2840	43.8	1960	30.2	4800	74.0	2940	45.4	2000	30.9	4940	76.3	0.97
G.R.	8.5.47	21	59.2	102	46.1	2310	39.0	1970	33.3	4280	72.3	2300	38.9	1970	33.3	4270	72.2	1.00
W.M.J.	9.5.47	20	62.3	95	43.1	2840	45.6	2150	34.5	4990	80.1	2580	41.4	1950	31.3	4530	72.7	1.10
P.J.R.P.	14.5.47	20	64.2	104	45.7	2840	44.2	2390	37.2	5230	81.4	2770	43.1	2330	36.3	5100	79.4	1.03
C.G.M.	15.5.47	20	67.7	105	44.2	2880	42.5	2280	33.7	5160	76.2	2690	39.7	2130	31.5	4820	71.2	1.07
W.W.	16.5.47	25	88.2	97	42.8	3330	37.8	2490	28.2	5820	66.0	3420	38.8	2560	29.0	5980	67.8	0.97
R.G.T.	21.5.47	20	70.6	114	47.2	2870	40.7	2560	36.3	5430	77.0	2910	41.2	2600	36.8	5510	78.0	0.99
H.	22.5.47	21	63.6	111	45.1	2570	40.4	2120	33.3	4690	73.7	2390	37.6	1960	30.8	4350	68.4	1.08
J.M.L.G.	28.5.47	22	60.5	109	47.2	2710	44.8	2420	40.0	5130	84.4	2490	41.2	2230	36.9	4720	78.0	1.09
J.M.L.G.	28.5.47	22	60.5	109	47.4	2580	42.6	2310	38.2	4890	80.8	2480	41.0	2230	36.9	4710	77.9	1.04
																	Mean	1.03
Exercise.																		
F.C.C.	3.6.47	36	66.4	103	40.8	2840	42.8	1960	29.5	4800	72.3	2720	41.0	1880	28.3	4600	69.3	1.04
R.W.G.	5.6.47	24	81.5	108	47.1	2890	35.5	2570	31.5	5460	67.0	2810	34.5	2500	30.7	5310	65.2	1.03
W.J.S.	13.6.47	28	61.4	-	45.5	2380	38.8	1980	32.3	4360	71.1	2170	35.3	1810	29.5	3980	64.8	1.10
T.J.M.	18.6.47	30	86.8	102	44.3	3090	35.6	2460	28.3	5550	63.9	3100	35.7	2470	28.5	5570	64.2	1.00
																	Mean	1.04

analytical methods used. Thus in the CO in blood estimations the blank CO content and the final CO content might have each been incorrect by 0.03 vols.%, making a total of 0.06 vols.% error in the final value of approximately 1.70 vols.% used in the calculation; i.e. 3.5%. The error of the dye in plasma determination was shown to be of the order of 3%. Disregarding physiological factors which will be discussed later, a discrepancy of 6.5% in the comparison of the two methods if their errors were opposite in sign could be explained by these analyses alone.

The average ratio of CO blood volume/Dye blood volume in the 16 observations at rest was 1.03.

The average plasma, red cell and total blood volumes of the twelve individuals are given below.

<u>Mean of 12 subjects at rest</u>

Wt. 69.6 Kgms.

CO	(Plasma	- 2903	c.c.	= 41.7	c.c.	per	Kgm.
	(Cell	- 2297	"	= 33.0	"	"	"
	(Total	- 5200	"	= 74.7	"	"	"
T-1824	(Plasma	- 2818	"	= 40.5	"	"	"
	(Cell	- 2226	"	= 32.0	"	"	"
	(Total	- 5043	"	= 72.5	"	"	"

In two subjects the blood volume estimation was done simultaneously by the CO and dye methods and then repeated by both methods 30 minutes later. Thus, 4 determinations of blood volume were

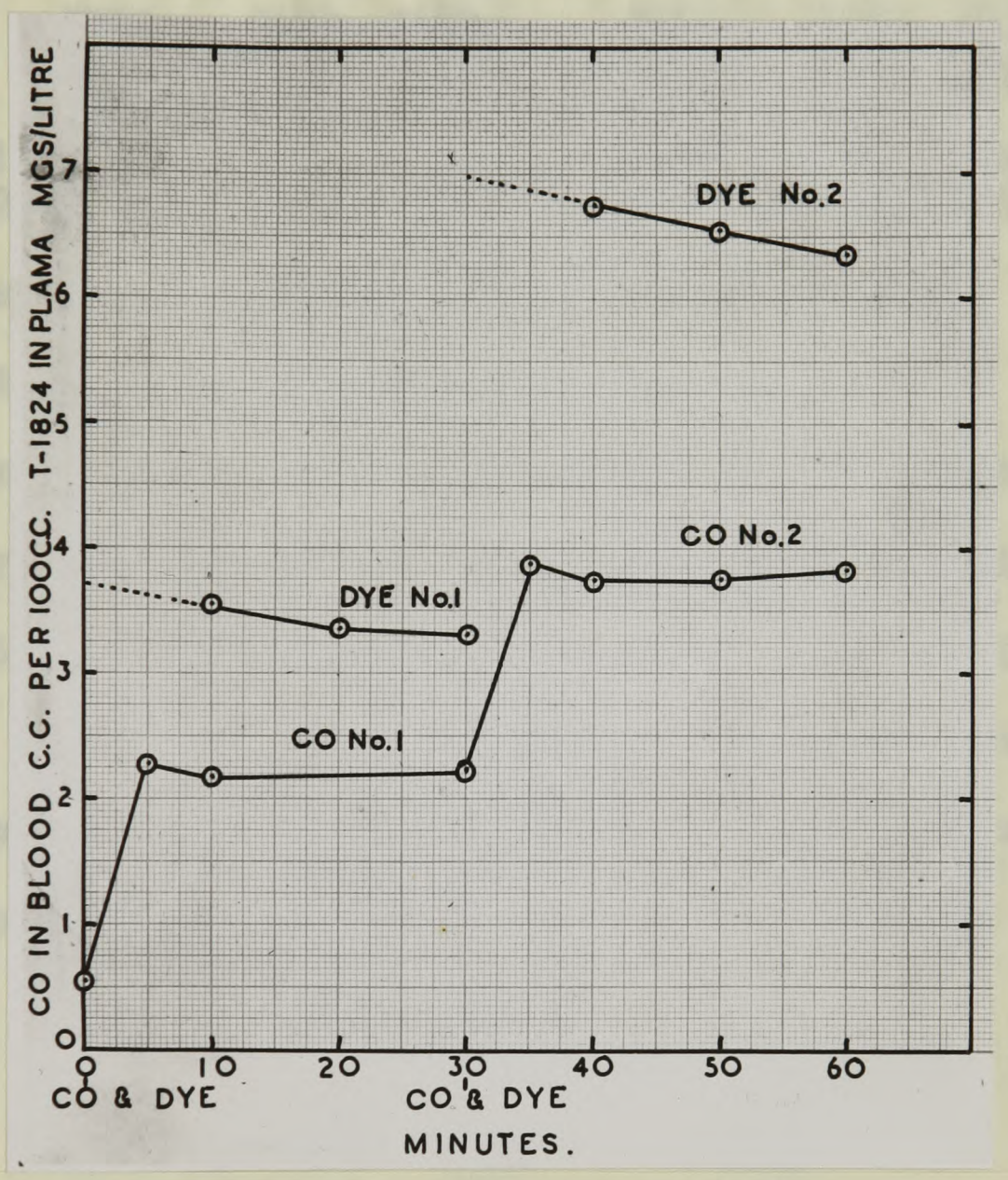


Figure 13. CO concentration in blood and T-1824 concentration in plasma of human subject after simultaneous administration of CO and dye at 0 and 30 minutes.

recorded in one subject at rest within a period of one hour.

		CO			T-1824		
Subject		Plasma	Cell	Total	Plasma	Cell	Total
R.W.G.	(1)	3070	2400	5470	3150	2480	5630
	(2)	3330	2620	5950	3290	2590	5880
J.M.L.G.	(1)	2710	2420	5130	2490	2230	4720
	(2)	2580	2310	4890	2480	2230	4710

The results agreed within the error of the methods. Data from the subject R.W.G. are presented in Fig. 13.

In some subjects, during the course of these experiments, the blood volume at rest was estimated on several different days over a period of a few months. The values obtained for F.C.C., R.W.G. and T.J.M. are presented below. These estimations were done by the CO method usually at about 10 A.M. at normal room temperature with the subject seated at rest in a deck chair.

Subject	Date	Hct.	Plasma Volume c.c.	Cell Volume c.c.	Total blood Volume c.c.
R.W.G.	28.2.47	44.8	3110	2530	5640
	18.3.47	43.4	3460	2650	6110
	7.3.47	44.8	3240	2640	5880
	21.4.47	44.0	3330	2620	5950
	6.10.47	44.9	3100	2530	5630
	16.10.47	43.3	3230	2460	5690
	23.10.47	43.9	3280	2570	5850

Subject	Date	Hct.	Plasma Volume c.c.	Cell Volume c.c.	Total blood Volume c.c.
F.C.C.	27.2.47	40.8	2750	1890	4640
	17.3.47	40.6	2900	1980	4880
	16.4.47	40.9	2760	1910	4670
	8.10.47	39.9	2830	1880	4710
	21.10.47	41.4	2700	1910	4610
T.J.M.	19.3.47	43.4	3420	2620	6040
	18.10.47	40.4	3430	2320	5750
	29.10.47	43.1	3140	2380	5520

The repeated estimations on R.W.G. and F.C.C. over a period of 8 months revealed constant values for the red cell volume. This did not vary by more than 100 c.c. from the mean figures in these two subjects. In the case of T.J.M. the first value was 300 c.c. greater than the two others done 7 months later. It would be expected that on an adequate diet, with no history of haemorrhage, transfusion or sojourn at a lower barometric pressure, the red cell volume of healthy subjects should remain constant. Only slight changes were observed in plasma volume and haematocrit.

Results: II Rabbits.

CO uptake and disappearance curves

The blood CO content was determined before and at intervals after the administration of approximately 3.00 c.c. pure CO @ S.T.P. to the closed circuit. Seven experiments on nembutalized

rabbits using the circuit with mixing chamber included appear below in Table 11.

Table 11. Uptake and disappearance of CO; nembutalized rabbit rabbits.

Date	CO admitted to circuit	Time in minutes. CO in blood c.c. per 100 c.c.							
		0'	3'	6'	9'	15'	30'	45'	60'
21.11.46	3.55 c.c.	0	1.52	2.05	2.33	2.24	2.02	1.96	1.89
27.11.46	2.59 "	0	1.63	1.95	2.22	2.23	2.13	2.00	1.97
3.12.46	1.59 "	0	0.94	1.11	1.26	1.26	1.06	1.05	0.93
21.1.47	4.11 "	0	1.52	2.38	2.94	3.05	2.87	2.70	2.51
25.1.47	3.41 "	0	1.34	1.94	2.23	2.35	2.19	2.07	2.10
29.1.47	3.22 "	0	1.99	2.62	2.75	2.98	2.86	2.82	2.62
30.1.47	2.19 "	0	1.13	1.44	1.52	1.35	1.33	1.26	1.20
Mean	2.94	0	1.44	1.93	2.18	2.21	2.07	1.98	1.89

The mean values from these 7 experiments are plotted on the graph in Fig. 14. The initial blood sample in rabbits contained no CO except in cases where the animal had been used recently for a blood volume determination. In these experiments the blood CO concentration showed a slow rise through the 3 min. and 6 min. samples to a maximum at 9 min. or 15 min. From 15 min. to 1 hour there was a slow fall which amounted to 14.3% of the maximum value taking average figures from all experiments.

It was shown in the human CO curves that the slow fall in CO concentration was not due to haemodilution. In rabbits the repeated removal of 1.5 c.c. samples of blood was not without

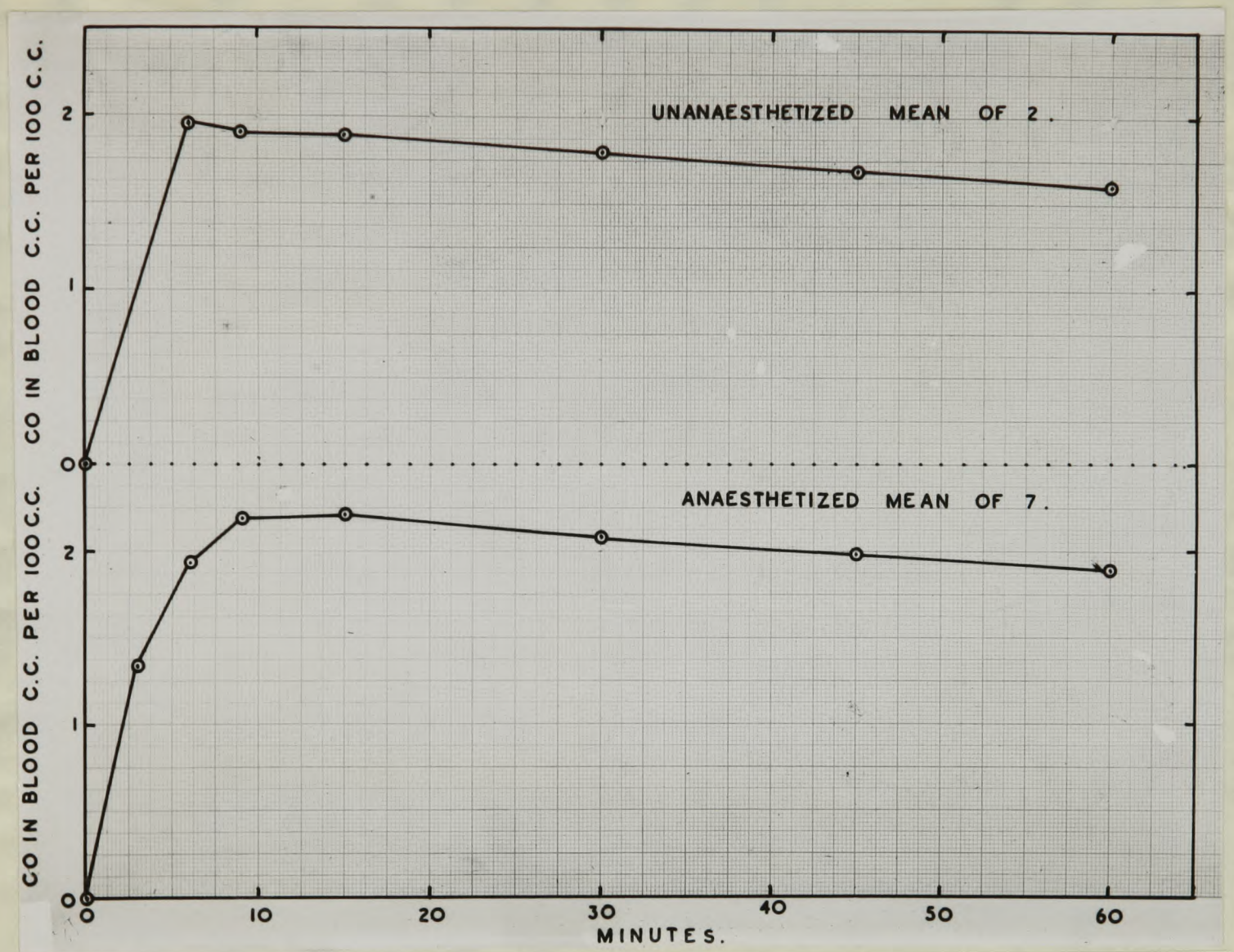


Figure 14. CO uptake and disappearance curves in rabbits breathing from the closed circuit for one hour after the administration of CO.

effect on the blood volume. This was demonstrated in another series of experiments on nembutalized rabbits where 1.5 samples of blood were removed from an ear vein at almost identical time intervals: 0, 2, 4, 6, 9, 15, 30, 45 and 60 min. Duplicate haematocrit determinations were done on the heparinized blood samples taken at 0, 15, 30, 45 and 60 minutes. Average results of the 5 experiments were:

	Time of sample in minutes				
	0'	15'	30'	45'	60'
Haematocrit	33.7	33.4	32.7	32.7	32.3

The haematocrit at 60 min. was 3.3% lower than the haematocrit at 15 min. Thus haemodilution had occurred during the course of the experiment. A similar degree of haemodilution during the study of the CO disappearance curves would have accounted for 3.3% of the observed fall of 14.3% from 15 min. to 1 hour.

The uptake and disappearance curve of CO in blood was then studied in unaesthetized animals with the mixing chamber removed from the circuit. The values from two experiments where the CO content of the blood was followed for 60 min. are given in Table 12 and mean values are plotted in Fig. 14.

Table 12. Uptake and disappearance of CO: unanaesthetized rabbits.

	CO admitted to circuit	Time in minutes. CO in blood c.c. per 100 c.c. 0'	6'	9'	15'	30'	45'	60'
	3.08 c.c.	0	1.81	1.74	1.80	1.77	1.68	1.62
	2.73 c.c.	0	2.09	2.06	1.97	1.83	1.67	1.58
Mean	2.91	0	1.95	1.90	1.89	1.80	1.68	1.60

These curves and others done in the course of blood volume estimations where the CO levels were followed for 15 minutes showed that the increased pulmonary ventilation of the unanaesthetized animals and the reduction in the dead space of the circuit caused the CO to be absorbed much more quickly. Maximum CO levels were usually attained at 6 min. or 9 min. and in the blood volume calculations the highest CO in blood value was used. These curves also showed a continuous disappearance of CO from the blood during the 1 hour rebreathing period. See Fig. 14.

The haemodilution resulting from removal of serial blood samples observed in nembutalized rabbits was even more pronounced in unanaesthetized rabbits. In the second experiment appearing in Table 12 haematocrit values at 0, 30 and 60 minutes were 31.0, 26.8, and 25.7% respectively. This 17% reduction in the haematocrit in the course of 1 hour was still not sufficient to account for the 25% reduction in the CO concentration in the blood observed in this experiment.

The most probable reason for the slow but continuous fall in CO blood concentrations observed in anaesthetized and unaesthetized rabbits was thought to be a leak in the mask. This was investigated in several ways. With the mask in position on the animal's head the tube leading to the breathing circuit was clamped off. The inspiratory efforts of the animal were sufficient to collapse the rubber in the region of the small dead space surrounding the nostrils despite the fact that in this position the fabric was re-inforced by inflexible bars of hard rubber tubing. Left in this state for several minutes the rabbit soon became asphyxiated. More important was a test for an expiratory leak. The mask was put on with a flexible rubber tube about 6 inches in length leading to the breathing circuit. The animal's head was then immersed in a water bath and breathing was continued for 10 minutes. No water was aspirated into the mask during inspiration. No bubbles of air appeared anywhere about the mask, head or neck during expiration. The possible sites of leakage were held just under the surface of the water in order that not more than 1 cm. of water pressure resisted the escape of air. The expiratory pressure within the lungs and mask was increased by placing a heavy rubber stopper on the spirometer which the animal had to raise with each expiratory effort. This did not cause the

appearance of any bubbles.

Despite these observations it was felt that no general conclusions regarding the rate of disappearance of CO from rabbits' blood were warranted. There was no positive evidence that during the determination of individual CO disappearance curves, a very slight expiratory leak had not occurred over the course of 1 hour. Such a leak would have affected the results in this work only if it had caused the escape of a significant quantity of CO in the first few minutes prior to the taking of the blood sample from which the blood volume was calculated. In two experiments the blood volume of a rabbit was estimated at one time using the mask and at another time with an intra-tracheal cannula tied tightly in place to eliminate possible leakage about the mask. The results are given below.

Rabbit (1) 29.1.47 with mask; blood volume = 108 c.c.
31.1.47 with tracheal cannula blood volume = 109 c.c.

Rabbit (2) 20.1.47 with mask; blood volume = 102 c.c.
19.2.47 with tracheal cannula blood volume = 104 c.c.

Measurement of CO remaining in closed circuit

In these experiments the usual 3.00 c.c. of pure CO were admitted to the breathing circuit and 10 minutes later an air sample was taken for CO analysis. The removal of the 80-100 c.c. sample

emptied the spirometer. It was quickly refilled with air and oxygen and the equilibrium between CO in the air of the circuit and the blood was allowed to be re-established. In some experiments a second air sample was taken at 30 minutes. Blood samples were drawn at the same time as the air samples for determination of CO content.

The results are presented in Table 13.

Table 13. CO and O_2 content of air in breathing circuit at equilibrium.

	CO%	O_2%
(10'	0.0558	79.1
(30'	0.0492	89.0
(10'	0.0581	75.4
(10'	0.0274	55.8
(30'	0.0384	62.0
(30'	0.0420	90.2
(10'	0.0467	62.1
(10'	0.0269	53.3
(30'	0.0306	77.6
Mean	0.0417	

There was considerable variation in the determined CO percentage between individual rabbits and even in the same rabbit at 10 min. and 30 min. This CO percentage was modified by several factors. In several cases the O_2 tension was significantly higher at 30

min. In all cases the blood CO content was slightly lower at 30 min. than at 10 min. According to the equation $pCO = \frac{HbCO}{HbO_2} \times \frac{pO_2}{K}$, the CO tension at equilibrium would be expected to vary as the other values contributing to the equilibrium varied.

In order to determine the correction factor to be applied to the blood volume calculations, average values from these experiments were used. The average CO percentage was 0.0417%. The volume of the breathing circuit with the spirometer half-filled, the lungs, and the dead space of the mask and tubing totalled 230 c.c. The quantity of CO unabsorbed $= \frac{0.0417}{100} \times 230$ $= 0.0959 = 0.10$ c.c. The usual volume of CO admitted was approximately 3.00 c.c. Therefore, the unabsorbed CO would be: $\frac{0.10}{3.00} \times 100 = 3.3\%$ of the CO admitted. A correction factor of of 0.967 was applied to the blood volume calculations.

This figure was obtained from average values. In experiments where the O_2 tension, HbCO saturation and volume of CO admitted to the circuit were different from these average figures, this factor would not be strictly correct. However, the most extreme variations observed in the course of this work would not have affected the final figure for the blood volume by more than

1% of itself.

The approximate value for K was calculated from the data of these experiments according to the equation $K = \frac{HbCO}{HbO_2} \times \frac{pO_2}{pCO}$ considering the equilibrium to have existed between the alveolar air and arterial blood. Partial pressures of CO and O_2 in the alveoli were estimated from their percentages in the air sample taken from the closed circuit with allowance for CO_2 and vapour pressure, and assuming a barometric pressure of 760 mm. Kg. The O_2 capacity of the blood was determined using a Haldane haemoglobinometer on which 100% = 19.5 vols.% O_2 capacity. HbCO was measured by the syringe capillary apparatus. HbO_2 in arterial blood at these high oxygen tensions was assumed to be 100 less COHb. These values and the calculated figure for K are presented in Table 14 below.

Table 14. Value for K in arterial blood of rabbits.

CO		O_2				HbCO			
%	mm.Hg.	%	mm.Hg.	Hb%	O_2 Cap.	Vols. %	% saturation	HbO_2 %	K
0.0558	0.398	79.1	514	72	14.0	2.16	15.4	84.6	235
0.0492	0.351	89.0	585	72	14.0	1.95	13.9	86.1	269
0.0581	0.414	75.4	488	78	15.2	2.04	13.6	86.4	186
0.0274	0.195	55.8	348	63	12.3	1.47	12.0	88.0	243
0.0384	0.274	62.0	392	63	12.3	1.38	11.2	88.8	180
0.0420	0.300	90.2	593	77	15.0	1.94	12.9	87.1	293
0.0269	0.192	53.3	330	76	14.8	2.05	13.9	86.1	278
0.0306	0.218	77.6	503	76	14.8	1.86	12.6	87.4	333

These values for K are considerably higher than those reported in the literature (Killick 1940). These were very crude experiments and would not suffer comparison with controlled in vitro procedures. In the calculations the assumption has been made that the CO tension of the air in the circuit is equal to the CO tension in arterial blood. This assumption disregards the effect of blood at the venous end of the lung capillary where the O_2 tension must have fallen from 300-500 mm.Hg. in arterial blood to 40-60 mm.Hg. in venous blood. The fall in O_2 tension would have resulted in a corresponding fall in CO tension. The figure for CO tension in the closed circuit would therefore represent the combined effect of the tension in arterial and venous blood; and the CO tension in arterial blood would have been higher than the value used in these calculations. This effect, the magnitude of which is unknown, would have lowered the K values. The results are included here for the purpose of completeness and to show that despite wide variations in O_2 tension in the circuit the equilibrium tension of CO has been maintained at approximately the same level.

Blood volume by CO method repeated.

In 3 experiments the administration of CO was repeated at 30 minutes with the rabbit continuing to breathe from the closed

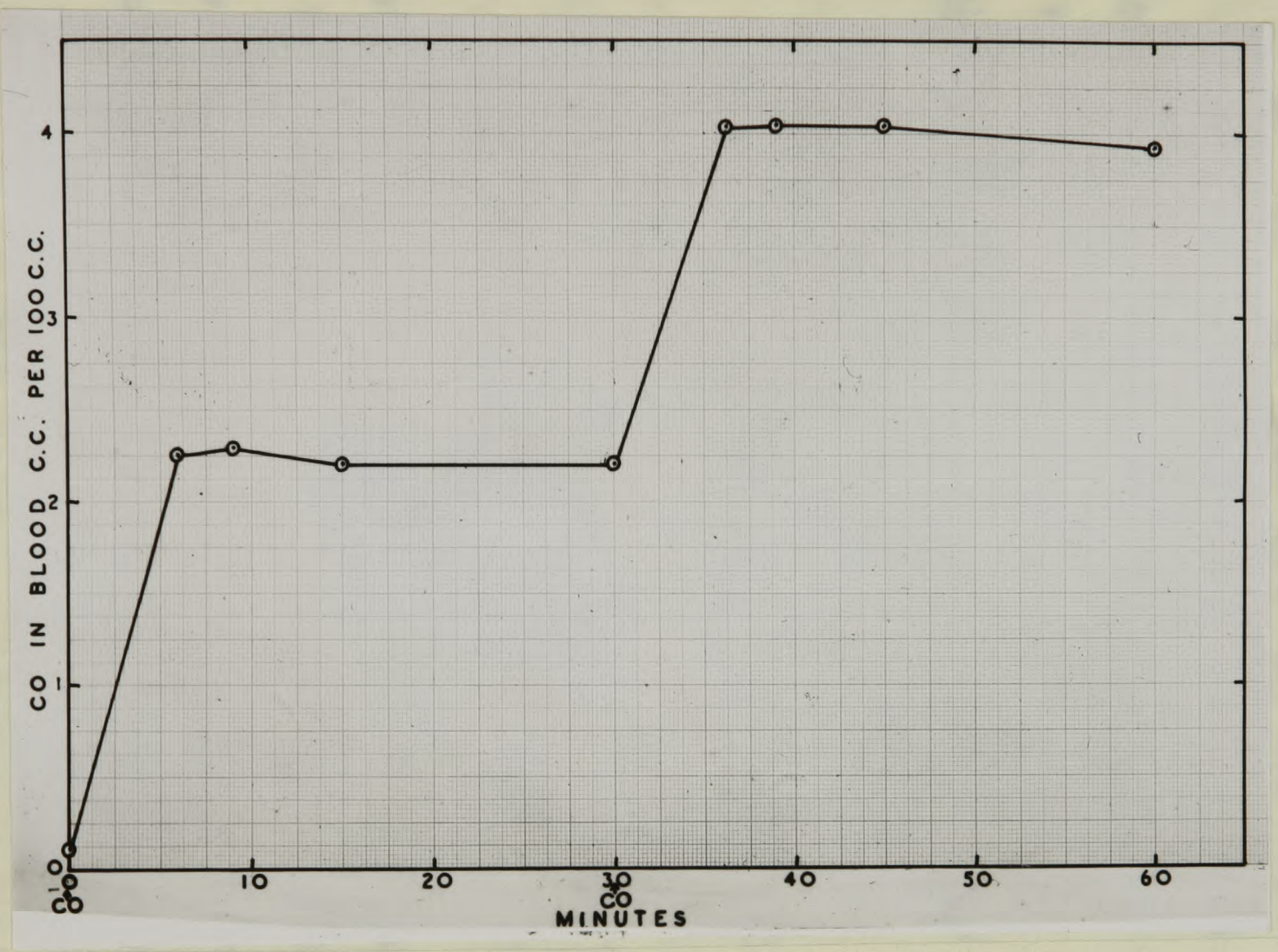


Figure 15. CO concentration in blood of rabbit continuing to breathe from closed circuit for one hour with CO admitted at 0 and 30 minutes.

circuit. The results of one experiment are presented below and in Fig. 15.

Time in minutes. CO in c.c. per 100 c.c.

0'	6'	9'	15'	30'	36'	39'	45'	60'
0.12	2.25	2.29	2.20	2.20	4.03	4.04	4.04	3.94

The blood volume values from these 3 experiments are given in Table 15.

Table 15. Blood volume of rabbits in c.c. by CO method repeated at 30 minutes.

	I			II		
Expt.	Total	Cell	Plasma	Total	Cell	Plasma
(1)	194	54	140	191	45	146
(2)	147	61	86	157	65	92
(3)	160	65	95	162	60	102

In another 3 experiments the blood volume of a rabbit was estimated by the CO method in the morning and again 3-4 hours later in the afternoon of the same day. In the interval the animal breathed off most of the CO which had been absorbed during the first determination. This type of experiment was important from the standpoint of providing a control blood volume determination from which subsequent changes could be observed. It showed the accuracy with which the estimation could be repeated

in a period of several hours. Protocol and results of a typical experiment will be given in detail below.

Rabbit: Chinchilla; male; Wt. 3.2 Kgms.

<u>9.30 A.M.</u> 3.00 c.c. CO Temp. 15.40) Bar. 761) Purity CO 98.4%) = 2.75 c.c. CO pure @ S.T.P.

CO in blood analyses.

0 min.	- 0.09	vols.	%
9 "	- 1.97	"	"
15 "	- 1.94	"	"

Haematocrit: 30.0) 30.2) 30.1

Total Blood Volume = 141 c.c.
Cell " = 40.8 "
Plasma " =100.2 "

<u>2.30 P.M.</u> 3.12 c.c. CO Temp. 19.6) Bar. 761) Purity 98.4%) = 2.80 c.c. CO pure @ S.T.P.

CO in blood analyses.

0 min.	- 0.20	vols.	%
9 "	- 2.11	"	"
15 "	- 2.11	"	"

Haematocrit 28.4) 28.5) 28.5

Total Blood Volume = 142
Cell " = 39.0
Plasma " =103

The CO in blood concentrations are plotted on the graph in Fig. 16. The results of two other similar experiments were:

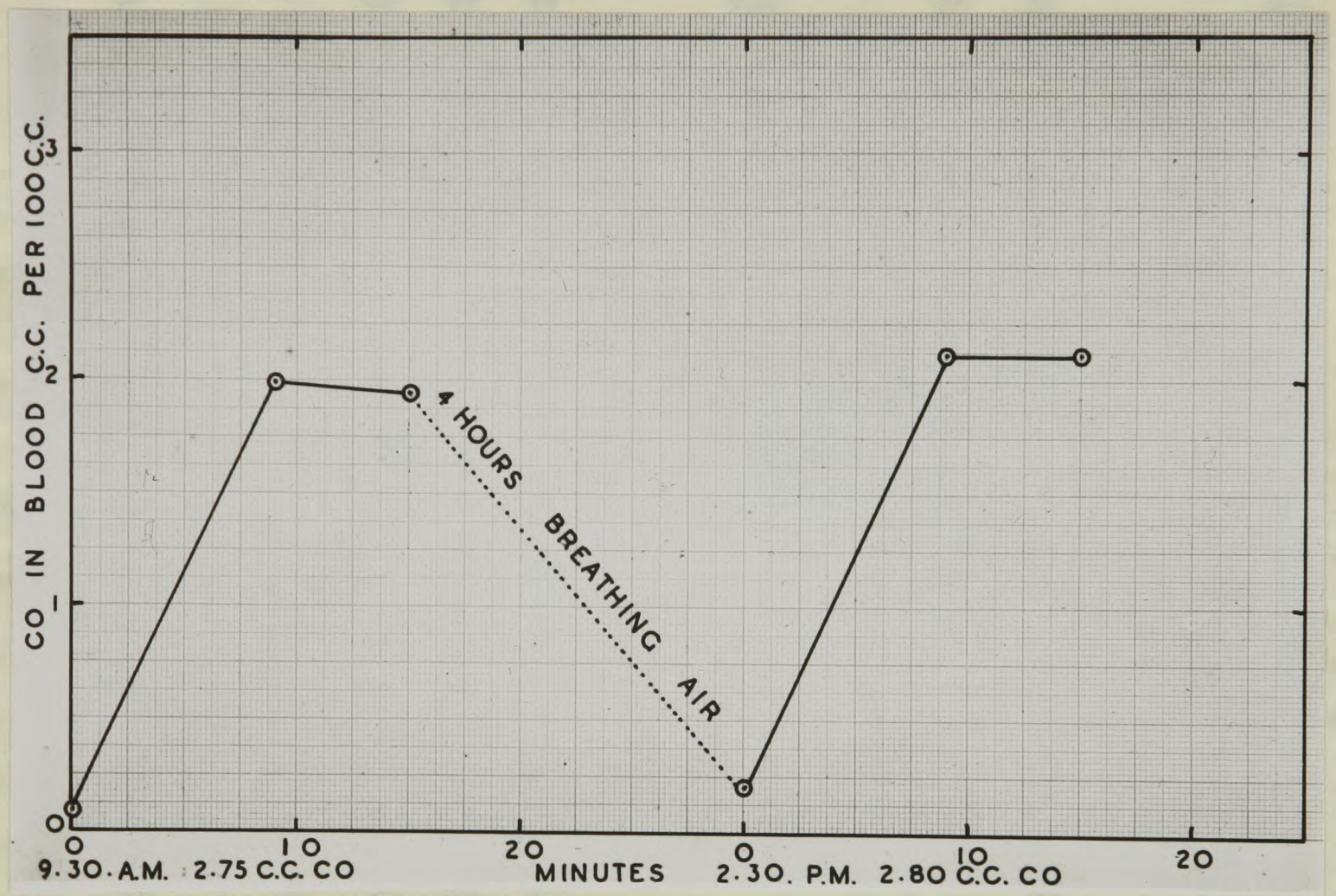


Figure 16. CO concentration in blood of rabbit during repeat estimation of blood volume in the morning and afternoon of the same day.

Rabbit. Black; male; Wt. 2.75 Kgms.

11.0 A.M.	Haematocrit 37-8%	
	Total Volume	= 136 c.c.
	Cell "	= 51 c.c.
	Plasma "	= 85 c.c.
4.00 P.M.	Haematocrit 34.3	
	Total Volume	= 148 c.c.
	Cell "	= 51 c.c.
	Plasma "	= 97 c.c.

Rabbit. Chinchilla; male (No.2); Wt. 3.0 Kgms.

9.45 A.M.	Haematocrit 37.0%	
	Total Volume	= 168 c.c.
	Cell "	= 62.3 c.c.
	Plasma "	= 105.7 c.c.
2.30 P.M.	Haematocrit 33.4%	
	Total Volume	= 174 c.c.
	Cell "	= 58.1 c.c.
	Plasma "	=115.9 c.c.

These results showed that slight haemodilution had occurred during the interval; the haematocrit values were lower at the second determination. This was probably a response to the removal of the blood samples. The red cell volume remained the same or several cubic centimeters lower than the initial level. Lower values were anticipated because 1-3 c.c. of red cells were removed during the first determination. On the whole, these repeat experiments showed very close agreement; there was no discrepancy exceeding 4% of the expected value.

Study of dye disappearance curve in the rabbit and repeat blood volume estimations by the dye method.

Injections of the usual amount of T-1824 (1.1816 mgs.) were made into the marginal vein of the ear of nembutalized rabbits. Blood samples (1.5-2.0 c.c.) were drawn before, and at 2, 4, 6, 9, 15, 30, 45 and 60 minutes after the first injection. The same volume of dye solution was injected again as soon as the 60 minute sample had been taken. Further samples were withdrawn at 62, 64, 66, 69, 75 and 90 minutes. Dye concentration in plasma was determined for each sample; haemoglobin and haematocrit were measured in the 0, 15, 30, 45, 60 and 75 min. samples. The results of 5 experiments are given in Table 16. The mean values are plotted on the graph in Fig. 17.

The dye disappearance curve following the first injection shows the initial rapid fall in dye concentration during the first 10 minutes and the succeeding slow linear disappearance slope. The dotted line represents the method of extrapolation of the curve from 10 min. to obtain the theoretical dye concentration at 0 min. Haemoglobin and haematocrit determinations showed a slow parallel fall throughout the hour indicating slow dilution of the blood by tissue fluid to compensate for the removal of the blood samples. The total observed fall in dye concentration in

Table 16. Data from T-1824 disappearance curves in 5 rabbits with repeat dye injection at 60 minutes. Changes in Hb. and Hct. as repeated small samples of blood are withdrawn.

ime of ample inutes	I			II			III			IV			V			Mean		
	Dye	Hct	Hb	Dye	Hct	Hb	Dye	Hct	Hb	Dye	Hct	Hb	Dye	Hct	Hb	Dye	Hct	Hb
0	0	27.9	69.5	0	39.4	95	0	33.9	75	0	33.2	71	0	34.2	72.5	0	76.6	33.7
2	11.7	-	68	14.8	-	97	14.1	-	-	14.3	-	-	12.3	-	-	13.44	-	-
4	11.6	-	70	14.5	-	94	13.8	-	-	14.0	-	-	11.7	-	-	13.12	-	-
6	11.3	-	69	14.1	-	94	13.6	-	-	13.6	-	-	11.6	-	-	12.84	-	-
9	11.3	-	68	13.9	-	91	13.3	-	-	12.8	-	-	11.6	-	-	12.58	-	-
15	10.8	27.8	67.5	13.7	41.7	91.5	12.5	33.0	74	12.3	32.3	71	10.8	32.4	72	12.02	75.2	33.4
30	10.5	27.1	65.0	13.0	39.9	92	11.6	32.2	75	11.2	31.9	70	10.3	32.4	72	11.32	74.8	32.7
45	9.6	26.8	65.5	12.6	40.5	90	11.6	32.5	73	10.6	32.4	70	9.9	31.4	71	10.86	73.9	32.7
60	9.3	26.3	65	12.4	39.8	91	11.3	31.8	72	10.1	32.2	68	9.2	31.5	70	10.46	73.2	32.3
62	21.2	-	65.5	27.8	-	-	26.0	-	-	24.7	-	-	22.7	-	-	24.32	-	-
64	20.8	-	65	27.2	-	-	26.2	-	-	24.3	-	-	22.1	-	-	24.12	-	-
66	20.8	-	62	26.8	-	-	26.0	-	-	23.6	-	-	21.6	-	-	23.76	-	-
69	20.1	-	63	26.1	-	-	25.5	-	-	23.3	-	-	21.4	-	-	23.28	-	-
75	19.5	25.3	60	25.5	40.7	88.5	25.3	29.5	69	22.5	30.8	68.5	21.1	31.3	68.5	22.78	70.9	31.5
90	-	-	-	-	-	-	-	-	-	21.6	-	-	21.1	-	-	-	-	-

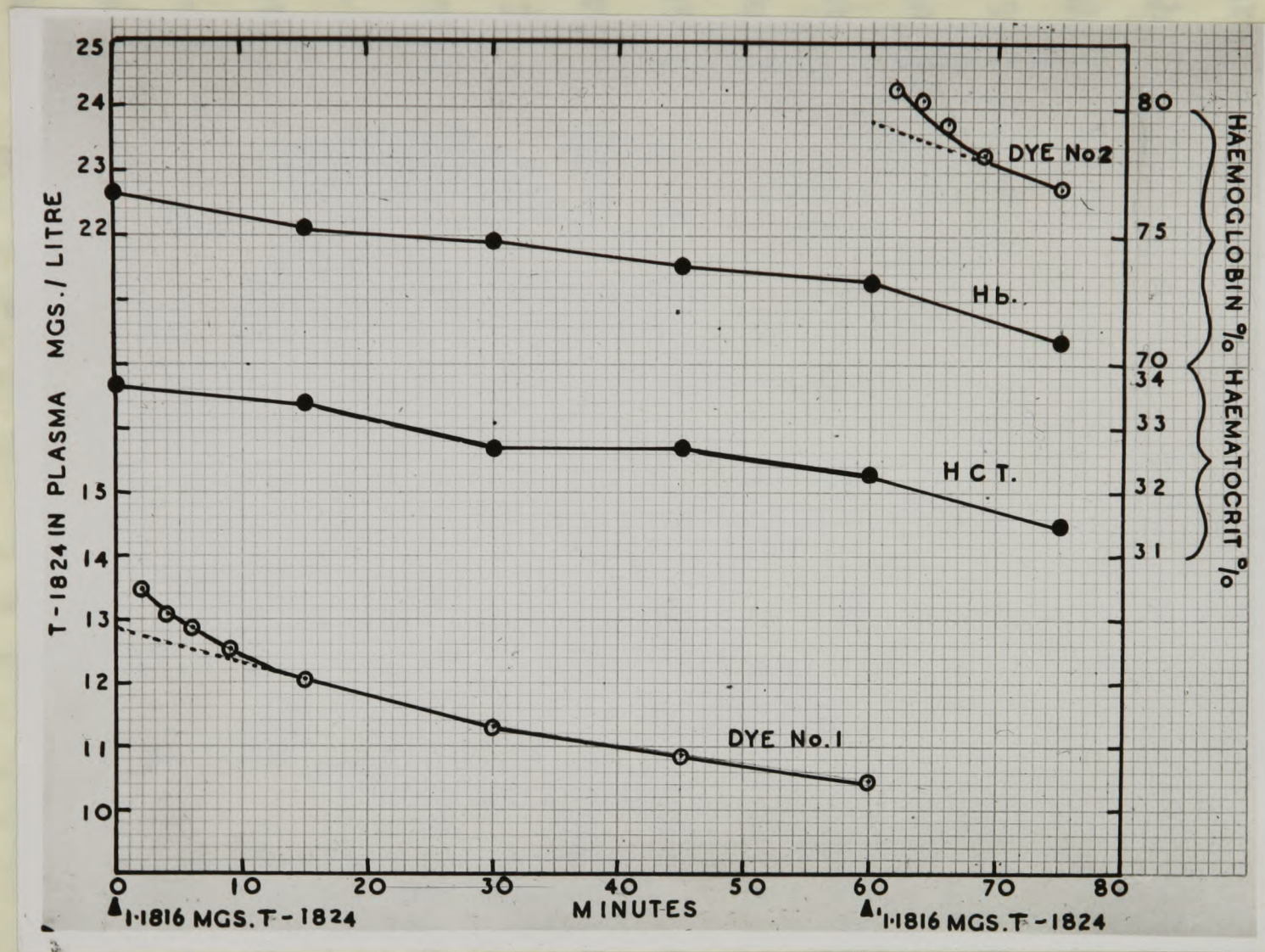


Figure 17. Disappearance curves of T-1824 in plasma of rabbits after single and repeat injection of dye with accompanying changes in haemoglobin and haematocrit resulting from removal of small serial blood samples. Average results from five experiments.

the 60 min. was 12.84 mgs. per litre to 10.46 mgs. per litre, i.e. 18.5%. The fall in haemoglobin for a corresponding time was 4.4% of the initial value; the fall in haematocrit 4.1%. The true disappearance rate of T-1824 from the circulating plasma was therefore approximately 14% per hour, allowing for 4% haemodilution.

The early part of the disappearance curve following the second injection of dye is shown as DYE NO. 2. in Fig. 17. It demonstrates the early rapid fall accupying the first 10 minutes followed by the slower disappearance slope. This effect was illustrated more clearly by Experiments IV and V where the dye concentration was followed to 90 min. The results from Experiment IV are plotted in Fig. 18.

These observations in the rabbit are not in agreement with those of Cruickshank who reported that in the cat the first injection of dye 'blocked' the reticulo-endothelial system so that after a second injection the disappearance curve was linear. He postulated that the initial rapid fall in dye concentration was due to 'gobbling' of T-1824 by the reticulo-endothelial cells. The results presented here suggest that the same circulatory factors responsible for the initial rapid fall after the first injection are operative after the second injection

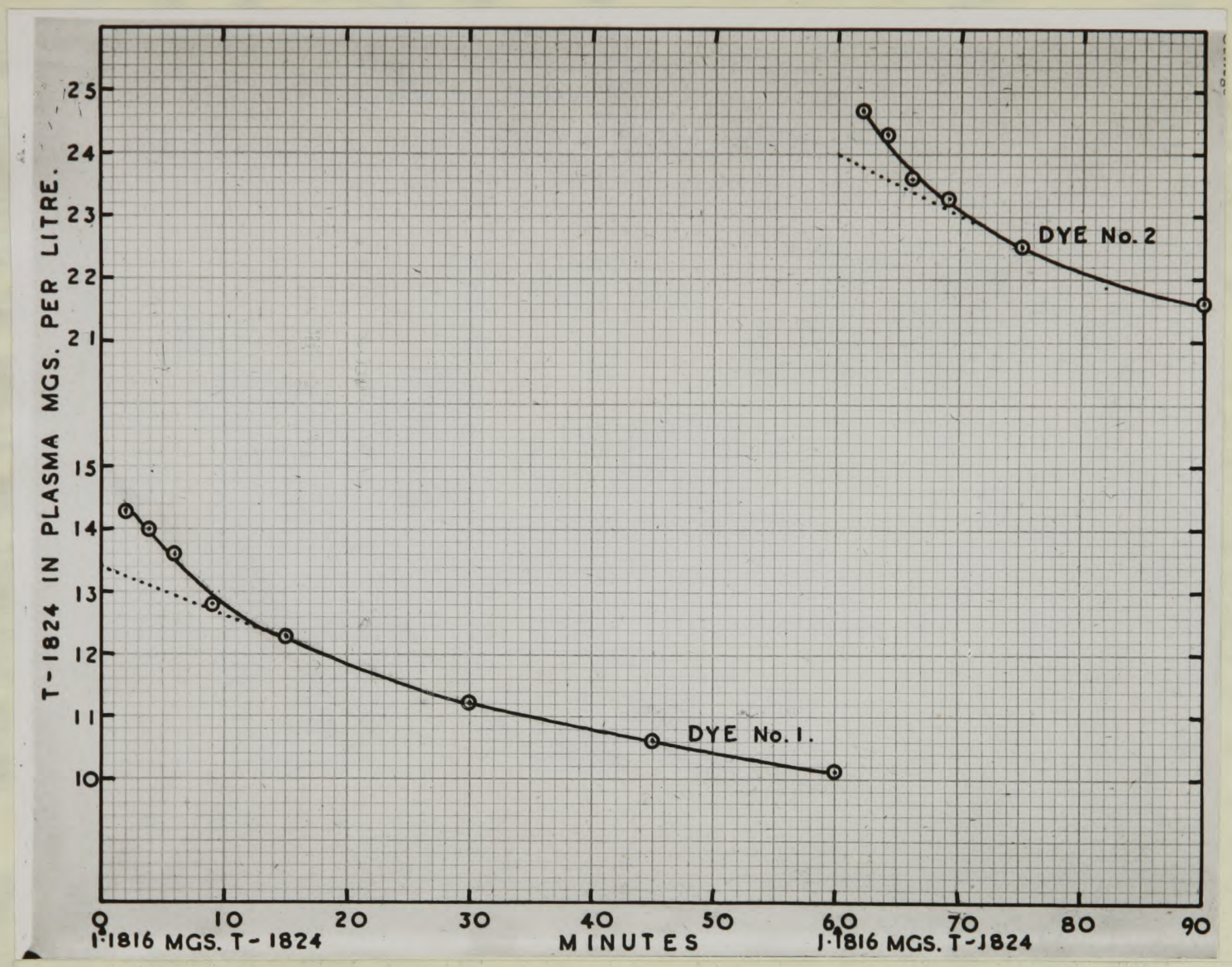


Figure 18. Disappearance curves of T-1824 in plasma of one rabbit followed for 60 minutes after the first dye injection and for 30 minutes after the second dye injection. The similarity in shape of the two curves is illustrated. The dotted lines show the method of extrapolation of the curves after mixing is complete.

The total red cell and plasma volumes in each of the 5 experiments where the dye injection was repeated have been calculated from the data in Table 16. The results are presented below in Table 17.

Table 17. Blood volume of rabbits by dye method repeated at 60 min.

	I				II			
Expt.	Hct. at 0 min.	Plasma Vol. c.c.	Cell Vol. c.c.	Total Vol. c.c.	Hct. at 60 min.	Plasma Vol. c.c.	Cell Vol. c.c.	Total Vol. c.c.
I	27.9	103	39	142	26.3	104	36	140
II	39.4	83	55	138	39.8	77	52	129
III	33.9	85	43	128	31.8	80	37	117
IV	33.2	88	44	132	32.2	85	40	125
V	34.2	102	53	155	31.5	95	44	139
Mean	33.7	92	47	139	32.3	88	42	130

In each of the experiments the blood volume calculated from the second dye injection was several c.c. lower than that calculated from the first. On the average it was 9 c.c. lower. This observation will bear further scrutiny. During the first 60 minutes a significant amount of blood was removed for the dye, haemoglobin and haematocrit determinations. In each experiment this was measured in small calibrated cups. The results were:

Experiment	Amount of blood removed in first hour
I	14 c.c.
II	12 c.c.
III	14 c.c.
IV	15 c.c.
V	15 c.c.
Mean	14 c.c.

If no replacement of fluid had occurred from the extravascular spaces, the blood volume should have been estimated 14 c.c. lower by the second dye injection and the haematocrit should have remained constant. The figures show that at the end of the hour the haematocrit and haemoglobin were 4% lower than the initial levels. This indicated that a volume of fluid equivalent to 4% of the original blood volume had been added to the circulation during the course of one hour. Using the average figures this would have been 4% of 139 c.c. = 5.5 c.c. That is, 14 c.c. of blood were removed and 5.5 c.c. fluid replaced to the moment of the second blood volume determination - a net reduction of 8.5 c.c. The average reduction measured by the second dye injection was 9.0 c.c.

Further confirmation of the accuracy of the repeat determination by the dye method was obtained by examining the red cell volume figures. In each experiment and on the average, a reduction in red cell volume of approximately 5.0 c.c. was

recorded by the repeat estimation. Since 14 c.c. of blood composed of ⅓ red cells had been removed in the first hour, a decrease in the red cell volume of 5 c.c. would be expected.

These results show that in the rabbit the blood volume determination by a repeat dye injection is valid. Cruikshank found that in the cat a second dye injection gave lower values for the blood volume. Failure to account for the amount of blood removed during the first estimation and the effects of haemodilution might cause erroneous interpretation in such small animals as the cat and rabbit.

Blood volume estimations on individual rabbits.

In Tables 18, 19 and 20 below, the results of blood volume estimations on 35 individual rabbits are given. Those in Table 18 were done by the CO method; those in Table 19 by the T-1824 method; and those in Table 20 by CO and T-1824 methods simultaneously. They are expressed as cell, plasma and total blood volume in absolute amounts and in c.c. per Kilogram of body weight.

The average total volume in c.c. per Kgm. by the CO method alone was 57.3; by the dye method 56.0. These were in different groups of animals and were not in large enough numbers to be statistically significant, although they suggest that the two

Table 18. Blood volume of individual rabbits by the CO method.

Date	Wt.	Hb	Hct.	Plasma vol. c.c.	Plasma vol. c.c./Kgm.	Cell vol. c.c.	Cell vol. c.c./Kgm.	Total Blood vol. c.c.	Total Blood vol. c.c./Kg.
20.11.46	2.50	-	30.0	91.0	36.4	39.0	15.6	130	52.0
21.11.46	2.80	-	24.1	112.3	40.2	35.7	12.7	148	52.9
22.11.46	2.45	-	27.8	93.9	38.3	36.1	14.7	130	53.0
27.11.46	1.70	-	21.4	88.0	51.8	24.0	14.1	112	65.9
28.11.46	1.95	-	21.8	95.4	48.9	26.6	13.6	122	62.5
29.11.46	2.20	-	24.7	90.4	41.1	29.6	13.5	120	54.6
3.12.46	2.20	-	27.2	88.8	40.4	33.2	15.1	122	55.5
17. 1.47	2.20	77	27.5	81.9	37.2	31.1	14.1	113	51.3
25. 1.47	1.90	62	19.5	112.7	59.3	27.3	14.4	140	73.7
6. 2.47	2.20	66	21.5	87.9	40.0	24.1	11.0	112	51.0
21. 1.47	2.20	54	20.5	104.1	47.3	26.9	12.2	131	59.5
24. 1.47	2.20	68	28.4	87.3	39.7	34.7	15.8	122	55.5
				94.5	43.4	30.7	13.9	125	57.3

Table 19. Blood volume of individual rabbits by dye method.

Date	Wt.	Hb	Hct.	Plasma vol. c.c.	Plasma vol. c.c./Kgm.	Cell Vol. c.c.	Cell vol. c.c./Kgm.	Total Blood vol. c.c.	Total Blood vol. c.c./Kg.
23.4.47	2.45	77	31.5	87	35.6	40	16.3	127	51.9
25.4.47	2.95	96	37.1	88	29.8	52	17.6	140	47.4
30.4.47	2.35	83	33.2	101	43.1	50	21.3	151	64.3
1.5.47	2.50	69	26.8	102	40.9	37	14.8	139	55.6
2.5.47	2.35	95	37.8	83	35.3	50	21.3	133	56.6
17.6.47	2.50	71	31.9	88	35.2	41	16.4	129	51.6
23.6.47	2.55	73	32.8	102	40.0	50	19.6	152	59.6
8.7.47	2.70	-	37.5	103	38.1	62	23.0	165	61.1
				94.3	37.3	47.8	18.8	142	56.0

Table 20. Blood volume of rabbits by CO and dye methods simultaneously.

				CO						T-1824						
Date 1947	Wt. Kgms.	Hb	Hct.	Plasma vol. c.c.	Plasma vol. c.c./Kgm.	Cell vol. c.c.	Cell vol. c.c./Kgm.	Total Blood vol. c.c.	Total vol. c.c./Kgm.	Plasma vol. c.c.	Plasma vol. c.c./Kgm.	Cell vol. c.c.	Cell vol. c.c./Kgm.	Total Blood vol. c.c.	Total vol. c.c./Kgm.	Tot. v CO / Tot. v dye
13.2	2.65	-	22.3	112.0	42.3	32.0	12.1	144	54.3	112	42.3	32	12.1	144	54.3	1.00
30.1	2.30	62	20.2	111.0	48.2	28.0	12.2	139	60.4	100	43.5	25	10.9	125	54.3	1.11
12.2	2.40	61	21.5	111.0	46.2	30.0	12.5	141	58.7	115	47.9	32	13.3	147	61.2	0.96
10.4	2.55	105	38.3	86.0	33.7	54.0	21.2	140	54.9	91	35.7	56	22.0	147	57.7	0.95
11.4	2.80	88	34.7	102.0	36.4	54.0	19.3	156	55.7	101	36.1	54	19.3	155	55.4	1.01
11.4	3.10	94	34.4	107.0	34.5	56.0	18.1	163	52.6	98	31.6	51	16.4	149	48.0	1.09
12.4	2.10	105	37.2	84.0	40.0	49.0	23.3	133	63.3	81	38.6	48	22.8	129	61.4	1.03
26.5	2.65	75	32.5	90.0	34.0	43.0	16.2	133	50.2	85	32.1	41	15.5	126	47.6	1.06
3.7	2.60	74	30.1	100.0	38.5	43.0	16.5	143	55.0	93	35.8	40	15.4	133	51.2	1.08
18.4	2.60	67	26.6	138.0	53.1	50.0	19.2	188	72.3	147	56.5	53	20.4	200	76.9	0.94
5.7	2.40	-	37.3	83.0	34.6	49.0	20.4	132	55.0	79	32.9	47	19.6	126	52.5	1.05
5.7	2.50	-	39.0	86.0	34.4	55.0	22.0	141	56.4	88	35.2	56	22.4	144	57.6	0.98
7.7	2.55	84	35.7	88.0	34.5	49.0	19.2	137	53.7	92	36.1	51	20.0	143	56.1	0.96
7.7	2.10	73	30.5	96.0	37.6	42.0	16.5	138	54.1	91	35.7	40	15.7	131	51.4	1.05
9.7	2.50	-	30.3	121.0	48.4	53.0	21.2	174	69.6	123	49.2	53	21.2	176	70.4	0.99
				101	39.8	45.1	18.0	146.8	57.7	99.7	39.3	45.3	17.8	14.5	57.1	1.02

methods give roughly equivalent values.

The comparisons of the CO & dye methods done simultaneously in the same animal show agreement of the same order as that obtained in humans. 11 of the 15 observations show agreement within 5%; in only one case was the discrepancy greater than 10%.

The average values for cell volume (18 c.c./Kgm.) and plasma volume (39 c.c. per Kgm.) are comparable in Tables 19 and 20 but in Table 18 the cell volume is considerably lower and the plasma volume higher. In these earlier experiments potassium oxalate was used as the anticoagulant and the haematocrit was falsely low.

Test of accuracy of CO method in rabbits by the removal and addition of known volumes of red cells.

In these experiments the blood volume of an unanaesthetized rabbit was determined in the morning. The animal was then bled or transfused a measured amount and the blood volume estimation repeated early in the afternoon of the same day. In the interval the animal was kept in the laboratory without food or water.

Bleeding was done from the marginal vein of the ear with the aid of an electric light to warm the ear and promote blood flow. The 40-50 c.c. usually removed took ½ - ¾ of an hour. The blood was collected in a small flask; coagulation was prevented by dry powdered heparin. The volume of whole blood removed was

measured in a 50 c.c. graduated cylinder. The volume of red cells removed was calculated from the haematocrit done on a mixed sample of all the blood collected.

Blood was transfused into the animals using a small hypodermic needle and a 30 c.c. record syringe. The needle was introduced into the marginal vein of the ear and held in position with thumb and forefinger. With the animal's head maintained in comfortable position, the plunger of the syringe was gradually shoved home. 30-40 c.c. of whole blood were usually injected in a period of 15-20 minutes.

The results of six experiments are given below.

Experiment I. Rabbit: Chinchilla; male, Wt. 2.90 Kgms.

9.30 A.M.	Total Blood volume	164 c.c.
	Cell "	56.6 c.c.
	Plasma "	107.4 c.c.
10.15-11.00 A.M.	Bled 53.0 c.c.	
3.15 P.M.	Total Blood volume	194 c.c.
	Cell "	42.6 c.c.
	Plasma "	151.4 c.c.

Haematocrits: 9.30 A.M. 34.4%
Bleeding. 26.6%
3.15 P.M. 22.0%

Total amount of blood removed = 53.0 c.c.
Haematocrit = 26.6%
Total amt. cells removed = 14.1 c.c.
Estimated reduction in cell volume = 56.6 - 42.6 = 14.0c.c

Experiment II. Rabbit: Black: male; Wt. 2.80 Kgms.

10.00 A.M.	Total	142 c.c.
	Cell	49.3 c.c.
	Plasma	92.7 c.c.

10.15-11.00 A.M. Bled 46.5 c.c.

2.45 P.M.	Total	166 c.c.
	Cell	38.9 c.c.
	Plasma	127.1 c.c.

Haematocrits:	10.00 A.M.	34.6%
	Bleeding	31.3%
	2.45 P.M.	23.5%

Total amount of blood removed = 46.5 c.c.
Haematocrit = 31.3%
Total amount cells removed = 14.6 c.c.
Estimated reduction in cell volume = 49.3 - 38.9 = 10.4 c.c.

Experiment III. Rabbit; Black; female; Wt. 3.65 Kgms.

10.15 A.M.	Total	180 c.c.
	Cell	58.2 c.c.
	Plasma	121.8 c.c.

10.30-11.00 A.M. Bled 56.0 c.c.

2.45 P.M.	Total	210 c.c.
	Cell	43.2 c.c.
	Plasma	166.8 c.c.

Haematocrits:	10.15 A.M.	32.4%
	Bleeding	27.5%
	2.45 P.M.	20.6%

Total amount of blood removed = 56.0 c.c.
Haematocrit = 27.5%
Total amount cells removed = 15.4 c.c.
Estimated reduction in cell volume = 58.2 - 43.2 = 15.0 c.c.

Experiment IV. Rabbit; Chinchilla; male; Wt. 2.80 Kgms.

9.30 A.M.	Total	196 c.c.
	Cell	33.8 c.c.
	Plasma	162.2 c.c.

10.00 A.M.-10.20 A.M. Transfused 43.0 c.c.

2.45 P.M.	Total	205 c.c.
	Cell	46.3 c.c.
	Plasma	158.7 c.c.

Haematocrits: 9.30 A.M. 17.2%
Transfused blood 27.0%
2.45 P.M. 22.6%

Total amount of blood added to circulation = 43.0 c.c.
Haematocrit = 27.0%
Total amount of cells added = 11.6 c.c.
Estimated increase in cell volume = 46.3 - 33.8 = 12.5 c.c.

Experiment V. Rabbit; Chinchilla; male; Wt. 3.2 Kgms.

9.45 A.M.	Total	167 c.c.
	Cell	48.3 c.c.
	Plasma	118.7 c.c.

10.15-10.30 A.M. Transfused 39.0 c.c.

12.30 P.M.	Total	194 c.c.
	Cell	61.9 c.c.
	Plasma	132.1 c.c.

Haematocrits: 9.45 A.M. 28.9%
Transfused blood 31.6%
12.30 P.M. 31.9%

Total amount of blood added to circulation = 39.0 c.c.
Haematocrit = 31.6%
Total amount of cells added = 12.3 c.c.
Estimated increase in cell volume = 61.9 - 48.3 = 13.6 c.c.

Experiment VI. Rabbit; Black; female; Wt. 3.65 Kgms.

9.45 A.M.	Total	246 c.c.
	Cell	45.2 c.c.
	Plasma	200.8 c.c.

10.15-10.35 A.M. Transfused 47.0 c.c.

2.45 P.M.	Total	233 c.c.
	Cell	58.8 c.c.
	Plasma	174.2 c.c.

Haematocrits: 9.45 A.M. 18.4%
Transfused blood 27.6%
2.45 P.M. 25.2%

Total amount of blood added to circulation = 47.0 c.c.
Haematocrit = 27.6%
Total amount of cells added = 13.0 c.c.
Estimated increase in cell volume = 58.8 - 45.2 = 13.6 c.c.

These experiments provided very satisfactory evidence that the CO method of determining blood volume in rabbits as used in this work was accurate. Except in the case of Experiment II the estimated reduction or increase in red cell volume was within 1.0-1.5 c.c. of the volume of cells known to have been removed from or added to the circulation. In a total red cell volume of approximately 50 c.c. this would indicate an accuracy of 2 to 3% in the determinations.

Several other interesting observations were made from the data of these experiments concerning the rate of replacement of fluid to the circulation in unaesthetized rabbits following

haemorrhage. In the 3 experiments where approximately 50 c.c. of blood were removed the haematocrit of the blood removed was lower than the haematocrit at the start of the experiment, indicating that considerable haemodilution had occurred during the taking of the blood. This haemodilution must have continued during the 4 hour interval because the total blood volume done in the afternoon was greater than the original total blood volume. The animals had overcompensated for the removal of 50 c.c. of blood from the circulation. They had replaced the 50 c.c. of fluid removed plus 20-30 c.c. of additional fluid in a period of 4 hours following the haemorrhage. This effect is shown below.

	Total blood volume A.M.	Volume of blood removed	Total blood volume P.M.	Volume of fluid replaced in 4 hours.
Experiment I	164 c.c.	53 c.c.	194 c.c.	83 c.c.
Experiment II	142 "	46.5 c.c.	166 "	70.5 c.c.
Experiment III	180 "	56.0 "	210 "	86.0 "

A shift of fluid in the opposite direction, from the intravascular to the extra-cellular compartment, followed transfusion of whole blood, as demonstrated by these results of the transfusion experiments.

	Total blood volume A.M.	Volume of blood transfused	Total blood volume P.M.	Volume of fluid lost in 4 hours.
Experiment IV	196 c.c.	43.0 c.c.	205 c.c.	34 c.c.
Experiment V	167 "	39.0 "	194 "	12 "
Experiment VI	246 "	47.0 "	233 "	60 "

These rapid adjustments in circulating fluid volume were illustrated by the results on a single animal. Experiments I and IV described above were done on the same rabbit - Chinchilla; male; Wt. 2.9 Kgms. These and other data from the same animal are presented in schematic form in Figure 19. The columns represent total blood volume in c.c. estimated by the CO method; the shaded areas represent red cell volume. The open circles in the upper part of the figure designate percentage change in the haematocrit, taking the original value of 40.6 obtained from this animal as 100%.

Four separate determinations in a two week period from 4.9.47 to 18.9.47 showed a total blood volume which fluctuated from 160 to 175 c.c. The red cell volume in this same period showed a slight downward trend from 64 to 57 c.c. due to the removal of red cells in the samples for the repeated determinations. On 18.9.47 the animal was bled 53 c.c. and as described

in Expt. I above, the total blood volume on the afternoon of the same day rose to 194 c.c. The haematocrit showed a steep fall corresponding to this degree of haemodilution. This overcompensation was not a transient phenomenon, for on the morning of the next day 19.9.47, the total blood volume remained at 196 c.c. The red cell volume on this occasion was 8.8 c.c. lower than it had been on the afternoon of 18.9.47, despite the fact that no blood had been removed in the interval. It was difficult to account for this disappearance of red cells. An error in the determinations was improbable because the removal and addition of known quantities of red cells, preceding and following respectively this determination, were estimated to within 1.0 c.c. The animal was then transfused with 43.0 c.c. of its own blood and the total blood volume rose only slightly to 205 c.c. on that afternoon, although the red cell volume was increased by the expected amount. On the morning of 20.9.47, 19 hours after this transfusion, the total blood volume had returned to its original level of 170 c.c. and the red cell volume remained constant. The haematocrit showed a rise corresponding to this shift of fluid back to the extra-cellular compartment.

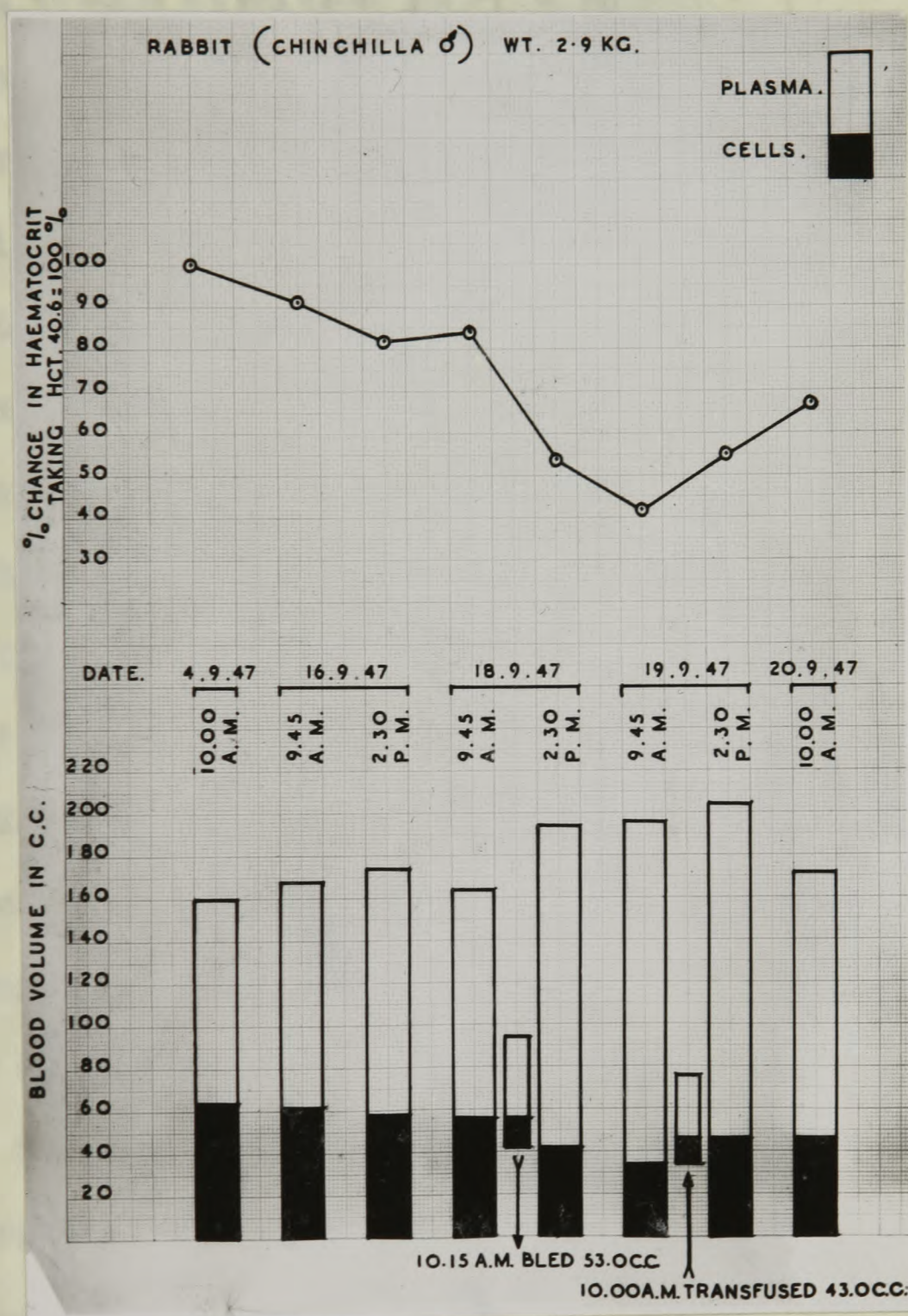


Figure 19. Blood volume and haematocrit determinations on a rabbit carried out over a period of two weeks, showing an increase in plasma volume following haemorrhage and a decrease in plasma volume following transfusion of whole blood.

DISCUSSION

CO uptake and disappearance curve in blood and its importance in blood volume determinations in humans

Any foreign substance method for the determination of blood volume will be accurate only if that substance is completely mixed with and has not disappeared from the circulating blood at the time the calculation of its dilution is made. This fact has been fully appreciated in the development of the dye method. The time required for complete mixing of T-1824 in plasma has been extensively studied in man and dogs, without complete unanimity of opinion but with general agreement that it requires at least 10 minutes under resting conditions. The disappearance of T-1824 from the plasma during this mixing period has been accounted for by the extrapolation method described in this work. Disappearance curves of T-1824 and certain red dyes have been studied by Dawson et al. (1920), Hooper, Smith, Belt and Whipple (1920), Sunderman and Austin (1936), Gibson and Evans (1937), Gregersen and Rawson (1943), Bowler, Crooke and Morris (1944), Noble and Gregersen (1946), Mather, Bowler, Crooke and Morris (1947).

Disappearance curves of CO from blood have not received the same attention despite the fact that the CO method has a longer

history. It has apparently been assumed that because CO is carried by the haemoglobin of the red blood cells which cannot leave the vascular system, the CO content of the blood of a subject breathing from a closed circuit remains constant from the time the equilibrium level is achieved. Haldane and Lorrain-Smith (1896) using a closed breathing circuit and the carmine titration method of determining COHb found that the blood CO content did remain constant. They took blood samples 3 min. after the administration of CO and again several minutes later and found no change in COHb percentage. From this Haldane concluded that CO did not undergo any oxidation in the human body. This fact has recently been authenticated by Tobias et al. (1945-46) using radio-active CO although the original belief that there is no disappearance of CO from the circulating blood can no longer be held.

Douglas (1910) found that in humans breathing CO from a rubber bag closed system, the CO content of a sample of blood taken 15-20 minutes after administration of the CO was considerably lower than in a sample taken 2-3 minutes after the administration. He concluded that this was due to more complete mixing of the blood at the later time and that such an effect could have explained the low values for blood volume obtained by Haldane

and Lorrain-Smith who took only one sample at 3 minutes. Chang and Harrop (1928) used the same type of closed circuit as Haldane and Smith and Douglas but found that the CO content of the blood rose slowly to a maximum at 15 minutes and then remained constant to 30 minutes. They reported the results of 3 experiments on humans. Asmussen (1941) in an unspecified number of human experiments, using again the rubber bag system, found that the CO concentrations fell rapidly during the first 5 min. and then remained at a plateau level to 30 min.

These reports in the literature were made on very few cases and show inconsistencies. The analytical methods for estimation of CO in blood were not of high degree of accuracy. In the present work an attempt has been made to standardize the conditions under which the CO was administered and the breathing continued. The use of the Krogh spirometer with respiratory valves has permitted a complete circulation of the air in the breathing system. The 90 c.c. volume of CO was always administered in a period of 1 minute to the inspiratory side of the circuit. The volume of the system was maintained at 7 litres, so that in a human subject of average size at rest with a pulmonary ventilation of 6-7 litres per minute, the entire volume of air in the circuit passed through the lungs 5 times before the first blood

sample was taken. The CO in blood analyses by the Scholander-Roughton apparatus were accurate to 0.03 - 0.05 vols. %.

The results of the 25 experiments at rest showed that the maximum CO concentration was achieved at 5 min.; it remained constant to 10 minutes and then fell gradually to 30 min. The degree of fall in this 20 min. period was slight, 0.08 c.c. per 100 c.c. and probably would not have been detected by the earlier analytical methods. Nonetheless it comprised 4.7% of the CO absorbed in these experiments. If the blood volume had been calculated from the 20 min. or 30 min. CO level it would have given a result which was several % higher than that calculated from the 10 min. value.

The 10 min. value was used for several reasons. In the first place it was considered desirable to use a blood concentration of CO which existed soon after absorption from the closed circuit was complete in order to avoid the error due to the slow disappearance observed from 10 to 30 min. But use of the 10 min. concentration would be valid only if complete mixing had occurred, in addition to complete absorption. It was shown by the analysis of residual CO in the circuit at equilibrium, that absorption was 98% complete in 5 minutes and it is a reasonable assumption that by 6 minutes it was complete. This left 4

minutes to ensure that the last traces of absorbed CO were evenly mixed in the blood by the time the 10 minute sample was taken. Complete mixing of an inhaled gas such as CO cannot be compared with mixing of a dye solution injected into a vein. In the case of the dye, the foreign material is distributed only to that volume of blood which passes along the vein during the time required for the injection. This would comprise a barely significant fraction of the total blood volume. In the case of CO, the foreign substance is being continually taken up by the large area of specially adapted capillary surface surrounding the pulmonary alveoli. The volume of blood which passes through the lungs in 1 minute is identical with the cardiac output and at rest is roughly equal to the total blood volume. The method of introduction of CO to the circulation by inhalation is thus in itself a natural mixing process. On these grounds alone it was felt that 4 minutes allowed ample time for complete mixing of all the CO absorbed. Corroborative evidence was supplied by the work of Hahn, Ross, Bale, Balfour and Whipple (1942) who reported that radio-actively tagged red cells were completely mixed with all other red cells in the circulation 4 minutes after injection. Because CO is carried by the red cells it may be inferred that 4 minutes after all

the CO has reached the circulating blood, it will be completely mixed. In addition, Tobias et al. (1945-46) in their work with radio-active CO demonstrated that 3-4 minutes after inhalation of the gas a uniform blood concentration had been reached. In the various reports in the literature on the determination of blood volume by the CO method, the CO concentration used in the calculation has been obtained from blood samples taken anywhere from 3 to 20 min. following administration of the gas to a closed circuit breathing system. It is suggested that failure to investigate the rate of uptake and disappearance of CO with the particular circuit used may have introduced errors in these determinations. Use of a blood sample at 20 min. would make the blood volume too high by reason of the disappearance reported here. Use of a blood sample at 3 or 4 minutes would not allow time for complete absorption and mixing. All these factors warrant as much consideration in the CO method as they have been accorded in investigations of the dye method.

Use of the 10 min. CO level is not exempt from criticism. Although absorption and mixing have been considered, it is possible that the slow disappearance observed from 10 to 30 min. was also taking place during the first 10 minutes. If this were the case and this disappearance occurred at the same rate during the first 10 minutes, the blood volume estimations in this work would be

2% too high. There would seem to be as much justification for this extrapolation procedure as for the determination of theoretical dye concentration at 0 min. by assuming the same rate of disappearance for the first 10 min. as for the succeeding 20 minutes. However, because these are only theoretical considerations, this correction has not been applied.

The disappearance of CO from the blood; its physiological significance.

The importance of the CO disappearance curve with relation to blood volume determination has been discussed in the preceding section. It remains to discuss the possible causes for this experimental observation.

The first possibility is that it represented leakage of air from the breathing circuit. To account for this fall in CO concentration in the blood it would have been necessary for approximately 35 litres of gas to have escaped in the course of 1 hour. The total fall in CO concentration in blood over the hour period was 0.13 c.c. per 100 c.c. In a subject with a blood volume of 5000 c.c. this would equal a loss of 6.5 c.c. of CO. At the equilibrium concentration of CO in the circuit of 0.0200% this amount of CO would be carried by $\frac{6.5}{0.02} \times 100 = 35$ litres of air. With an expiratory leak of this magnitude it would have been necessary to replace in the spirometer 35 litres of O_2 in

addition to the 18 litres used by an average subject at rest in the course of an hour. This would have resulted in almost pure oxygen in the circuit for the nitrogen would have been washed out by these frequent replacements. It was actually found necessary to replace only a litre of O_2 every 4-5 minutes, an amount just sufficient to meet the O_2 needs of the subject; the O_2 % at the end of 1 hour was always roughly equal to that at 5 min. These points of evidence were strongly against any expiratory leak.

The CO concentration in blood at the end of the hour did not fall as steeply as in the earlier stages. This effect was not compatible with an external leak; in the latter case the fall would have been continuous and uniform. Finally, it was shown during the repeat CO administration that higher CO tensions in the air in the circuit did not accelerate the rate of disappearance; and during muscular exercise when 3 times the volume of air was being moved around the circuit, the disappearance rate was not increased.

Another possibility was that this fall in CO concentration represented taking up of CO in true physical solution by the fluids of the body. Assuming that the body is 60% water, the volume of available fluid in a 70 Kgm. man would be $\frac{60}{100} \times 70 =$

42 litres. The tension of CO in the air of the circuit and therefore in the blood and tissue fluid was 0.148 mm. The co-efficient of solubility of CO in water at 37° C is 0.01837. The total volume of CO which could be present in the body water if it were completely saturated would be:

$$\frac{0.148}{760} \times 0.01837 \times 42000 = 0.15 \text{ c.c.}$$

It has been shown that the total disappearance of CO from the circulating blood in 1 hour was of the order of 6.5 c.c. The volume of CO in true physical solution comprises an insignificant fraction of this amount.

The question of haemodilution has already been discussed under the results of the haematocrit determinations on serial blood samples taken for the CO disappearance curve experiments. It was shown that there was no statistical difference between the haematocrit of samples taken at any time during the 30 minute period. Thus, the observed fall in CO concentration does represent disappearance and is not a dilution effect.

In all probability the disappearance of CO from the blood represents slow chemical combination with some haemoglobin like substance outside the circulation. The observations included in this work are in agreement with those of Roughton and Root (1945-46). These authors studied the rate of disappearance

of CO from the blood of human subjects breathing air from an open circuit after the absorption of approximately 100 c.c. of CO (i.e. sufficient to raise the blood CO content to 2.0 vols.%). They found that 15% of the CO absorbed disappeared from the blood in 1 hour. They measured the amount of CO in the expired air during this period by precise analytical methods and found that it comprised only 60% of the CO lost from the blood in a corresponding time. They repeated the experiments breathing pure O_2 at sea level and breathing pure O_2 at a simulated altitude of 35,000 ft. (180 mm. Hg.) with identical results. Their observations meant that 40% of the CO which disappeared was not collected in the expired air and must have been lost to some substance in the body outside the circulating blood. The amount so lost would be 40% of the 15% total loss during a one hour period, i.e. 6% of the CO originally absorbed. This figure, obtained by very different methods is comparable with the 7.7% disappearance of CO recorded in this work for a one hour period with the subject breathing from a closed system. To show that this disappearance was due to reversible combination of CO with some haemoglobin like substance outside the circulating blood, Roughton and Root measured the CO in the expired air with the subject breathing pure O_2 at sea level for 4 hours. They obtained almost complete recovery of all the

CO which had been lost from the circulating blood. At higher O_2 tensions CO is liberated more quickly from haemoglobin and similar iron containing pigments.

Extra-circulatory substances most likely to be responsible for this effect are stagnant red cells in the spleen and bone marrow, and muscle haemoglobin. Smith et al. (1921) from perfusion and extraction experiments in dogs reported that the haemoglobin in these stagnant areas comprised only 2% of the body's total haemoglobin. More recently Hahn et al. (1942) using radioactively tagged red cells have shown that there are no depots of stagnant red cells in man; all the red cells of the body are in active circulation. This leaves muscle haemoglobin (myoglobin) to account for the disappearance effect. This substance is known to be present in vertebrate muscles which undergo repeated, slow, powerful contractions. Smith et al. have reported that in dogs it comprises 15% of the total body haemoglobin. The analyses of McCance and Widdowson (quoted by Root et al. 1946) suggest that it is present in similar amount in humans.

Myoglobin is similar to haemoglobin in its reactions with O_2 and CO except that it has a higher affinity for O_2 and a lower affinity for CO. The amount of CO which would be combined with myoglobin at equilibrium conditions of the experiments described

in this work can be calculated from the equation:

$$MHbCO = \frac{K \times pCO \times MHbO_2}{pO_2}$$

which obtains for myoglobin as well as for ordinary haemoglobin.

In skeletal muscle:

pO_2 = 40 mm. Hg.

pCO = 0.15 mm. (from data on CO concentration in closed circuit)

$MHbO_2$ = 94%(at 40 mm. Hg. O_2 tension. cf. Millikan 1939)

K for MHb = 13.8 (Theorell; quoted by Millikan, 1939)

$$MHbCO = \frac{13.8 \times 0.15 \times 94}{40} = 4.8\%$$

In a normal subject with a total blood volume of 5000 c.c. and an O_2 capacity of 20.0 c.c. per 100 c.c., the total O_2 and CO capacity of the circulating blood would be: 1000 c.c.

If it be accepted that myoglobin has a CO capacity of 15% of the circulating haemoglobin, the total myoglobin CO capacity would equal $\frac{15}{100} \times 1000 = 150$ c.c.

Under the conditions of these experiments with a saturation of 4.8% it would contain at equilibrium

$$\frac{4.8}{100} \times 150 = 7.2 \text{ c.c. CO}$$

It will be noted that this figure 7.2 c.c. is very near the figure

6.7 c.c. which was the average amount of CO which disappeared from the circulation in the 11 human subjects after 1 hour of re-breathing. It is worthy of speculation to suggest that the slow disappearance observed during the one hour at rest was due to the slow equilibration of the CO in the blood with the myoglobin of the resting muscles. During muscular exercise, all the factors which would accelerate the achievement of this equilibrium; cardiac output, pulmonary ventilation and blood supply to the muscles, were increased. Average values from the 8 CO curves during exercise did show that the equilibrium was achieved earlier than at rest.

Root et al. (1946) believe that the equilibration of CO with myoglobin takes place within 15 minutes after inhalation of the gas with a subject at rest. They suggest that the slower disappearance of CO may be due to its combination with certain iron containing pigments in the liver. One of these, pseudo-haemoglobin of Barkan, is intermediate between haemoglobin and bile pigments and is believed to have 10 time greater affinity for CO than haemoglobin.

It is a very attractive hypothesis that the slow fall in CO concentrations observed in this work is due entirely to muscle myoglobin. The volume of CO lost in this manner in 1 hour at

rest is equal to the amount of CO which should be required by the myoglobin to satisfy equilibrium conditions in the muscle. Acceptance of this hypothesis would mean that no appreciable quantity of CO had disappeared from the circulating blood in the first 5 minutes and that no error had been included thereby in the blood volume determinations. Certain other facts suggest that this equilibration is a slow process. At rest the cardiac output per minute is approximately equal to the blood volume. Yet only about 1/5 of the cardiac output is probably distributed to the skeletal muscles. It would take several minutes for the entire blood volume to be brought in contact with muscular tissue. Further time would be required for complete absorption of CO from the circuit. No positive decision can be laid down from this indirect evidence. But it seems fairly certain that the effect of myoglobin in the first 5 minutes is very small.

Comparison of CO and dye methods of determining blood volume

The reason for comparing the results of CO and dye methods of determining blood volume performed simultaneously on the same animal or human subject was to establish that these two indirect procedures gave identical values. Disagreement in the results would indicate that one or both of the methods was incorrect.

Agreement of results would provide strong evidence that the values obtained truly represented the actual blood volume.

Haldane and Smith in 1900 using the CO method reported the blood volume of humans to be 47.8 c.c. per Kgm. of body weight. Salvesen in 1919 also using the CO method reported a figure of 59.5 c.c. per Kgm. Keith et al. in 1915 using the dye method found the value to be 85 c.c. per Kgm. It was obvious that all three of these sets of results could not be right. Smith, Arnold and Whipple (1921) were the first workers to compare CO and dye methods simultaneously. They found that in dogs their CO method gave a value of 82.7 c.c. per Kgm. and their dye method (brilliant vital red) a value of 103.9 c.c. per Kgm. McKintosh (1929) and Bazett et al. (1941) found that the dye method gave higher values in humans than the CO method.

Whipple and his co-workers on the basis of their results, suggested that the reason the dye method gave a higher value for total blood volume was not technical error in the determination of plasma volume but the incorrect assumption that the venous haematocrit represented the true ratio of the body's total red cell volume and plasma volume. They pointed out that in smaller blood vessels in the peripheral part of the circulation there is a central 'axial stream' of rapidly moving cells surrounded by

a slower moving layer of plasma which is impeded in its flow by contact with the wall of the vessel. The cross-sectional area of this plasma component is greater than the area of the cell component; and the volume of plasma in these peripheral 'still spaces' therefore comprises a greater proportion of the blood than in the larger central vessels. This concept is supported by the work of Fahraeus and Lindquist (1931) on blood flow in capillary tubes. Whipple concluded that since the cell to plasma ratio in peripheral blood was lower than the ratio in mixed venous blood, the 'body' haematocrit was lower than the central venous haematocrit. Red cell volume calculated from plasma volume and the central venous haematocrit would therefore give too high a value. He proposed that the true blood volume would be given by the sum of the red cell volume estimated by the CO or tagged cell method and the plasma volume determined by the dye method. In later work Hahn et al. (1942) using dogs, estimated red cell volume directly by the injection of red cells containing radio-active iron, and plasma volume by brilliant vital red. They found that the 'body' haematocrit given by the ratio

$$\frac{\text{Red cell volume (radio-iron)}}{\text{Red cell vol. (radio-iron)} + \text{Plasma vol. (dye)}}$$

was 20% lower than the haematocrit obtained from central venous

blood. This was evidence in support of Whipple's earlier view that total blood volume calculated from plasma volume and the venous haematocrit gives a falsely high value. Further support was provided by the work of Gibson, Peacock, Seligman and Sack (1946) who determined the body haematocrit in man and dogs using radio-iron for red cell volume and T-1824 for plasma volume. They found that the body haematocrit in both species was 91% of the observed venous haematocrit.

An opposite point of view has been taken by Gregersen and his co-workers. Root, Roughton and Gregersen (1946) using an elaborate and carefully controlled open circuit method for determining red cell volume by CO, and T-1824 for determining plasma volume, found in man and dogs that the body haematocrit was approximately equal to the central venous or arterial haematocrit. They found that total blood volume by the CO method agreed with total blood volume by the dye method to within ± 10%.

It cannot be argued that the cell to plasma ratio in the small peripheral vessels is not lower than that in the larger arteries and veins. Various theoretical considerations and actual microscopic observation indicate that this is so. But the volume of blood concerned constitutes a small percentage of the total blood volume. Bazett (1941) estimates that capillary blood comprises 10-15% of the total blood volume. Gibson, Seligman, Peacock, Aub

and Fine (1946) give evidence that in dogs under light morphine anaesthesia the blood in small vessels is 17% of the total blood volume. The haematocrit in this quantity of peripheral blood would have to be reduced to ½ that in central blood to produce a significant discrepancy in blood volume determination by the two methods.

In this work, comparison of simultaneous determinations of blood volume by CO and T-1824 in 20 human and 15 rabbit experiments showed agreement between the two methods usually within ± 5%. These results agree with those of Root et al. in man and dogs and confirm the fact that there is no significant difference between the central venous and 'body' haematocrit.

General.

The principles for the determination of blood volume by T-1824 used in this work are those described by Gibson and Evans, and Noble and Gregersen (1946) in which the theoretical dye concentration at 0 min. is obtained by extrapolation of the disappearance curve from the 10 min. dye concentration. The disappearance curves studied in the human and the rabbit show the rapid fall in dye concentration during the first 10 minutes described by these authors. One difference in technique has been the extraction of dye from plasma by the butyl alcohol method of Harington et al. This has obviated the necessity of having the human subjects

in a post-absorptive state to avoid lipemia. It has rendered the estimation of plasma dye concentration in the photo-electric colorimeter more reliable.

Most recent work on blood volume in human subjects has been done by the T-1824 method and has been expressed as plasma volume in c.c. per Kgm. of body weight. The results of the plasma volume determinations in this work are presented in Table 21 together with results by other authors. All series were done on young male subjects at rest at normal room temperature.

Table 21. Plasma volume in human subjects using T-1824.

Author	Number of subjects	Plasma volume c.c. per Kgm.
Present series	12	40.5
Davis (1942)	11	40.5
Gibson and Evans (1937)	49	43.1
Noble and Gregersen (1946)	51	44.7
Mather, Bowler, Crooke and Morris (1947)	53	54.8
Gibson, Peacock, Seligman and Sack (1946)	40	48.0

It will be noted that there is fairly close agreement between the results of the present series and those of Davis, Gibson and Evans, and Noble and Gregersen. The results of Crooke and Morris are very much higher. It was pointed out that these authors used oxalate as an anti-coagulant and that this caused a dilution effect on dye concentration. The series of Gibson, Peacock, Seligman and Sack is different from the original series of Gibson and Evans. It was from the series with higher plasma volumes that the discrepancy between venous and 'body' haematocrit was postulated.

The study of repeat T-1824 injections in the rabbit reported here has failed to confirm the findings of Cruikshank and Whitfield in the cat that the second dye injection is followed by a linear disappearance curve and that the plasma volume calculated therefrom is lower than from the first injection.

More emphasis has been placed in this work on the CO method for determining blood volume in man and rabbits. Use of standard closed-circuit breathing systems and the syringe-capillary method of CO analysis have made the procedure simple and accurate. The accuracy has been tested in both species by comparison with the dye method, by repeat determinations on the same and different days; under different physiological conditions as muscular

exercise in humans; and by the removal and addition of known amounts of blood in the rabbit. It has been found that the CO method offers certain advantages over the dye method. Only two small blood samples are required. Haemolysis in these is of no importance. The method can be repeated several times in one day with the same degree of accuracy. In human subjects repeated determinations by the dye method sometimes result in discoloration of the skin. CO, being 'physiological' in its behaviour, is breathed off in several hours. When the apparatus is set up a blood volume determination can be carried out in 10-15 minutes.

These considerations recommend the CO method for use in clinical or laboratory investigations where it is desired to perform repeated determinations of the blood volume in a short period of time. It has been employed in this manner in Part II of this thesis for haemorrhage experiments in cats and rabbits.

PART II. Haemorrhage experiments in the rabbit and cat.

Introduction.

In Part I during the test of accuracy of the CO method of determining blood volume in rabbits by the removal of a known quantity of red cells, it was observed that in 3-4 hours after the bleeding, the total blood volume had been completely restored, and, in fact, was greater than the original value before bleeding. This rapid restoration of circulating fluid volume in the rabbit is not found in dogs and man. Elman, Lischer and Davey (1943) reported that after a single, severe, non-fatal haemorrhage in the unanaesthetized dog fluid continued to be added to the circulation for 72 hours. Ebert, Stead and Gibson (1941) found in man that after removal of 20% of the blood volume, the total circulating fluid was replaced in 72 hours. Wallace and Sharpey-Schafer (1941) removed approximately the same quantity of blood from humans and observed that maximum dilution occurred in 3 to 90 hours, with an average value of 32 hours. These authors found that 3 of their 27 subjects had replaced more fluid than was removed during the haemorrhage. Boycott and Douglas (1909) studied the effect of a single haemorrhage on the blood volume of rabbits and observed that in 4 hours the circulating fluid volume had returned to its original level, while at 4-5 days it was increased

above this value. These observations suggested that it would be profitable to investigate in more detail the effect of haemorrhage on the blood volume of rabbits.

During the study of CO and T-1824 disappearance curves in the rabbit it was noted that nembutal influenced the rate of haemodilution after the removal of small blood samples at intervals during the course of 1 hour. In the series of nembutalized animals a fall in the haematocrit of approximately 5% occurred. In cases where the animal was unanaesthetized a decrease up to 20% of the original value was noted, although identical amounts of blood at the same time intervals had been removed. This inhibitory effect of nembutal on haemodilution had been observed by Courtice (1944) during haemorrhage experiments on dogs. It was decided to compare the experiments on haemorrhage in unanaesthetized rabbits with others on rabbits anaesthetized with nembutal.

This study has some practical importance in that experiments on production of and recovery from shock are often carried out under nembutal anaesthesia and it would be valuable to know the effect this anaesthetic exerts on restoration of fluid volume.

One effect of nembutal on the circulating blood has already been reported. This is the fall in haematocrit which occurs

after administration of this drug. This fall is due to the taking up of circulating red cells by an enlarged spleen and the addition of plasma to the circulation. These effects are most marked in the dog, which has a large spleen relative to body weight and have been reported by several investigators; (Bollman, Svirbley and Mann, 1938), (Hamlin and Gregersen, 1939), (Hahn, Bale and Bonner, 1943). The changes occur quickly and remain fairly constant through a period of several hours. They have not been studied further in this work. In the experiments on nembutalized animals described here the control values for blood volume, haematocrit and haemoglobin have been taken after the administration of nembutal. Because of the small size of the spleen in rabbits, the effect of this drug on the circulating red cell volume of rabbits would be negligible. Courtice (1944) has shown that spleens of rabbits removed under nembutal weigh 1.0 gm. per Kgm. of body weight (compared with 11.7 gms. per Kgm. in the dog). In a 3 Kgm. rabbit this organ could contain only approximately 2.0 c.c. of red cells.

Procedure.

In the experiments on rabbits 10 animals were used; 5 were unanaesthetized and 5 were anaesthetized with intravenous nembutal (sodium pentobarbital), 1 grain per 5 pounds of body weight

initially, with smaller doses at intervals thereafter. The depth of anaesthesia was such that the respirations were slow and deep, and pinching of a hind foot caused a very slight withdrawal response. It was maintained for 4 hours. The animals were fed on their ordinary diet of oats, bran, and water up to the time of the experiment. Blood volume was estimated by the CO method within 15 minutes of the time the anaesthetic was given. As soon as the original blood volume determination had been made the animals were bled from an ear vein. This usually required 10-20 minutes and an amount of blood equal to approximately 30% of the blood volume was removed in each case. This blood was collected in a flask containing dry powdered heparin to prevent coagulation and its volume was measured in a graduated cylinder. Using the mid-point of the bleeding period as zero time, small samples for haemoglobin and haematocrit determinations were taken at 1, 2, 6, and 24 hours. The amount of blood removed in samples for the original blood volume determination and the 1 hour sample were measured and included in the calculation for total volume of the bleeding. The blood volume determination was repeated at 2 and 24 hours in each of the 10 animals, and in 5 of these it was repeated at 72 hours and 14 days. No food or water were given for the first 6 hours after

haemorrhage. After this time the animals were returned to their cages and given their normal feedings. The accuracy of the blood volume determinations was checked in each case by comparing the estimated reduction in red cell volume with the volume of red cells known to have been removed and directly measured.

Results.

The protocol of a typical experiment on an anaesthetized rabbit with the results will be given.

Rabbit 20 A. Chinchilla, female, Wt. 2.5 Kgms.

9.45 A.M.	Nembutal I.V. 1.2 c.c.	
10.00 A.M.	Blood volume by CO method.	
	Blood removed in samples . . .	2.0 c.c.
10.20-10.40 A.M.	Bled from ear vein	35.0 c.c.
11.30 A.M.	Sample for Hb & Hct.	1.0 c.c.
12.30 P.M.	Blood volume by CO method.	
4.30 P.M.	Sample for Hb. & Hct.	

Total volume of blood removed in first 2 hours . . . . . 38.0 c.c.

Haematocrit of bleeding: 32.3%.

Therefore volume of cells removed . . . 12.3 c.c.

" " " plasma " . . . 25.7 c.c.

Blood volume estimation repeated at 24 hrs., 72 hrs. and 14 days.

Table 22. Results of haemorrhage experiment on rabbit 20A.

				Blood volume c.c.				Blood removed c.c.		
Date	Time	Hb	Hct.	Cell	Plasma	Total	c.c. per Kgm.	Cell	Plasma	Total
3.3.48	10.00 A.M.	79	34.1	48.8	94.2	143	57.2	-	-	-
	10.30 A.M.	-	-	-	-	-	-	12.3	25.7	38.0
	11.30 A.M.	75	28.9	-	-	-	-	-	-	-
	12.30 P.M.	71	30.1	38.8	90.2	129	51.6	-	-	-
	4.30 P.M.	67	28.0	-	-	-	-	-	-	-
4.3.48	10.00 A.M.	55	20.0	36.0	142	178	71.2	-	-	-
6.3.48	10.00 A.M.	46	20.4	35.0	137	172	68.9	-	-	-
17.3.48	10.00 A.M.	70	31.0	44.3	98.7	143	57.2	-	-	-

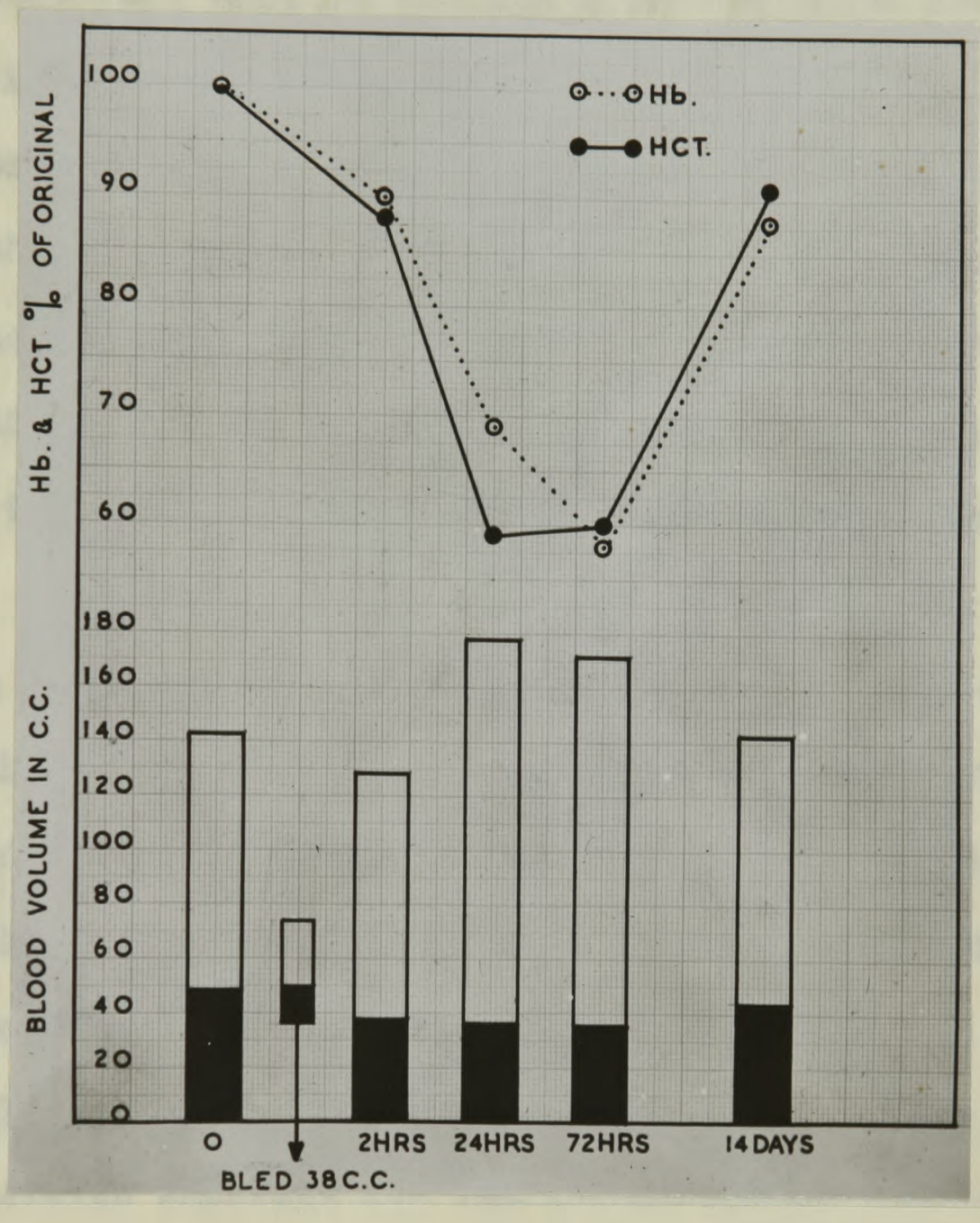


Figure 20. Changes in blood volume, haemoglobin and haematocrit in Rabbit 20A following haemorrhage. Shaded parts of columns represent cell volume; unshaded parts plasma volume. Over-compensation at 24 and 72 hours is illustrated.

The results of the experiment on rabbit 20A are presented in Table 22 and represented graphically in Figure 20. It will be noted that 12.3 c.c. of red cells were removed and that the estimated reduction in cell volume 2 hours later was 10.0 c.c. This degree of discrepancy is within the error of the experimental method and represents an error in the total blood volume estimations of only 5-6%. It indicates that no appreciable quantity of red cells was added to the circulation as an immediate result of the haemorrhage. The original plasma volume was 94.2 c.c. Despite the fact that 25.7 c.c. of plasma were removed, the plasma volume at 2 hours had risen almost to the original value, although this was not sufficient to cause a return of the total blood volume to its original level (see Fig. 20). At 24 hours the cell volume remained at the same level but the plasma volume rose from 90.2 to 142 c.c. and the total blood volume was considerably increased over the control value. With the cell volume remaining constant and the plasma volume increased, the haematocrit fell steeply. In Figure 20 haemoglobin and haematocrit values are expressed as per cent of the original. At 72 hours this condition of overcompensation was still present, but at 14 days with the cell volume regenerated to near normal value the plasma and total blood volumes had returned to the

original level.

All the experiments on anaesthetized and unanaesthetized animals were carried out and recorded in a similar manner. For the sake of brevity these results will be presented in tabular form.

Table 23. Unanaesthetized rabbits. Changes in haematocrit and haemoglobin following haemorrhage. Time in hours.

Rabbit	0	1	2	6	24	
19A	24.6	20.0	18.1	15.7	16.0	
8A	32.6	24.8	22.9	23.8	23.4	
12A	31.3	23.1	21.6	20.4	18.5	Hct.
16A	34.9	27.6	27.1	27.0	27.6	
15A	40.8	30.1	28.2	27.0	27.6	
Mean	32.8	25.1	23.6	22.8	21.7	
19A	60	48	46	42	41	
8A	75	60	54	56	53	
12A	66	51	47	51	47	Hb.
16A	76	62	59	61	52	
15A	92	72	66	62	63	
Mean	74	59	54	54	51	

Table 24. Unanaesthetized rabbits. Changes in blood volume following haemorrhage. Time in hours. Cell, plasma, and total blood volume in c.c.

Rabbit	0	Vol. removed	2	24	72	14 days
			Cell			
19A	44.0	12.4	33.5	32.6	36.2	53.8
8A	57.7	15.1	40.1	39.7	48.8	50.9
12A	42.9	9.3	35.2	26.5	27.0	29.1
16A	49.6	12.7	32.9	31.1	-	-
15A	52.4	14.9	34.1	37.3	-	-
Mean	49.3	12.9	35.2	33.4		
			Plasma			
19A	135.0	41.6	151.5	171.4	162.8	150.2
8A	119.3	32.9	134.9	130.3	151.2	110.1
12A	94.1	24.7	127.8	116.5	93.0	78.9
16A	92.4	25.3	88.1	103.9	-	-
15A	76.6	24.1	86.9	97.7	-	-
Mean	103.5	29.7	117.8	124.0		
			Total			
19A	179	54	185	204	200	204
8A	177	48	175	170	200	161
12A	137	34	163	143	120	108
16A	142	38	121	135	-	-
15A	129	39	121	135	-	-
Mean	153	42.6	153	157		

Table 25. Anaesthetized rabbits. Changes in haematocrit and haemoglobin following haemorrhage. Time in hours.

Rabbit	0	1	2	6	24	
20A	34.1	28.9	30.1	28.0	20.0	
3B	34.0	25.9	23.3	21.3	19.0	Hct.
11A	40.1	31.6	32.0	26.3	23.9	
9A	32.0	25.1	25.7	19.2	21.7	
10B	40.0	30.9	31.0	25.4	23.6	
Mean	36.0	28.5	28.4	24.0	21.6	
20A	79	75	71	67	55	
3B	71	62	55	57	44	
11A	85	70	69	58	51	Hb.
9A	76	63	60	47	49	
10B	86	72	69	62	54	
	79	68	65	58	51	

Table 26 Anaesthetized rabbits. Changes in blood volume following haemorrhage. Time in hours. Cell, plasma, and total blood volume in c.c.

	Cell					
Rabbit	0	Vol. removed	2	24	72	14 days
20A	48.8	12.3	38.9	36.0	35.0	44.3
3B	44.2	14.8	30.5	29.3	-	-
11A	56.7	13.6	41.9	44.0	48.9	48.8
9A	40.4	11.3	32.7	27.8	-	-
10B	55.2	13.0	35.4	35.3	-	-
Mean	49.1	13.0	35.9	34.5		

Table 26 contd.

Plasma

Rabbit	0	Vol. removed	2	24	72	14 days
20A	94.2	25.7	90.1	142	137	98.7
3B	86.1	32.2	100.2	125	-	-
11A	85.3	25.4	89.0	140	146.1	99.2
9A	85.6	29.7	94.3	100	-	-
10B	82.8	24.0	78.6	114	-	-
Mean	86.8	27.4	90.4	124.2		

Total

20A	143	38	129	178	172	143
3B	130	47	131	154	-	-
11A	142	39	131	184	195	148
9A	126	41	127	128	-	-
10B	138	35	114	149	-	-
Mean	136	40.4	126	159		

In Tables 23 and 24 the haemoglobin, haematocrit, cell, plasma and total blood volume changes in unanaesthetized rabbits following haemorrhage of 30% of the total blood volume are given. In Tables 25 and 26 the values for anaesthetized rabbits are given.

In the unanaesthetized series the average original red cell volume was 49.3 c.c. and the volume 2 hours after haemorrhage was 35.2 c.c. The estimated reduction was thus 14.1 c.c. The volume of cells actually removed was 12.9 c.c. In the

anaesthetized series the original red cell volume was 49.1 c.c. and the volume 2 hours after haemorrhage was 35.9 c.c. The estimated reduction in cell volume was 13.2 c.c., while the volume of cells actually removed was 13.0 c.c. The individual figures show slightly wider variation but in both series it may be reasonably concluded that there was no increase in circulating red cell volume 2 hours after the haemorrhage. Likewise the values at 24 hours show no increase. In the unanaesthetized series the red cell volume had fallen by a further 1.8 c.c. and in the anaesthetized series by 1.4 c.c. These amounts of red cells were known to have been removed in the samples required for the 2 hour blood volume determination and the 6 hour haemoglobin and haematocrit.

It was not expected that any increase in circulating red cell volume would be demonstrated because, as pointed out in Part I, all the red cells are in active circulation. Even if the spleen were granted a blood reservoir function its size in the rabbit is so small that it could make only a negligible contribution.

Considering average figures for plasma volume, in the unanaesthetized series the original value was 103.5 c.c. During the bleeding 29.7 c.c. of plasma were removed and yet at 2 hours

the plasma volume had risen above its original level to 117.8 c.c. That is, in 2 hours 44.0 c.c. of plasma had been added to the circulation. This was sufficient to bring the total blood volume back to its original level. At 24 hours in the unanaesthetized animals, the plasma volume was raised only slightly more - to 124 c.c., representing a total addition of 50.2 c.c.

In the nembutalized series the original plasma volume was 86.8 c.c. During the bleeding 27.4 c.c. of plasma were removed and at 2 hours the plasma volume was 90.4 c.c. This represented a replacement of 31.0 c.c. which was not sufficient to return the total blood volume to normal. During this period these animals were anaesthetized. The values at 24 hours after recovery from the anaesthetic showed that more haemodilution had taken place. The plasma volume rose to 124.2 c.c. representing a total addition of 64.8 c.c. in the 24 hours. The total blood volume was much higher than the original value.

In the unanaesthetized group the major part of the haemodilution occurred in the first 2 hours following haemorrhage with only slight further addition of fluid to the circulation by 24 hours. In the nembutalized group haemodilution in the first 2 hours was not great enough to return the total blood

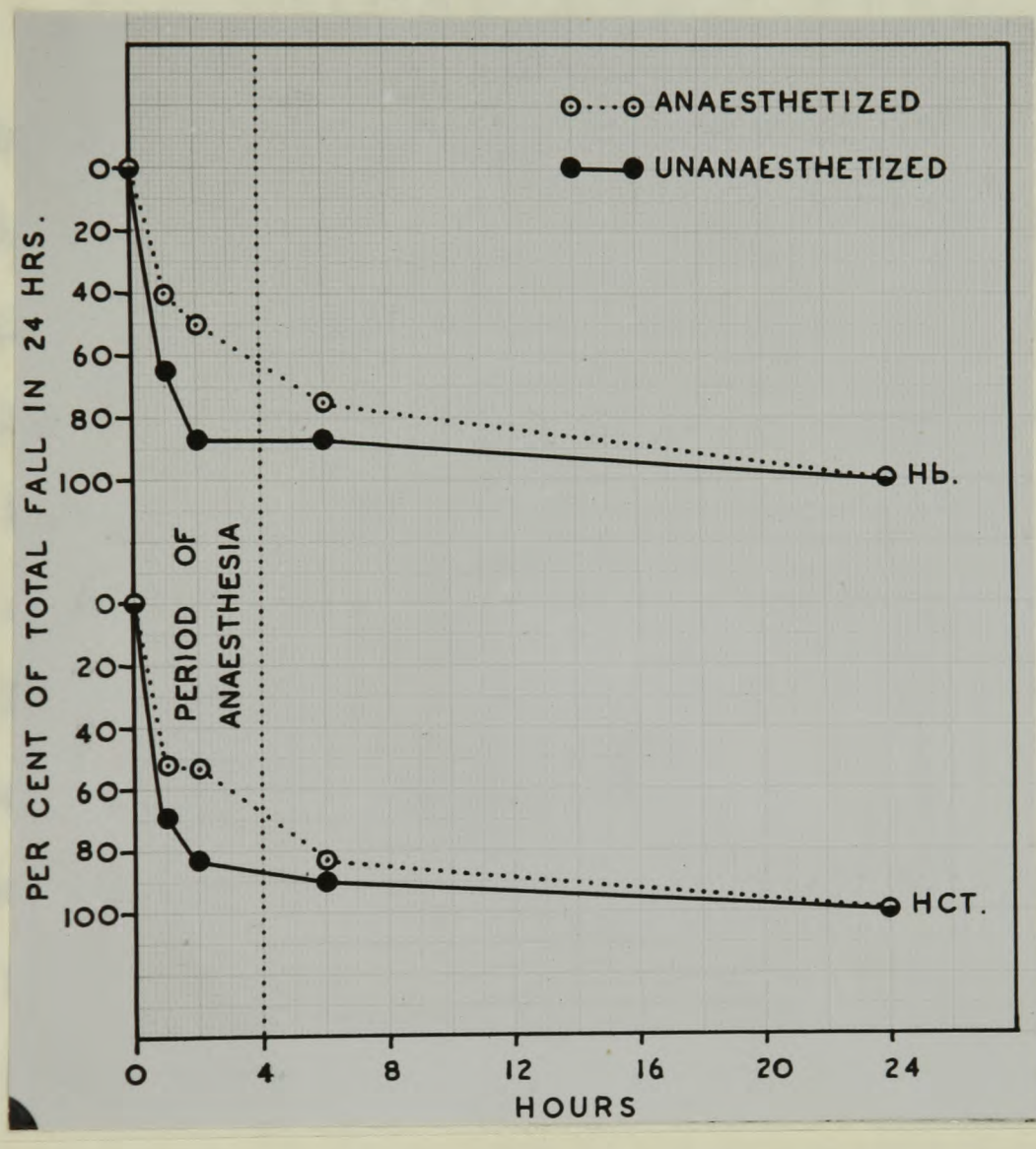


Figure 21. Fall in haemoglobin and haematocrit following haemorrhage in unanaesthetized and anaesthetized rabbits. Curves show the inhibitory effect of nembutal anaesthesia on haemodilution.

volume to normal but at 24 hours with recovery from the anaesthetic there was further addition of fluid equal to that which had occurred in the first 2 hours.

The inhibitory effect of nembutal on haemodilution is shown clearly by the haematocrit and haemoglobin changes in the 24 hour period. These are represented graphically in Figure 21. Hb. and Hct. are plotted as % of total fall in 24 hours against time in hours. Continuous lines represent the unanaesthetized group; interrupted lines the nembutalized group. The former shows a sudden fall in the first 2 hours; the latter a more gradual fall. In the unanaesthetized group there is little change between 2 and 6 hours. In the anaesthetized group this period corresponds to recovery from the anaesthetic and there is further haemodilution.

In rabbits 19A, 8A, 12A, 20A, and 11A the blood volume was determined at 72 hours and 14 days in addition to 0, 2, and 24 hours. Average results of this series of 5 are presented below and graphically in Figure 22.

	0	1	3	14 days
Haematocrit %	32.5	20.4	22.1	29.8
Cell vol. c.c.	50	36	39	45
Plasma vol. c.c.	105	140	138	107
Total vol. c.c.	155	176	177	152

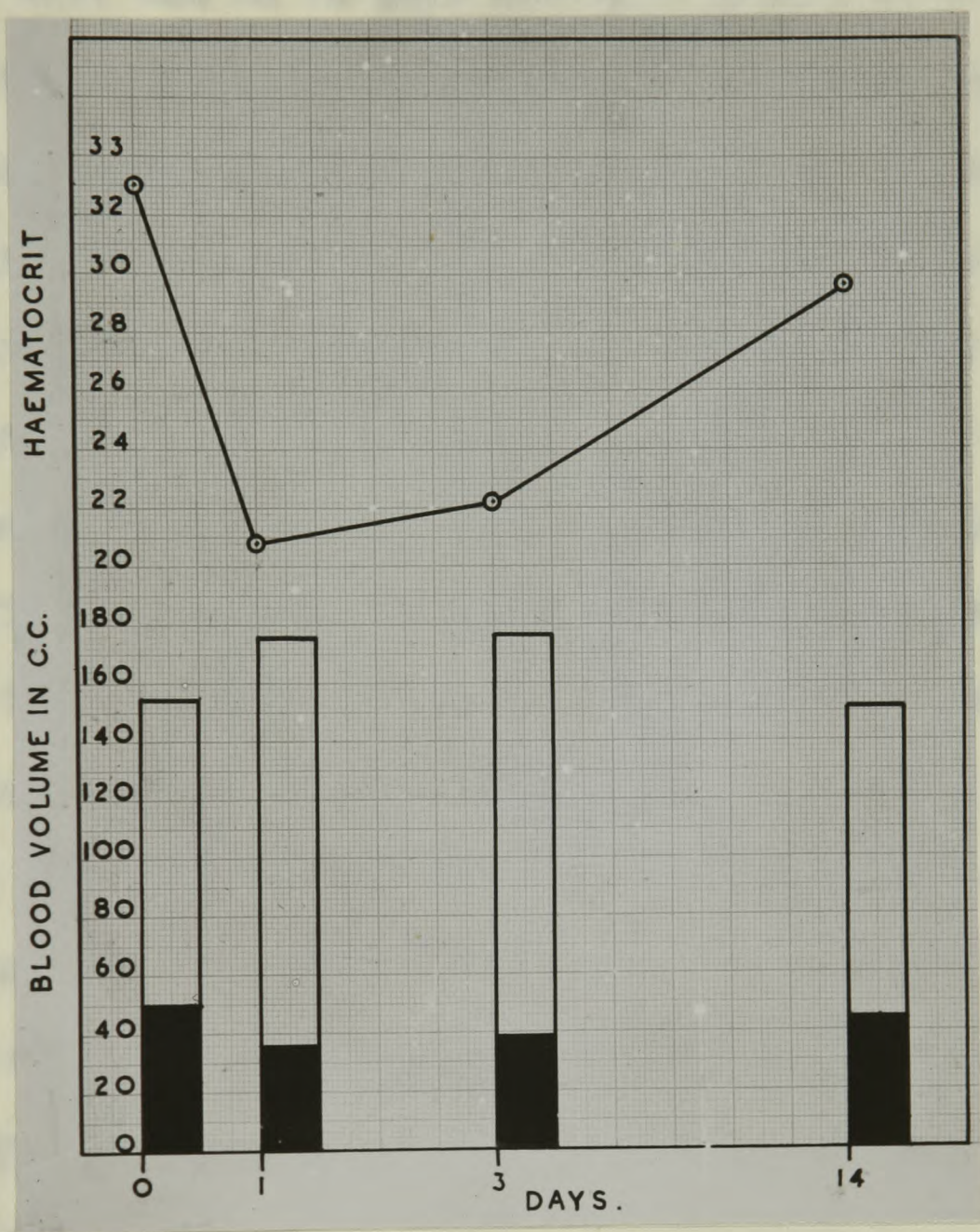


Figure 22. Average values from blood volume determinations on five rabbits before and 1, 3 and 14 days after haemorrhage of 30% of the blood volume. Shaded parts of columns represent cell volume; unshaded parts plasma volume. Overcompensation of plasma volume at 1 and 3 days is illustrated.

It will be noted that the cell volume 1 day after the haemorrhage was 36 c.c. In 3 days regeneration had increased this to 39 c.c. and in 14 days the cell volume of 45 c.c. was very near the original value of 50 c.c. During this period no attempt was made to provide any extra iron in the diet. The animals were maintained on their usual feeding of oats, bran and water. In this series the total blood volume 1 day after haemorrhage averaged 176 c.c. compared with 155 c.c. before. These animals had overcompensated for the loss of blood. In order to find out whether this was merely a transient overdilution, the estimations were repeated at 3 days and 2 weeks. In 4 of the 5 animals studied this overdilution was still present at 3 days. But at 2 weeks 4 out of 5 showed a fall in total blood volume to the original value. This return to normal coincided with the restoration of red cell volume to normal.

Haemorrhage Experiments in the cat.

The rabbits studied in the preceding experiments demonstrated the inhibitory influence of nembutal on haemodilution, but because these animals replace plasma volume so rapidly the results obtained cast little light on the probable behaviour of human subjects sustaining haemorrhage under nembutal anaesthesia. The cat and dog resemble the human more closely in function of

the vascular system and fluid exchanges. It was decided to study rate of haemodilution in the nembutalized cat following haemorrhage.

Procedure.

The blood volume determinations in the cat were done by the CO method using the apparatus already described for the rabbit. The animals were anaesthetized with intra-peritoneal nembutal, (1 grain per 5 pounds of body weight). A glass cannula was tied tightly in the trachea and connected with the spirometer unit. By this procedure all possibility of leak was excluded. Blood samples were taken from the femoral artery which was cannulated distal to a large branch, ensuring that the samples obtained represented mixed arterial blood. These preliminary operative procedures were carried out with minimal blood loss.

The blood volume was then estimated in the ordinary manner by the CO method. Samples were taken at 10, 20 and 30 min. after administration of CO for determination of haematocrit and CO content. The animal was then bled 25-30% of its total volume from the femoral artery. The amount collected was measured in a graduated cylinder; to this figure was added the amount removed in the various samples. At 1, 2, and 4 hours after the bleeding, samples were removed for Hb. and Hct. determinations.

The blood volume estimation was repeated at 2 hours. Throughout this period the animal was kept under anaesthetic and at the conclusion of the experiment it was killed. Protocol and results of a typical experiment will be described.

Results.

Cat No. 3. Black, male; Wt. 4.0 Kgms.

10.00 A.M. Anaesthetized with intra-peritoneal nembutal. Trachea and femoral artery cannulated.

10.45 A.M. Blood volume. 4.01 c.c. CO pure @ S.T.P. admitted to breathing circuit. Samples (2.0 c.c.) taken at 0, 10, 20, 30 min.

11.20 A.M. Bled 31.0 c.c.

12.20 P.M. Sample for Hb. and Hct. (3.0 c.c.)

1.20 P.M. Blood volume. 3.07 c.c. CO @ S.T.P. Samples taken at 0, 10, 20, 30 min.

3.20 P.M. Sample for Hb and Hct.

Total volume of blood removed to the time of 2 hr. blood volume determination = 31 + 8 + 3 = 42 c.c.

Analyses

Sample		Hct.		Hb	CO
10.45 A.M.	0 min.	-		-	0.18 c.c. per 100 c.c.
	10 min.	33.6		76	2.02 " " "
	20 min.	33.0	33.8	-	2.06 " " "
	30 min.	34.9		-	2.07 " " "

Sample		Hct.	Hb	CO
12.20 P.M.	-	32.9	71	-
1.20 P.M.	0 min.	-	-	0.72 c.c. per 100 c.c.
	10 min.	31.0	71	2.53 " " " "
	20 min.	31.4 31.8	-	2.41 " " " "
	30 min.	33.1	-	2.40 " " " "
3.20 P.M.	-	32.5	71	-

Cell, plasma, and total blood volume were calculated from the average Hct. of the 10, 20 and 30 min. samples and from the CO content of the blood at 10 min.

10.45 A.M. Total: 211 c.c.
Cell: 71.2
Plasma: 139.8

1.20 P.M. Total: 164 c.c.
Cell: 52.2 c.c.
Plasma: 111.8 c.c.

The estimated reduction in cell volume was 19.0 c.c. The volume of cells actually removed was 42.0 x 35.5 (Hct.) = 150 c.c. The marked difference from the rabbit in rate of haemodilution is shown by the figures for plasma and total blood volume. The original plasma volume was 139.8 c.c. Bleeding removed 27.0 c.c. of plasma. At 2 hours the plasma volume was 111.8 c.c., i.e. 28 c.c. less than the original value. During this period there had been no restoration of fluid to the circulation.

CO disappearance curve in the cat.

In each of the 4 animals used the CO concentration in the blood after administration of CO to the closed circuit was estimated at 10, 20, and 30 min. The results of one of these curves is given above and plotted on the graph in Figure 23. In this case the CO blood levels were measured for 30 min. during both estimations of blood volume. In Table 27 the results from the 4 animals are presented, where CO curves were done only during the first blood volume determination.

Table 27. CO disappearance curves; anaesthetized cats breathing through tracheal cannula. CO in c.c. per 100 c.c. Time in minutes.

	0'	10'	20'	30'
Cat I	0.09	1.83	1.77	1.68
" II	0.06	2.24	2.04	1.96
" III	0.18	2.02	2.06	2.07
" IV	0.12	1.81	1.76	1.76
Mean	0.11	1.98	1.91	1.87

These curves showed that in 3 of 4 cases studied the CO concentration was highest at 10 min. and that there was a slow fall throughout the remaining 20 minutes. Average values demonstrated a fall of 0.11 c.c. per 100 c.c. This was of the same order as the CO disappearance observed in humans and rabbits. In these cat experiments the possibility of a leak in the breathing

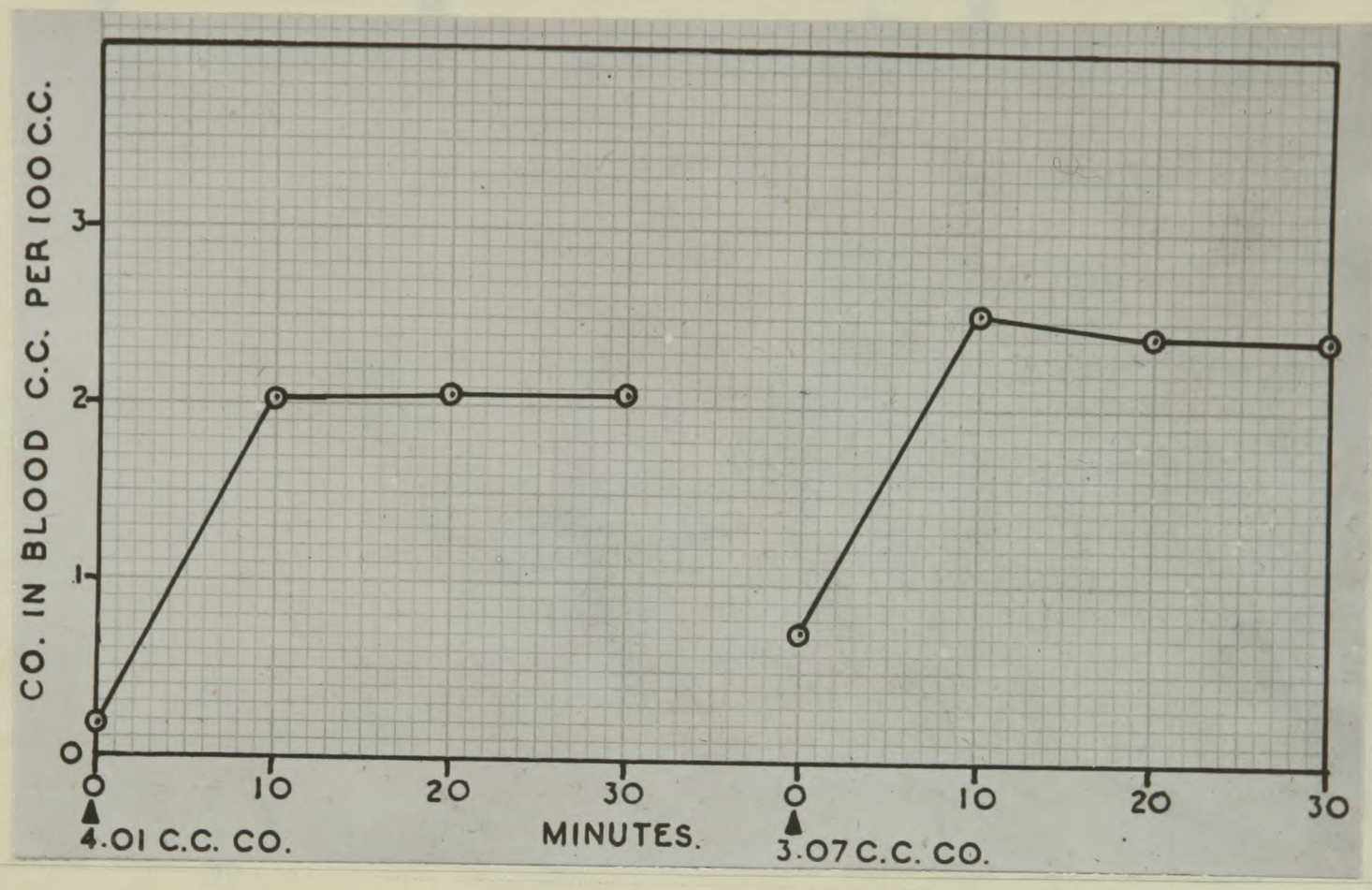


Figure 23. CO concentration in blood of cat breathing from closed circuit through intra-tracheal cannula, followed for 30 minutes. Interval of 2 hours between two administrations of CO

system was excluded because the animals breathed through a tracheal cannula tied tightly in place. The significance of this fall has already been discussed. These results in the cat serve to corroborate those already obtained in humans and rabbits.

Rate of haemodilution in nembutalized cats following haemorrhage. Table 28 gives the changes in haemoglobin and haematocrit, and Table 29 the changes in cell, plasma and total blood volume after removal of 25-30% of the blood volume in 4 nembutalized cats.

Table 28. Nembutalized cats. Changes in haematocrit and haemoglobin after haemorrhage. Time in hours.

Cat	0	1	2	4	
I	27.0	30.4	29.7	27.3	
II	35.4	37.6	35.5	35.4	Hct.
III	33.8	32.9	31.8	32.5	
IV	34.5	32.1	31.9	31.3	
Mean	32.6	33.3	32.2	31.6	
I	62	69	69	66	
II	81	86	80	78	
III	76	71	71	71	Hb
IV	84	80	79	75	
Mean	76	77	75	73	

Table 29. Nembutalized cats. Changes in blood volume after haemorrhage. Cell, plasma and total blood volume c.c. Time in hours.

Cell

Cat	0	Vol. removed	2
I	58	22	35
II	49	17	38
III	71	15	52
IV	63	12	48
Mean	60	17	43

Plasma

I	160	54	83
II	88	31	71
III	140	27	112
IV	118	24	102
	127	35	92

Total

I	218	76	118
II	137	48	109
III	211	42	164
IV	181	36	149
	187	52	135

Examination of average figures for cell volume changes reveals that removal of 17 c.c. of red cells from an original volume of 60 c.c. resulted in a cell volume of 43 c.c. 2 hours later. There had been no addition of red cells to the circulation following the haemorrhage. This was true in all the individual results except in the case of Cat No. II in which an increase of 6 c.c. over the expected value was obtained.

The figures for plasma volume changes at 2 hours demonstrate the striking difference in response to haemorrhage of nembutalized cats and rabbits. In 2 hours the rabbits had replaced all the plasma which had been removed. Average figures for the series of cats showed that no plasma at all had been replaced. Removal of 35 c.c. of plasma from an original volume of 127 c.c. resulted in a plasma volume of 92 c.c. at 2 hours. These average figures were not truly representative of individual results. In Cat I the plasma volume at 2 hours was actually lower than the expected value assuming no replacement. In this animal a relatively larger fraction of the total blood volume had been removed and this effect may have been due to the onset of shock. In Cat II, 14 c.c. of plasma were replaced; in Cat III, 0 c.c.; and in Cat IV 10 c.c. Yet in none of these animals had more than half of the plasma removed been restored to the circulation in

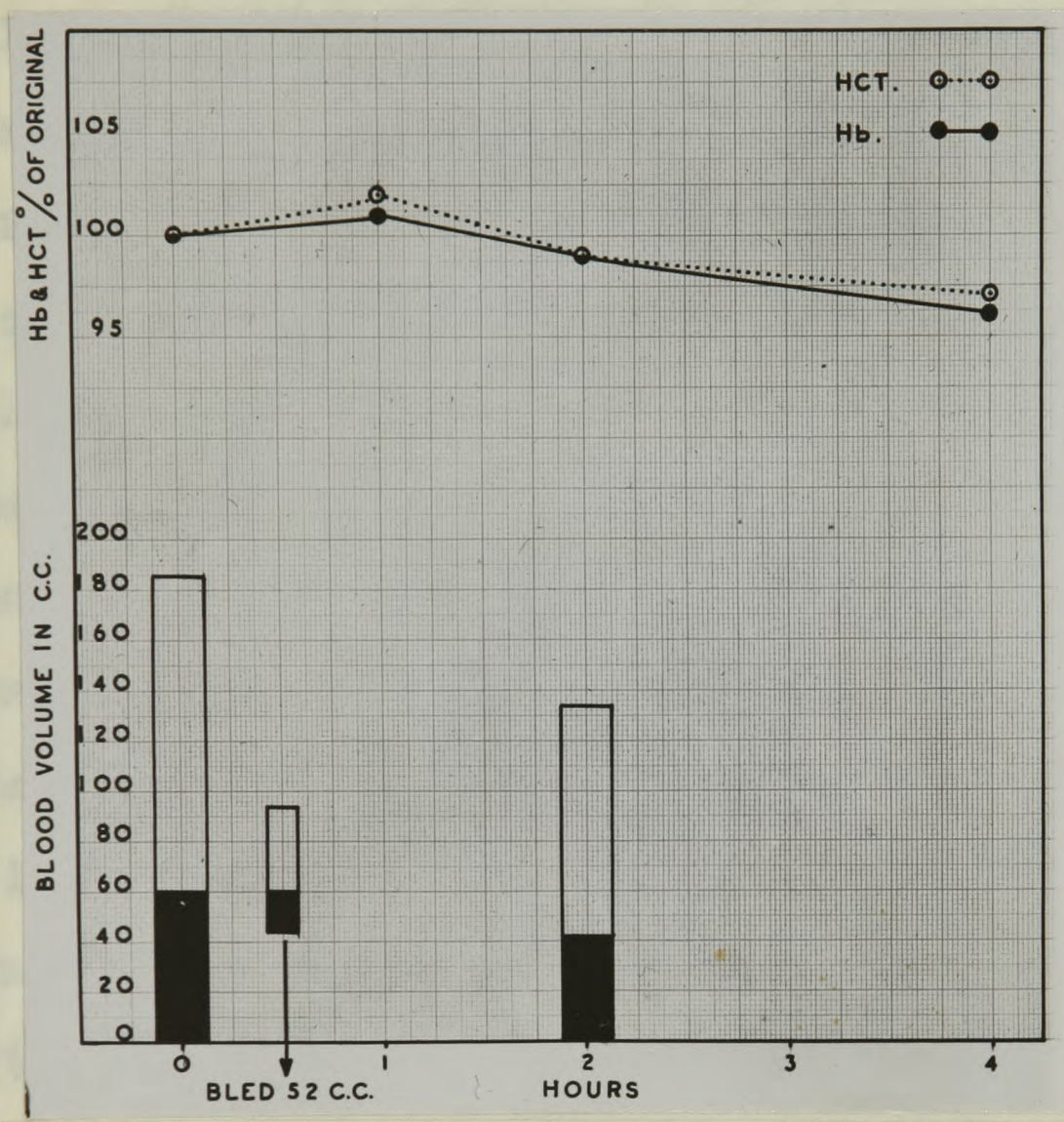


Figure 24. Blood volume, haemoglobin and haematocrit changes following haemorrhage in cats maintained under nembutal anaesthesia; mean of 4.

2 hours. The haematocrit values at 4 hours show that in the next two hours there was no further haemodilution.

With no restoration of red cell or plasma volume, the figures for total blood volume show parallel values. In the series of 4 cats removal of 52 c.c. of whole blood from an original volume of 187 c.c. left 135 c.c. at 2 hours. The results of these haemorrhage experiments on nembutalized cats are presented graphically in Figure 24.

Because these studies were done on animals breathing through a tracheal cannula it was not possible to compare them with others on the rate of haemodilution in unanaesthetized cats following haemorrhage of a similar degree. Courtice (1944) carried out such an investigation in unanaesthetized and nembutalized dogs. He determined blood volume by the T-1824 method before, and 4 hours after haemorrhage of 20% of the blood volume. His results are presented in Table 30. They show that the cell volume after haemorrhage in both groups was not higher than the expected value. There had been no release of red cells from the spleen or any other possible depot in response to bleeding. This was comparable to the results obtained in this work on rabbits and cats. Likewise in nembutalized dogs there was no restoration of plasma 4 hours after the haemorrhage. Removal of 215 c.c.

from an original plasma volume of 974 c.c. resulted in a plasma volume of 761 c.c. at 4 hours; i.e. the 4 hour plasma volume was still 213 c.c. lower than the original value. On the other hand, in the unanaesthetized group after removal of 143 c.c. from an original plasma volume of 724 c.c. the value at 4 hours was 648 c.c. If no addition of fluid had occurred the plasma volume at 4 hours would have remained at 581 c.c.

Table 30. Effect of bleeding 20% of the total blood volume on the cell, plasma and total blood volume in unanaesthetized and nembutalized dogs. (Courtice) Blood volume in c.c. Time in hours.

		0	Vol. removed	4
Unanaesthetized dogs Mean of 4				
	Total	1204	238	1013
	Plasma	724	143	648
	Cell	480	95	365
Nembutalized dogs Mean of 4				
	Total	1586	351	1224
	Plasma	974	215	761
	Cell	611	136	463

Discussion

The experiments on haemorrhage suggest several points of physiological interest. The first of these concerns the changes in circulating red cell volume following bleeding. It has been taught that while a large proportion of the body's total red cells are in active circulation, there are reserve depots of red cells in such tissues as the spleen, liver sinusoids and bone marrow where blood flow is sluggish or even stagnant. (Wiggers 1944). In times of emergency, especially those producing anoxaemia, these reserve stores are thought to contribute their red cells to the active circulation. The conditions at high altitude, muscular exercise, and haemorrhage are important emergencies producing anoxaemia. Barcroft and his associates (Barcroft, Harris, Orahovats, and Weiss 1925) focussed attention on the spleen as one of the most important blood reservoirs. They demonstrated radiologically and by actual observation that the spleen contracted during muscular exercise and haemorrhage. Other workers have observed splenic contraction and a rise in the haematocrit following injection of adrenaline. (Cannon and Izquierdo 1928). There can be little doubt that these phenomena actually occur but the fact that the spleen contributes a significant volume of red cells to the circulation has largely been

inferred. Direct measurement of the red cell volume would provide the only satisfactory proof. It is possible that contraction of the spleen and a rise in the haematocrit could occur without any change in circulating red cell volume.

The results presented in the preceding sections do not demonstrate any increase in circulating red cell volume following haemorrhage in the rabbit, cat, or dog. The observations on rabbits and cats were made by the CO method; those on the dog by the T-1824 method. The red cell volume after haemorrhage was equal to the original red cell volume less the volume of cells removed during the haemorrhage. It would appear that the reserve depots either do not exist or they are not brought into play by bleeding. These observations in animals are supported by the report of Ebert et al. in humans that 2 hours after removal of approximately 1000 c.c. of blood, the red cell volume was found to be equal to its original value less the amount of cells taken away. It is possible that the changes produced are so slight that they cannot be measured by the methods employed for blood volume determinations. In that case the reserve depots are too small to be of any physiological significance. Concerning muscular exercise, it was shown in Part I that moderate muscular exercise in humans was not attended by any change in haematocrit

or circulating red cell volume. Nylin (1947) using radioactively tagged red cells has reported that there is no increase in circulating red cell volume in humans during heavy muscular work.

These observations suggest that the concept of actively circulating and reserve red cells is open to question. The evidence from blood volume determinations is that all red cells are in active circulation.

Another point of interest concerns the rate of replacement of fluid to the circulation following haemorrhage in unanaesthetized and nembutalized animals. Since there is no replacement of cells the plasma fraction of the blood is responsible for the return of circulating volume to normal. In Figure 25 the changes in plasma volume in the cat and rabbit at 2 hours, and in the dog at 4 hours, after haemorrhage are presented schematically. The vertical volumns represent plasma volume as % of the original value before bleeding. The solid black columns represent the volume of plasma removed; the cross-hatched sections represent the volume of plasma which has been replaced. There is a very striking difference between the species; the rabbit replaces in 2 hours more plasma than was removed in both nembutalized and unanaesthetized groups, although this over-

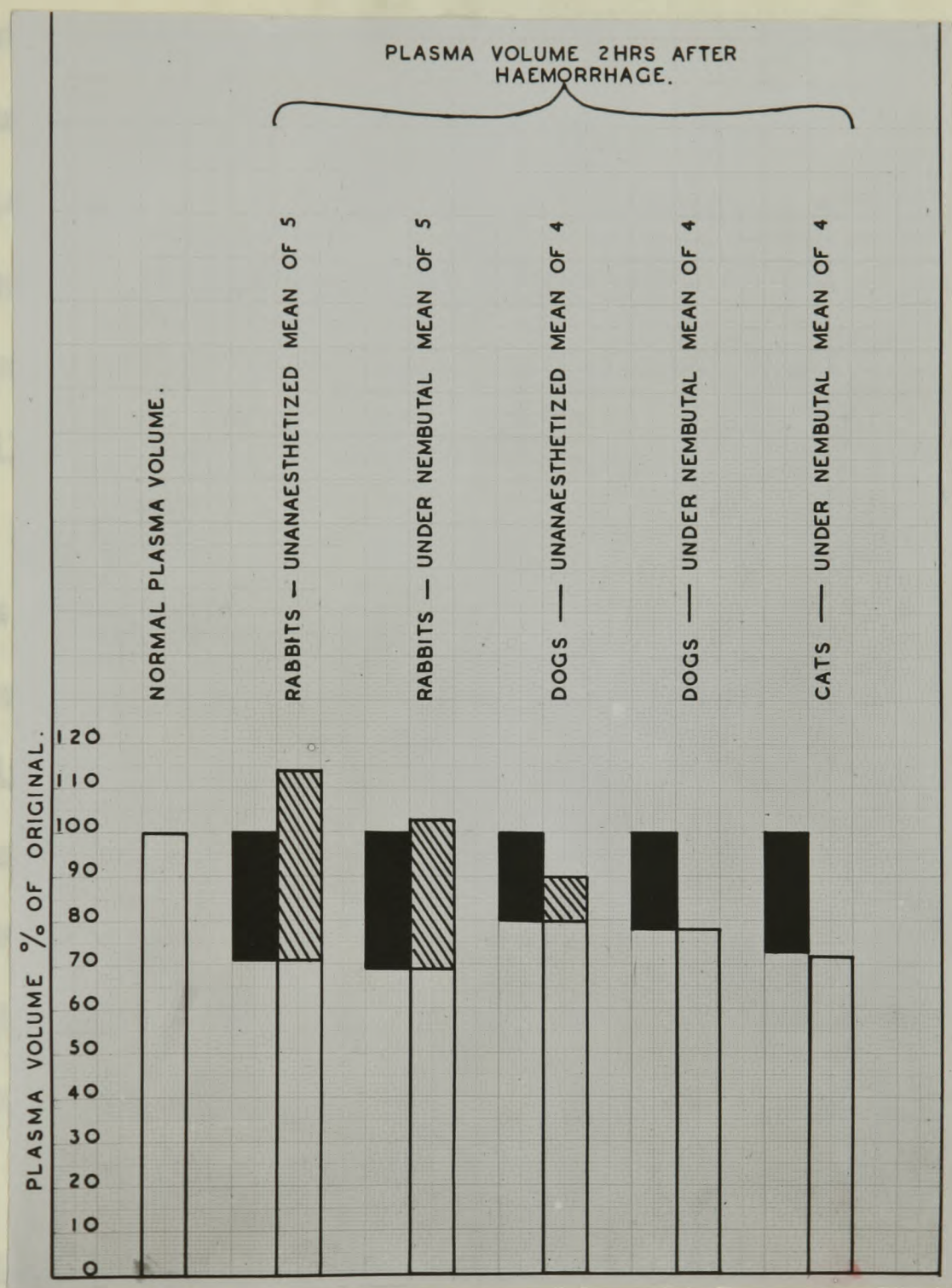


Figure 25. Diagram to show relative changes in plasma volume 2 hours after haemorrhage in rabbits, cats, and dogs. Densely shaded parts represent volume of plasma removed; cross-hatched parts represent volume of plasma restored to circulation. Striking features are: the difference in species response to haemorrhage between rabbits and cats and dogs; and the effect of nembutal anaesthesia.

compensation is greater in the unanaesthetized animals. The unanaesthetized dogs have replaced part of the plasma removed, but the nembutalized cats and dogs have restored no fluid. It is probable that human subjects resemble cats and dogs in rate of replacement of circulating fluid volume. Blood loss in hospital patients under nembutal anaesthesia would not be compensated until recovery from the anaesthetic. This could be of practical importance in the choice of anaesthesia for surgical procedures in traumatic cases.

The mechanism by which nembutal inhibits haemodilution is not clearly understood. There is only one reference in the literature on this subject (Elman, Weiner and Cole 1935) in which it was simply observed that the red blood counts of dogs maintained under sodium amytal did not fall after haemorrhage whereas an unanaesthetized group demonstrated the expected haemodilution. Restoration of fluid to the circulation following haemorrhage in unanaesthetized animals is believed to be due to a fall in capillary pressure with the result that fluid is drawn into the capillaries from the extra-vascular tissue spaces. The fall in capillary pressure is brought about by peripheral arteriolar constriction, a reaction which maintains arterial blood pressure as blood is lost during haemorrhage. It is known

that barbiturates cause arteriolar relaxation through depression of sympathetic tone. Arteriolar relaxation would result in a raised capillary pressure. The osmotic pressure of the plasma proteins tending to draw fluid into the capillaries would then be counterbalanced by the increased hydrodynamic pressure in the capillaries and haemodilution would be slowed or stopped altogether.

PART III The effect of diet on the blood volume of rabbits.

Introduction.

Cameron and Courtice (1946) studied the effect of phosgene on the production of lung oedema, plasma volume, and blood concentration in rabbits. They found that in rabbits maintained on a normal diet the average excess oedema fluid in the lungs 6 hours after exposure to the irritant gas was 31 c.c. Although this fluid had been derived from the circulating plasma, they found that the plasma volume had not fallen and there had been no haemoconcentration in this 6 hour period. They concluded the rabbit normally has a readily available store of fluid with which lost plasma can be replaced. These observations are corroborated by the work on haemorrhage reported here in which it was shown that rabbits can replace 30 c.c. of plasma in 2 hours. Cameron and Courtice studied these same changes in

response to phosgene on rabbits whose fluid intake had been restricted for several days prior to the experiment. They found that lung oedema developed as in the other cases but it was accompanied by haemoconcentration, indicating failure to restore plasma volume because of depletion of the animal's fluid stores. Wood and Blalock (1941) observed that simple restriction of fluid intake in dogs resulted in haemoconcentration. Gibson and Evans (1937) reported that diuresis is accompanied by a decrease in plasma volume. Hopper, Winkler and Elkington (1944) produced dehydration in dogs by peritoneal lavage with hypertonic saline solution and demonstrated a decrease in plasma volume. In all these examples fluid had been removed, or its intake restricted, with resulting fall in plasma volume or depletion of fluid stores.

It was felt that it would be of interest to study the effect of variations in the water content of the food on the plasma volume of rabbits.

Procedure.

Six animals were studied in these experiments, two groups of three. One group was fed on dry oats and bran with water ad lib. The second group was given the same diet with the addition of an excess of cabbage. After 3 days on these diets the blood

volume of all six rabbits was determined. Then, excess of cabbage was given to the first group and withdrawn from the second group. After a further 3 days on these diets the blood volume of all six rabbits was again determined. Although water was freely available, the animals drank very little. Because the water content of cabbage is high and rabbits eat this green food voraciously, the fluid intake on the cabbage diet was increased considerably. This has been shown by the increased urine output of rabbits fed on cabbage.

Results.

The results of the diet experiments on 6 rabbits are presented in Table 31. There was no significant change in the red cell volume between the dry and the cabbage diet. However in 5 of the 6 animals there was an increase in the plasma and total blood volume. The increase averaged 15 c.c., i.e. 10% of the original blood volume. A corresponding fall in the haematocrit was recorded.

Table 31. Effect of diet on the blood volume of rabbits. Cell, plasma, and total blood volume c.c.

	Dry diet					Cabbage diet				
Rabbit	Hct	Cell	Plasma	Total	c.c./Kgm	Hct	Cell	Plasma	Total	c.c./Kgm
1	34.3	43.7	83.3	127	52.9	30.2	44.1	101.9	146	60.8
2	33.2	38.6	77.4	116	46.4	28.7	38.7	96.3	135	54.0
3	29.9	61.1	142.9	204	70.3	29.5	59.9	143.1	203	70.0
4	29.0	45.0	110.0	155	55.4	28.9	50.2	123.8	174	62.1
5	32.4	48.6	101.4	150	55.8	28.5	51.7	129.3	181	71.0
6	31.6	44.6	96.4	141	52.2	30.0	44.6	104.4	149	55.2
Mean	31.7	46.9	101.9	149	56.0	29.3	48.2	116.5	165	62.2

Discussion.

Although the series of animals studied in these experiments was small, the fact that 5 of the 6 animals showed an increase of plasma volume provides reasonable evidence that increased fluid intake will exert an effect on the plasma volume of rabbits. Undoubtedly this increase of plasma volume was paralleled by an increase in the fluid stores of the body so that the compensatory mechanism for restoration of circulating fluid in haemorrhage and shock are greater on a wet diet. It is not possible to say whether this effect would be present in other species such as the cat, dog, and human without further study. Rabbits appear to be capable of wide variations in fluid adjustment.

SUMMARY

1. The circulating blood volume of man and animals has been determined chiefly by two methods: the CO method which measures red cell volume; and the dye method which measures plasma volume. Total blood volume is calculated in each case by the cell to plasma ratio given by the central venous or arterial haematocrit.

2. Both methods have been criticised on the ground that neither accurately measures total blood volume, (a) because the central venous or arterial haematocrit may not represent the ratio of the body's total red cell volume to total plasma volume, (b) because the CO method may include an unknown quantity of muscle myoglobin, (c) because the injected dye disappears from the circulating plasma and its degree of dilution is estimated by extrapolation of the disappearance curve - a procedure which is open to various theoretical objections.

3. In this work both methods have been studied in humans and rabbits. Total blood volume has been determined simultaneously by CO and the blue dye T-1824, and the results compared.

4. CO was administered by having the subject or animal breathe from a closed respiratory circuit consisting of a Krogh

spirometer, inspiratory and expiratory valves, and a soda-lime container, with O_2 admitted at intervals to maintain the volume of the circuit constant.

5. CO in blood analyses were done using the syringe-capillary apparatus of Scholander and Roughton. The accuracy of this instrument was tested and it was found that in 36 individual estimations the standard error of the mean of duplicate determinations was ± 0.037 c.c. per 100 c.c. blood.

6. Uptake and disappearance curves of CO in blood were followed in 11 human subjects at rest breathing from the closed circuit for 1 hour after the administration of approximately 90 c.c. of CO. These curves showed that absorption was complete in 10 minutes and that there was a slow fall in the CO blood levels thereafter which amounted to 0.13 vols.% in 1 hour when the original CO concentration was 2.00 vols.%. In a larger series of 25 human experiments where the CO in blood levels were followed for 30 minutes there was an average fall of 0.09 vols. %.

7. Haematocrit determinations on the blood samples used in these experiments showed that the fall was not due to haemodilution. It was also shown that this effect could not be due to leakage in the breathing circuit or taking up of CO in physical

solution by the body fluids.

8. On the assumption that the slow fall was due to combination of CO with the myoglobin of inactive muscles, the CO curves were followed in subjects performing moderate muscular exercise on the Krogh electric-brake ergometer. The respiratory and circulatory effects of this degree of exercise (420 Kgm. metres/min.) were determined in 3 subjects. It was found that the pulmonary ventilation, O_2 consumption and cardiac output were increased to 3 times the resting value.

9. Despite the increased blood flow to active muscles the CO curves during 30 minutes of muscular exercise showed no greater fall than corresponding curves in the same subjects at rest. From this it was concluded that the total effect of myoglobin on CO disappearance could not be greater than that observed at rest unless there had been a very rapid uptake of CO by the active muscles during exercise before the first blood sample was taken.

10. In 4 subjects blood volume was determined by the CO method at rest and repeated 30 minutes later during exercise. The values agreed to within 3% so that muscular exercise could not have caused the rapid uptake of a significant amount of CO.

11. On the basis of the CO uptake and disappearance curves the blood volume was calculated from the CO concentration in blood 10 minutes after administration of CO to the circuit. This allowed time for complete absorption and mixing in the blood and reduced to a minimum the disappearance effect

12. In order to measure the amount of CO left behind in the circuit and dead space of the lungs at equilibrium a method was devised for measuring CO in air. This involved de-oxygenation of the air sample by Krogh's hydrosulphite-borate solution, use of reduced blood as the active reagent to absorb CO from the de-oxygenated air, and calculation of CO content of the original sample by determining the increase in CO content of the reagent blood and the percentage of O_2 absorbed by Krogh's solution.

13. Using this method of analysis it was found that equilibrium between the CO tension in the blood and the air of the circuit was reached within 10 minutes. Calculating from the CO percentage and the total volume of the lung-spirometer system it was found that 1·6% of the CO admitted was not absorbed. A correction factor of 0.984 was applied to allow for this unabsorbed fraction in the human experiments.

14. Co uptake and disappearance curves in rabbits showed that

absorption was complete and equilibrium had been achieved 9-15 minutes after administration of CO. At equilibrium 3.3% of the CO admitted was left unabsorbed. In the rabbit experiments a correction factor of 0.967 was therefore applied in the blood volume calculations.

15. Blood volume was determined by the dye method by injecting a known amount of T-1824 in aquaeous solution, estimating dye concentration in plasma at 10, 20 and 30 minutes after injection, and extrapolating the disappearance curve so obtained to zero time to obtain the theoretical dye concentration at the moment of injection. From the degree of dilution of the dye the plasma volume was calculated, and from the haematocrit, the total blood volume.

16. The effect of varying concentrations of potassium oxalate and a mixture of potassium and ammonium oxalate as anticoagulants, on the haematocrit value were studied. It was shown that a concentration of potassium oxalate as low as 0.1% resulted in a haematocrit value 3.5% too low because the tonicity of these salts caused shrinkage of the red cells. In this work heparin was used as the anti-coagulant to avoid this error.

17. In the T-1824 method in humans the dye was extracted from plasma by the butyl alcohol method of Harington, Pochin and

Squire to avoid the difficulties in colorimetry caused by plasma pigments and varying degrees of lipemia in the samples.

18. Dye concentration was measured in a photo-electric colorimeter from calibration curves obtained by plotting values for known concentrations of T-1824 in human and rabbit plasma.

19. Investigations of dye disappearance curves in rabbits after single and repeat dye injections demonstrated that the shape of the disappearance curve does not alter after a repeat injection and that the plasma volume calculated from the second determination is accurate.

20. Simultaneous estimations of blood volume by CO and T-1824 methods were carried out in 20 human experiments. Of these 20 comparisons, 16 showed agreement within 5% and the remaining 4 within 10%.

21. Use of the same two methods simultaneously in 15 rabbits showed agreement in 11 cases within 5%, in 3 cases within 10% and in 1 case a discrepancy greater than 10%.

22. The CO method measures directly red cell volume; the dye method plasma volume. In the results of simultaneous determinations the total blood volume was calculated in each case from the central venous haematocrit, so that if this value did not represent the true ratio of the body's total

cell volume to plasma volume the two methods would not give equal values for total blood volume. The close agreement in results indicates that the central venous haematocrit and the 'body' haematocrit are identical.

23. Average values from 12 human subjects showed a total blood volume of 74.7 c.c. per Kgm. of body weight; cell volume of 33.0 c.c. per Kgm. and plasma volume of 41.7 c.c. per Kgm.

24. Performance of moderate muscular exercise in 4 human subjects was not accompanied by any increase in the circulating red cell volume or venous haematocrit.

25. Average values from 15 rabbits showed a total blood volume of 57.7 c.c. per Kgm. of body weight, a cell volume of 18.0 c.c. per Kgm. and a plasma volume of 39.8 c.c. per Kgm.

26. The accuracy of the CO method was tested in rabbits by the removal and addition of known volumes of red cells.

27. Haemorrhage experiments in rabbits demonstrated that 2 hours after the removal of 30% of the blood volume, the circulating fluid volume had returned to normal; 24 hours after haemorrhage it was in many cases higher than the original value; this condition of overcompensation was still present 72 hours after haemorrhage but at 14 days with regeneration of the red cell volume, the total blood volume had returned

to its original level.

28. The inhibitory effect of nembutal anaesthesia on haemodilution following haemorrhage was demonstrated in the rabbit and cat. In the rabbit the replacement of fluid was merely slowed; in the cat there was no significant replacement 4 hours after bleeding.

29. Haemorrhage in the rabbit, cat, and dog was not followed by any detectable contribution of reserve red cells to the circulation from such storage depots as the spleen, bone marrow and liver sinusoids.

30. Variation in the fluid content of the diet of rabbits was shown to have an effect on plasma volume. On a cabbage diet the plasma volume was 10% greater than on a dry diet of oats, bran and water.

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