BLOOD VOLUME STUDIES IN CHILDREN.

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by

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Preface.

During the years 1945 and 1946, the following studies on blood volume were carried out in the wards and biochemical laboratories of the Royal Hospital for Sick Children, Glasgow. The latter part of the work was done during the tenure of a Muirhead Scholarship.

To Dr. Stanley Graham I wish to accord my thanks for permission to study the patients under his care. Throughout the investigations, his unfailing interest and advice have been a constant source of encouragement.

My gratitude is also due to Dr. H. E. C. Wilson and Miss Olive D. Peden of the Biochemistry Department, who not only gave advice on technical matters, but also placed all the available laboratory facilities at my disposal.

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Introduction.

Since the introduction of experimental methods in clinical medicine, the study of blood volume has aroused considerable interest. Numerous methods of estimating the blood volume have been introduced, and by their use, attempts have been made to define the limits of variation in normal adults, and the changes occurring in pathological states.

The research was given considerable impetus by the two world wars, particularly the second, the urgent problem being to establish the degree and manner of restoration of blood volume after haemorrhage and shock, and the type and amount of intravenous fluid which should be administered, if the vis medicatrix naturae were judged inadequate.

In children, however, very little work has been done. The present investigation was therefore undertaken, in order to define if possible the limits of normality of the blood volume at various ages throughout infancy and childhood, and to investigate various conditions such as, the dehydration occurring in gastro-enteritis, and the anaemias of childhood.

PART I

Throughout the years, numerous methods of blood volume estimation have been employed, and these have been listed as follows:-

A. Direct. The exsanguination method of Welcker.

B. Indirect.

1. Carbon monoxide inhalation.
2. Injection of physiological fluids.
   (a) Water and saline.
   (b) Serum and plasma.
   (c) Concentrated red cells.
3. Injection of foreign substances.
   (a) Antitoxin.
   (b) Colloids.
   (c) Dyes.
   (d) Radio-active substances.

Historical.

Since the beginning of the nineteenth century, physiologists have been interested in the study of the blood volume. Probably the earliest published work is that of Herbst in 1822 with an account of earlier methods. These fell into two groups the one using measurement of the outflowing blood of a corpse as an indication of the true blood volume, the other relying on the amount of "stiffening substance" required to fill the vessels of a corpse on injection. These methods gave such varying results as to be completely unreliable. Valentin (1847) described a method of estimating various of the blood solids in samples taken before and after an injection of sterile water. This work, however, was done on animals alone, and the results obtained applied to man by analogy. Valentin deserved credit for showing that the absolute blood volume was of little significance, as it varied with size, but that the blood volume when related to body weight should be used for comparison. Next in chronological sequence came the washing out methods, carried out on human corpses. Lehmann and Weber in 1853 advocated the estimation of solids on two samples of blood, the one taken from the freely flowing blood following an execution - which volume was accurately measured - and the other from the measured washings collected after injection of the vessels with water. By proportion the blood volume was calculated. Lehmann and Weber themselves gave up the method, as salt was liable to be washed out of the tissues, and thus lead to error.

The Welcker method (Welcker, 1858) was the first to give consistent results. The technique, described originally by Welcker in 1854 for animals, was carried out on three human corpses,on one suicide by Welcker in 1858, and on two executed criminals by Bischoff in 1856 and 1858. The method consisted of collecting the entire blood of the body obtained by bleeding, to which was added the water used to wash out the vessels till the return was colourless, and also the pigmented water obtained from the maceration of the muscles and bones. A filtered sample of the total was matched against various dilutions of the subject's blood taken prior to the experiment. Welcker warned that any pigmented tissues, such as the gall-bladder, must be removed prior to maceration. The method as applied to the human body was exceedingly laborious. According to the details given by Welcker, two days were occupied in washing out the blood vessels, a task involving the handling of 254 litres, and then two more days were required for mincing and maceration, for which 152.5 litres were used. In all, for one experiment, he required four days and a vast amount of water, the total volume of which had to be accurately recorded. The average for the three experiments of Welcker and Bischoff gives the blood volume as $\frac{1}{13.1}$ or 7.63 per cent of the "Reingewicht", i.e. body weight minus weight of stomach and intestines. Similar results were obtained by Heidenhain (quoted by Welcker) using the same method. A variation in the technique was suggested in 1870 by Brozeit, the washing out and collection of the blood

being similar, but instead of colorimetric matching, the samples were extracted with acid ether, and the amount of acid haematin obtained was estimated by weighing.

In 1874, Malassez described the first attempt to perform experiments on blood volume on living human subjects. One method involved the withdrawal by venesection of a measured quantity of blood whose red cell count was estimated, and after an interval for dilution, the erythrocytes were counted in a second sample. His other method required the giving of a blood transfusion, with red counts of the recipient's blood before and after, and of the donor blood. Using these results, a formula was evolved to calculate the original blood volume. Malassez quoted no results for his experiments, which is not surprising, considering the inconsistent results which must have been obtained when using blood transfusions in the days before grouping was known.

Further investigations on living subjects were carried out by Tarchanoff (1880) who evolved a most complicated method. The patient was weighed before and after a turkish bath, and the total body loss known. From this, were subtracted the weight of urine, sweat and saliva lost during the period, and allowance was also made for gases lost from the lungs and skin. The residual loss in weight was regarded as being due to water loss from the blood stream. Using this figure (p) and the haemoglobin values before (a), and after the bath (a'), the original volume x was obtained from the formula $x = \frac{pa'}{a' - a}$.

The method was almost certain to be inexact in the measurement of the various fluids lost, and also in the fact that water would be lost from the tissues as well as from the blood stream.

In considering these earlier methods, in particular those of Welcker and Tarchanoff, which were so laborious and time-consuming, one agrees wholeheartedly with the dictum of Valentin, that "the exact estimation of the blood volume is one of the most difficult tasks in physiology."

Fortunately, the introduction of more modern methods has brought greater precision within reach of the clinical worker wishing to investigate the blood volume.

Review of Previous Work.

The methods already described are purely of historical interest, except the direct method of Welcker, which is still used for laboratory animals. As the exsanguination method involves the death of the individual, however, it is clearly of no practical importance in clinical medicine. Numerous indirect methods have been described for application to the human subject, and these will be discussed in some detail.

Indirect Methods.

1. The Carbon Monoxide Inhalation Method.

This method was first introduced by Gréhant and Quinquaud in 1882. The technique was based on the fact that carbon monoxide combines with haemoglobin volume for volume as does oxygen. A known amount of carbon monoxide was introduced into the air to be respired, and the animal left to inhale the mixture for fifteen minutes. The volume of carbon monoxide remaining in the breathing apparatus and lungs was then determined, by absorbing the carbon dioxide from a measured sample of this residual "air" by means of soda lime, oxidising the carbon monoxide to the dioxide, which in turn was absorbed by a different trap of soda lime. This last specimen of soda lime was then decomposed in a vacuum, and the carbon dioxide evolved, measured accurately, was taken to correspond to an equal volume of the monoxide. Hence, by subtraction of this figure from the original volume introduced, the amount of carbon monoxide absorbed into the circulation was ascertained. Blood samples taken before and after the inhalation were analysed for oxygen capacity, and

from these figures was calculated the amount of carbon monoxide which had been absorbed by one hundred cubic centimetres of blood. By simple proportion, the volume of circulating blood was then calculated. Grehant and Quinquaud described experiments on laboratory dogs only.

In 1900, Haldane and Smith applied a modification of the former method to human subjects. These authors noted that the oxygen capacity of any specimen of blood is directly proportional to the amount of haemoglobin contained. This observation was utilised to determine the percentage oxygen capacity of the patient, by matching a solution of his blood against a one per cent solution of blood of known oxygen capacity, determined by the ferricyanide method. After respiration of a known amount of carbon monoxide in a re-breathing apparatus - the time allowed for full absorption being two to three minutes - a specimen of the subject's blood was analysed for the percentage carbon monoxide saturation by the carmine titration method. By proportion, the blood volume was then calculated. Haldane and Smith found the average blood mass (i.e. weight of the blood volume) of fourteen normal subjects to be 4.8 per cent of the body weight. This figure is very low compared with the results of more recent work, which may be due to the fact that the time allowed for saturation of the blood with the carbon monoxide was inadequate.

Chang and Harrop in 1928 revised the carbon monoxide method, and the form it took in their hands, is that in use

at the present day. A measured amount of carbon monoxide, usually 100 cc., was introduced into a re-breathing apparatus with a constant slow supply of oxygen, and the subject allowed to breathe for twenty minutes, The expired air was passed through a soda-lime bottle, ice-cooled, in order to remove both carbon dioxide and water-vapour. At the end of twenty minutes, a sample of venous blood was removed and its carbon monoxide content determined by the Van Slyke apparatus. The gases - oxygen, nitrogen, carbon monoxide and carbon dioxide - were driven off by means of acid ferricyanide in a vacuum, the oxygen and carbon dioxide were removed with alkaline pyrogallol, and the monoxide was absorbed by Winkler's cuprous chloride solution.

The following criticisms have been levelled at the carbon monoxide method.

1. For clinical use, the prolonged period of re-breathing is trying for a sick person. As the inspiration of the gas produces a mild condition of anoxia, it is unwise to use the method when anoxaemia already exists, e.g. in anaemia and cardiac failure.

2. Theoretically, it should give results which are too high as the carbon monoxide is attached also to myohaemoglobin, and the red cells in the splenic pulp which are not in active circulation. In the hands of Boycott and Douglas (1909) this objection was substantiated, as carbon monoxide experiments gave higher blood volume figures than the

Welcker technique in the same animals - in spite of the fact that the latter method has the same sources of error. In actual fact, the method gives lower results than the dye method. Smith, Arnold and Whipple (1921) insisted that the total blood volume could not be estimated accurately by carbon monoxide, but only the red cell volume or total body haemoglobin. These authors pointed out that the haematocrit value of blood from the veins was not representative of that of the blood as a whole, as the capillaries contained relatively more plasma, surrounding an axial stream of cells. Consequently, the plasma volume as calculated from the carbon monoxide blood volume and venous haematocrit reading, would only represent that portion of the plasma in rapid circulation in the large vessels. On the other hand, the cell volume and total blood volume estimated by the dye method and venous haematocrit reading would be too high. To eliminate these errors it was suggested that, as the carbon monoxide gave a reasonable estimate of the cell volume, and the dye method of the plasma volume, the two methods should always be carried out in conjunction, and the results added to obtain the true total blood volume.

2. Injection of Physiological Fluids.

Many variations of the same basic method are included in this group, all depending on the introduction of a considerable volume of fluid into the circulation, and the estimation of some constituent of the blood

before and after transfusion.

(a) Water and Saline.

Some of the earlier attempts with this method may be mentioned briefly. Valentin in 1866 injected a volume of water, and calculated the resulting dilution of the blood from determinations of the blood solids. Cohnstein and Zuntz (1884) used saline infusions and red cell counts, while Sherrington and Copeman (1893), also using saline, observed the change in specific gravity. Haematocrit values before and after saline injections formed the basis of Kottmann's method (1906). Kottmann emphasised that the saline must be injected rapidly, and that the mixing was complete at the end of five minutes, by which time the second sample could safely be taken. The unreliability of these methods has been proved conclusively, as normal saline leaves the blood stream very readily, even during the course of a rapid transfusion (Bogert et al.,1916; Chanutin et al., 1924; Hill et al., 1940). The effect of this transference of fluid to the tissues is to give too high a result for the original blood volume, e.g.

If x = original blood volume, and

a and b = haemoglobin concentration before and after infusion respectively, and

V = volume injected,

then by equating the total amounts of haemoglobin,

$$x = \frac{bV}{a - b}.$$

It is obvious, that the more the saline leaves the circulation, the higher b will become, with resultant increase in the value of the fraction $\frac{b}{a - b}$, and therefore in the value calculated for x. Despite this important source of error, the average for Kottmann's four results in human subjects gives the blood mass: body weight ratio as 1 : 12.1 - a figure which compares favourably with the results of the Welcker method.

(b) Serum and Plasma.

Following the rejection of crystalloid solutions as blood diluents, the suitability of serum and plasma was investigated. It was thought that as plasma was the actual matrix of the blood cells, it would tend to be retained by the circulation indefinitely. Among others, Bushby, Kekwick and Whitby (1940) advocated the use of measured injections of plasma, and the observation of the haematocrit or haemoglobin readings, or red cell counts, before and after the conclusion of the experiment. Plasma specific gravity changes were observed in the methods of Beattie (1942a) and Phillips et al. (1946). The formulae used are similar to that given above for the saline injection. These formulae are true, only under the following circumstances.

1. That no red cells are added to the circulation during the experimental period.

2. That there is no loss of fluid from the circulation, or entry of fluid into the circulation.

3. That all the transfused fluid is used to dilute the blood.

1. This statement is probably true in man, as it has been shown that normal human subjects do not possess reservoirs of blood, whether in the spleen or other part of the body (Ebert and Stead, 1941a), i.e. changes in red cell count, haemoglobin concentration, or haematocrit reading in man are due to variation in the volume of circulating plasma (excluding blood regeneration of course).

2. The question of fluid loss from the circulation following infusions of serum and plasma must next be considered. On this point, many investigations disagree. All are agreed, however, that the added fluid eventually does leave the blood stream, but the speed of removal has been found to vary. In cats, serum may be removed almost as quickly as saline (Robertson, 1938), while in rabbits elimination is also rapid (Scimone, 1940). The results in man show some divergence of opinion. In normal subjects, transfused serum may be retained for long periods (Hill et al., 1940), it may be retained for several days so that the blood volume is restored in two to five days (Hayward and Jordan, 1942), or finally elimination may be so rapid that the haemoglobin value reaches the pre-transfusion level in one to two hours (Sharpey-Schäfer and Wallace, 1942a).

Entry of fluid into the blood stream from the tissues occurs less often, and probably only when the

protein concentration of the transfused fluid is greater than that of the circulating plasma (Metcalf, 1944).

It may be concluded, that as there is a definite possibility of rapid fluid elimination, the post-transfusion observations should be made within ten minutes of finishing the transfusion. Nevertheless, as with saline, fluid may start to leave the blood vessels even during the injection. To minimise this source of error, the injection should be rapid, as it has been shown that the most rapid rates of injection, cause the greatest increase in blood volume, using the same volume of fluid (Gilligan et al., 1938). The dangers of rapid intravenous injections, however, are now well known, and preclude the use of this method in human subjects (Sharpey-Schäfer and Wallace, 1942b).

3. Assuming that fluid loss does not occur, is all the transfused volume available to dilute the blood? Fåhraeus in 1929 demonstrated that the blood circulating in a capillary had a lower cell:plasma ratio than that flowing from its cut end, the phenomenon being explained by the division of the blood flow through the capillaries into two component parts, a peripheral layer, consisting mainly of sluggishly moving plasma, and an axial stream of more rapidly moving cells and plasma. The fraction of the total plasma volume required to form the peripheral lining for the blood vessels has been estimated to be 21 per cent (Hahn et al., 1942). During a transfusion, the increased blood volume may be accommodated

in two ways, firstly by the dilatation of existing vessels, secondly by the opening up of closed capillaries. If the latter adjustment takes place, 21 per cent (approx.) of the transfused plasma will be"lost" and only 79 per cent available to dilute the circulating blood (Beattie, 1942b). In addition to this error introduced by the lining cuff of plasma if new capillaries are opened up, one must consider the possibility of the great increase in vascular surface area facilitating further fluid loss to the tissues.

Further criticism of Bushby's method of haematocrit readings in plasma transfusions may be adduced. Serum and plasma transfusions in dogs have been shown to cause a decrease in mean corpuscular volume, thereby causing an alteration in the haematocrit reading (Metcalf, 1944), though it is difficult to see why this should have occurred if the solutions used were isosmotic. A similar finding has been recorded in man (Hayward and Jordan, 1942).

Another variation in the plasma transfusion method advocates the use of plasma protein concentrations as the factors for the blood volume calculation. Besides the errors inherent in the fluid shifts and plasma loss for lining capillaries, which have been discussed, this method has the added disadvantage that the blood constituent to be determined is not a constant. According to Madden and Whipple (1940), "a steady state of ebb and flow exists between it (plasma protein) and a

portion of cell and tissue body protein." The ease with which plasma protein shifts take place has been demonstrated also by Beattie and Collard (1942 a and b).

Although the above methods are worthless in determining the absolute blood volume, they are of some value in gauging the direction of change in the volume of the blood.

(c) Concentrated Red Corpuscles.

(1). Concentrated Corpuscle Haemoglobin Method.

The transfusion of concentrated red cells was introduced as an improvement on the Bushby plasma technique (Hill, 1941). The blood volume is obtained by equating the total amounts of haemoglobin concerned, the data required being the exact volume and haemoglobin content of the transfused blood, and the haemoglobin readings of the recipient's blood before and after transfusion. The formula for calculation is as follows:-

$$x = \frac{V(Hb_v - Hb_2)}{Hb_2 - Hb_1}$$

where x = initial blood volume

V = volume transfused

Hb_v = haemoglobin concentration of transfused blood.
Hb_1 = haemoglobin concentration of recipient before transfusion.
Hb_2 = haemoglobin concentration of recipient after transfusion.

Hill claimed the superiority of this method over plasma transfusion on the grounds that the red cells, in contrast to plasma, could not leave the circulation, at least

over such a short period as the duration of the experiment. Although erythrocytes cannot leave, however, plasma may either be added to or withdrawn from the circulation, which factor adds a considerable error to the technique (McMichael et al., 1943).

2. Concentrated Corpuscle Differential Agglutination Method.

This method consists of transfusing concentrated corpuscles of Group O, to a recipient of group A, B, or AB, the data required being the exact volume transfused, and the red cell counts of the donor blood and of the recipient's blood after transfusion, after the recipient's own cells have been agglutinated out of the sample by the use of the appropriate serum (McMichael et al., 1943). As a small number of the recipient's cells may not become agglutinated, it is more accurate to perform a "blank" agglutination count before the experiment. This method estimates the final blood volume, which equals

$$\frac{\text{volume transfused} \times \text{red count of donor blood.}}{\text{count of donor cells in recipient's blood.}}$$

The concentrated corpuscle methods are of limited value clinically. They are applicable only in conditions of anaemia, and the transfusions require to be rapid to minimise the error introduced by fluid shifts.

3. Injection of Foreign Substances.

In this section, the introduction of various substances foreign to the circulation will be discussed,

the blood volume being calculated from the dilution attained by the substance in the blood stream, after a suitable time has been allowed for mixing.

(a) Antitoxin.

Von Behring in 1912 evolved a technique requiring the injection of a known amount of tetanus antitoxin of proved potency. This method never seems to have enjoyed any popularity.

(b) Colloids.

Gum acacia was first employed by Meek and Gasser in 1918, for experiments on animals. The substance was injected in 20 per cent solution, allowing 4 cc. per kilo of body weight, and after the ten minutes allowed for mixing, the amount of acacia in the plasma was estimated as furfurolphloruglucid. The disadvantages of this method are two in number.

1. A bulky injection is required

2. The acacia solution probably raises the plasma osmotic pressure, thus inducing an inflow of fluid from the tissues. Meek and Gasser maintained, however, that within the time of mixing, no such osmotic effect took place.

In 1920, McQuarrie and Davis described a modification involving the use of either acacia or gelatin solutions. Their presence in the non-protein fraction of the serum was determined refractometrically. By the precipitation of proteins in the serum specimens by means of an equal volume of N/2 acetic acid, followed by

boiling for two minutes, errors due to the presence of haemolysis and lipaemia were simultaneously removed. The method, if repeatedly used, is not without danger (Heckel et al., 1938). Acacia appears to be deposited in the spleen and liver, interfering with the function of the latter, and causing diminished formation of plasma proteins. These authors noted swelling and vacuolation of the liver cells on histological examination.

(c) Dyes.

1. Haemoglobin.

First in this group may be mentioned briefly a method using the injection of a haemoglobin solution obtained by the laking of packed red cells by distilled water (Lee and Whipple, 1921), the resultant colour of the plasma being matched against a standard haemoglobin solution. If any haemolysis took place during the withdrawal of the blood samples, a considerable error might be introduced. This method has been deservedly unpopular.

2. Benzidine Dyes.

The benzidine group of the azo dyes has been extensively used in blood volume studies. These dyes fall naturally into two groups, the red and the blue, of which the former, including vital red, brilliant vital red, and congo red, were the more popular until 1935. The method in its original form is simple. A known amount of the dye chosen is injected after withdrawing a specimen of blood, time is allowed for mixing, and a second specimen

withdrawn. Standards are made up with some of the dye solution as used for injection, and the subject's undyed plasma or serum. By comparison of the dyed plasma, with the standards in a colorimeter, the dilution attained by the dye in the circulating plasma can be calculated, and hence the plasma volume. By using the haematocrit reading, the total blood volume may then be ascertained.

(1). Red Dyes.

In 1915, Keith, Rowntree and Geraghty gave tremendous impetus to the study of blood volume, by their experiments on human subjects using the dye vital red. They formulated the precautions required for accuracy in the technique, and these have been observed in every more recent dye method.

a. Specimens must be taken from the veins without stasis, in order to obtain a haematocrit reading consistent with that obtaining in the general circulation.

b. The dyed specimen should be removed from a different vein from that used for the dye injection.

c. Precautions must be taken against haemolysis.

d. Lipaemia alters the colour and transparency of the plasma, and therefore the time of estimation should be arranged so that food has not been taken for four hours beforehand.

This method was used later in the First World War (Robertson and Bock, 1919) to investigate the state of the blood volume following haemorrhage in wounded soldiers.

Hooper, Smith, Belt and Whipple in 1920, modified the original method of Keith, Rowntree and Geraghty, by using brilliant vital red. The unsuitability of dry oxalate as

an anti-coagulant was also shown, as it caused shrinkage of the red cells. They advised the use of plasma, rather than serum, for comparison, as clotting removed about 7 per cent of the dye. In experimental controls of the method, they proved that after rapid haemorrhage of a known amount of blood, the immediate application of the dye method would show close correlation between the observed blood volume, and that calculated from the original volume minus the volume lost by haemorrhage.

In a critical survey of the methods then in use, Lamson and Rosenthal (1922) showed that the dye methods could not give an absolute accurate figure for the blood volume, but that they were of value in a series of estimations. These authors also cast doubt on the current practice of accepting the mixing time as three to four minutes, since the curve of vital red concentrations in the plasma plotted from their results for the first five minutes, showed great variation in form.

The next dye to be brought into use was congo red, its advantages being that it is non-toxic, and less rapidly eliminated than vital red. Keith, Rowntree and Geraghty, in their original work, had shown that vital red could cause a transient albuminuria, and that "unpleasant effects" were sometimes observed following the injection. Congo red has no such disadvantages.

As a group the red dyes have one great drawback. With the ordinary laboratory colorimeter, including the photo-electric, it is impossible to detect, or correct for,

haemolysis. Consequently, any results giving a low plasma volume, must fall under a certain amount of suspicion. With the Koenig-Martens spectro-photometer alone, can one estimate the true dilution of a red dye in the presence of haemoglobin, as the reading can be taken at the wavelength of maximum absorption for the dye in question, which in most instances, is different from that of haemoglobin (Heilmeyer, 1929).

(2). Blue Dyes.

The blue benzidine dyes were first recommended in 1920, although it seems strange that they find no further place in the literature until fifteen years later. Dawson, Evans and Whipple, (1920) reviewing a large series of dyes, showed that the blue dyes, in particular T1824, or Evans blue, were superior to the red, as they were slower in elimination, and showed the presence of haemolysis by changing colour. T1824 was later shown (Gibson and Gregerson, 1935) to be non-toxic for rats in doses up to 20 mg. per kilo.

Up till then, and even in the present day, one of the most controversial points of the dye method, has been the time required for mixing, and the time at which elimination started. Gibson and Evans (1937a) using the dye Evans blue, and taking blood samples every minute for the first ten minutes, and thereafter, every four to five minutes, plotted the results so obtained, and showed in every graph a "mixing curve" lasting 7½ minutes on the average, joined to a "disappearance slope" which was a straight line. They advocated this multiple sampling for every blood volume estimation, the finding of the disappearance slope, and the extrapolation of

this slope back to the ordinate, the point of intersection giving the theoretical dye dilution in the plasma supposing mixing to be complete immediately after injection. By reading the optical density of the dyed plasma in the spectrophotometer, set at the wavelength 620$\mu\mu$ - i.e. that of maximum absorption of T1824 - the error due to haemolysis was largely minimised, as the maximum absorption of haemoglobin takes place at a lower wavelength. Further correction for haemolysis, however, was advised by the authors, by reading the optical density of the dyed plasma first at 620$\mu\mu$ where the dye would be largely responsible for the value obtained, and secondly at 540$\mu\mu$, where the reading would be due largely to the haemoglobin. By applying factors worked out for the two substances in varying dilutions in pure solution on these two wavelengths, the small reading due to haemoglobin could be defined and subtracted from the reading at 620$\mu\mu$. This method has done much to improve the accuracy of the dye technique, but the taking of so many samples makes it unsuitable for ordinary use, particularly in paediatrics. An adaptation of the same method was later described for the photo-electric colorimeter (Gibson & Evelyn 1938).

A few years later, a simplification, so called, of the Gibson and Evans method was described (Harington, Pochin and Squire, 1940). whereby the haemoglobin, if haemolysis had taken place, plasma pigments, and lipides were extracted by butyl alcohol, after digestion of the proteins by pepsin. Thirty cubic centimeters of blood were said to be required. The procedure recommended is too laborious for clinical use.

Another method was based on the removal of plasma proteins, lipaemic opalescence, and the colour of the plasma pigments, by means of a mixture of concentrated hydrochloric acid and alcoholic phosphotungstic acid (Crooke and Morris, 1942; Morris, 1944). The presence of more than slight haemolysis, however, ruined the estimation, as the haemoglobin became converted to acid haematin, which has significant light absorption in the same part of the spectrum as Evans blue.

Yet another method of circumventing the effect of haemolysis has been suggested - namely, the reduction of the dye in the plasma specimen to a colourless compound by $Na_2S_2O_4$ in alkaline solution. The optical densities of the plasma before and after reduction are recorded. The absorption of light by haemoglobin and lipides is unaffected by $Na_2S_2O_4$. This method requires the use of a spectro-photometer, and is unsuitable for clinical use (Phillips, 1943).

Further investigation into the question of mixing time was carried out in 1945 (Cruikshank and Whitfield). By taking simultaneous samples from the femoral and jugular veins, they proved that mixing must really be complete at the end of one minute, as shown by identical concentrations of the dye reached simultaneously in the two veins. They suggested that the fall in concentration between one and six minutes, called the "mixing curve" by Gibson and Evans, and others, was in reality an "absorption curve" due to the rapid removal of the dye by the reticulo-endothelial system, which became saturated at the end of six minutes.

To remove this phase of rapid absorption, an ingenious device was proposed - that of injecting, half an hour prior to the blood volume estimation, either a small dose of the dye to be used, or else Indian ink, in order to "block" the reticulo-endothelial system. The "disappearance slope" then obtained was slow and gradual. One may argue, however, that even although mixing is complete throughout the large vessels at the end of one minute, the dye probably has not penetrated to the minute capillaries, where it is known that the plasma flow is very sluggish.

To summarise, the main difficulties inherent in the dye method are as follows:-

1. The presence of "foreign" colouring matter, e.g. haemoglobin, lipides, plasma pigments.
2. The determination of the mixing time.
3. The speed and manner of excretion.

The first two points have already been discussed in the various methods. The dyes in common use are eliminated at an average rate of 13.3 per cent in forty minutes (Dawson et al. 1920), while Evans blue disappears at rates varying from 5 to 8 per cent in forty minutes (Gregersen et al., 1935). They are taken up, partly by the reticulo-endothelial system and excreted via the biliary tract, and partly by the tissues, whence they pass slowly into the lymph and are returned to the blood stream. The return of dye via the lymph can be ignored in most experiments, as it has been shown to be negligible during the first hour after injection (Courtice, 1943; Price and Longmire, 1942; Gregersen and Rawson, 1943).

Of all the dyes, Evans blue may be said to be the most reliable, for the following reasons:-

1. It has the slowest rate of elimination.
2. Haemolysis can be detected readily.
3. The dye is absolutely non-toxic in the doses required for blood volume estimations.

(d) Radio-active Substances.

Several methods of utilising the property of radio-activity in blood volume estimations have been described in the literature.

1. Iron.

Radioactive isotopes of iron can be prepared by the bombardment of iron with deuterons in a cyclotron. The substance then owes its radio-activity to the emission of electrons or β rays. By including radio-active iron in the diet of the subject, production of corpuscles carrying radio-active iron can be stimulated, these erythrocytes being known as "tagged" red cells. The subject is then bled until all the tagged red cells have been removed, i.e. until no radio-activity can be detected in the circulating blood, and the volume withdrawn is noted. By comparison of the radio-active property of the packed cells of the blood before haemorrhage, and of the blood removed by haemorrhage, the original cell volume may be estimated. The radio-activity of the specimens is estimated by means of a Geiger-Müller counter (Hahn et al. 1942).

A variation of the method consists of injecting "tagged" red cells of known radio-activity, bled from a

subject who has been fed radio-active iron, and estimating the activity of a unit volume of the recipient's cells, after a reasonable time has been allowed for mixing. The cell volume is then obtained by proportion. The above authors emphasise that these methods estimate the true red cell volume, which in their hands is 10 to 40 per cent lower than the cell volume calculated from the plasma volume (dye method), and the jugular haematocrit reading. This discrepancy is similar to that described by Smith, Arnold and Whipple (1921) between the carbon monoxide and dye methods, and has the same explanation - namely, that the jugular or antecubital venous haematocrit reading is 25 per cent higher, on the average, than the mean haematocrit value for the entire circulation.

2. Iodine.

"Tagged" plasma proteins have also been used, in experiments investigating capillary permeability in shock, the tagging substance being radio-active iodine (Fine and Seligman, 1944).

3. Phosphorus.

Disodium hydrogen phosphate containing the radio-active isotope P^{32} has been used in rabbit experiments, with methods similar to those described for radio-active iron (Anderson, 1942).

Methods involving the use of radio-active substances are probably the most accurate of all the blood volume techniques so far described. The means of preparation, however, and the apparatus required, are too elaborate for ordinary clinical application.

Results of Previous Investigations.

The results for total blood volume obtained by the methods already discussed have been gathered together in the form of a table for comparison. The average blood volume per kilo is given for each series of estimations, with the range beneath in brackets.

Table 1.

Investigators.	Method.	Blood Volume ml. per kilo
Welcker (1858)	Direct	76.4
Bischoff (1856 and 1858)	Direct	74
Haldane and Smith (1900)	Carbon monoxide	47.8 (39.5-62.7)
Keith, Rowntree and Geraghty (1915)	Vital red	85 (78 - 97)
Salvesen (1919)	Carbon monoxide	59.5 (52.3-69.9)
Bock (1921)	Vital red	81
Plesch (1922)	Carbon monoxide	53.2 (46.9-60.5)
Brown and Rowntree (1925)	Vital red, and congo red	86 (72 - 100)
Chang and Harrop (1928)	Carbon monoxide	66.6 (60.4-75.5)
Giffin and Brown (1929)	Congo red	87.7 (70 - 100)
Uhlenbruck and Leyendecker (1931)	Congo red	80 (66 - 93)
Maltreider, Hurtado and Brooks (1934)	Brilliant vital red	79.1 (62.1-91.1)
Goldbloom and Libin (1935)	Trypan red	78.4 (66 - 90)

Investigators.	Method.	Blood Volume ml. per kilo
Gibson and Evans (1937b)	Evans blue Males Females	77.7) 66.1)±15%
Bennett, Dow, Lander, and Wright (1938)	Congo red	85 (78.9 - 99)
Ebert, Stead and Gibson (1941)	Evans blue	81.8 (71.9 - 93.5)
Davis (1948)	Evans blue	76.7
Hopper, Tabor and Winkler (1944)	Evans blue	80.5 (71.9 - 89.1)
	Carbon monoxide	80.2 (74.9 - 85.5)
Noble and Gregersen (1946b)	Evans blue	85.1 (65 - 100)
2. Children up to 2 years.		
Lucas and Dearing (1921)	Brilliant vital red	109 (90 - 126)
Bakwin and Rivkin (1924)	Brilliant vital red	101 (71 - 148)
McIntosh (1929)	Carbon monoxide	77 (55.6 - 112.1)
	Brilliant vital red	93 (58.3 - 168.6)
Brines, Gibson and Kunkel (1941)	Evans blue	73.6 (46.5 - 95.9)
3. Older Children.		
Seckel (1936)	Congo red	83 (73 - 93)
Brines, Gibson and Kunkel (1941)	Evans blue	69.8 (52.5 - 88.9)

Part II

Introduction.

The plasma and blood volumes have been estimated in five groups of children. The first group comprises healthy children in each year of childhood up to thirteen years of age (Section 2). The other groups include marasmic children, children with cardiac disease, infants with gastro-enteritis, and infants and children suffering from anaemia (Sections 3 - 6). For convenience, a short summary of the results and conclusions has been given at the end of each section.

The results were obtained by methods using the dyes Congo red and Evans blue. No differentiation has been made between the results, but for purposes of reference, the congo red investigations have been marked with an asterisk throughout the tables.

When required for relating blood and plasma volumes to body measurements, the surface area was calculated from the formula of Du Bois (Du Bois and Du Bois, 1916)

Section 1.

Methods employed in Present Series.

For the estimation of the plasma volume, the dye dilution method has been used throughout. Using the same specimens of blood as were withdrawn for the plasma volume estimations, investigations were also done, (a) by a photo-electric method on the haemoglobin level of the blood using oxyhaemoglobin (Bell, Chambers and Waddell, 1945),(b) on the red cell count, and (c) on the packed cell volume; and finally, in the children with disease, on the blood non-protein nitrogen and total plasma proteins by micro-Kjeldahl methods. In all, 156 blood volume estimations have been successfully completed, part of the work being done during tenure of a Muirhead Scholarship.

(1) Congo red was used for the first forty estimations. The dye was prepared as a 1 per cent solution in sterile water and was injected in the following dosage:-

Infants, 2ml. of a 1 per cent solution i.e. 20mg.
1-4 yrs, 3ml. " " " " " " i.e. 30mg.
4-12 yrs, 4ml. " " " " " " i.e. 40mg.

As the colour of congo red masks the presence of haemoglobin, the utmost precautions were taken to prevent haemolysis of the samples. Needles and syringes were rinsed in normal saline before use, and samples were withdrawn and discharged without frothing.

One ml. of the 1 per cent dye solution was used to prepare varying dilutions of the dye to be read on the photo-electric colorimeter, using the green filter (Ilford No.3)

these dilutions ranging from 1 in 30,000 to 1 in 100,000, and the readings were plotted on a graph. Although it was realised that congo red in water and congo red in plasma gave different absorption curves (Heilmeyer 1929) water was used for congo red solution in all instances as in the case of small children one could not remove enough blood to make up four or five dilutions each requiring 3 ml. of plasma.

(2) Evans Blue Method.

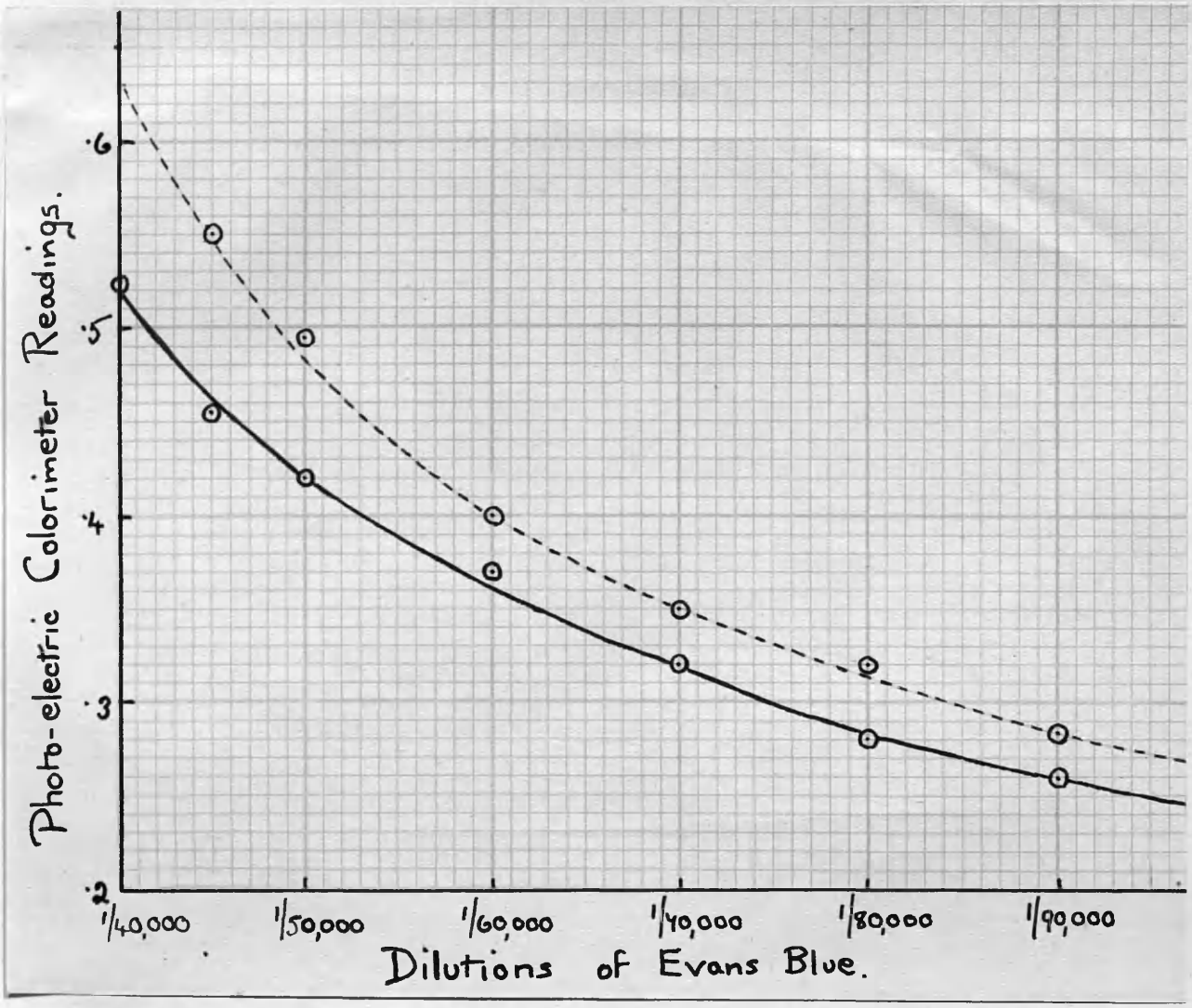
Evans blue was substituted for congo red in all the recent experiments as it possessed the advantages of being more slowly excreted and showed the presence of haemolysis, by changing colour. The dye was prepared in 0.5, 0.2, and 0.1 per cent solutions in distilled water, and the solutions were sterilised by Seitz filtering. The amount injected varied between 0.5 and 0.8 mg. per kilo. of body weight, as amounts within these limits were found to give suitable dilutions for reading on the photo-electric colorimeter. The syringe used for injecting the dye was graduated in fifths of a ml. up to 5 ml., and was found to be accurately calibrated. The same syringe was used throughout for all the experiments.

Standards.

Fresh dye solutions were prepared every four to five weeks, and with each batch, standards were prepared in blood bank plasma, and the photo-electric colorimeter readings graphed. The 5 ml. syringe, used for the dye injections, was used to measure out 5 ml. of each Evans blue solution

Graph 1.

Light absorption curves of Evans blue dilutions (a) in normal saline (b) in plasma.



⊙---⊙---⊙ Dilutions of Evans blue in normal saline.

⊙—⊙—⊙ Dilutions of Evans blue in plasma.

as used for injection, and was always rinsed out once. Thereafter the dilutions were prepared with a standard volumetric flask and an accurate burette.

As the absorption curve of Evans blue dilutions in plasma differs from that of the same dilutions in normal saline, it is clear that plasma must always be used for the standards to obtain accurate readings. The small reading due to plasma alone, was of course deducted from the reading obtained for each dilution, before construction of the graph (Graph 1). (Kennedy and Millikan, 1938; Gregersen and Gibson, 1937; Graff and Clarke, 1931).

Mixing Time.

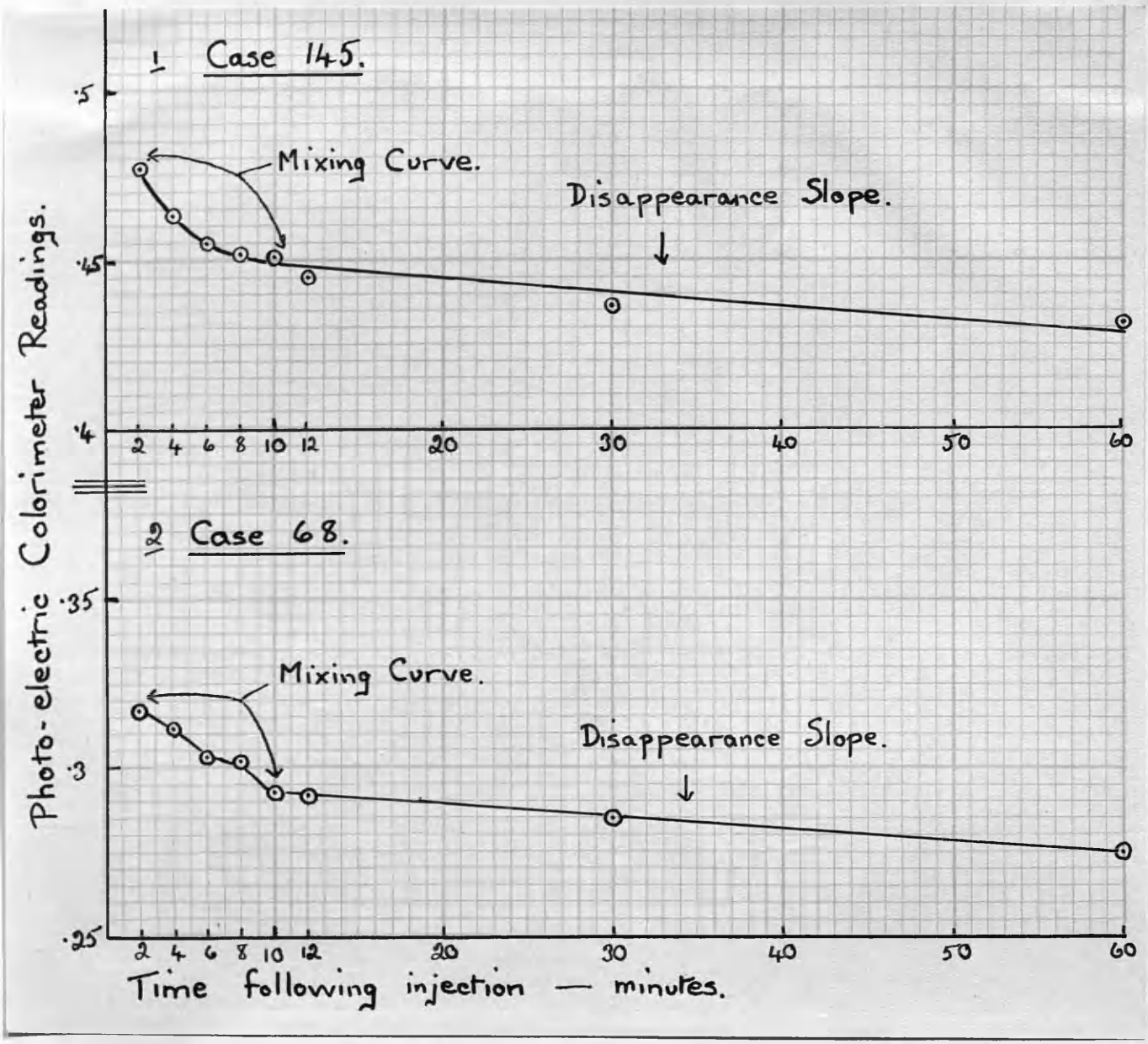
The mixing time chosen for the experiments was 10 minutes, the choice being based on the results of two experiments on big children, from whom blood was withdrawn at regular intervals (Graph 2). The form of the curves agrees with the form of those found by Gibson and Evans (1937a), and may be said to consist of a mixing curve covering 8 to 10 minutes, and a disappearance slope. That the mixing time allowed should be 10 minutes is advocated frequently in the modern literature (Davis, 1942; Gregersen, 1944; Noble and Gregersen, 1946a). Earlier workers gave conflicting opinions:- 2 min. (Dawson et al., 1920); 4 min. (Keith, Rowntree, Geraghty, 1915, and others); 5 min. (Kottmann, 1906); 6 min. (Graff, D'Esopo and Tillman, 1931).

Distribution of dye in body.

In the plasma, both congo red and Evans blue are bound to the albumin fraction of the plasma proteins, as it was

Graph 2.

Time-excretion graphs for Evans blue.



observed that after precipitation of fibrinogen and globulin, the remaining solution was dye stained. This was also demonstrated by Rawson in 1943 by electrophoresis experiments and ultra-centrifugation of albumin solutions.

The dye does not enter the cellular elements of the blood in any appreciable amount (Gregersen and Shiro, 1938), neither is it adsorbed by the red cells (Haizels, 1945).

After circulating for several hours, the dye, especially if Evans blue had been used, could be detected in the skin.

The dyes do not enter the cerebro-spinal fluid and do not appear in the urine, which findings were also recorded by Gibson and Gregersen (1935).

In traumatised regions, escape from the cardio-vascular system may be abnormally rapid (Price and Longmire, 1942). This phenomenon was observed in the case of a child who had severe weeping eczema of the scalp, and whose dressings showed blue staining within an hour of injection.

Excretion.

Excretion takes place via the reticulo-endothelial system and the biliary tract. Several of the infants of the group examined, caused alarm to members of the nursing staff, by passing bright pink stools following congo red injections.

From the results shown in Graph 2, the average rate of excretion of Evans blue was calculated to be 9 per cent in the first hour. Gregersen and Rawson (1943), found the average rate of excretion in dogs to be 8.8 per cent in

the first hour.

Toxicity.

No toxic effects have been observed after the injection of congo red or Evans blue. Two children had a slight rigor fifteen minutes after the injection, followed by a sharp rise in temperature to 101°F., lasting only a few hours. This was attributed to pyrogens in the water.

Procedure.

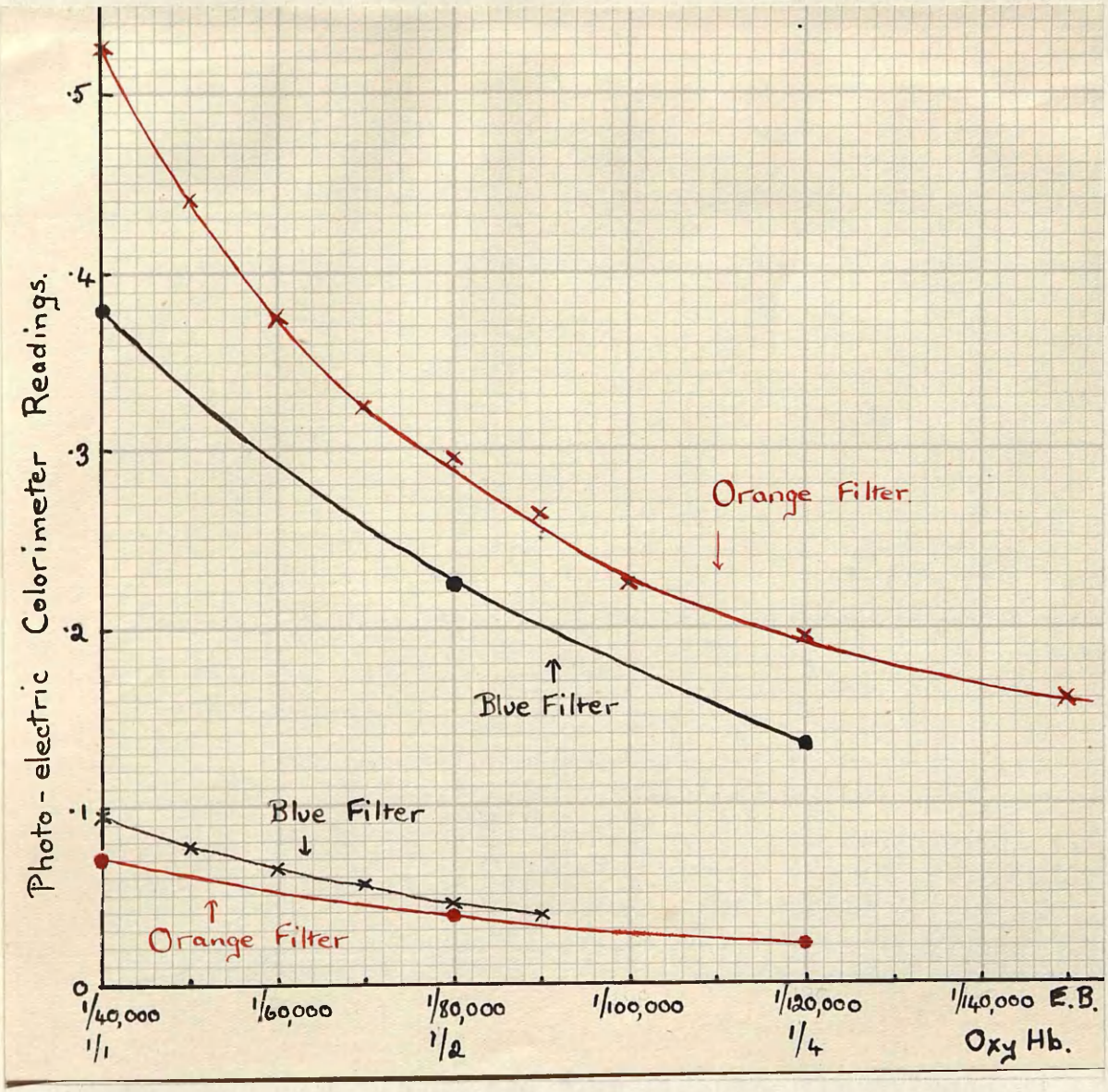
On the morning of the experiment, the children were kept in bed, and given nothing to eat or drink after 9 a.m. save sips of water. Estimations were usually carried out five hours later, between 2 and 3 p.m. Immediately prior to the injection, the weight and height were recorded.

1. 6-8 ml. of blood were removed, and through the same needle, the dye was injected. Blood was withdrawn into the dye syringe, and the syringe washed out once.
2. Mixing time of 10 minutes was allowed.
3. 6-8 ml. of blood were removed by venepuncture. If at all possible a different vein was used, from that into which the dye had been injected.

Samples were withdrawn into dry syringes with the minimal venous stasis, and obstruction released as soon as the vein had been entered. The blood was ejected gently into graduated haematocrit centrifuge tubes containing 0.3 ml. of a mixture of 2 per cent potassium oxalate and 3 per cent ammonium oxalate - a solution which does not alter the osmotic pressure of the plasma (Wintrobe and Landsberg, 1935). The tubes were then covered with a light aluminium cap to prevent evaporation, and centrifuged at

Graph 3.

Light absorption curves of solutions in plasma (a) of Evans blue, and (b) of oxyhaemoglobin.



}--- Evans blue dilutions.

}--- Oxyhaemoglobin dilutions.

With Evans blue solutions, maximal light absorption was obtained with the orange filter and minimal with the blue. With oxyhaemoglobin solutions, the converse was found.

2800 r.p.m. for thirty minutes. The haematocrit reading was taken as the average of the two tubes, after allowance was made for the dilution. The haemoglobin, red cell counts, and plasma proteins estimations, were carried out on the first sample of blood.

After "setting" the photo-electric colorimeter, the undyed plasma was placed in the specimen cup and a reading taken, to be followed by a reading for the dyed plasma. By subtracting the former from the latter result, the colour effect of natural plasma was removed, and the reading due to dye alone obtained. From the graph of standard dilutions, the dilution attained by the dye in the plasma was then read.

For the Evans blue method, readings were taken routinely on the orange filter (Ilford No.5) as this was found to give the maximum deviation of the galvanometer with Evans blue, and the minimum with haemoglobin. (Graph 3). The graph also demonstrates that with the blue filter (Ilford No.1) maximum light absorption is obtained for haemoglobin, whereas Evans blue gives minimal results. These facts were utilised in the presence of haemolysis in the dyed specimen in the following manner. Readings were taken on both orange and blue filters, the values due to the undyed plasma subtracted, and the error due to dissolved haemoglobin corrected with this formula.-

$$\text{Corrected } R_O = \frac{5.66R_O - R_B}{5.66 - 0.169}$$

where R_O and R_B were the readings on the orange and blue

Table 2.

Case	Date	Dye	Weight kilos.	Plasma Volume Total ml.	Plasma Volume per kilo ml.	Per cent deviation Total	Per cent deviation Per kilo
52	31. 1.47	E.B.	21.32	1058	49.6		
	7. 2.47	E.B.	21.52	1066	49.6	+0.7	0
66	5.10.45	C.R.	28.4	1659	58.4		
	1.11.45	C.R.	27.68	1618	58.4	−2.4	0
139	8. 3.46	E.B.	17.4	1191	68.4		
	13. 3.46	C.R.	17.5	1256	71.7	+5	+4
102	9. 5.46	E.B.	23.5	1288	54.8		
	23. 5.46	C.R.	22.2	1216	54.8	−5.5	0
62	15. 4.46	E.B.	22.4	1149	51.3		
	23. 5.46	C.R.	22.6	1272	56.3	+10	+9

E.B. = Evans blue C.R. = congo red

filters respectively. These factors were the averaged ratios of readings on the blue filter to readings on the orange, 5.66 applying to haemoglobin solutions, and 0.169 to Evans blue. The latter factor required to be defined with each fresh batch of dye.

The formula was obtained as follows:-

Let x = reading due to haemoglobin on the orange filter

Then $R_O - x$ = reading due to Evans blue on the orange.

$\therefore$ $0.169(R_O - x)$ = reading due to Evans blue on the blue filter.

$$= R_B - 5.66x$$

$$\therefore x = \frac{R_B - 0.169R_O}{5.66 - 0.169}$$

$\therefore$ Corrected $R_O = R_O - x$

$$= \frac{5.66R_O - R_B}{5.66 - 0.169}$$

If haemolysis was present in the first blood sample (undyed plasma) the experiment was discarded.

The final calculation is simple -

$$\text{Plasma volume} = \frac{D \times a}{y}$$

where D = the dilution of dye in the plasma,

a = amount of dye injected in grams,

y = diluting factor involved by the addition of anticoagulant.

Accuracy.

Owing to the objection of children to "jags", and in the case of Evans blue, to the temporary staining of the skin which occurs after repeated estimations, only five children were submitted to more than one injection (Table 2).

The first two experiments show that identical results were obtained with repeat estimations with both Evans blue and congo red, allowing for change in weight. The second three were carried out to ascertain if results by the two methods were comparable. As the figures agreed within plus or minus ten per cent, it was decided to tabulate the congo red results along with those obtained with Evans blue. The large deviation of ten per cent was found in a child in whom the two experiments were separated by an interval of six weeks.

Experimental Error.

In many duplicate readings, the photo-electric absorptiometer used gave a maximum error of two per cent. Errors involved in weighing out the Evans blue and in making up the three solutions were compensated by the fact that standards were prepared from each of the dye solutions as used for injection. As regards the actual dye injection, the syringe was washed out once, which was also the procedure used in making up the standards. If any dye was injected round the vein, the experiment was of course abandoned. The haematocrit method had a maximum error of $\pm$ 2 per cent.

Section 2.

Blood Volume in Health.

As previous investigations in normal children gave varying results, due mainly to the use of the red dyes, and the inherent fallacies of the technique, it was judged advisable to define the range of normality in healthy children before proceeding to study the blood volume in disease. The only other large series published is that of Brines, Gibson and Kunkel (1941) comprising fifty children from infancy to sixteen years of age. Table 3 showa an analysis of the results of the present series tabulated along with those of Brines, Gibson and Kunkel, and of other authors using the Evans blue method in adults.

Table 3.

Results with the Evans blue technique.

1. Plasma Volume.

	No. of Cases	ml. per kilo Plasma Volume		
		Range	Mean	S.D.
(a) Adults.				
Gibson and Evans, 1937b	49 ♂ 41 ♀	32 - 58 27 - 52	43.1 ? 41.5	-
Noble and Gregersen, 1946b	51	34 - 58	44.7	4.9
Davis, 1942	11	?	40.5	5.7
Crooke and Morris, 1942	10	41 - 55	48.7	-
(b) Children.				
Brines, Gibson and Kunkel, 1941	50	32 - 55.4	41.8	-
Present Series	80	35 - 58.4	47.8	5.83

Table 3 contd.

2. Blood Volume.

	No. of Cases.	Range	Mean	S.D.
Brines, Gibson and Kunkel, 1941	50	46.5 - 95.9	69.8	-
Present Series	80	62 - 113	83.5	10.39

S.D. = standard deviation.

The present studies included 80 children, from infancy up to thirteen years, 42 being females, and 38 males. The limits of normality decided upon were rather wide, due to the fact that one was dealing with a hospital population. The child chosen for investigation had to be not less than 80, and not more than 110 per cent of the expected weight as judged from Holt's tables, he had to be on full diet and convalescent from his illness, and the haemoglobin level of the blood had to be over 10 grams per cent. Two infants, aged three and nine months, however, were included with haemoglobin values of 9.5 grams per cent, as that level could not really be regarded as indicating anaemia at that age (Mackay, 1933). The studies have been grouped according to age, the number being as follows:-

a. Ten infants, aged three to eleven months.

b. Six children in each year of life up to eleven years.

c. Five children in each of the two remaining years, eleven to twelve, and twelve to thirteen.

The individual results are shown in Tables 6 - 12, which have been inserted at the end of section to avoid encumbering the text. The average figures for absolute plasma and blood volumes, taken from these tables, are

Table 4.

Normal Children.

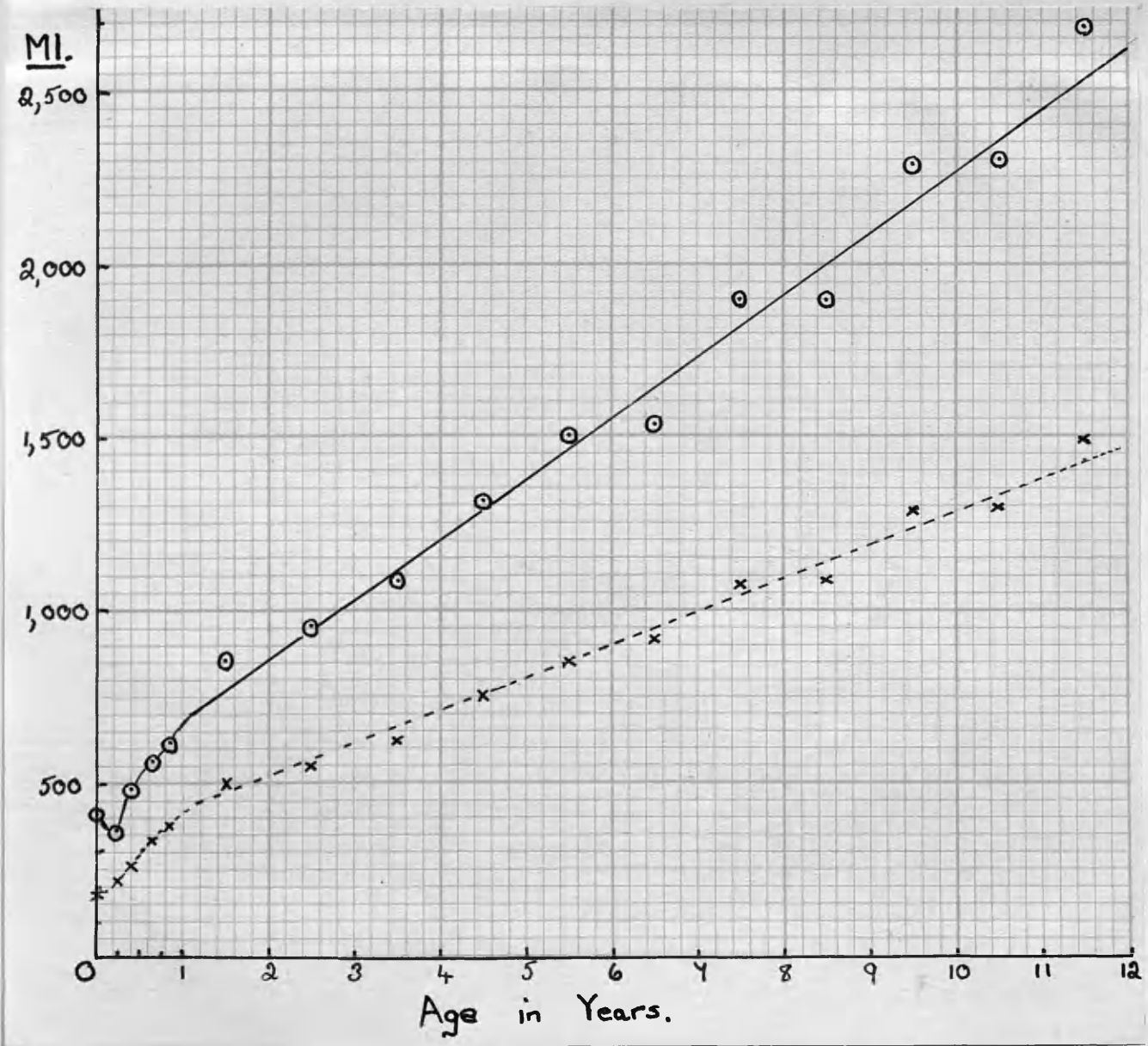
(Average values)

Age years	No. of Cases	Plasma Volume ml.	Blood Volume ml.
$\frac{1}{365}$	1	180	418
- $\frac{3}{12}$	1	213	359
$\frac{4}{12}$ - $\frac{6}{12}$	3	273	487
$\frac{7}{12}$ - $\frac{9}{12}$	3	338	574
$\frac{10}{12}$ - $\frac{12}{12}$	3	379	623
1-2	6	502	857
2-3	6	547	956
3-4	6	625	1090
4-5	6	752	1316
5-6	6	855	1500
6-7	6	913	1535
7-8	6	1083	1902
8-9	6	1092	1898
9-10	6	1292	2288
10-11	6	1297	2317
11-12	5	1495	2682
12-13	5	1304	2397

The one-day old baby included in the table was not a healthy child, but had erythroblastosis foetalis (Case 143). As the haematocrit reading was high (57 per cent) and haemolysis only slight, the blood volume result has been used for the above table to complete the series of normal children.

Graph 4.

The increase of absolute plasma and blood volumes with age, plotted from the average values for each age group. (Table 10)



⊙ ⊙ ⊙ Blood volume.

× × × Plasma volume

gathered together in Table 4.

Discussion.

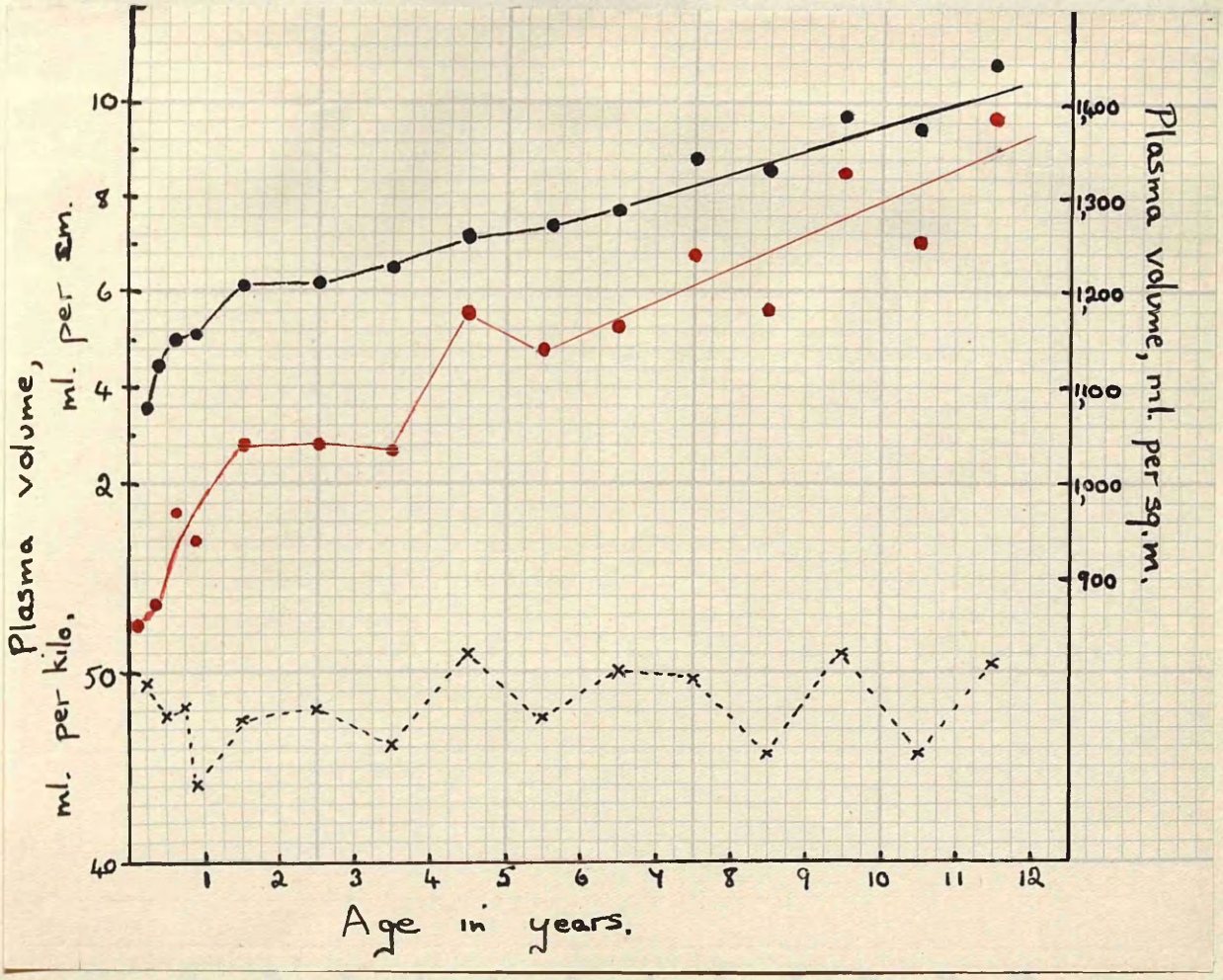
1. The influence of age.

The total blood volume was relatively high at birth, fell in the following two or three weeks and had reached 300 ml. at 3 months, was doubled by the end of the first year, and thereafter showed a steady increase with advancing age, to about 2½ litres at 11 years. The plasma volume followed the trend of the total blood volume fairly closely after the first few months of life, but in the first few months after birth it increased steadily, in contrast to the blood volume, which decreased because of the destruction of the excessive red cells in the circulation. Seckel (1936) was of the opinion that relatively high values were obtained at two periods during childhood, namely between three and six years, and between eleven and thirteen. There is no indication of this in the present series (Graph 4). The irregular mode of increase in the later age groups after 6 years was probably due to the overlapping of weights and heights in the consecutive ages.

Unit plasma and blood volumes related to body weight fluctuated from age group to age group, but showed no consistent tendency to increase or decrease (Graphs 5 and 6). The plasma volume in ml. per kilo. in the group of infants gave an average figure of 47.1, ranging from 39.8 to 50.6 ml. per kilo. These results agree with those of Brines, Gibson and Kunkel (1941), but are at variance with

Graph 5.

The relationship of unit plasma volume, to age plotted from the average values for each age group.



Plasma volume, ml. per cm.

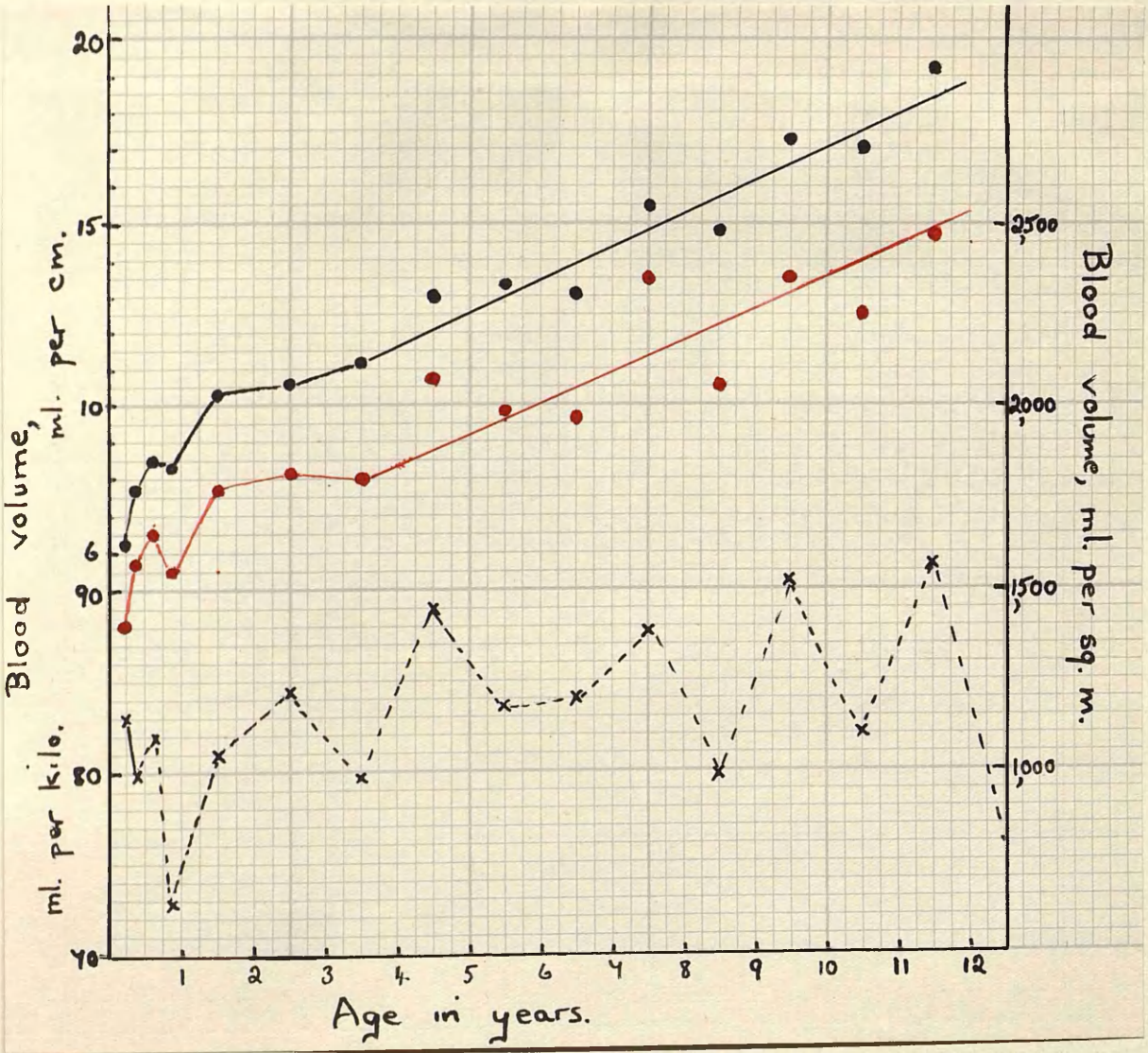
Plasma volume, ml. per sq.m.

Plasma volume, ml. per kilo.

Unit plasma volume related to height and surface area increased with advancing age.
Unit volume per kilo showed no consistent tendency to increase or decrease.

Graph 6.

Relationship of unit blood volume to age plotted from the average values for each age group.



x---x---x Blood volume, ml. per kilo.

Blood volume, ml. per cm.

Blood volume, ml. per sq.m.

Unit blood volume related to height and surface area increased with advancing age.
Unit volume per kilo showed no consistent tendency either to increase or decrease.

those of Bakwin and Rivkin (1924), who found an average of 60.5 ml. per kilo for the plasma in normal infants, with a range of 38 to 72 ml. These authors explained their results as being due to the fact that fluctuations in body weight at that age were often due to changes in the water content of the tissues and not to real tissue loss or growth. In a healthy infant, however, on an adequate caloric and fluid intake, and in the absence of diarrhoea, vomiting, and fever, there is no justification in the present results at least, for postulating an unstable system of fluid exchange between tissues and plasma. Unit volume related to height and surface area showed a general tendency to increase with advancing age. During the first eighteen months, the rise in both plasma and blood volumes was very rapid in relation to both height and surface area. Between 1½ and 3 years, the plasma volume expressed as ml. per cm. and ml. per sq. m. remained stationary around 6.2 ml. and 1040 ml. respectively (Graph 5). To a lesser degree, unit blood volume related to height and surface area showed the same tendency towards a decrease in the rate of growth. A similar "halt" in the rate of increase of plasma per square metre was recorded by Darrow, Soule, and Buckman (1928), but no attempt was made to explain the finding. When Holt's tables of normal average weights and heights were graphed against age, a flattening in the curve of weight gain could be discerned between the ages of one and two years. It was concluded that the rate of synthesis of

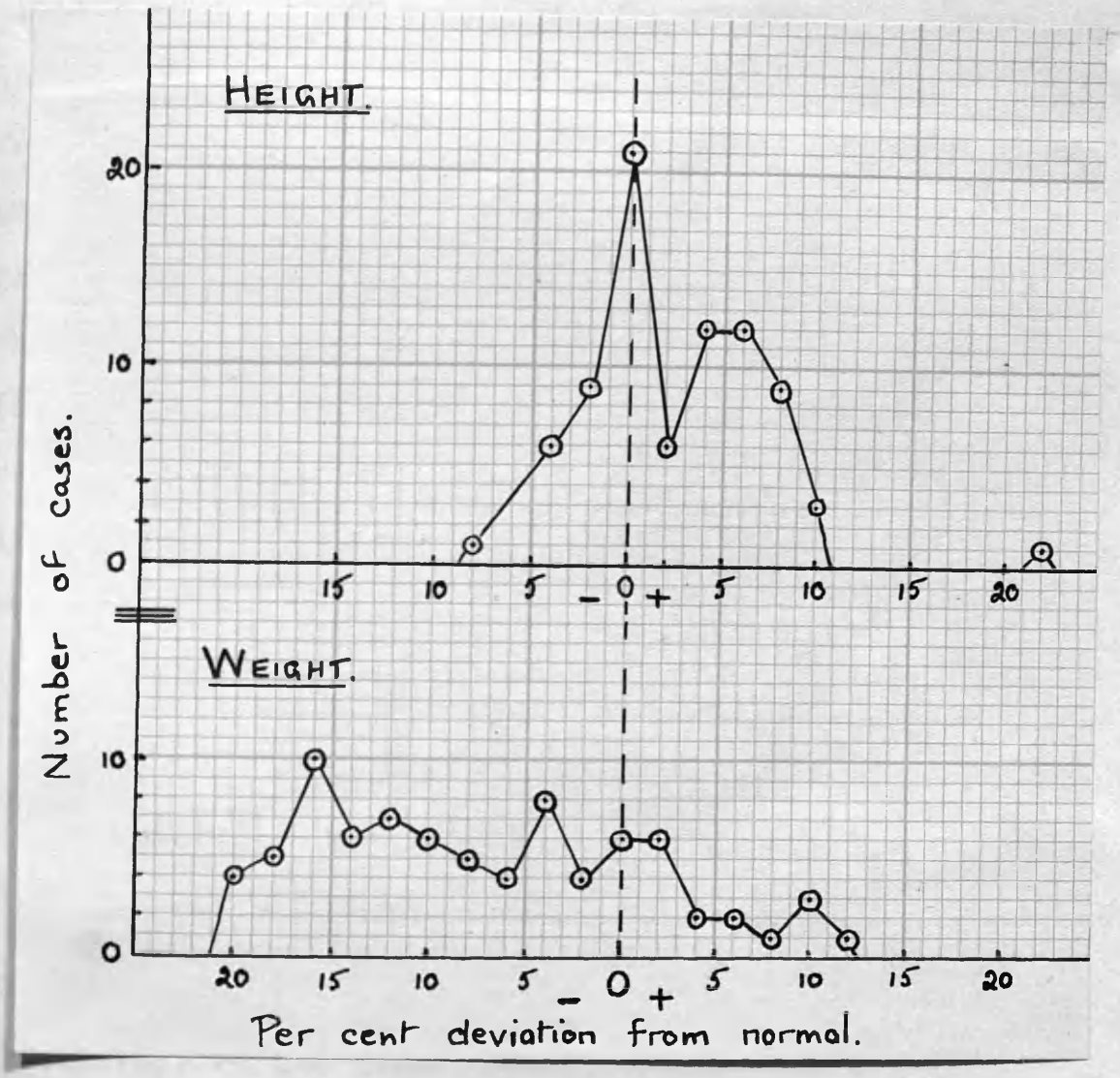
new tissue was slower at that period, and that probably the rate of increase of the blood volume was correspondingly slower. Consequently, since the rate of height gain showed less tendency than the rate of weight gain to decrease between one and two years, unit plasma volume per cm., and also per sq. m. (since height is involved in the calculation of surface area) will show a tendency to remain constant.

2. The influence of increase in body measurements.

As it was felt that attempts to relate blood volume results to age might be fallacious, due to variation of weight within each age group, the results have been rearranged according to increasing weight, height and surface area. The absolute plasma volume increased with growth as regards all three measurements, the relationship giving straight line graphs for weight and surface area, and a curved line graph for height. A fairly wide scatter existed of the individual results (Graphs 14 - 16). These graphs are inserted at the end of the section for purposes of reference. The results for absolute blood volume gave a similar picture (Graphs 17 - 19). A comparison of these graphs shows that there was no striking difference in the correlation with the three factors. By the use of the graphs to read the expected values for plasma and blood volumes, it was found that equally good approximations to the actual findings with the dye method were obtained from all three.

Graph 7.

Frequency distribution of the series of normal children, showing percentage deviation from expected weight and height.

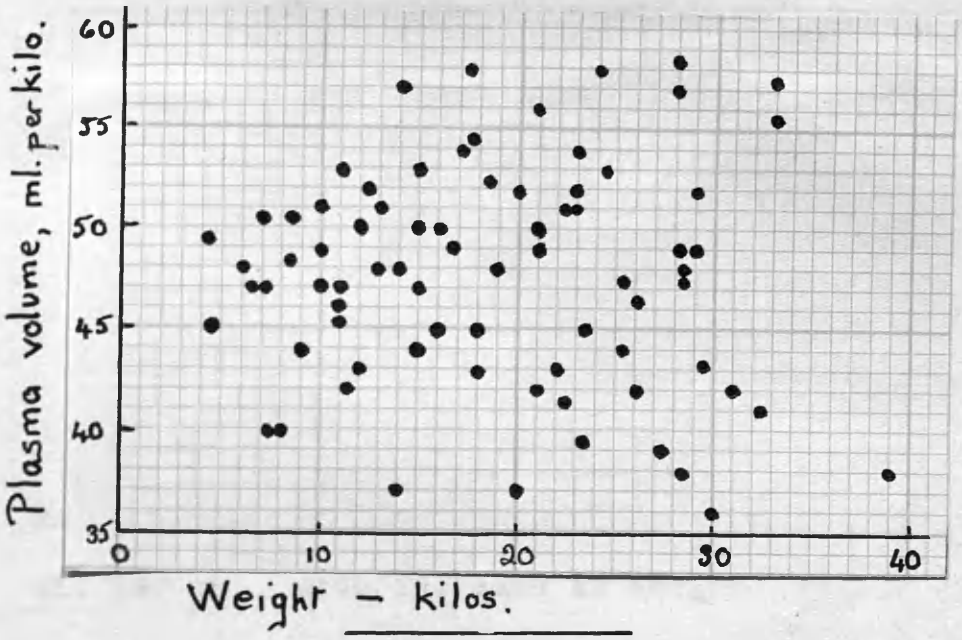


It is clear that in the healthy children greater variation existed as regards weight than as regards height.

Previous workers have held diverse opinions about the correlative factor of choice for blood volume. Dreyer and Ray (1910) advocated surface area, after their studies of animals of varying size, and stated "that it is absolutely unjustifiable to consider blood volume as a function of the weight of an animal, since the body weight divided by the blood volume is never a constant for animals of different weight". Gibson and Evans (1937b) found that the blood volume of an individual could be predicted on the basis of height, weight, or surface area, only within wide limits. Investigations in children have also led to conflicting opinions - McIntosh (1929) and Darrow, Soule and Buckman (1928) advocated weight as a basis of correlation; while Brines, Gibson and Kunkel (1941) came to the conclusion that correlation with the three body measurements appeared to be similar. In consideration of the fact that weight, and therefore also surface area, was such a variable factor in children, even in health (Graph 7), and that in dehydration and oedema such gross changes could take place in the weight of the soft tissues, it was decided to correlate the results with the child's height, which was a constant factor (Gibson and Evans, 1937b; Brines et al. 1941; Perera, 1946). Height was chosen in preference to ideal weight, advocated by some authors, as the former was a property of the child which could actually be measured, whereas the latter was the average weight calculated from a number of children of a certain age, and might differ

Graph 8.

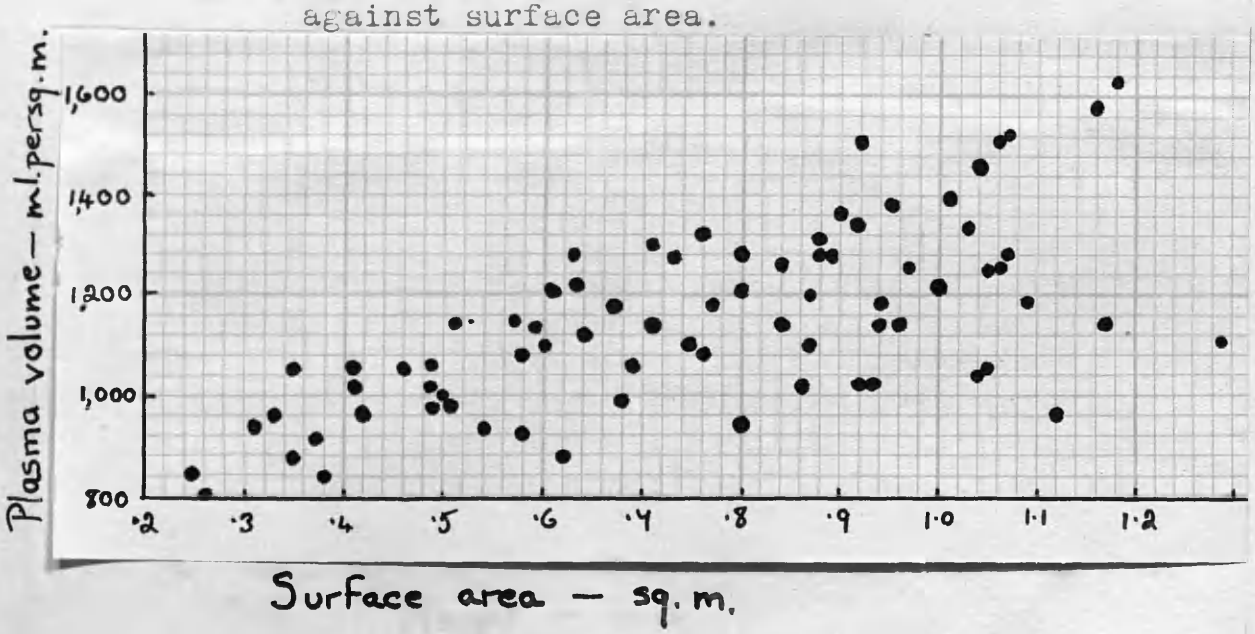
Plasma volume, in ml. per kilo, graphed against weight.



Unit plasma volume bears no relationship to increase in weight.

Graph 9.

Plasma volume, ml. per sq.m., graphed against surface area.



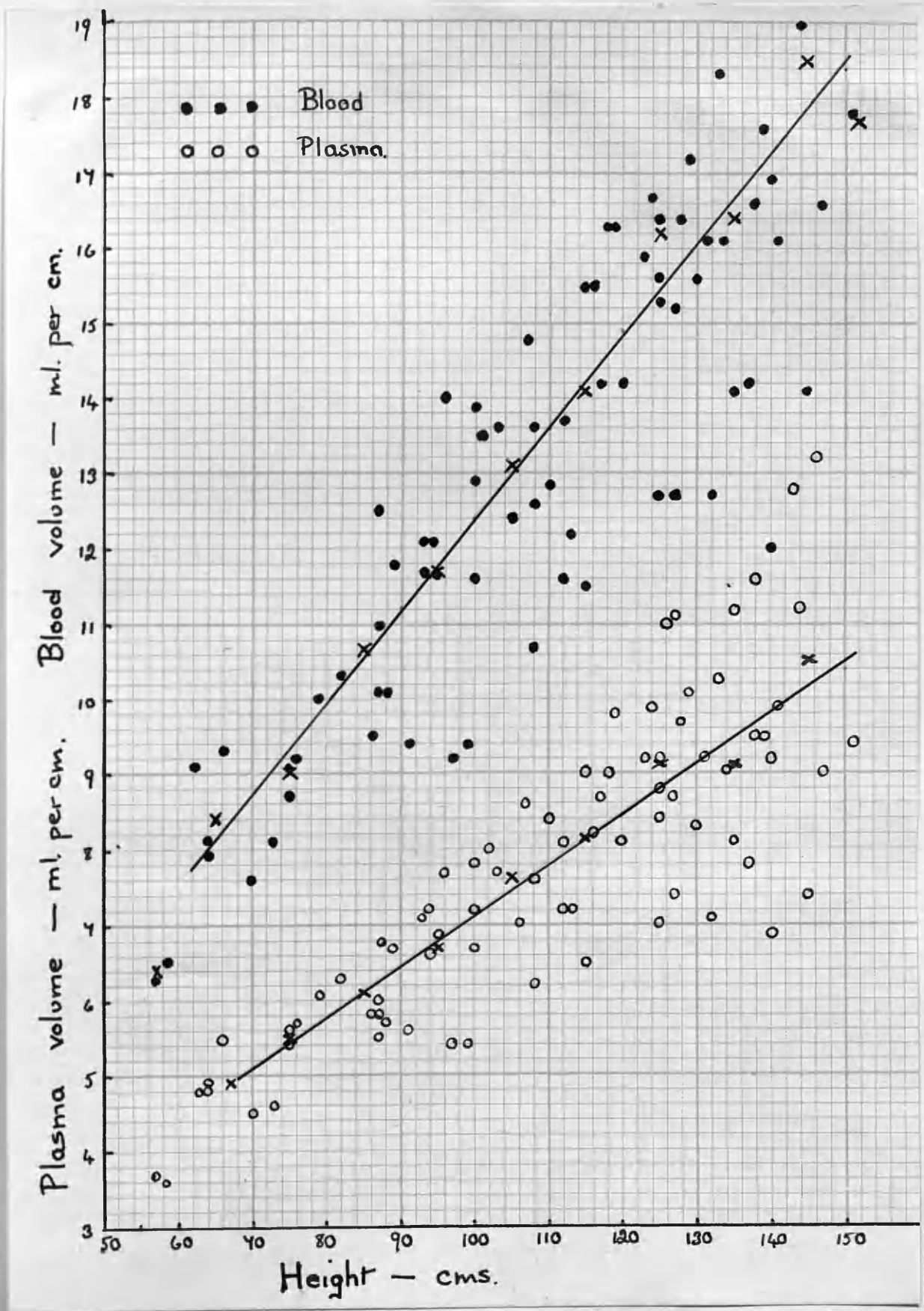
Unit plasma volume increases with increase in body surface.

Graph 10.

The increase of plasma and blood volumes, in ml. per cm., with increase in height.

The lines drawn through the average results have been used in the sections on disease to calculate the expected normal values related to height.

Graph 10.


Blood
Plasma.
Blood volume — ml. per cm.
Plasma volume — ml. per cm.
Height — cms.
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
50
60
70
80
90
100
110
120
130
140
150

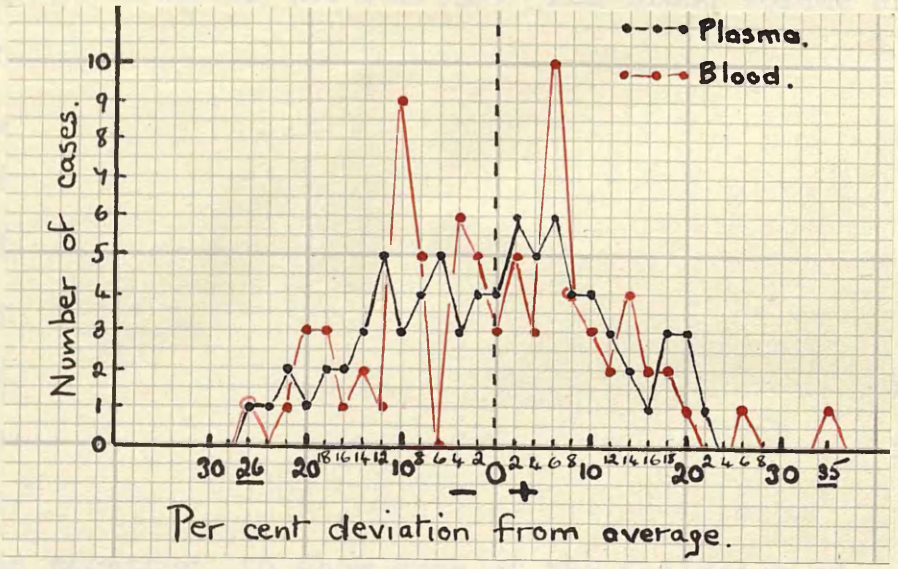
considerably from the weight in health of the particular child under observation.

Unit plasma volume when graphed against increasing weight, merely showed an indeterminate scatter within the range of the series. Plasma volume expressed as ml. per square metre, and ml. per cm., showed a definite increase with increase of surface area and height respectively (Graphs 8 - 10). Of these last two factors, height appeared to show the better correlation (i.e. for unit volume). The graphs for unit blood volume were similar in form to those for unit plasma volume, and again, height gave the best correlation.

The frequency distribution of the series has been worked out as regards two findings (a) unit volume expressed as ml. per kilo, and (b) unit volume expressed as ml. per cm. (a) For each observation the percentage deviation from the average of the series for unit volume per kilo was calculated, and a graph drawn of the distribution, (Graph 11) - the average unit volume per kilo being 47.8 for plasma and 83.5 for blood. (b) From the curves of unit volume per cm., drawn through the average values for the series when grouped according to each 10 cm. of height, the expected plasma and blood volumes per cm. were read for each child's height (Graph 10). The percentage deviations of the actual findings from these average values were then calculated, and the distribution of the cases graphed (Graph 12). A comparison of Graphs 11 and 12 shows that as regards both methods of expressing unit

Graph 11.

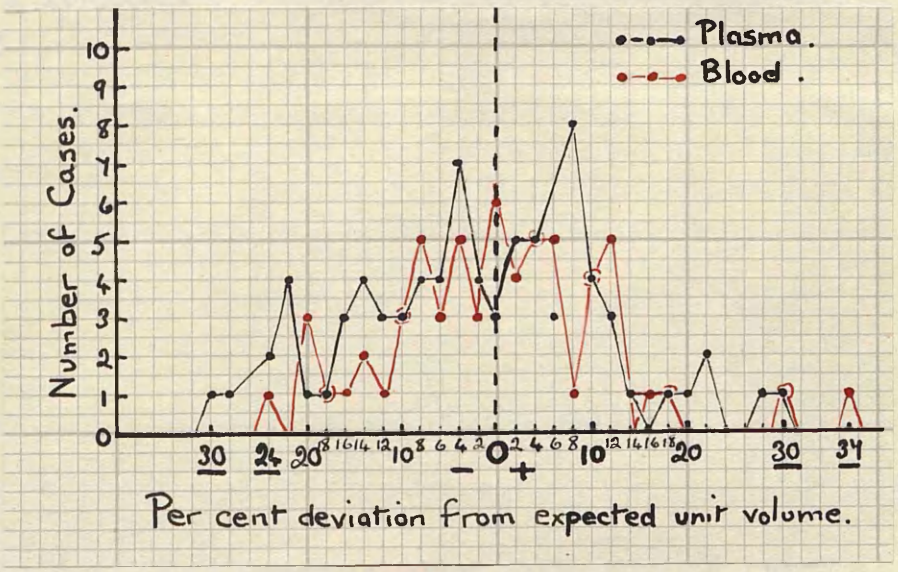
Distribution of the series round the average unit volume related to weight.



P.V. per kilo varies between +22 and −26 per cent of average.
79 per cent of the results are within ±15 per cent of average.
B.V. per kilo varies between +35 and −26 per cent of average.
80 per cent of the results are within ±15 per cent of average.

Graph 12.

Distribution of the series round the expected unit volume related to height.



P.V. per cm. varies between ±30 per cent of expected values.
80 per cent of the results are within ±15 per cent of average.
B.V. per cm. varies between +35 and −26 per cent of average.
80 per cent of the results are within ±15 per cent of average.

volume, 80 per cent of the results fell within plus or minus 15 per cent of the average. For blood volume alone, the range of variation was the same for weight as for height, while plasma volume showed greater limits of percentage deviation on the height basis. The reason for this difference was not apparent.

Children outwith the Range of Normality.

Three children who were at the extremes of normality as regards body measurements, and three who were outwith the range chosen for the healthy group, have been examined together, to show some of the fallacies of relating blood volume to weight alone. The per cent deviations of the expected values for weight, height and surface area from the actual findings with the dye method are listed below. (The expected values were read from Graphs 14 - 19).

Case	Per cent of expected		Absolute blood volume. Percentage deviation.		
	weight	height	Weight	Height	Surface area.
4	110	101	+25.6	-9.8	+12.8
81	130	106	+34.2	+2.9	+18.5
82	120	115	+13.4	-2.5	+11.2
83	111	105	+15.1	+0.1	+9.6
63	110	110	-12.7	-18.6	-15.8
41	82	122	-12.0	+49.0	+16.8

It is clear that for cases 4, 81, 82 and 83, height was the correlative factor of choice. All of these children were overweight, and all were less abnormal as regards height than as regards weight. In cases 63 and

41, however, weight gave a closer approximation than height. It is probable that for the usual deviations from normal size, height is the better basis for the comparison of blood volume with normal children. Cases 63 and 41 however, had deviations from normality in height of +10 and +22 per cent respectively, both of which represent a greater departure from average than similar deviations regarding weight.

3. The influence of sex.

Although it has been proved that adult females have a lower absolute blood volume than males, and also a lower unit volume when related to weight due to their greater proportion of fat (Gibson and Evans 1937b), it was unexpected to find this difference in children also.

Table 5 shows the analysis of the results gathered in two groups, male and female.

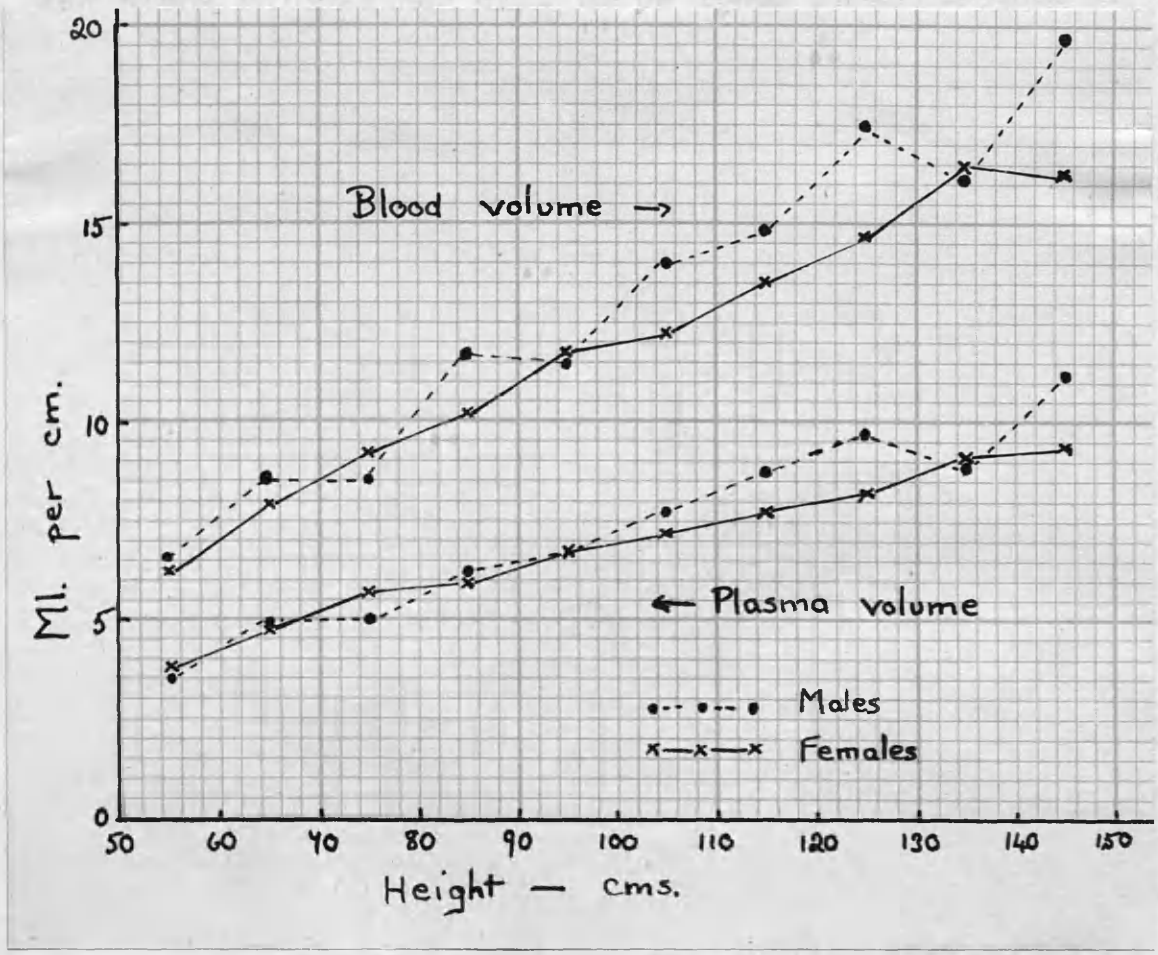
Table 5.

	Plasma Volume ml. per kilo			Blood Volume ml. per kilo		
	Mean	Range	S.D.	Mean	Range	S.D.
38 boys	48.8	36.8 to 58.4	5.9	86.0	67.4 to 113.0	9.95
42 girls	46.9	35.8 to 58.4	5.9	81.3	62.0 to 105.2	9.38
Difference	1.9			4.7		
Standard error of difference	1.32			2.17		

S.D. = standard deviation.

Graph 13.

The sex difference in plasma and blood volumes per cm., when graphed against height.
The readings are the average values for the two sexes, when grouped according to each 10 cm. of height.



There was a definite tendency for both plasma and blood volumes to be higher in boys than in girls of the same height.

The figures show that the female children had lower plasma and blood volumes per kilo of body weight than the males. The difference for the total blood volume per kilo is statistically significant, as the difference is more than twice the standard error of difference. These lower values in the female group are not merely due to the fact that the girls of the group were fatter than the boys. A definite tendency for the unit plasma and blood volumes per cm., to be lower in girls than in boys of the same height, was also observed (Graph 13). In the adult series of Gibson and Evans (1937b), the increase in the absolute blood volume of males over that of females, was due mainly to the difference in cell volume, although the plasma volume was also higher in males. The following table shows the average values in the present series for three blood estimations.

	Hb g.per cent	P.C.V. per cent	R.B.C. Millions per c.mm.
42 girls	12.02	42.5	4.532
38 boys	11.7	43	4.536

The average haematocrit reading and erythrocyte count was only slightly higher in the group of boys, but these values probably did not reflect the difference in cell volume adequately, since, as shown in Graph 13, the absolute plasma volume as well as blood volume tended to be higher in boys than in girls.

Summary.

Blood volume investigations have been performed

in eighty normal children.

Increase in age was accompanied by increase in absolute plasma and blood volumes. Unit volumes related to height and surface area showed a rise with increasing age, while unit volumes related to weight remained unchanged.

Increase in weight, height and surface area also led to rise in absolute volumes, but as with age, unit volumes per kilogram showed no definite trend, while unit volumes per centimetre and per square metre showed a gradual increase.

While the correlation of blood volume with each of the three body measurements appeared very similar when graphed, height was chosen as the correlative factor for clinical reasons.

In boys, both plasma and blood volumes showed a tendency to be higher than in girls.

Table 6.

Normal Infants, 3 - 11 months old.

	Case	Sex	Age mths.	Weight kilos	Weight per cent	Height cms.	Height per cent	Hb.	RBC	PCV	Plasma Volume, ml. Total	Plasma Volume per kilo	Plasma Volume per cm.	Plasma Volume per sq.m.	Blood Volume, ml. Total	Blood Volume per kilo	Blood Volume per cm.	Blood Volume per sq.m.
*	1	F	3	4.305	84	57	99	9.5	4.6	41	213	49.5	3.74	850	359	83.4	6.30	1433
*	2	M	4	4.68	84	58.5	96	10.6	4.27	45	212	45.4	3.62	805	383	81.9	6.55	1454
*	3	M	5	6.22	100	62.5	97	10.2	4.92	47	300	48.2	4.80	954	569	91.4	9.10	1811
	4	F	6	7.675	110	64	101	10.9	4.30	40	306	39.8	4.78	880	510	66.4	7.97	1467
	Average		4-6								273	47.8	4.30	879	487	79.9	7.87	1577
	5	M	7	6.73	92	64	99	11.0	4.30	39	316	47.0	4.94	961	518	77.0	8.09	1574
	6	M	7	7.16	98	66	100	10.1	4.40	41	362	50.6	5.49	1047	614	85.7	9.30	1776
	7	M	9	7.16	86	73	106	10.7	4.50	43	337	47.1	4.62	906	591	82.6	8.10	1588
	Average		7-9								338	48.2	5.02	971	574	81.8	8.50	1646
	8	M	10	9.095	109	75	108	9.4	3.80	41	402	44.2	5.36	958	681	74.9	9.08	1623
	9	F	10	8.635	103	75	108	10.4	4.20	36	419	48.6	5.59	1021	655	75.9	8.73	1596
	10	M	11	7.91	87	70	95	10.0	4.50	40	317	40.1	4.53	842	533	67.4	7.62	1416
	Average		10-12								379	44.3	5.16	940	623	72.7	8.47	1545

Hb. = haemoglobin in g.per cent. RBC = red blood count, millions per c.mm.
PCV = haematocrit reading, per cent.

Table 7.

Normal Children, 1 - 3 years of age.

Case	Sex	Age yrs.	Weight kilos	Weight per cent	Height cms.	Height per cent	Hb.	RBC	PCV	Plasma Volume, ml. Total	Plasma Volume, ml. per kilo	Plasma Volume, ml. per cm.	Plasma Volume, ml. per sq.m.	Blood Volume, ml. Total	Blood Volume, ml. per kilo	Blood Volume, ml. per cm.	Blood Volume, ml. per sq.m.
11	F	1 11/12	11.49	102	87	107	12.8	4.06	46	479	41.7	5.51	970	880	76.6	10.11	1775
12	F	1 2/12	8.61	93	76	103	11.0	4.2	38	435	50.5	5.72	1051	701	81.4	9.23	1694
13	F	1 6/12	10.26	102	79	103	10.9	4.2	39	483	47.1	6.12	1052	793	77.3	10.03	1728
14	F	1 4/12	11.08	110	82	105	10.0	4.2	39	516	46.6	6.29	1058	846	76.4	10.31	1735
15	F	1 11/12	10.75	95	86	105	12.5	4.6	39	499	46.4	5.80	1001	817	76.0	9.50	1639
16	M	1 9/12	11.31	100	87.5	110	12.2	4.65	46	598	52.9	6.84	1161	1108	98.0	12.66	2150
Average 1-2							11.9	4.32	41	502	47.5	6.05	1049	857	80.9	10.31	1787
17	F	2 6/12	11.92	94	89	103	11.2	4.54	43	599	50.3	6.73	1124	1052	88.2	11.81	1969
18	F	2 5/12	10.24	84	87	103	11.8	4.40	41	520	50.7	5.97	1057	880	86.0	10.12	1791
19	F	2 1/12	10.12	87	88	105	13.4	4.95	44	500	49.4	5.68	1015	892	88.1	10.14	1811
20	M	2 9/12	11.2	86	87	100	13.3	4.85	47	506	45.2	5.82	991	954	85.2	10.97	1868
21	M	2 9/12	12.52	95	95	105	12.1	4.60	41	653	52.2	5.88	1143	1108	88.4	11.65	1937
22	M	2 2/12	11.8	95	90.5	107	12.9	4.70	41	503	42.6	5.56	935	852	72.2	9.42	1584
Average 2-3							12.4	4.67	43	547	48.4	6.17	1044	956	84.7	10.68	1827

Table 8.

Normal Children, 3 - 5 years of age.

	Case	Sex	Age yrs.	Weight kilos	Weight per cent	Height cms.	Height per cent	Hb.	RBC.	PCV.	Plasma Volume, ml. Total	Plasma Volume, ml. per kilo	Plasma Volume, ml. per cm.	Plasma Volume, ml. per sq.m.	Blood Volume, ml. Total	Blood Volume, ml. per kilo	Blood Volume, ml. per cm.	Blood Volume, ml. per sq.m.
*	23	M	3 6/12	15.24	102	100	108	10.3	4.2	44	723	47.4	7.20	1120	1289	84.6	12.90	2001
*	24	F	3	13.08	96	94	106	12.1	4.6	45	623	47.7	6.63	1079	1141	87.3	12.14	1976
*	25	M	3	13.9	98	93	105	10.4	4.14	40	673	48.4	7.20	1140	1127	81.1	12.06	1909
	26	M	3 8/12	13.06	85	100	107	12.5	4.88	42	671	51.4	6.71	1113	1156	88.6	11.56	1916
	27	M	3 3/12	12.32	84	99	108	10.1	4.2	42.5	535	43.4	5.41	918	931	75.5	9.40	1596
	28	F	3 9/12	14.42	96	97	102	10.2	4.5	41	527	36.6	5.44	855	894	62.0	9.21	1450
	Average 3-4							10.9	4.42	42.4	625	45.8	6.43	1037	1090	79.8	11.21	1808
	29	F	4 5/12	12.98	80	93	92	12.1	4.7	39	662	51.0	7.12	1157	1085	83.6	11.67	1897
	30	M	4 6/12	14.64	84	100	99	12.8	4.9	44	777	53.0	7.77	1226	1388	94.7	13.88	2191
	31	F	4 7/12	14.24	85	101	100	13.4	4.3	41	806	56.6	7.95	1277	1367	96.0	13.47	2163
	32	F	4 1/12	16.24	104	105	107	13.1	4.8	44	735	45.3	6.97	1068	1312	80.8	12.44	1905
	33	F	4	14.62	95	96	97	13.0	4.7	45	737	50.4	7.68	1202	1341	91.7	13.96	2187
	34	M	4 6/12	15.96	100	103	103	13.0	4.98	44	793	49.7	7.69	1182	1403	87.9	13.62	2091
	Average 4-5							12.9	4.73	43	752	51.0	7.53	1185	1316	89.1	13.17	2072

Table 9.

Normal Children, 5 - 7 years of age.

	Case	Sex	Age yrs.	Weight kilos	Weight per cent	Height cms.	Height per cent	Hb.	RBC	PCV	Plasma Volume, ml. Total	Plasma Volume per kilo	Plasma Volume per cm.	Plasma Volume per sq.m.	Blood Volume, ml. Total	Blood Volume per kilo	Blood Volume per cm.	Blood Volume per sq.m.
*	35	M	5 11/12	18.4	90	111.5	100	10.1	3.9	38	797	43.3	7.15	1055	1288	70.0	11.55	1706
*	36	M	5	16.74	90	108	102	13.0	4.96	44	818	48.9	7.57	1153	1466	87.6	13.57	2068
	37	M	5 9/12	16.98	84	107	99	11.6	4.75	42	920	54.2	8.64	1299	1587	93.4	14.83	2242
	38	F	5 1/12	15.17	85	108	103	13.0	4.6	42	672	44.3	6.22	989	1158	76.4	10.73	1704
	39	F	5 4/12	18.54	100	120	110	12.6	4.85	43	971	52.4	8.09	1213	1703	91.9	14.19	2127
	40	F	5 8/12	22.12	110	116	105	14.9	4.98	47	954	43.1	8.22	1133	1799	81.3	15.51	2139
	Average 5-6							12.5	4.67	42.7	855	47.7	7.65	1140	1500	83.4	13.40	1998
*	41	M	6 4/12	17.54	82	140	122	10.0	4.54	43	960	54.7	6.86	1100	1678	95.7	11.99	1923
*	42	F	6 11/12	19.96	92	114.5	98	11.4	4.23	44	739	37.0	6.45	926	1316	66.0	11.50	1650
*	43	F	6 4/12	17.2	83	109.5	96	11.9	4.4	45	921	53.6	8.41	1271	1406	81.8	12.84	1939
	44	F	6	17.52	88	117	105	10.5	4.1	39	1016	58.0	8.69	1328	1666	95.1	14.24	2178
	45	M	6 6/12	19.74	93	114.5	100	11.1	4.45	42	1027	52.0	8.97	1293	1770	89.7	15.46	2230
	46	F	6	17.88	89	113	101	10.3	4.1	41	812	45.4	7.18	1079	1376	76.9	12.17	1828
	Average 6-7							10.9	4.3	42.3	913	50.1	7.76	1166	1535	84.2	13.03	1958

Table 10.

Normal Children, 7 - 9 years of age.

	Case	Sex	Age yrs.	Weight kilos	Weight per cent	Height cms.	Height per cent	Hb.	RBC	PCV	Plasma Volume, ml. Total	Plasma Volume, ml. per kilo	Plasma Volume, ml. per cm.	Plasma Volume, ml. per sq.m.	Blood Volume, ml. Total	Blood Volume, ml. per kilo	Blood Volume, ml. per cm.	Blood Volume, ml. per sq.m.
*	47	M	7 3/12	20.68	90	118.5	100	11.2	4.0	40	1161	56.1	9.80	1315	1935	93.5	16.32	2192
	48	F	7 8/12	25.06	108	125	104	12.7	4.7	46	1105	44.0	8.84	1181	2046	82.0	16.37	2189
	49	F	7 3/12	18.96	85	112	95	11.9	4.6	41	904	47.7	8.07	1177	1532	80.8	13.68	1994
	50	F	7 6/12	21.4	95	125	105	13.6	4.9	46	1050	49.1	8.40	1201	1945	90.9	15.55	2225
	51	M	7	23.32	102	124	103	13.6	4.75	41	1222	52.4	9.86	1357	2071	88.8	16.70	2299
	52	M	7 6/12	21.32	91	118	99	13.6	4.85	45	1058	49.6	8.97	1263	1923	90.2	16.30	2296
	Average 7-8							13.3	4.63	43	1083	49.8	8.99	1249	1902	87.7	15.82	2365
*	53	F	8 10/12	20.8	81	124.5	99	10.7	4.43	45	869	41.8	6.98	1008	1576	75.8	12.66	1828
	54	M	8	26.0	105	127	104	12.5	4.85	43	1101	42.4	8.67	1147	1932	74.3	15.21	2010
	55	F	8	23.64	98	126.5	103	11.6	4.8	42	933	39.5	7.38	1014	1609	68.1	12.72	1747
	56	F	8 6/12	25.36	100	131	105	13.6	4.94	42.5	1211	47.7	9.24	1245	2105	83.0	16.07	2164
	57	F	8 9/12	22.28	87	123	98	12.6	4.61	42	1132	50.8	9.20	1287	1952	87.6	15.87	2219
	58	M	8 1/12	24.56	99	129	102	14.2	4.8	41	1307	53.2	10.14	1378	2216	90.2	17.18	2336
	Average 8-9							12.5	4.74	42.6	1092	45.9	8.60	1180	1898	79.8	14.95	2051

Table 11.

Normal Children, 9 - 11 years of age.

	Case	Sex	Age yrs.	Weight kilos	Weight per cent	Height cms.	Height per cent	Hb.	RBC	PCV.	Plasma Volume, ml. Total	Plasma Volume per kilo	Plasma Volume per cm.	Plasma Volume per sq.m.	Blood Volume, ml. Total	Blood Volume per kilo	Blood Volume per cm.	Blood Volume per sq.m.
*	59	M	9 9/12	25.8	88	133.5	102	10.2	4.24	44	1204	46.6	9.02	1210	2156	83.6	16.14	2158
*	60	M	9	23.76	89	126	99	12.3	4.27	48	1388	58.4	11.02	1509	2695	113.0	21.39	2930
*	61	F	9 6/12	22.56	82	132	103	10.6	4.62	44	940	41.7	7.12	1009	1681	74.5	12.74	1804
	62	F	9 7/12	22.4	81	124.5	96	10.0	4.1	40	1149	51.3	9.23	1293	1900	84.8	15.26	2136
	63	M	9 6/12	32.84	110	142.5	110	10.8	4.7	43	1822	55.5	12.78	1577	3196	97.3	22.43	2766
	64	M	9 6/12	22.92	82	128	100	13.4	4.8	41	1239	54.0	9.68	1354	2099	91.6	16.40	2295
	Average		9-10					11.2	4.45	43	1290	51.2	9.81	1325	2288	90.8	17.39	2348
*	65	F	10 8/12	28.6	92	135	100	12.0	4.75	42	1088	38.1	8.06	1040	1890	66.1	14.01	1804
*	66	F	10 10/12	27.68	87	144	106	11.6	4.68	41	1618	58.4	11.24	1520	2737	99.0	19.01	2570
*	67	F	10 1/12	30.96	106	138	104	10.2	4.62	43	1304	42.1	9.45	1242	2296	74.1	16.64	2184
	68	M	10	29.1	96	127	96	12.7	4.63	45	1416	48.6	11.15	1404	2575	88.5	20.28	2554
	69	M	10	23.68	80	130	98	12.5	4.7	47	1074	45.4	8.27	1145	2027	85.6	15.60	2160
	70	F	10 9/12	29.64	94	140	100	13.8	4.8	46	1283	43.3	9.17	1180	2378	80.2	16.98	2184
	Average		10-11					12.1	4.69	44	1297	45.9	9.55	1255	2317	82.2	17.09	2243

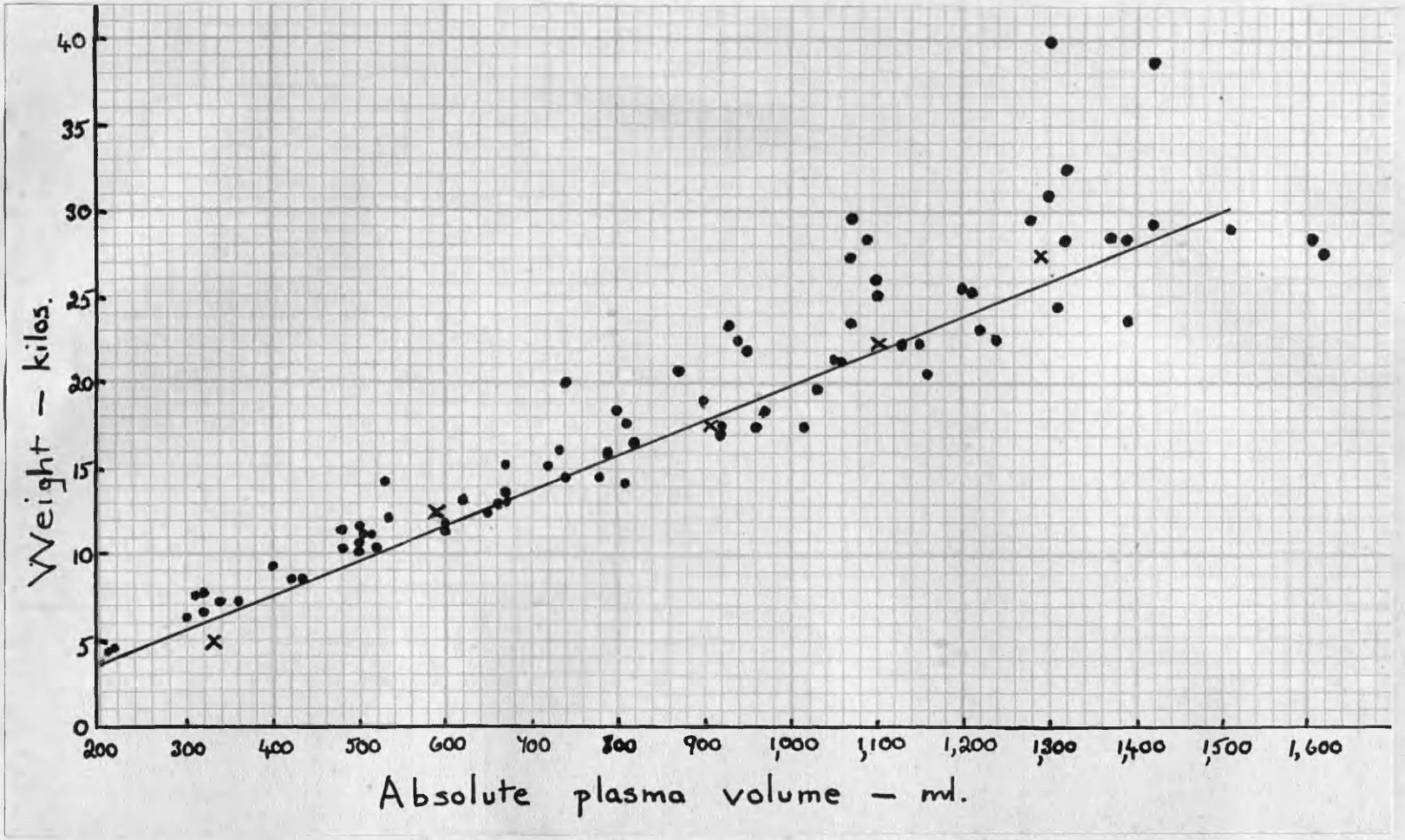
Table 12.

Normal Children, 11 - 13 years of age.

	Case	Sex	Age yrs.	Weight kilos	Weight per cent	Height cms.	Height per cent	Hb.	RBC	PCV	Plasma Volume, ml. Total	Plasma Volume per kilo	Plasma Volume per cm.	Plasma Volume per sq.m.	Blood Volume, ml. Total	Blood Volume per kilo	Blood Volume per cm.	Blood Volume per sq.m.
*	71	F	11 3/12	27.32	83	137	99	11.8	4.2	45	1068	39.1	7.81	1032	1945	71.2	14.23	1879
	72	M	11 4/12	33.08	98	146	105	11.7	4.6	42	1918	57.9	13.15	1629	3296	99.6	22.57	2800
	73	F	11 6/12	28.28	83	138	98	13.8	4.8	46	1606	56.8	11.63	1516	2974	105.2	21.55	2806
	74	F	11 4/12	29.0	87	135	97	14.6	4.9	45	1514	52.2	11.21	1460	2752	94.9	20.39	2641
	75	M	11 6/12	28.46	84	133	97	13.1	4.85	44	1367	48.0	10.28	1325	2441	85.8	18.35	2367
	Average 11-12							13.0	4.67	44	1495	50.8	10.82	1392	2682	91.3	19.42	2499
*	76	M	12	28.4	80	139	98	12.0	4.70	46	1322	47.5	9.51	1250	2441	87.7	17.56	2308
*	77	M	13	38.75	102	151	104	12.2	4.2	47	1424	36.8	9.43	1103	2683	69.2	17.77	2076
	78	M	12 11/12	32.46	90	147	100	13.5	4.86	46	1320	40.7	8.98	1125	2445	75.3	16.63	2083
	79	F	12 6/12	29.8	80	145	100	14.0	4.9	48	1066	35.8	7.35	950	2049	68.8	14.14	1828
	80	F	12	28.28	80	141	98	12.9	4.6	39	1390	49.2	9.86	1293	2278	80.6	16.15	2120
	Average 12-13							12.9	4.65	45	1304	42.0	9.05	1210	2379	76.3	16.45	2207

Graph 14.

The increase of absolute plasma volume with increase in weight.

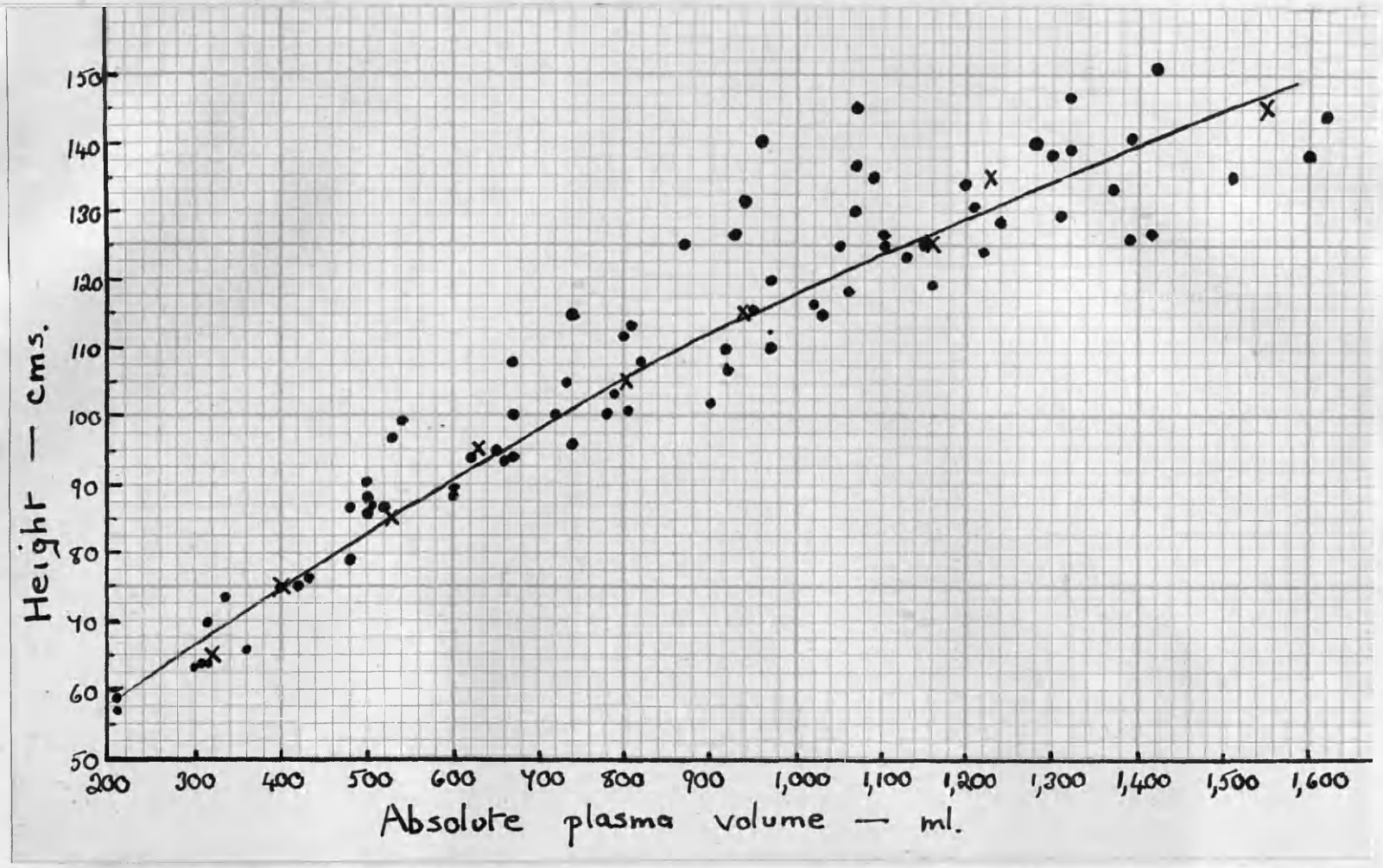


• • • individual observations

× × × average values for each group of 5 kilos.

Graph 15.

The increase of absolute plasma volume, with increase in height.

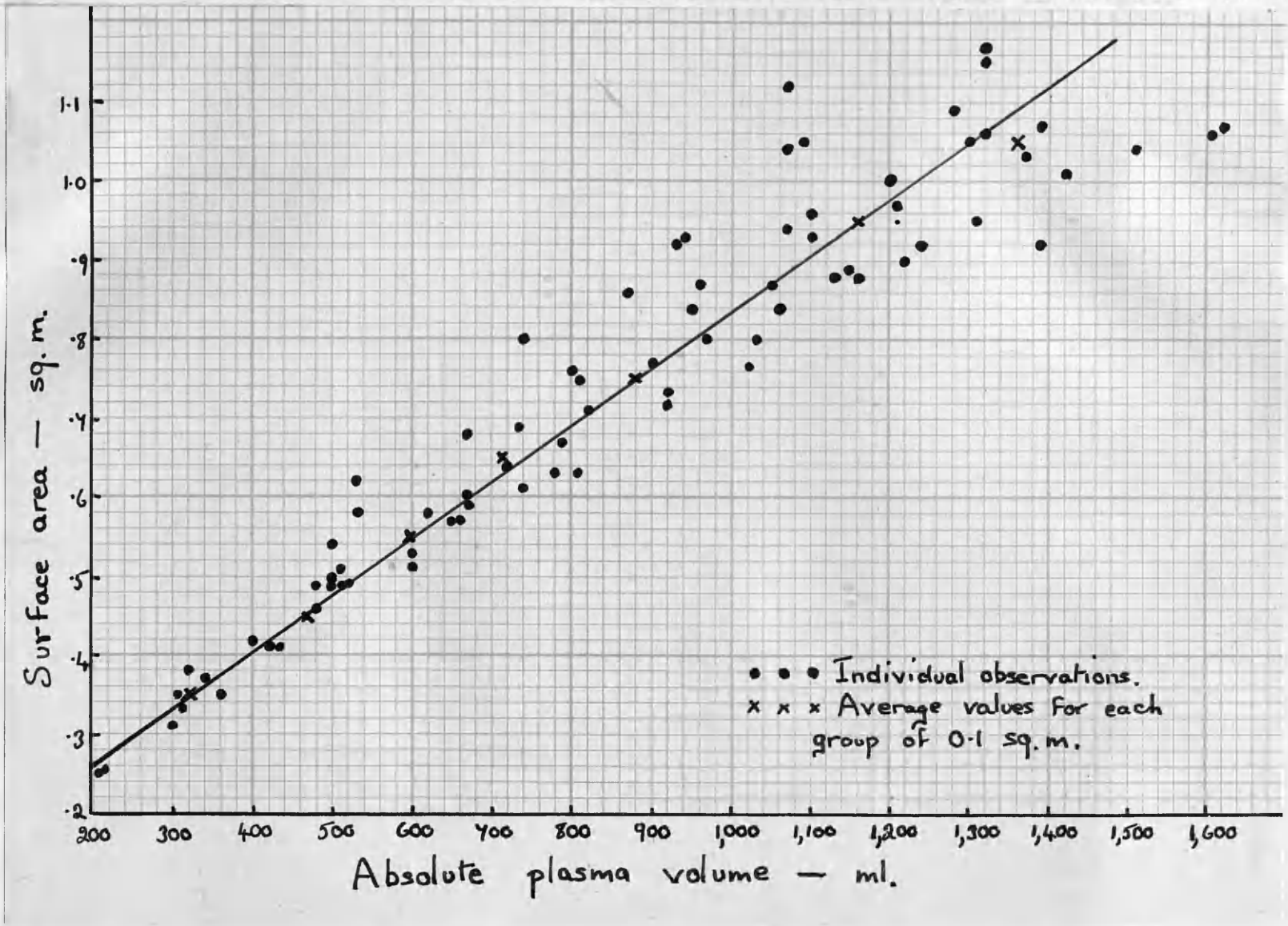


• • • individual observations.

× × × average values for each group of 10 cms. of height.

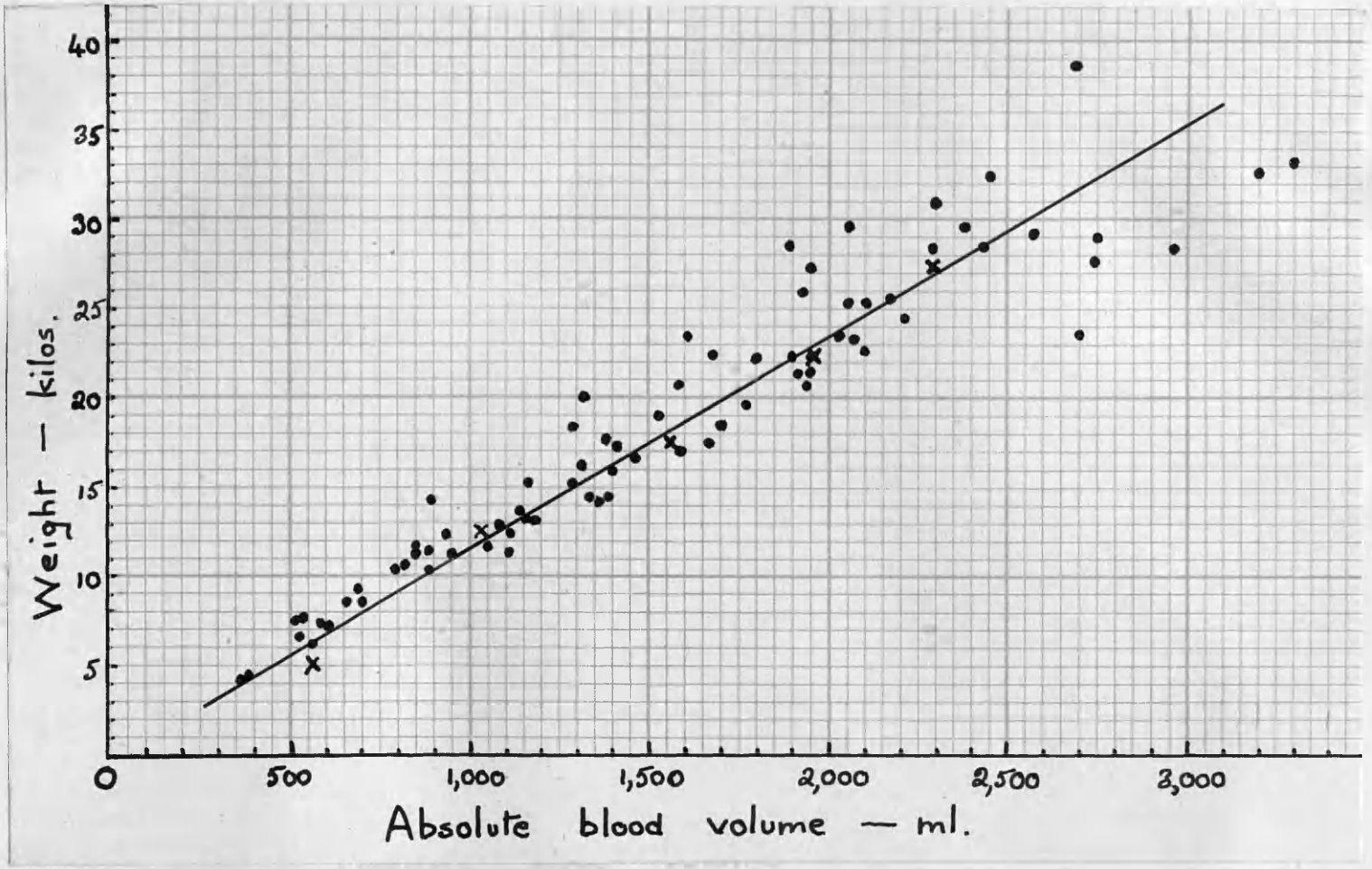
Graph 16.

The increase of absolute plasma volume, with increase in body surface.



Graph 17.

The increase of absolute blood volume, with increase in weight.

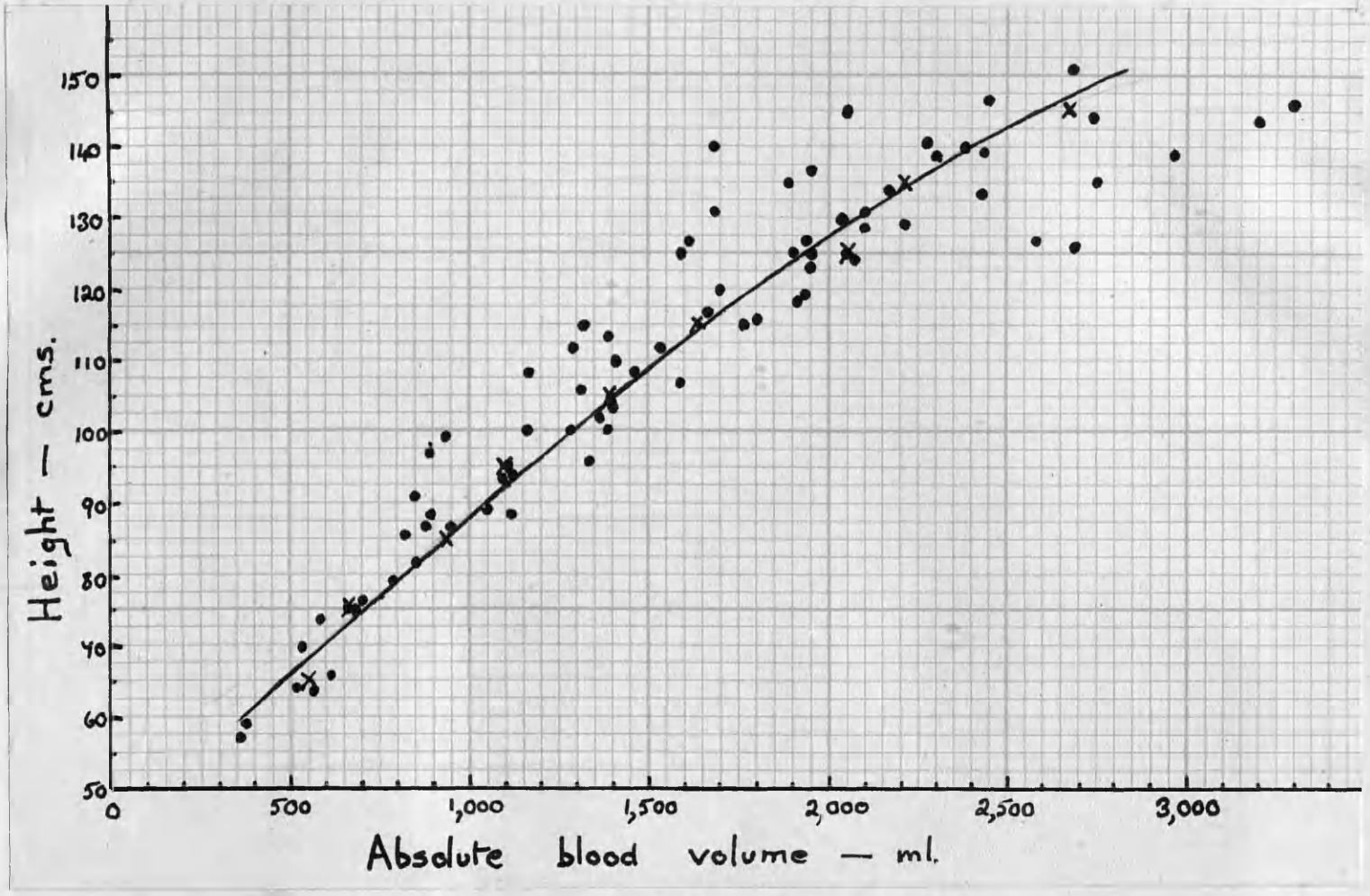


• • • individual observations.

× × × average values for each group of 5 kilos of weight.

Graph 18.

The increase of absolute blood volume, with increase in height.

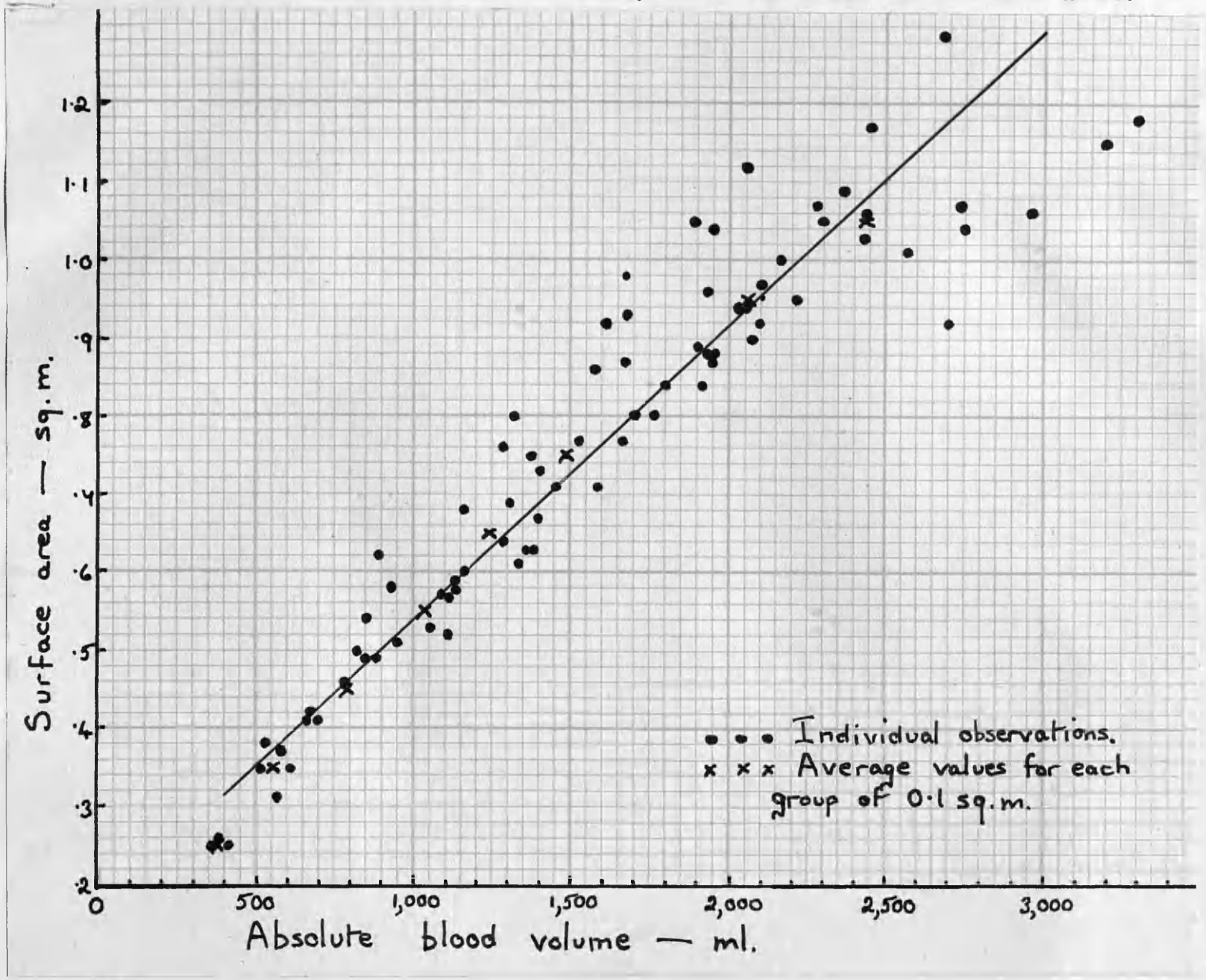


• • • individual observations.

× × × average values for each group of 10 cms. of height.

Graph 19.

The increase of absolute blood volume, with increase in surface area.



Section 3.

Blood Volume in Marasmus.

References to the blood volume in marasmus by previous workers are very few. Marriott and Perkins (1920-21) found that the blood volume in seven "normal" infants ranged from 8 to 10.8 per cent of the body weight, while in eleven infants showing extreme wasting, the range was 4 to 10.4 per cent. They concluded that the blood volume in marasmus was low. Bakwin and Rivkin (1924) on the other hand, were of the opinion, that for the same age period, the blood volume in infants with marasmus was relatively higher than in normal infants. Similar findings were recorded by Darrow, Soule and Buckman (1928).

The present investigation includes eight marasmic children varying from 53 to 66 per cent of their expected weight for age (Table 13). It will be seen that the plasma and blood volumes related to body weight tended to be high, in one instance reaching 76 and 103 ml. per kilo respectively. When plasma volume was related to body weight however (Table 14), the majority had low values, the two exceptions being children with anaemia, which itself is associated with increase of plasma volume (Cases 88 and 91). Although Cases 89 and 90 were also suffering from anaemia, they were found to have low plasma volumes, the deviations from normal being minus 3 and minus 18 respectively. Examination of the case record revealed that Case 90, at the time of investigation, had had frequent loose stools for two days. As gastro-enteritis

Table 13.

Marasmic Children.

Case	Sex	Age yrs.	Weight kilos	Weight per cent	Height cms.	Height per cent	Hb.	RBC	PCV	Plasma Volume, ml. Total	Plasma Volume, ml. per kilo	Plasma Volume, ml. per cm.	Plasma Volume, ml. per sq.m.	Blood Volume, ml. Total	Blood Volume, ml. per kilo	Blood Volume, ml. per cm.	Blood Volume, ml. per sq.m.
* 84	M	3 1/2 / 12	2.88	53	54	90	12.9	3.81	42	158	55.0	2.93	780	274	95.2	5.08	1353
* 85	M	2/12	2.65	60	53	93	12.8	3.9	44	128	48.4	2.42	665	230	86.6	4.34	1191
86	F	1 1/12	4.8	53	66	89	11.8	4.6	43	290	60.0	4.40	997	504	105.0	7.66	1732
87	F	1 5/12	5.74	57	73	96	10.7	3.9	40	342	59.6	4.69	1009	570	99.4	7.81	1683
* 88	F	3/12	2.70	54	52	90	7.6	4.0	34	205	76.0	3.94	1070	278	103.0	5.35	1446
89	M	3/12	3.08	58	51.5	87	7.5	3.9	33	176	57.2	3.42	873	262	85.0	5.09	1299
90	F	8/12	4.57	58	62	94	7.5	4.01	41	236	51.7	3.81	865	401	87.6	6.46	1470
91	F	2 6/12	8.12	66	78.5	92	7.5	3.9	33	535	65.9	6.82	1292	799	98.3	10.17	1929

Table 14.

Marasmic children, arranged in order of increasing percentage of expected weight.

Case	Hb. g.per cent	N.P.N. mg.per cent	T.P.P. g.per cent	Plasma, ml.per cm.			Blood, ml.per cm.		
				Actual	Expected	per cent deviation	Actual	Expected	per cent deviation
84	12.9	-	-	2.93	3.7	−20	5.08	5.8	−12
86	11.8	62	8.2	4.40	4.95	−12	7.66	8.0	−5
88	7.6	55.5	7.16	3.94	3.5	+12	5.35	5.6	−5
87	10.7	31.3	6.41	4.7	5.4	−13	7.81	8.9	−12
89	7.5	38	8.3	3.42	3.5	−3	5.09	5.6	−8
90	7.5	43	6.4	3.81	4.6	−18	6.46	7.2	−11
85	12.8	-	-	2.42	3.6	−33	4.34	5.8	−25
91	7.5	32	6.1	6.82	5.7	+19	10.17	9.6	+6
Average			7.08			−9			−8.5

N.P.N. = Blood non-protein nitrogen, mg. per cent.

T.P.P. = Total plasma proteins, grams per cent.

causes a reduction in plasma volume (Section 5), the tendency of the existing anaemia towards increase of plasma may have been obscured. Blood volume in relation to body height also showed a tendency to be decreased, except in one of the anaemic children. The magnitude of the deviations from expected volumes was not outwith the range of normality found in the healthy children. Nevertheless, from the fact that all showed a change in the direction of decrease, apart from the two anaemic children where there was an obvious explanation for an increase, it may be concluded that both plasma and blood volumes tend to be low in marasmus. The average figures for the eight children were as follows:- 9 per cent decrease in plasma volume, and 8.5 per cent decrease in blood volume.

On the basis of the limited data available in the present group of marasmic children, there was no evident relationship between either the degree of wasting and the plasma protein level, or between the plasma proteins and the degree of reduction of the plasma volume (Table 14). Taking as a basis the figures published by Rennie (1935) for the serum protein concentrations of healthy children, the average and range of the plasma protein concentrations of the marasmic group did not show any departure from normal. Since the plasma volume was reduced however, the total quantity of protein in circulation would be lower than normal. In recent investigations on a group of emaciated, mal-nourished,

prisoners of war, Walters, Rossiter and Lehmann (1947) found reduction in plasma and blood volume associated with low serum proteins, the average serum protein concentration being 5.24 ± 0.74 g. per cent. Although the finding of a low protein concentration is not necessarily associated with a reduction in plasma volume - in the haemodilution following haemorrhage the blood volume may be fully restored and the plasma protein concentration low - experimental evidence exists that elevation of the plasma protein level causes a rise in plasma volume, once the protein stores have become saturated (Beattie and Collard, 1942b). From these conflicting reports, it is apparent that the exact rôle played by the plasma proteins in blood volume regulation has yet to be defined.

In the reduction of plasma volume in marasmus, the basal metabolic rate is probably a more important factor than the level of the plasma proteins. The basal metabolic rate in children does not fall appreciably until the weight reduction has reached 66 per cent of expected weight. Of the eight children under discussion, only one was at this level, and all the others were appreciably lower. In diseases of the thyroid, where the basal metabolic rate is altered, it should be noted that Blumgart, Gargill and Gilligan (1931), and Gibson and Harris (1939) found the total blood volume increased in hyperthyroidism and decreased in myxoedema, the deviation from normality being due to

changes in both cell and plasma volumes. The latter authors suggested that the level of oxygen consumption had a direct influence on the blood volume.

Summary.

In eight children studied, a general tendency was observed for the plasma and blood volumes to be low. The average percentage deviations from normality were -9 and -8.5 for plasma and blood respectively.

No obvious correlation existed between the plasma protein concentration and the reduction in plasma volume. It is suggested that decrease in the basal metabolic rate may be an important factor in causing blood volume reduction.

Section 4.

Blood Volume in Cardiac Disease.

The group of children investigated in this section, comprised eight patients with rheumatic carditis, four of whom had congestive cardiac failure, and three patients with congenital heart disease. As the circulation time in cardiac failure is considerably prolonged, the mixing time allowed in all these children was fifteen minutes (Gibson and Evans, 1937c; Meneely and Kaltreider, 1943).

Rheumatic Carditis.

The results obtained are shown in Table 15.

The first four children (Cases 92 - 95) all had acute rheumatism with carditis. At the time of investigation, they had been in bed for at least a week, under salicylate therapy. The cardiovascular signs were essentially similar in every child.

1. The pulse rate was elevated and often swinging.
2. The heart was enlarged to the left, the apex beat being on an average half an inch beyond the left nipple line, and varying murmurs were present.

The second four (Cases 96 - 99) in addition to the presence of active rheumatism, were showing signs of congestive cardiac failure, shown by more marked cardiac enlargement, orthopnoea and cyanosis, venous congestion, enlarged liver and, in Case 96, oedema. Case 99 had a very marked degree of decompensation, with the heart enlargement extending to 2½ inches outside the left

Table 15.

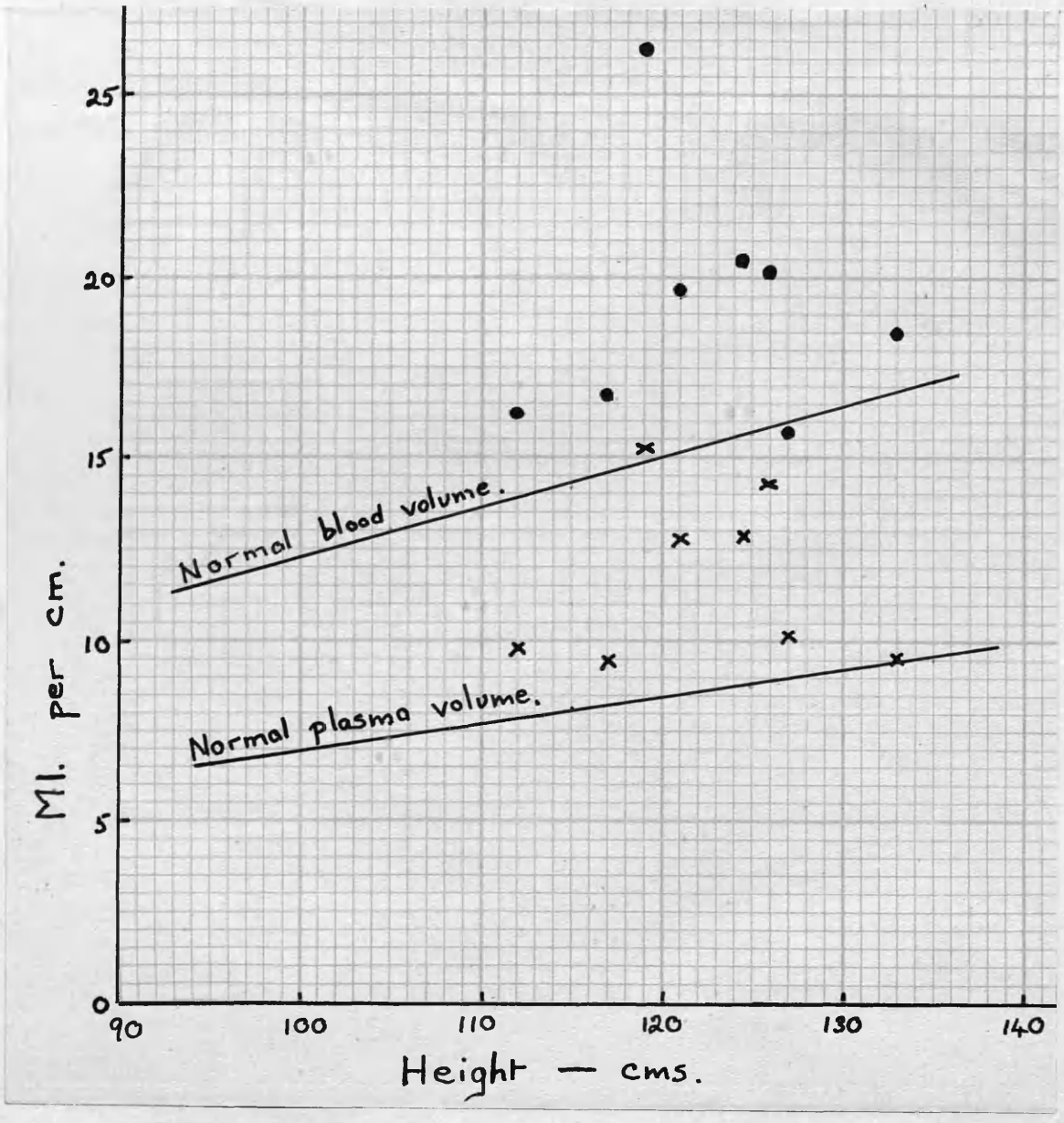
Cardiac Disease.

	Case	Sex	Age Yrs.	Weight kilos	Weight per cent	Height cms.	Height per cent	Hb. g. per cent	RBC mills per c.mm.	PCV per cent	NPN mg. per cent	TPP g. per cent	Plasma Volume ml. Total	Plasma Volume ml. Per cm.	Blood Volume ml. Total	Blood Volume ml. Per cm.
Carditis.																
	92	M	11 4/12	23.04	68	132.5	96	10.8	4.63	49	58	8.5	1256	9.48	2453	18.51
	93	F	10	23.52	81	127	97	11.2	4.1	35	-	-	1288	10.14	1982	15.61
*	94	M	5 6/12	16.88	86	117	107	10.7	4.02	44	48	7.3	1106	9.45	1967	16.81
*	95	M	8 3/12	21.26	84	121	98	8.2	3.6	35	-	-	1550	12.80	2385	19.71
Congestive Cardiac Failure.																
	96	M	7	19.28	86	111.5	94	8.9	4.5	39	60	7.75	1103	9.89	1808	16.22
*	97	M	8 10/12	20.1	76	124.5	100	9.6	3.35	37	76	6.94	1612	12.95	2555	20.52
	98	M	8 4/12	23.48	94	126	102	4.5	3.1	29	36	6.47	1797	14.27	2530	20.07
	99	F	9 2/12	21.32	80	119	93	11.5	4.9	42	22	5.5	1800	15.19	3103	26.19

N.P.N. = blood non-protein nitrogen.
T.P.P. = total plasma proteins.

Graph 20.

Plasma and blood volumes in cardiac disease.



● ● ● Cardiac blood volumes.
× × × Cardiac plasma volumes.

The continuous lines represent the average increase in plasma and blood volume per cm. for the series of 80 normal children. (Graph 10)

In carditis, and to a more marked degree in cardiac failure, both plasma and blood volumes showed elevations above the normal average values.

nipple line, and the liver palpable six finger breadths below the right costal margin. These four children were all suffering from their second or third attack of acute rheumatic infection.

Graph 20 has been constructed to show the deviation from normal of the group, plasma and blood volume per centimetre being graphed against the height of the patient in centimetres. The continuous line represents the average increase in height, for the series of eighty normal children. Six of the eight children under discussion had high total blood volumes, while the remaining two were within normal limits. As regards the plasma volume, elevation was observed in seven children, and was most marked in those with the greatest degree of clinical failure i.e. Cases 98 and 99.

From this small series of cases in children, it would appear that in congestive failure, the plasma and blood volumes are raised, while in carditis alone elevation of the plasma volume may precede signs of failure. Case 95, had a marked rise in plasma volume (Table 16) and at the time of the estimation he seemed to be on the verge of failure clinically - he had a massive rheumatic infection, with toxaemia and numerous subcutaneous nodules, and in addition to cardiac enlargement and rapid heart action, showed jugular distension, and mild orthopnoea.

The reversion of plasma and blood volume to within

Table 16.

Cardiac Disease.

Case	M.C.Hb.C. per cent	M.C.V. c.μ	Plasma, ml.per cm. Actual	Expected	D.	Cells, ml. per cm. Actual	Expected	D.
92	22	105	9.48	9.4	0	9.03	6.95	+29
93	32	85	10.14	9.1	+11	5.47	6.6	−15
94	24	109	9.45	8.5	+12	7.36	5.9	+24
95	23	97	12.80	8.8	+49	6.91	6.15	+8
96	23	87	9.89	8.2	+21	6.33	5.6	+13
97	25	110	12.95	8.95	+44	7.57	6.45	+19
98	15	93	14.27	9.1	+59	5.80	6.5	−12
99	27	85	15.19	8.6	+76	11.0	6.2	+77

M.C.Hb.C. = mean corpuscular haemoglobin concentration.
M.C.V. = mean corpuscular volume, expressed in cubic microns.
D. = percentage deviation of the actual observation from the expected value for the child's height, taken from Graph 10.

normal limits after recovery from decompensation was found in Case 97, while an intermediate stage was demonstrated in Case 98, at a time when his general condition had improved, but when jugular distension and hepatic enlargement were still marked (Table 17).

Table 17.

	Case 97		Case 98	
Date	(1) 5.10.45	(2) 20.12.45	(1) 25.11.46	(2) 24.1.47
Haemoglobin, g. per cent	9.6	11.0	4.5	10.7
R.B.C., millions per c.mm.	3.35	4.32	3.1	4.37
P.C.V., per cent	37	42	29	38
Plasma volume, ml.				
Total	1612	1100	1797	1588
Per cm.				
Actual	12.95	8.87	14.27	12.60
Expected	8.95	8.95	9.1	9.1
Blood volume, ml.				
Total	2555	1902	2530	2561
Per cm.				
Actual	20.52	15.34	20.07	20.32
Expected	15.4	15.4	15.6	15.6
Blood N.P.N., mg. per cent	76	47	36	39
Total plasma proteins, g. per cent	6.94	7.71	6.47	7.38

Discussion.

These results find some support in recently published work, but are somewhat at variance with the conceptions of earlier authors. Wollheim (1951) using the dye Trypan red, found the blood volume reduced in compensated

cardiac disease to an average of 70ml. per kilo. (The average normal for his series was 83.9 per kilo). To explain his results in decompensation, he postulated two types of cardiac failure, plus or minus, the classification depending on whether the blood volume was increased above, or decreased below, the compensation figures. The plus type was found to be more common, but both types could occur in the same individual at different stages of his illness. Whether the plus or minus form occurred was considered to depend, not on the type of heart lesion, but on the immediate causative factor e.g. overwork would precipitate decompensation with a high blood volume, whereas infection, by altering the tonus of the peripheral capillaries, would lead to a reduced blood volume. Similar results were found by Goldbloom and Libin (1935) who further stated that if the minus form was observed in a patient who had been shown previously to have a high blood volume, coronary occlusion should be suspected. One criticism of this work is that the minus form of cardiac decompensation should not be called cardiac failure at all, but simply peripheral circulatory failure occurring in response to an infection in a patient who happens to have a cardiac lesion. The explanation of the low volume found in coronary thrombosis following the plus decompensation could lie in the fact that the onset of weaker muscle contractions and therefore reduced cardiac output

would lead to an ever greater part of the blood volume remaining stagnant in the capillary plexuses in the muscles, lungs, liver etc.

Other investigators have found the plasma and blood volumes to be high in all cases of congestive cardiac failure (Uhlenbruck and Vogels, 1931; Gibson and Evans, 1937c; Meneely and Kaltreider, 1943). Gibson and Evans observed that in valvular disease of the heart with no signs nor symptoms of cardiac failure, the blood volume was within normal limits, while in the group with symptoms but no signs, a small increase in the blood volume could be detected. Finally, in the group with frank congestive cardiac failure, the average deviation from the normal value was +55.5 per cent. The percentage increase in plasma volume was thought by Perera (1945), to bear a loose correlation to the size of the liver, and the latter writer also suggested that the increased plasma volume was due to dilatation and engorgement of vascular channels in the liver and portal circulatory beds. He observed that the plasma was augmented to a greater degree in right sided heart failure than in left. The finding of a reduction in plasma volume before any clinical signs of recovery were noticeable, was recorded by Waller, Blumgart and Volk (1940). The changes in plasma volume in adults so far discussed, have found parallels in the group of children investigated. Where cell volume was concerned, the results of the present series differed somewhat from the findings in adults.

In patients with congestive cardiac failure, Gibson and Evans (1937c) found an increase in cell volume, which was always proportionately greater than the plasma volume increase, and which therefore resulted in apparent haemoconcentration. The haematocrit reading was also raised during recovery, due to the rate of plasma volume reduction being more rapid than the rate of cell destruction. In Table 16 the percentage deviations from normal for both plasma and cell volumes have been tabulated, unit volume related to height being used for the comparison. Only two children (Cases 92 and 94) showed an increase in cells proportionately greater than the increase in plasma, while two (Cases 93 and 98) showed a decrease in cell volume below the normal average. Cardiac failure in children, however, differs from the mechanical failure in adults, in that active rheumatic infection is usually the precipitating factor. The presence of this infection, by disturbing the normal utilisation of iron, and possibly by exercising a toxic effect on the bone marrow, may prevent the usual erythropoietic response of the marrow to anoxaemia. This cannot be the only factor concerned however, as Case 98 doubled his total circulating haemoglobin in a period of eight weeks, during which time his blood sedimentation rate was persistently raised (50-60 mm. in the first hour, Westergren) (Table 17). Table 16 shows that seven of the eight children did in fact have a hypochromic anaemia as judged by the mean corpuscular haemoglobin concentration. The mean corpuscular volume was normal or high in all the

children, though Whitby and Britton (1939a) state that in acute infections, including rheumatic fever, a hypochromic microcytic anaemia is the rule. Paxton however (1935), found that children with acidosis had a high mean corpuscular diameter, which fact might account for the raised corpuscular volume in the children with cardiac failure.

The failure of Gibson and Evans (1937c) to find an increase in blood volume in compensated valvular lesions is confirmed in Case No. 97 (2) (Table 17). This child had classical aortic disease, in which condition one might have expected an increase in blood volume, to compensate for the regurgitation through the aortic valve with each diastole. The maintenance of cardiac output in this condition must depend entirely on the presence of cardiac hypertrophy and increased diastolic filling, and appears to be independent of the state of the blood volume. Plesch on the other hand, (1922), considered that increase in blood volume was part of the compensatory mechanism.

In Table 15, it will be seen that the plasma proteins tended to be low in the children with congestive cardiac failure, a finding which is characteristic of adults with the same condition.

The mechanism of production of the increase in blood volume in cardiac failure appears to consist of two parts. The cell volume is augmented in response to the effect of anoxia on the bone marrow (Starling, 1909), while the

Table 18.

Congenital Heart Disease.

Case	Age years	Weight kilos	Height cms.	Hb. g. per cent	R.B.C. mills. per c.mm.	P.C.V. per cent	Plasma Volume, ml. Total	Plasma Volume, ml. Per cm. Actual	Plasma Volume, ml. Per cm. Expected	Blood Volume, ml. Total	Blood Volume, ml. Per cm. Actual	Blood Volume, ml. Per cm. Expected
* 100	6/12	4.15	59	15.1	7.5	63	254	4.3	4.3	690	11.7	7.1
101	10/12	5.63	66	9.3	4.5	40	525	7.95	5.0	879	13.31	7.7
* 102	9	21.68	124	13.8	4.9	49	1094	8.86	8.9	2132	17.27	15.3

plasma volume increases as a result of decreased excretion of sodium and water by the kidneys due to impaired cardiac function (Warren and Stead, 1944). The exact mechanism causing the impaired excretion is unknown.

2. Congenital Heart Disease.

Three children with congenital heart lesions were studied in this group, with results as shown in Table 18.

Case 100 was a baby with morbus caeruleus. Case 102 had a patent ductus arteriosus, confirmed at operation. These two had essentially normal plasma volumes, with high cell volumes, which finding agrees with results recorded in three cases by Seckel (1936). Case No. 101 was found to have a marked increase in both cell and plasma volumes. This child however probably had congestive cardiac failure. The lesion was thought to be a patent interventricular septum, and at the time of investigation she was cyanosed, and slightly dyspnoeic, and in addition to enlargement of the liver, had moist râles at both lung bases.

Summary.

The blood volume has been investigated in four children with rheumatic carditis, in four with carditis and congestive cardiac failure, and in three children with congenital heart lesions.

In carditis, the plasma volume showed a tendency to be raised, increasing as cardiac failure approached. In congestive failure the rise in plasma volume averaged 50 per cent.

The cell volume in carditis and congestive failure was not found to be consistently raised, in contrast to the findings in adults.

In congenital heart disease the cell volume was markedly increased, and the plasma volume normal, except in one child who was considered to have developed congestive cardiac failure, and showed a marked increase in both cell and plasma volumes.

Section 5.

Blood Volume in Gastro-enteritis.

It is now well recognised, that the dehydration which is the predominant feature of gastro-enteritis in infants, is far from being a simple problem of withdrawal of fluid from a closed system of vascular channels. The concentration of the various blood constituents does not necessarily show a rise in proportion to the fluid loss. The assessment of the degree of dehydration either by clinical or chemical means is a difficult problem, and so far remains unsolved. It is not within the scope of this thesis to discuss the problem fully, but it can be pointed out that clinically the signs of dehydration and toxaemia often become inextricably mixed, and the problem becomes even more confusing when peripheral circulatory failure (shock) supervenes. If the blood volume tends to fall, some of the interstitial fluid, along with the plasma, forms what is known as the extra-cellular fluid (Marriott, 1947). Under certain conditions, the intra-cellular fluid (i.e. fluid within the cells) may share in the fluid withdrawal, as judged by the considerable loss of potassium which may occur in the diarrhoeal stools (Butler, McKhann and Gamble, 1933). It should be pointed out that potassium is held in solution in the intracellular fluid, and sodium in the extracellular fluid. Previous workers however, have obtained varied results for plasma volume in gastro-enteritis. Darrow and Buckman (1928) in five cases, found the plasma

Table 19.

Gastro-enteritis.

	Case	Sex	Age mths	Weight kilos	Weight per cent	Height cms.	Height per cent	Hb.	RBC	PCV	Plasma Volume, ml. Total	Plasma Volume, ml. per kilo	Plasma Volume, ml. per cm.	Plasma Volume, ml. per sq.m.	Blood Volume, ml. Total	Blood Volume, ml. per kilo	Blood Volume, ml. per cm.	Blood Volume, ml. per sq.m.
*	103	F	4½	3.56	60	56	92	12.7	4.5	42	206	57.8	3.68	944	355	99.7	6.34	1556
*	104	M	4	3.5	63	55	91	13.5	4.58	46	197	56.2	3.58	883	367	104.8	6.67	1644
	105	M	9	7.14	86	70	100	10.7	5.35	44	353	49.5	5.03	979	630	88.4	9.0	1748
	106	M	6	7.08	97	67	102	13.2	5.1	45	306	43.3	4.57	881	557	78.7	8.31	1604
	107	M	6	4.87	67	63	97	6.5	3.71	32	255	52.4	4.05	900	375	77.1	5.96	1323
	108	M	2	2.83	60	53	93	11.6	4.2	37.5	171	60.4	3.23	862	273	96.5	5.15	1377
	109	M	6	5.15	76	63.5	97	10.6	4.7	43	243	47.1	3.82	832	426	82.7	6.70	1457
*	110	F	2½	3.56	74	55.5	96	8.6	3.3	33	170	48.0	3.06	750	255	71.5	4.59	1125
	111	F	9	8.23	98	74	109	11.5	4.55	45	289	35.1	3.91	725	521	63.3	7.04	1308
	112	M	7	6.92	94	66	100	12.2	5.05	44	242	34.9	3.67	710	432	62.4	6.55	1268
	113	F	7	5.08	71	63	98	14.1	5.4	53	185	36.4	2.94	641	394	77.5	6.25	1366
	114	F	4	4.09	73	60	100	13.2	5.6	51	154	37.6	2.57	606	317	77.4	5.28	1248
	115	F	8	5.46	69	70	104	12.4	5.15	41	237	43.4	3.38	737	409	75.0	5.84	1272

Table 20.

Gastro-enteritis.

Case	Plasma Volume, per cm.			Blood Volume, per cm.		
	Actual ml.	Expected ml.	per cent deviation	Actual ml.	Expected ml.	per cent deviation
103	3.68	3.9	−6	6.34	6.15	+3
104	3.58	3.8	−6	6.67	6.0	+11
105	5.03	5.3	−5	9.0	8.5	+5
106	4.57	5.1	−11	8.31	8.1	+2
107	4.05	4.7	−14	5.96	7.3	−19
108	3.23	3.6	−10	5.15	5.7	−10
109	3.82	4.75	−20	6.70	7.4	−10
110	3.06	3.9	−22	4.59	6.0	−24
111	3.91	5.5	−29	7.04	9.0	−22
112	3.67	5.0	−27	6.55	7.9	−18
113	2.94	4.7	−38	6.25	7.3	−15
114	2.57	4.3	−41	5.28	6.8	−23
115	3.38	5.3	−36	5.84	8.5	−32

Expected plasma volumes per cm. were read from Graph 10 (healthy children).

Per cent deviation = percentage difference of the actual from the expected values.

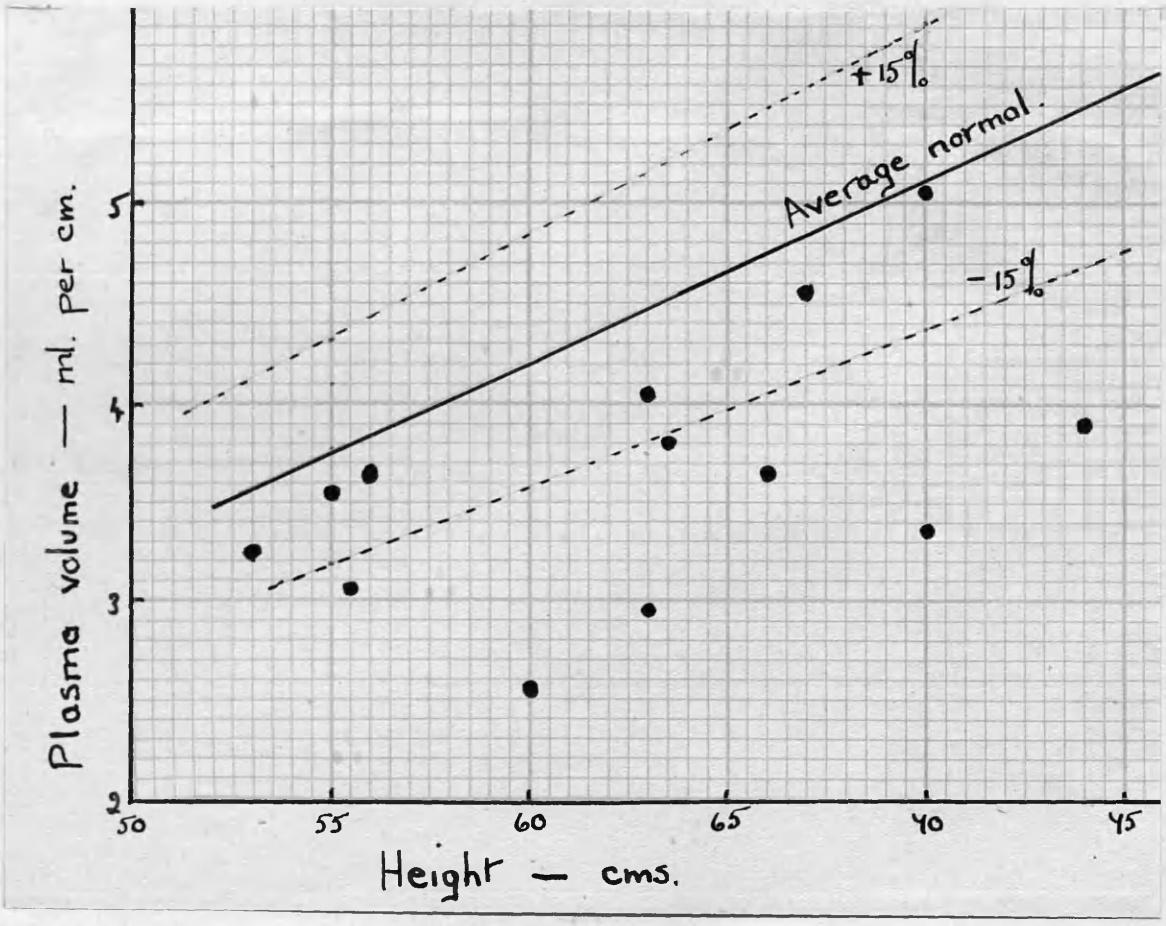
volumes to be low in the acute stage of gastro-enteritis, with increase on recovery, and in another case, a high plasma volume decreasing on recovery. In a group of eleven dehydrated infants, McIntosh, Kajdi and Meeker (1931) demonstrated plasma volume increase on recovery in five cases, no change in three, and an actual decrease in the remaining three.

The number of children investigated in the present series, thirteen in all, is unfortunately small, because of the difficulty of finding suitable cases. If the children were severely dehydrated, the method was carried out only when an intravenous drip was going to be given, as the procedure might have been prejudicial to their chances of recovery. Severely dehydrated infants were chosen, as they were more likely to show blood volume changes than the milder cases. Blood was withdrawn from the anterior fontanelle in the first three babies, but in the others, from the external jugular veins, as the fontanelle blood was open to the criticism of being diluted with cerebro-spinal fluid. The difficulties of withdrawing blood in dehydrated patients are too well-known to require elaboration.

In Table 19 the results obtained in the thirteen dehydrated infants are detailed. Table 20 shows the percentage deviation of the plasma and blood volumes in ml. per cm. of height, from the average for normal children of corresponding height. These average or expected values were read from Graph 10. The figures

Graph 21.

Plasma volume in gastro-enteritis.



• • • Gastro-enteritis cases.

——— Average normal curve of plasma volume per cm. related to height. (Graph 10)

In every infant with gastro-enteritis, the plasma volume lay below the normal average, although in some instances, it did not lie outwith the limits of normality.

for plasma volume have been drawn out in Graph 21. The plasma volume in all cases showed a reduction which in seven was below minus 15 per cent, The three children (Cases 113, 114, and 115) whose plasma volumes were reduced to levels below 70 per cent of normal, all died shortly after admission, despite the administration of intravenous fluid by continuous drip.

Discussion.

On first examining the results of the investigations in these dehydrated infants, one was impressed by the lack of conformity between the biochemical findings, the reduction in the plasma volume, and the degree of dehydration judged by clinical methods. An attempt has therefore been made to assess the conditions influencing each of these factors, and thereby explain the apparent anomalies in the results.

1. Degree of Clinical Dehydration.

Probably the most important problem in gastro-enteritis, from the viewpoint of the practical paediatrician, concerns the question of correlation between the degree of reduction of the blood volume, and the degree of dehydration which can be estimated by clinical examination. Darrow and Buckman (1928) found low plasma volumes in all the infants of their series showing clinical dehydration. McIntosh, Kajdi and Meeker (1931) found no correlation between the two, and concluded that the volume of circulating blood need not necessarily show a decrease, even in the presence of marked dehydration. Eight of

Table 21.

Gastro-enteritis.

Case	Age mths	Plasma Volume	Clinical Dehydration	Days of Illness	N.P.N. mg.per cent	T.I.F. g.per cent	Hb. g.per cent	R.B.C. mills. per cmm.	P.C.V. per cent	M.C.V. c.μ	M.C.Hb.C. per cent
103	4½	±	+++	14	39	7.1	12.7	4.5	42	93	30
104	4	±	+	2	-	-	13.5	4.58	46	100	29
105	9	±	+++	22	69	6.4	10.7	5.35	44	82	24
106	6	—	+++	2	112	9.7	13.2	5.1	45	88	29
107	6	—	+	5	90	9.8	6.5	3.71	32	86	20
108	2	—	+++	9	74	7.4	11.6	4.2	37.5	89	31
109	6	—	+++	7	63	8.3	10.6	4.7	43	91	24
110	2½	—	+	5	66	7.5	8.6	3.3	33	100	26
111	9	— —	++	21	50	5.9	11.5	4.55	45	98	25
112	7	— —	+	5	120	6.9	12.2	5.05	44	87	28
113	7	— — —	+++	2	140	7.6	14.1	5.4	53	98	26
114	4	— — —	+++	25	151	6.0	13.2	5.6	51	91	25
115	8	— — —	+++	7	-	- -	12.4	5.15	41	79	30

Clinical Dehydration, the number of pluses indicates the severity.
Plasma Volume, ± indicates figures within normal limits.
— indicates degree of reduction below normal.

the present series (Cases 103,105,106,108,109,113,114 and 115) showed three pluses of clinical dehydration, yet five of these (Cases 103,105,106,108 and 109) were found to have only slight reduction of the blood volume (Tables 20 and 21). Of these five, there was no apparent reason for the discrepancy in Case 103. In Cases 106 and 108 the estimations were not done until the day after admission, by which time each infant had drunk 500 ml. of half-strength saline, and thereby had probably increased the plasma volume. This would suggest that the plasma "hydration" is completed first, and that only then do the interstitial and intracellular fluids have their volumes restored. Experimental work with dogs subjected to water and salt depletion, separately and combined, led Hopper, Elkington and Winkler (1944) to conclude that there was a definite tendency for the plasma volume to be maintained at normal levels. The remaining two infants of the group showing severe clinical dehydration, with only slight reduction of plasma volume (Cases 105 and 109), both had iron deficiency anaemia, as judged by the mean corpuscular haemoglobin concentration, and therefore probably had high plasma volumes before the onset of the gastro-enteritis (see Section 6). In addition, Case 109 had a remittent pyrexia, and fever tends to induce a rise in plasma volume (Pinkston and Gregersen, 1935). The three children who died (Cases 113,114 and 115), were those who showed the greatest reduction in plasma volume, all over 36 per cent. The fact that in two, the fatal outcome was not prevented by adequate intravenous fluid (plasma and 10 per cent glucose in 0.5 per cent sodium

chloride solution) suggests that when the plasma volume has been reduced to a certain level, probably below 70 per cent of normal, mere restoration of the blood volume is insufficient. By that time, profound changes have probably taken place in the intracellular protoplasm, partly due to actual breakdown of proteins because of dehydration and inanition, and partly due to the withdrawal of intracellular fluids and loss of potassium, which has been shown to occur in severe gastro-enteritis (Butler et al., 1933).

The degree of reduction of the plasma volume should be regarded as a measure of the loss of fluid from the extracellular fluid compartment, which consists of plasma plus interstitial fluid. In this respect, the previous nutrition of the child will have some influence on the magnitude of the drain on the available body fluids, which can be withstood. It has been shown that chronic food and water insufficiency causes a decrease in both extra and intracellular fluid volumes (Hopper et al., 1944).

2. Plasma Proteins.

If the process of dehydration were simply one of contraction of the blood volume, with resultant increase in the concentration of each constituent, one would expect a very different series of results from that seen on studying Table 21. Cases 111 to 115 had the greatest reductions in plasma volume, yet did not have high plasma protein concentrations. The serum protein concentration of "normal" infants in hospital averaged 7.08 grams per cent, with a range of 6.04 to 8.0 grams per cent (Rennie, 1935).

At the other extreme, Cases 106 and 107 had definite elevations in plasma protein levels in the presence of only slight dehydration. In contrast to these findings, Bridge, Cohen and Scott (1941) in a study of the dehydration in diarrhoeal diseases, considered that in a general way, the serum protein concentration reflected the degree of dehydration. Unfortunately, one had no means of forming an estimate of the plasma protein level prior to the onset of the dehydration, other than by deduction from the history of the feeding given by the mother. Among the hospital class of patients, the practice of giving diluted feeds still continues, and this was found to hold for Cases 103 and 111. Estimations of the proteins carried out after full hydration are of little value as an index of the plasma protein level before the onset of the illness, as by that time the protein metabolism has probably been influenced by the treatment of the gastro-enteritis i.e. administration of water and half-strength saline only, followed by small, gradually increasing milk feeds.

There appeared to be some correlation however, between the length of the illness, and the degree of plasma protein elevation (Table 21). The three longest illnesses, Cases 105, 111 and 114, had the lowest protein concentrations, though the converse did not hold true, namely that all the children with a short illness had high values (see Cases 112 and 113). With regard to the length of illness, Marriott, Hartmann and Senn (1935) believed that

Table 22.

Gastro-enteritis.

Estimations on two children

(a) while dehydrated (b) after recovery.

	Case 111		Case 112	
	6.9.46	25.9.46	16.8.46	22.8.46
Plasma volume, ml.	289	419	242	362
Blood volume, ml.	521	655	432	593
Plasma proteins, g. per cent	5.94	6.26	6.86	6.01
Blood N.P.N., mg. per cent	50	25	120	48
Total circulating protein, grams	17.67	26.23	16.6	21.76
Haemoglobin, g. per cent	11.5	10.4	12.2	10.11
Haematocrit reading, per cent	44.5	36	44	39
Erythrocyte count, millions per c.mm.	4.55	4.2	5.05	4.4
Total haemoglobin, grams	59.9	68.12	52.7	59.95
M.C.V., c. microns	98	85	87	89
M.C.Hb.C., per cent	25	28	28	26

anhydraemia existing for some time caused plasma protein destruction. Darrow and Buckman (1938) stated that an actual "loss" of protein occurred secondary to reduction of plasma volume, and that the protein was returned to the blood stream during recovery. Experimental work on dogs, has produced evidence of a similar nature, that during salt depletion, protein disappears from the circulating plasma (Elkington, Danowski and Winkler, 1946). In two of the children of the present series, the plasma volume and protein concentration were estimated, firstly during severe dehydration, and secondly, when hydration had been accomplished (Table 22). Both showed an increase in total circulating protein on recovery. In one child, Case 111, this increase may have been due to normal protein synthesis, as a gap of three weeks intervened between the estimations. In the other infant, Case, 112, the experiments were separated by an interval of only six days, and the baby had had no plasma transfusions which might have disturbed the normal distribution of proteins in the body.

To summarise, the factors influencing the plasma protein concentration in children with gastro-enteritis are as follows:-

(a) Simple contraction of the blood volume with resultant haemoconcentration.

(b) The degree of inanition existing prior to the illness.

(c) The duration of the illness.

To these may be added a fourth -

(d) The effect of toxaemia on the normal synthesis of proteins by the liver.

It may be concluded from the above studies that the concentration of the plasma proteins forms a most unreliable index of the degree of dehydration in infants; McIntosh, Kajdi and Meeker (1931) and Aldridge (1941) came to similar conclusions.

3. Haemoglobin, Erythrocyte Count, and Packed Cell Volume.

The haemoglobin concentration did not indicate haemoconcentration proportionate to the amount of plasma volume contraction. Mackay (1933) found that the average haemoglobin curve for artificially fed infants rose from 70 per cent (Haldane) at three months to 75 per cent at six months and remained around that level for the rest of the first year. (In terms of absolute haemoglobin values the rise was from 9.66 grams per cent. to 10.35 grams per cent.). This low haemoglobin range in normal infants must be remembered when assessing the degree of haemo-concentration in dehydration. In the present series, two children were recognised as being very definitely anaemic, even in the presence of dehydration - Cases 107 and 110 (Table 21). Other two (Cases 105 and 109) had haemoglobin values just above the normal average, and on the basis of haemoglobin level alone, the question of dehydration might have been dismissed. In actual fact, both had moderate degrees of plasma volume reduction. The

existence of the milder degrees of anaemia obviously could not be diagnosed from examination of the haemoglobin concentration alone in gastro-enteritis. The calculation of the mean corpuscular haemoglobin concentration, however, was of value, as seen in the two cases of mild anaemia under discussion, in both of which the result was 24 per cent. (The normal range for adults is given as 32 to 38 per cent. by Whitby and Britton, 1939b. For infants however, the range is probably lower, and using Mackay's figures for haemoglobin (1933) and Cleland's results (1941) for haematocrit readings in healthy infants, the figures for mean corpuscular haemoglobin concentration lie between 26 and 30 per cent.) Aldridge (1941) also concluded that the haemoglobin concentration was only of value in assessing dehydration, if the degree of nutritional anaemia present could be estimated.

Except in the two severely anaemic infants (Cases 107 and 110) haematocrit readings lay at levels above normal, as judged by the findings of Cleland (1941), who stated that from the age of three months till about eight months, the haematocrit reading fluctuated between 35 and 38 per cent. The ten healthy infants of the present series, however, had an average packed cell volume of 41 per cent. Even with this latter figure as a basis for comparison, all, except the two severely anaemic infants already mentioned and Case 108, had readings above normal. The packed cell volume has the

advantage over haemoglobin as an index of dehydration, in that in nutritional anaemia, despite marked reduction in haemoglobin concentration, the number of erythrocytes may be only slightly decreased. Consequently the haematocrit reading may show no reduction as a result of the anaemia, except when the latter is microcytic. Apart from microcytosis, another factor which may lead to erroneous interpretations of the haematocrit reading, is the swelling of individual cells which may take place under the influence of acidosis (Paxton, 1935). Evidence of this increase in cell size was found in the present series, where the average mean corpuscular volume was 91 cubic microns, four of the values being above 94 cubic microns (Table 21). (Whitby and Britton 1939c, give the normal average as 86 cubic microns, with a range of 78 to 94).

4. Blood Non-Protein Nitrogen.

In eleven of the thirteen children with gastro-enteritis, estimations of the blood non-protein nitrogen were made (Table 21). All except one, (Case 103) were found to have a definite elevation above normal. In Cases 112, 113, and 114, i.e. the infants who had the greatest reduction in plasma volume, the levels ranged from 120 to 151 milligrams per cent. Young and McCance (1942) reported that the high levels of blood urea found in gastro-enteritis were due not to definite renal damage, but to the fact that only a very small amount of water

was available for excretion. On this account, the amount of urea excreted was very small, since the kidneys of infants had a very poor concentrating power. The results of the present series seem to follow these conclusions closely, as the highest blood non-protein nitrogen levels were found in the children with the lowest plasma volumes and therefore probably the lowest urinary minute volumes.

The problems which have already been discussed, are related to dehydration, which however, is not the only outstanding clinical manifestation of gastro-enteritis. Many infants are judged to be toxic, as well as dehydrated, this quality of toxicity probably depending on the severity of the precipitating infection. Where this factor predominates, the plasma volume will probably be reduced to a lesser degree than in dehydration alone, as Ebert and Stead (1941c) found that the plasma and blood volumes were not reduced in the circulatory failure caused by infections. Case 103, for example, was thought to be very dehydrated clinically, but in actual fact showed only slight reduction of blood volume. For two weeks prior to admission, the child had had an acute infection of one maxillary sinus, and was diagnosed in the ward as having broncho-pneumonia with secondary gastro-enteritis. On retrospect, the clinical description should probably have been - "a severely toxic infant, with slight dehydration".

Apart from the presence of toxaemia, peripheral circulatory collapse is common in gastro-enteritis, and

its severity may be related to the fact that the "dehydration", so called, is a mixture of salt and water depletion. Pure salt depletion in untraumatised animals has been shown to cause a form of peripheral vascular collapse like that seen in traumatic shock (Elkington et al., 1946). In simple water depletion, sufficient to produce a similar reduction in extracellular fluid volume, peripheral collapse was not found. The chief difference in the two conditions would appear to lie in the different electrolyte concentrations of the extracellular fluid compartment. The hypertonicity induced by water depletion is corrected by withdrawal of water from the cells. The hypotonicity of the extracellular fluid induced by salt depletion remains uncorrected, since the cell membrane is relatively impermeable to electrolyte ions (Marriott, 1947). One of the present series, Case 111, probably approached the condition of pure salt depletion as nearly as is possible for an infant with gastro-enteritis, where in addition to chloride loss, there is always a marked loss of base in the stools. This child had been given boiled water only, in adequate amounts for a week before admission, and for the week previous to that, had had one ounce feeds of milk which were mostly vomited, i.e. for fourteen days, her intake of salt was practically nil. The striking features of the clinical condition on admission were the extreme hypotonia of the muscles and mental state approaching coma-vigil. Although the child had been given a plentiful water intake, she was still

dehydrated clinically, and had a low plasma volume.

Summary.

Thirteen infants suffering from gastro-enteritis have been studied, and the findings recorded for plasma volume, blood volume, haemoglobin concentration, haematocrit reading, blood non-protein nitrogen and total plasma proteins.

The plasma volumes in the infants with gastro-enteritis were found to be consistently below the normal average for healthy children of the same height. In three very severe cases, the plasma volume reduction averaged 38 per cent.

An attempt has been made to correlate the above findings with one another, and with the degree of clinical dehydration. In general, the severity of the latter gave no indication of the degree of reduction in the plasma volume. The plasma protein concentration was found to be unreliable as an index of the degree of dehydration; more reliance could be placed on the haematocrit reading. The haemoglobin percentage occupied a position mid-way between these two in importance.

Section 6.

Blood Volume in Anaemia.

Since blood volume estimations were first practised, many divergent opinions have been held regarding the state of the blood volume in anaemia. Haldane and Smith (1900) using the carbon monoxide inhalation method, found that the blood volume was markedly increased in anaemia, and concluded (a) that the blood mass increased as the percentage oxygen capacity diminished, and (b) that anaemic blood was one to which plasma had been added, and that it did not contain a smaller total quantity of haemoglobin. In another publication (1900) Smith altered their previous conclusion, by stating that the decrease in number of erythrocytes and in haemoglobin concentration could not be regarded as due merely to increase in plasma. "Serous plethora" in anaemia was also recorded by Keith, Rowntree and Geraghty (1915), along with rather low figures for total blood volume, in secondary anaemia, and also in two out of three cases of pernicious anaemia. Bock (1921) confirmed these findings. Further work with the carbon monoxide method (Plesch, 1922) gave support to the theory of inverse relationship existing between the percentage oxygen capacity and the total blood volume. Plesch even stated categorically, "Je geringer der Hämoglobingehalt, um so grösszer ist die Blutmenge." In several patients with secondary anaemia, he found a true "thinning" of the blood i.e. high total blood volume associated with normal total

haemoglobin content. It is difficult to understand why Haldane and Smith, and Plesch, using the carbon monoxide method, should have obtained such high total blood volume results, as the error in the method tends to give apparently low values (see Part I). Later workers, with the dye methods, were all agreed (1) that in secondary anaemias regardless of aetiology, the plasma volume was increased, and the total blood volume slightly below normal and (2) that in pernicious anaemia, the plasma volume was again increased, but the total blood volume showed a greater reduction than in secondary anaemia. For example Gibson, 1939, found the total blood volume in Addisonian anaemia to be 14 per cent below the normal average. (Brown and Rowntree, 1928; Gibson, 1939; Gibson, Harris and Swigert, 1939.)

Results.

The present series included twenty-six children suffering from anaemia of various types, and also two babies with congenital haemolytic anaemia whose findings will be discussed at the end of the section. The various conditions investigated and the number of cases in each, are as follows:-

Diagnosis.	No. of Cases.	Table.
Iron deficiency anaemia	15	23
Leukaemia	3	24
Hodgkin's Disease	1	24
Aplastic anaemia	3	24
Idiopathic haemolytic anaemia	2	25
Post-haemorrhagic anaemia	1	25
Macrocytic anaemia	1	25

Table 23.

Iron Deficiency Anaemia.

Case	Sex	Age yrs.	Weight kilos	Weight per cent	Height cms.	Height per cent	Hb.	RBC	PCV	Plasma Volume, ml. Total	Plasma Volume per kilo	Plasma Volume per cm.	Plasma Volume per sq.m.	Blood Volume, ml. Total	Blood Volume per kilo	Blood Volume per cm.	Blood Volume per sq.m.
* 116	F	1 7/12	7.83	78	75.2	99	4.5	2.6	28	476	60.8	6.33	1210	661	84.4	8.79	1688
117	M	1 5/12	8.63	85	71	93	5.8	3.02	31	473	54.8	6.66	1198	685	79.4	9.65	1737
118	M	11/12	8.08	91	70	99	8.8	4.02	36	455	56.3	6.50	1197	711	87.9	10.15	1870
119	M	10/12	7.93	95	71	100	7.0	4.4	32	466	58.8	6.56	1225	685	86.4	9.65	1800
120	M	1 9/12	10.48	95	87	110	4.2	3.2	23	673	64.2	7.73	1355	874	83.4	10.04	1760
Coeliac Disease.																	
121	F	6 1/12	11.54	58	92.5	83	7.2	3.7	32	652	56.5	7.05	1206	959	83.1	10.36	1774
122	M	2 4/12	8.42	64	82	95	9.3	4.4	38	482	57.2	5.88	1111	777	92.3	9.48	1791
123	M	2 2/12	10.82	87	91	109	9.0	4.52	35	734	67.8	8.06	1412	1133	104.7	12.46	2180

Table 23, (continued.)

Iron Deficiency Anaemia.

Case	Sex	Age yrs.	Weight kilos	Weight per cent	Height cms.	Height per cent	Hb.	RBC	PCV.	Plasma Volume, ml. Total	Plasma Volume, ml. per kilo	Plasma Volume, ml. per cm.	Plasma Volume, ml. per sq.m.	Blood Volume, ml. Total	Blood Volume, ml. per kilo	Blood Volume, ml. per cm.	Blood Volume, ml. per sq.m.
Secondary anaemia.																	
* 124	M	2 6/12	10.18	78	82	95	4.5	4.51	37.5	549	53.9	6.70	1150	878	86.3	10.71	1841
125	M	2 3/12	12.92	102	91	105	7.0	3.0	31	751	58.2	8.25	1340	1081	83.8	11.90	1931
126	M	6	15.8	77	112	100	6.3	3.29	32	1136	72.0	10.14	1600	1659	105.0	14.81	2332
127	F	1 3/12	8.63	94	89	120	5.9	2.74	30	510	59.1	5.73	1100	717	83.1	8.06	1543
128	F	5	13.6	75	102	99	7.6	3.99	36	798	58.7	7.82	1285	1247	91.7	12.23	2008
129	M	2 6/12	12.08	92	89	106	5.6	4.1	35	748	61.9	8.40	1396	1150	95.2	12.92	2146
Ulcerative colitis.																	
130	M	11	36.36	101	144	102	7.0	3.3	29	2510	69.0	17.43	2070	3535	97.2	24.55	2916

Table 24.

Leukaemia, Hodgkin's Disease, Aplastic Anaemia.

Case	Sex	Age yrs.	Weight kilos	Weight per cent	Height cms.	Height per cent	Hb.	RBC.	PCV.	Plasma Volume, ml. Total	Plasma Volume, ml. per kilo	Plasma Volume, ml. per cm.	Plasma Volume, ml. per sq.m.	Blood Volume, ml. Total	Blood Volume, ml. per kilo	Blood Volume, ml. per cm.	Blood Volume, ml. per sq.m.
Leukaemia.																	
* 131	M	2 3/12	13.6	109	87	104	3.7	1.21	9.4	1039	76.4	11.94	1870	1147	84.3	13.18	2067
132	M	7 11/12	22.6	92	122	100	4.2	2.05	18	1355	60.0	11.10	1539	1652	73.1	13.54	1878
133	M	5 3/12	20.2	105	107.5	100	3.3	1.19	13	1565	77.5	14.52	2049	1801	89.2	16.71	2358
Hodgkin's Disease.																	
134	M	6	14.22	66	102	89	7.3	3.40	31	1086	76.4	10.65	1710	1570	110.5	15.39	2473
Aplastic Anaemia.																	
135	M	2 5/12	10.69	82	88	102	6.9	1.90	25	708	66.2	8.04	1405	944	88.3	10.73	1873
136	F	9/12	6.16	75	65	95	4.8	1.50	16	389	63.1	5.98	1213	463	75.1	7.12	1444
137	F	2 6/12	12.75	103	95	110	4.6	1.44	12.5	722	56.6	7.60	1254	825	64.7	8.68	1433

Table 25.

Haemolytic, Post-haemorrhagic, and Macrocytic Anaemias.

	Case	Sex	Age yrs.	Weight kilos	Weight per cent	Height cms.	Height per cent	Hb.	RBC	PCV	Plasma Volume, ml. Total	Plasma Volume per kilo	Plasma Volume per cm.	Plasma Volume per sq.m.	Blood Volume, ml. Total	Blood Volume per kilo	Blood Volume per cm.	Blood Volume per sq.m.
	Haemolytic Anaemia.																	
*	138	F	8	15.46	64	106	87	2.9	1.35	14.7	1136	73.4	10.72	1676	1331	86.1	12.56	1964
	139	M	6	17.4	79	111	96	6.3	3.46	28.4	1191	68.4	10.73	1619	1663	95.6	14.98	2261
	Post-haemorrhagic Anaemia.																	
	140	M	11	29.22	90	141	100	5.0	2.20	19	1996	68.3	14.16	1832	2465	84.4	17.48	2262
	Macrocytic Anaemia.																	
*	141	M	6	11.38	54	95	84	3.4	1.35	15.5	712	62.6	7.49	1298	843	74.1	8.87	1537

The results obtained showed that in children the average plasma volume was high in anaemia, and the average total blood volume was normal.

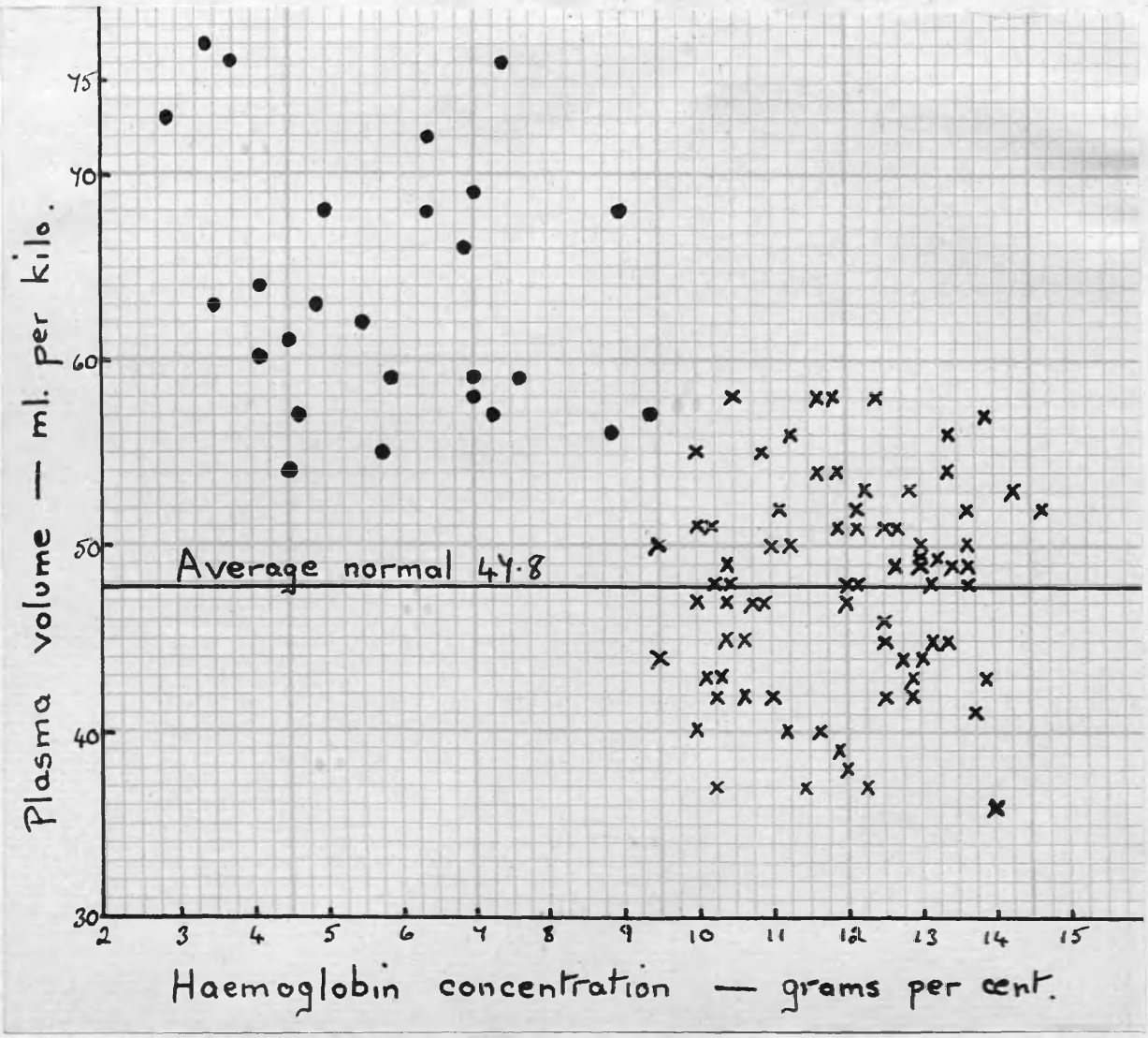
	Plasma ml. per kilo	Blood ml. per kilo
1 Normal children		
Mean	47.8	83.5
Range	35.8-58.4	62-113
Standard deviation	5.83	10.39
2 Anaemic children		
Mean	63.8	87.3
Range	53.9-77.5	64.7-110.5
Standard deviation	7.0	9.9
Difference between means	16.0	3.8
Standard error of difference	1.52	2.26

It is evident that on a weight basis, there was a very significant increase in the plasma volume, while the blood volume average was essentially normal. Without exception, the anaemic children had plasma volumes definitely above the average normal (Graph 22).

As the children in the group varied greatly as regards percentage of expected weight, two of the coeliac cases being only 54 and 58 per cent, the results were re-calculated in terms of deviation per cent from the average normal unit volume per cm. at each individual height (taken from Graph 10), and will be expressed as such throughout the section. For plasma volume,

Graph 22.

Plasma volume, in ml. per kilo, graphed against the blood haemoglobin concentration, in grams per cent.



● ● ● Anaemic children.

× × × Normal children.

In every child with anaemia, the plasma volume lay above the normal average, although in some cases it was not outwith the limits of normality.

similar results on the whole, were obtained, while as regards blood volume several interesting points of difference were made manifest.

Average Values.	Per cent deviation from average, normal, unit volume per cm. Plasma	Cells	Blood	Haematocrit per cent
Entire series	+29.0	-37	+1.7	
1. Iron deficiency	+20.7	-20.1	+4	32.4
2. Leukaemia	+68	-65	+2.9	17.8
Hodgkin's Disease	+44	-12	+21	31
3. Aplastic anaemia	+21	-62	-14	17.8
4. Haemolytic anaemia	+35.5	-46	+1.5	21.5
5. Post-haemorrhagic anaemia	+43	-56	+1	19
6. Macrocytic anaemia	+10	-72	-25	11.3

It is clear from the above analysis that the plasma volume showed a marked increase in all types of anaemia in children, the percentage rise varying from +10 per cent in the macrocytic anaemia to +60 per cent in the leukaemia cases. The average total blood volume for the entire series again showed no marked or consistent change from normal.

1. Iron deficiency group.

The anaemias concerned fell into two divisions, those purely dietetic in origin, and those secondary to infection. The two types have been classified together as it was felt that the blood pictures were essentially similar, i.e. both had a normal or low mean corpuscular volume with a

low mean corpuscular haemoglobin concentration. It should be pointed out that the infection group did not include children with fever, but merely children who had a history in the previous 6 to 12 months of recurrent infections, with the exception of Case 127, a female aged one year and three months, who had pyuria with dehydration and acidosis.

In the following tables, and throughout the section,

Hb. = haemoglobin concentration, in grams per cent.

R.B.C. = red blood count expressed in millions per c.mm.

P.C.V. = haematocrit reading, per cent.

M.V.C. = mean corpuscular volume, in cubic microns.

M.C.Hb.C. = mean corpuscular haemoglobin concentration, per cent.

Plasma, cell, and blood volumes are expressed as deviations per cent from the normal average unit volumes per cm. of height (Graph 10).

No.	Hb. g.per cent	R.B.C. mills per c.mm.	P.C.V. per cent	M.C.V. c.μ	M.C.Hb.C. per cent	Per cent deviation from normal: Plasma	Cells	Blood
Nutritional anaemia.								
116	4.5	2.6	28	107	16	+15	-31	-3
117	5.8	3.02	31	102	19	+24	-14	+10
118	8.8	4.02	36	90	24	+22	+5	+16
119	7.0	4.4	32	72	22	+22	-11	+9
120	4.2	3.2	23	72	18	+26	-51	-7
Coeliac Disease.								
121	7.2	3.7	32	86	23	+7	-32	-9
122	9.3	4.4	38	86	24	+1	-18	-6

No.	Hb.	R.B.C.	P.C.V.	M.C.V.	M.C.Hb.C.	Plasma Cells	Blood
Coeliac Disease.							
123	9.0	4.52	35	77	25	+24	-18 +9
Secondary anaemias.							
124	4.5	4.51	37	83	12	+15	-6 +5
125	7.0	3.0	31	100	22	+27	-26 +4
126	6.3	3.29	32	97	20	+23	-18 +7
127	5.9	2.74	30	109	19	-9	-52 -27
128	7.6	3.99	36	90	21	+6	-18 -4
129	5.6	4.1	35	85	16	+33	-6 +16
Ulcerative colitis.							
130	7.0	3.3	29	88	24	+74	-6 +40

This anaemia was probably of mixed aetiology, partly dietetic in origin and partly post-haemorrhagic.

Conclusions.

The plasma volumes were increased in all the iron deficiency anaemias with one exception, (Case 127) a child who was acidotic at the time of estimation. The total blood volume in the majority varied between +10 and - 10 per cent of normal.

In adults, using the Evans blue method, Gibson, Harris and Swigert (1939) obtained similar results in hypochromic anaemias of varied aetiology. They found the plasma volume to be high in all their patients, while the blood volume was raised in 33 per cent, and in 67 per cent was decreased as much as 18 per cent below normal. McMichael, Sharpey-Schäfer, Mollison and Vaughan (1943) in four adults with hypochromic anaemia found marked reduction of the total

blood volume. Their method however was the concentrated corpuscle haemoglobin technique, which, in their hands, also gave much lower volumes for pernicious anaemia than those generally found with the dye method. Results with this method in the present series, and the inherent fallacies involved, will be discussed later. Unfortunately the only available references to children have been in isolated cases. Seckel (1936) found the plasma volume low in "constitutional" anaemias, and high in infective anaemias, while Lucas and Dearing (1921) reported a very high plasma volume and high blood volume in an older child with anaemia, the aetiology not being stated, and increase of plasma volume with normal blood volume in three infants with secondary anaemia.

Response to treatment.

Under treatment with iron a gradual reversal of the changes noted in the anaemic stage took place. Unfortunately, several estimations were not possible with the small children, but two had repeat estimations performed once the haemoglobin was within normal limits.

	Case 128		Case 130		
Age, in years	$2\frac{1}{2}$		1 9/12		
Date	4.10.46	28.10.46	13.12.46	20.1.47	
Haemoglobin	5.6	10.8	4.2	12.2	g. per cent
Erythrocytes	4.1	4.88	3.2	4.65	mills per c.mm.
Haematocrit	35	43	23	46	per cent
M.C.V.	85	88	72	98	c.μ.

	Case 128		Case 130		
Age, in years.	$2\frac{1}{2}$		1 9/12		
Date	4.10.46	28.10.46	13.12.46	20.1.47	
M.C.Hb.C.	16	25	18	26	per cent
M.C.Hb.	13.6	22.1	13.1	26.2	$\gamma\gamma$.
Absolute C.V.	402	450	201	510	ml.
Absolute P.V.	748	597	673	598	ml.
Absolute B.V.	1150	1047	874	1108	ml.
Total Hb.	64.4	113.1	36.7	135.2	grams.

M.C.V. = mean corpuscular volume, in cubic microns
M.C.Hb.C. = mean corpuscular haemoglobin concentration per cent.
M.C.Hb. = mean corpuscular haemoglobin, micro micrograms.
C.V.,P.V.,B.V., = cell, plasma, and blood volumes respectively.

These estimations showed how important it was to know the state of the blood volume before one could judge adequately of the progress in anaemia. In Case 128, the haemoglobin percentage did in actual fact give a true indication of cure, as it was doubled, and so also was the total circulating haemoglobin. The cell volume, however, was <u>not</u> markedly increased, but each cell contained one and a half times the amount it held previously. Case 130 showed the total circulating haemoglobin increasing to about 400 per cent of the previous level, yet the haemoglobin percentage was only trebled - this anomaly being due to the fact that the absolute blood volume had been increased by about one third of the original value. The final absolute cell volume was only 250 per cent of the original, and seemed to contradict the 400 per cent

increase in total haemoglobin, until one examined the mean corpuscular haemoglobin, which was finally doubled in each cell.

From the analysis of these two cases, the futility of expecting full knowledge of the progress of an anaemia from the usual test-room estimations, was made unmistakably manifest.

2. Leukaemia group.

Case	Hb. g.per cent	R.B.C. mills per c.mm	P.C.V. per cent	M.C.V. c.μ.	M.C.Hb.C. per cent	Per cent deviation from normal. Plasma	Cells	Blood
131	3.7	1.21	9.4	78	36	+95	-74	+22
132	4.2	2.05	18	81	23	+26	-61	-10
133	3.3	1.19	13	110	25	+83	-60	+24
Hodgkin's Disease.								
134	7.3	3.4	31	91	23	+44	-12	+21

The striking feature of this group was the great increase of plasma, particularly in two of the cases, which raised the question of whether the dye particles might have been removed by the abnormal leucocytes in the circulation. One cannot exclude this possibility, but there is some evidence against it in the fact that Cases 131 and 132 were aleukaemic, and that in addition Case 131 could have had very few deposits of leukaemic tissue in his body, as neither liver, spleen nor lymphatic glands were enlarged at the time the blood volume was estimated, nor did marrow puncture reveal typical leukaemic infiltration until several weeks later. Keith (1923)

found a high plasma volume in adults, in six cases of myeloid leukaemia, and in one out of four cases of lymphatic leukaemia, and put forward the suggestion that the presence of the enlarged spleen and liver, presumably by furnishing a greater area of sinuses and sinusoids, might explain the rise in plasma volume.

3. Aplastic anaemia.

No.	Hb. g.per cent	R.B.C. mills per c.mm	P.C.V. per cent	M.C.V. c.μ.	M.C.Hb.C. per cent	Per cent deviation from normal Plasma	Cells	Blood
135	6.9	1.9	25	131	28	+29	-38	-1
136	4.8	1.5	16	101	30	+22	-68	-15
137	4.6	1.44	12.5	86	36	+12	-79	-26

Results in this group showed a definite tendency for the total blood volume to be reduced, in spite of increased plasma volume. In this respect, they differed from the iron deficiency group, and the contrast was obviously associated with the state of the absolute cell volume. In the iron deficiency group, although several cases had comparable reductions in haemoglobin level, the circulating cell volume was only slightly decreased (average haematocrit reading 32 per cent), the reaction of the bone marrow to iron deficiency being to produce cells slightly smaller than normal, and poorly filled with haemoglobin. In aplastic anaemia, on the other hand, the cells are well filled but are produced in ever decreasing numbers (average haematocrit reading 17.8 per cent).

The difference between the total blood volumes in

aplastic anaemia and leukaemia could not be explained so readily. Here the cell volume decreases were of similar magnitude. Why there should be the marked difference in plasma volume results was not obvious, though the rate of progress of the disease might have some influence. Judged by the history given with the children on admission, the leukaemias were all of much shorter duration than the aplastic anaemias when the blood volume was estimated. It may be that an anaemia of rapid onset evokes an increase in circulating plasma sufficient to maintain the blood volume, more readily than a chronic aplastic type, in which there is some evidence that the cardio-vascular system reacts to reduction of total haemoglobin by contracting the total blood volume slightly (Gibson, 1939).

4. Haemolytic anaemia.

No.	Hb. g.per cent	R.B.C. mills per c.mm	P.C.V. per cent	M.C.V. c.μ.	M.C.Hb.C. per cent	Per cent deviation from normal		
						Plasma	Cells	Blood
138	2.9	1.35	14.7	109	20	+39	-67	-5
139	6.3	3.46	38.4	82	22	+32	-25	+8

Case 138 had a subacute haemolytic anaemia, of the Lederer type, which had been progressive for one month following extensive impetigo of the scalp, while Case 139 had a history extending over some years, and was classified as chronic idiopathic haemolytic anaemia. From these two estimations, it was clear that in the children with haemolytic anaemia, the plasma increase was such that total

blood volume was maintained at about the normal level. Recent work on malaria (Feldman and Murphy, 1945) demonstrated that the plasma volume was uniformly increased during the active phases of the disease, when blood destruction was occurring, and that it remained high and even increased after the paroxysms, thus proving that the increase was not due to fever alone (Soule et al. 1928; Pinkston and Gregersen, 1935).

5. Post-haemorrhagic anaemia.

No.	Hb. g.per cent	R.B.C. mills per c.mm.	P.C.V. per cent	M.C.V. c.μ.	M.C.Hb.C. per cent	Per cent deviation from normal. Plasma	Cells	Blood.
140	5.0	2.2	19	86	27	+43	-56	+1

This child, a boy aged eleven years, was admitted following an attack of haematemesis and melaena due to a peptic ulcer. The blood volume was estimated on the fifth day after haemorrhage, which must have ceased on the day of admission, judged by serial tests for occult blood in the faeces. By that time the blood volume had been fully restored, requiring about a fifty per cent increase in the plasma fraction. In a series of adults, following haemorrhage from the stomach and duodenum, Bennett, Dow, Lander and Wright, (1938) found that dilution had become maximal in the majority a few hours after cessation of the haemorrhage. The time required however, for blood volume restoration may be much longer in some instances, as shown by Wallace and Sharpey-Schäfer in controlled haemorrhage (1941), and by Dacie and Homer in battle casualties (1946).

The process of blood dilution may continue for seven days following cessation of the haemorrhage.

6. Macrocytic anaemia.

No.	Hb. g.per cent	R.B.C. mills per c.mm.	P.C.V. per cent	M.C.V. c.μ.	M.C.Hb.C. per cent	Per cent deviation from normal Plasma	Cells	Blood
141	3.4	0.99	15.5	115	21	+10	-72	-25

This child was a coeliac dwarf with macrocytic anaemia, which responded to treatment with parenteral liver extract. He showed the same volume adjustments as do adults with pernicious anaemia, namely moderate increase in plasma volume accompanied by reduction in total blood volume. After six weeks, once the anaemia had responded to treatment with intramuscular liver extract, the complete blood investigation was repeated with the following results.

	20.10.45	6.12.45	
Haemoglobin	3.4	11.3	grams per cent
Erythrocytes	1.35	3.94	millions per c.mm.
Haematocrit reading	15.5	47	per cent
M.C.V.	115	119	c. microns
M.C.Hb.C.	21	24	per cent
Absolute cell volume	131	520	ml.
Absolute plasma volume	712	584	ml.
Absolute blood volume	843	1104	ml.
Total haemoglobin	28.7	124.8	grams.

The results of the final estimation of the plasma and blood volumes were within normal limits for the child's height. One interesting feature was that the mean corpuscular volume was still high at the second estimation, whereas in pernicious anaemia there is a steady decrease in the size of the erythrocytes produced during recovery (Gibson, 1939).

Congenital haemolytic anaemia.

Case	Hb. g.per cent	R.B.C. mills per c.mm.	P.C.V. per cent	Plasma Absolute ml.	Plasma Per cm. ml.	Blood Absolute ml.	Blood Per cm. ml.
142(a)	9.4	3.7	35	197	4.28	303	6.58
(b)	8.9	2.6	28	228	4.96	319	6.93
143	13.6	4.8	57	180	3.47	418	8.03

The results in these infants could not be expressed as per cent deviation from normal, because no standards at this age were available. Case 143 was only 36 hours old and had no clinical sign of haemolysis at that time, although the diagnosis from the blood picture was definitely erythroblastosis foetalis. In view of the high haematocrit reading one can assume that haemolysis was slight. This baby's blood volume has therefore been taken as being within normal limits for the second day of life.

Case 142, was investigated (a) when four days old and (b) when thirteen days old, each time immediately before transfusion. Compared with Case 143, the plasma volume was high on the first estimation, and had increased on the second investigation by which time the infant's condition had deteriorated. The increase was probably more marked

Table 26.

Plasma Proteins in Anaemia.

Case	Diagnosis.	N.P.N. mg. per cent	T.P.P. g. per cent
116	Iron deficiency anaemia	44	7.8
118	Iron deficiency anaemia	40	6.4
119	Iron deficiency anaemia	57	4.6
120	Iron deficiency anaemia	31	6.5
121	Iron deficiency anaemia	39	7.6
122	Iron deficiency anaemia	21	6.8
128	Iron deficiency anaemia	36	7.0
129	Iron deficiency anaemia	34	8.0
131	Leukaemia	53	7.35
132	Leukaemia	63	6.4
133	Leukaemia	53	4.9
135	Aplastic anaemia	29	6.1
136	Aplastic anaemia	39	6.4
137	Aplastic anaemia	26	6.8
138	Haemolytic anaemia	31	5.7
139	Haemolytic anaemia	38	6.2
140	Post-haemorrhagic anaemia	36	5.4
141	Macrocytic anaemia	70	4.35

N.P.N. = blood non-protein nitrogen

T.P.P. = total plasma proteins.

than was apparent, as Case 142 was only 46 cm. long and Case 143 was 52 cm. i.e. normally, in health, Case 142 would have a smaller blood volume than Case 143.

From these figures one may deduce that the cardio-vascular system of new-born infants reacts to anaemia in a similar manner to that of older children, namely by augmenting the plasma volume.

Plasma Proteins in Anaemia.

The blood non-protein nitrogen and total plasma proteins were estimated in eighteen of the anaemic children (Table 26). The average figure for the group was low, 6.3 grams per cent, and the range 4.35-8.0 grams per cent. (Rennie (1935) obtained for normal children an average serum protein level of 7.42 grams per cent in older children, and 7.08 grams per cent in children under two years of age).

The lowest level, 4.35 grams per cent, was found in Case 141, the coeliac dwarf, who probably had a true dietary protein deficiency. It is interesting that no oedema existed at this very low level. The patient with post-haemorrhagic anaemia (Case 140) had also very low proteins - 5.4 grams per cent - indicating that the fluid inflow into the blood stream to restore the blood volume was poor in protein. Ebert, Stead and Gibson (1941) suggested that the initial dilution of the blood following haemorrhage was effected by fluid with a low protein content, and that once the blood volume was restored to normal, proteins were added to the circulation,

the amount added being dependent on the protein stores (Beattie and Collard, 1948a). The aplastic and haemolytic anaemias were also associated with low proteins in this series, while the iron deficiency group showed no definite tendency, the levels being spread over a wide range.

Although the general average result for plasma protein concentration was low, it is unlikely that any child had a protein deficiency sufficient to prevent haemoglobin formation - with one possible exception. It has been shown in anaemic dogs, that a low protein intake will limit the production of haemoglobin, even in the presence of excess of iron (Hahn and Whipple, 1939). The exception was the child with macrocytic anaemia, whose plasma protein concentration was 4.35 grams per cent. For ten days, he failed to respond to treatment with liver extract and iron. He was then given a blood transfusion, and ten days later, while still on liver and iron, his blood regeneration started. It is possible that the daily injections of liver extract, and the protein from the blood transfused, were all required to raise his protein stores to a level at which haemoglobin formation could begin. Unfortunately, there is no verification of this hypothesis, as the plasma proteins were not estimated again until the child was cured of his anaemia.

Discussion.

1. Comparison with other results.

The results quoted above for anaemias in children are

somewhat at variance with those of McMichael, Sharpey-Schäfer, Mollison and Vaughan (1943), who found that the total blood volume was reduced in chronic anaemias regardless of aetiology. Their series, however, did not include any patients with nutritional anaemia, and moreover, only adults were investigated. Sharpey-Schäfer later correlated the reduction of the blood volume with the increased right auricular pressure and increased cardiac output which he found with experiments using cardiac catheterisation, and with the increased percentage oxygen utilisation seen in all cases of anaemia (Sharpey-Schäfer, 1944). In view of the fact that the resting minute oxygen consumption in anaemia was not decreased, he argued that the minute oxygen supply must therefore be maintained at normal levels, probably by a physiological adjustment of the cardio-vascular system involving the factors mentioned above, (a) increased cardiac output (b) increased oxygen utilisation at the periphery and probably (c) reduction in blood volume, since, at a given rate of flow, the available oxygen depended on the concentration of haemoglobin i.e. the reduced amount of haemoglobin in circulation would thus be concentrated. If the above findings are correct, how is the increase in cardiac output effected in the presence of reduced blood volume? The cardiac output is governed by three main factors (1) the venous return to the heart (2) the force of each systolic contraction and (3) the heart rate. The state of the peripheral blood pressure also plays a part in

the regulating mechanism (Samson Wright, 1938). With regard to the first factor, venous return, it would seem that diastolic filling would be more readily maintained with a normal blood volume than with one markedly reduced. (Some of the patients reported by McMichael et al. had blood volumes reduced to about 50 per cent of normal). If however, the blood volume were in actual fact, reduced in anaemia, the cardiac output would have to be increased by means of a marked rise in venous pressure, a marked acceleration of the heart, or both. Increase of pulse rate is of course a well-known concomitant of anaemia, but in chronic anaemia, the cardiac acceleration is only of moderate degree. Right auricular pressure, on the other hand, was found to be increased by Sharpey-Schäfer (1944), the level being regarded as a high normal. Figures published later (Sharpey-Schäfer, 1945) gave results for right auricular pressure in anaemia varying from the normal level of minus 4 cc. of saline to about plus 20 cc. of saline measured from the sternal angle. Brannon, Merrill, Warren and Stead (1945) however, found no significant rise in right auricular pressure in chronic anaemia, and no correlation between the former and cardiac output. Indeed, in two of their patients, the right auricular pressure tended to rise during recovery, and was accompanied by increase in the red cell count, and by decrease in the cardiac output. With regard to the second factor, the force of each systolic contraction depends on the stretch to which the cardiac muscle fibres are subjected, and will therefore increase, with increase

in diastolic filling up to a certain limit, beyond which increased filling leads to reduced cardiac output (Starling's denervated heart lung preparation). Results published by Sharpey-Schäfer (1945) on the response of anaemic patients to transfusion, showed decrease in cardiac output, subsequent to the increase in right auricular pressure caused by the extra filling of the vascular bed. This could be regarded as evidence that these patients in their anaemic state before transfusion, must have been very near the stretch limit of their cardiac muscle fibres. Once again it would seem that a decreased blood volume would with difficulty cause the requisite degree of diastolic filling and distension, unless in the presence of a very high right auricular pressure, which has been shown not to exist.

The method used by McMichael, Sharpey-Schäfer, Mollison and Vaughan, when the low blood volume results were recorded in anaemia, was the concentrated corpuscle haemoglobin method (vide Part 1). It was accordingly decided to try some experiments with this technique, immediately following an estimation with Evans blue. A small transfusion of packed red cells was injected fairly rapidly, during thirty to forty minutes, by means of a 20 cc. syringe connected by stopcock and rubber tubing to the bottle of blood. The exact volume injected was noted. The other data required were the haemoglobin values of the blood transfused, and of the patient's blood immediately before and after the transfusion. The amount injected varied of course with the size of the patient. The formula used for

the calculation was obtained in the following manner.

If x = initial blood volume before transfusion,

V = volume of blood transfused,

Hb_V = haemoglobin concentration of the transfused blood,

Hb_1 = haemoglobin concentration before transfusion,

Hb_2 = haemoglobin concentration after transfusion,

then by equating the total quantities of haemoglobin;

$$xHb_1 + VHb_V = (x + V)\,Hb_2$$

$$\text{Hence, } x = \frac{V(Hb_V - Hb_2)}{Hb_2 - Hb_1} \qquad \text{(Hill, 1941)}$$

The following table shows the injected volumes and haemoglobin values, from which the calculations were made.

Table 27.

	Blood Transfused		Blood of Recipient.	
Case	Amount	Hb_V	Hb_1	Hb_2
	ml.	g. per cent	g. per cent	
141	175	7.01	3.4	4.42
	+ 80	9.83		
131 (1)	230	11.6	1.7	3.74
(2)	165	15	3.03	6.02
130	160	12.98	8.05	9.73
133	125	17.37	3.3	4.50
144	210	18.79	7.54	9.54

The first four children have already been discussed. Case 131 had the procedure done on two occasions. Case 144 was a child with nephrosis, on whom the dye estimation could not be performed because of opacity caused by the lipoid content of his plasma.

The results obtained by the two methods are contrasted below.

Table 28.

Case		Evans blue Absolute B.V.	Evans blue Ml.per kilo	C.C.Hb. method Absolute B.V.	C.C.Hb. method Ml.per kilo
* 141		843	74.0	869	76.0
* 131	(1)	1147	84.3	896	65.9
	(2)	1366	103.5	496	39.0
130		2730	76.4	309	9.0
133		1801	89.2	1341	66.3
144		-	-	971	81.1

* congo red.

Of the five sets of results with the two methods, only one gave comparable figures, (Case 141), while in two, (Cases 131 (2) and 130) the results obtained with the concentrated corpuscle method were obviously ridiculous. McMichael and his co-workers themselves pointed out, that a serious source of error in the formula proposed by Hill, was the occurrence of plasma shifts, as the red cell volume in man was constant apart from the normal removal of effete cells, and the addition of new cells from the bone marrow (Ebert and Stead, 1941a). They accordingly modified the formula in the following manner. If y cc. of plasma leave the blood before the final estimation of haemoglobin, then by equating the total amounts of haemoglobin,

$$xHb_1 + VHb_v = (x + V - y)Hb_2$$

$$\text{Hence } x = \frac{V(Hb_v - Hb_2)}{Hb_2 - Hb_1} + \frac{yHb_2}{Hb_2 - Hb_1}$$

If there is a shift of fluid into the circulation during the transfusion, then the y fraction becomes a

negative quantity. As McMichael and his colleagues demonstrated, if y = 100 ml. and $\frac{Hb_2}{Hb_2 - Hb_1} = 5$, then the original blood volume calculated would be too low by as much as 1 litre. As the fraction $\frac{Hb_2}{Hb_2 - Hb_1}$ approaches unity, the error is reduced to its minimum. This is most likely to occur in anaemic bloods. Even with the last proviso however, there may be a large error, which is indeed seen in Case 131 (2) (Tables 27 and 28). In this instance $\frac{Hb_2}{Hb_2 - Hb_1} = 2$, the lowest value for the series of five estimations, and yet the calculated blood volume was only one third of that found by the dye estimation, due to the large value for y, the fluid shift. In a series of eight anaemias, in whom the plasma volume was investigated before and after a concentrated cell transfusion of 450 ml., Dyson, Plaut and Vaughan (1944) found the expected post-transfusion level for plasma volume in only one, an increase of 262 to 442 ml. in four, and a decrease up to 253 ml. in the remaining three.

In conclusion one may state that owing to the ease with which fluid shifts can occur, and the magnitude of the error thus introduced, the concentrated corpuscle haemoglobin method cannot be relied upon to give accurate results.

2. Blood Volume Regulation in Anaemia.

From the results reported in the various types of

anaemia, one may conclude that in children there is a definite tendency for the blood volume to be maintained at, or but slightly below, the normal level for each individual.

The correction of blood volume decrease following haemorrhage could take place theoretically in two ways - firstly by the discharge into the circulation of red cells from a "reservoir", or secondly, by the addition to the blood stream of plasma from a "plasma pool".

With regard to the existence of an erythrocyte reservoir, Ebert and Stead (1941a) investigated the effect on the blood volume of exercise, of injections of epinephrine, and of controlled haemorrhage, in human subjects, several of whom had undergone splenectomy for therapeutic reasons. No evidence was found of the existence of blood reservoirs in man, unless in patients with pathological enlargement of the spleen (Ebert and Stead, 1941a; Giffin and Brown, 1929).

The fact is well established, that the plasma volume may be markedly increased following haemorrhage, and also in chronic anaemia. This increase probably depends in part on an inflow of extracellular fluid, and in part on a redistribution of circulating plasma. The latter adjustment would seem to be effected by capillary constriction in areas of great vascularity e.g. muscles, liver (Robertson, 1935), pulmonary bed (Glaser and McMichael, 1940) and subpapillary plexus of the skin (Wollheim, 1931). By means of this constriction,

the major part of the "dead" plasma which lines the capillaries and which normally amounts to about 20 per cent of the total body plasma (Hahn et al., 1942; Ebert and Stead, 1941b) will be returned to the active circulation.

Analysis of the plasma and blood volume results in the series of anaemias has suggested several factors which probably influence the degree of plasma volume increase.

(a) Speed of onset of the anaemia.

Following a sudden reduction in haemoglobin as in haemorrhage, the plasma inflow is such that the total blood volume is restored, if the available body fluid is adequate (Boycott and Douglas, 1909; Robertson and Bock, 1919a and b; Wallace and Sharpey-Schäfer, 1941).

In chronic anaemias also, the speed of onset may play a part, since in the haemolytic anaemia group one child gave a much shorter history than the other, and had a higher plasma volume increase.

(b) Available body fluid.

In this connection, it is known that in haemorrhage, restoration of the blood volume is more quickly effected if fluids are given orally in unrestricted amount (Robertson and Bock, 1919b). Thirst probably comes into play only when the available interstitial fluid has already been drained to augment the plasma volume. It is interesting, that Case 148, the child who had subacute haemolytic anaemia of one month's duration, and in whom destruction

of 67 per cent of her cell volume had occurred, was reported by her mother to have been very thirsty during the period of increasing pallor. In pernicious anaemia, Gibson (1939) noted that there appeared to be certain limitations to the increase of plasma volume when compared to hypochromic anaemias, and that larger increases could be obtained by forcing fluids. He inferred that the tissues in pernicious anaemia were in a state of chronic mild dehydration.

(c) Degree of reduction in cell volume.

Excluding the children with leukaemia, one might suggest that the total blood volume is fully maintained only when the cell volume decrease does not exceed minus 60 per cent. Beyond this limit, plasma volume increase is only moderate (cf. aplastic anaemia and macrocytic anaemia, where the plasma volume increase was plus 21 and plus 10 per cent, accompanying deviations from normal in cell volume of minus 62 and minus 72 per cent respectively). In the iron deficiency group, on the other hand, where the reduction in cell volume was slight the total blood volume was fully maintained. Robertson and Bock (1919a), suggested that in anaemia, two distinct tendencies in relation to blood volume could be observed, namely the restoration of the blood mass to ensure efficient working of the cardiovascular system, and the inhibition of dilution to prevent lowering of the haemoglobin concentration. These authors stated that the restoration of the blood volume prevailed until the haemoglobin concentration was reduced

to about 20 per cent.

(d) Increased oxygen utilisation.

The increased oxygen utilisation at the periphery which accompanies anaemia, will ensure greater reduction of venous blood, and the relatively higher osmotic pressure thus induced, may cause a greater return of extracellular fluid to the vascular channels.

Summary.

Blood volume estimations were performed on twenty six children with anaemia of various types. The plasma volume was found to be increased in all the classes of anaemia investigated. The total blood volume was maintained at normal levels in iron deficiency anaemia, leukaemia, and in haemolytic and post-haemorrhagic anaemias. In aplastic and macrocytic anaemias, the total blood volume was reduced. Explanations of these differences have been suggested.

The results of total plasma protein estimations in eighteen cases, gave an average below normal.

The different findings of McMichael, Sharpey-Schäfer, Mollison and Vaughan (1943) and of Sharpey-Schäfer (1944 and 1945) in chronic anaemia in adults have been discussed, and an attempt has been made to justify the results of the present series on physiological grounds.

The concentrated corpuscle haemoglobin method advocated by McMichael et al. (1943) was carried out in five children with anaemia, the results obtained demonstrating clearly the fallacies of the technique.

Analysis of the results for the different types of anaemia has suggested various factors concerned in the regulation of the plasma volume in anaemia (a) speed of onset of the disease (b) amount of available body fluid (c) degree of reduction in cell volume and (d) increased oxygen utilisation at the periphery.

Section 7.

Fluid Shift in Two Cases of Anaemia following Transfusion.

The following two cases of anaemia have been described in view of the completely different response to blood transfusion, the one reacting by the addition of fluid to the blood stream, and the other by the loss of fluid. The initial blood volumes were determined by the congo red method after which the concentrated corpuscle method was applied following the technique described in the previous section.

Case Histories.

(1) Case 141 (Table 25) aet. 6 years, was a coeliac dwarf, 53 per cent of expected weight and 86 per cent of expected height. He had a severe macrocytic anaemia, which failed to respond initially to liver and iron. The blood picture was as follows (20.10.45):-

Haemoglobin	3.4 g. per cent
Erythrocyte count	1.35 millions per c.mm.
Leucocyte count	7,000 per c.mm.
Haematocrit reading	15.5 per cent
Mean corpuscular haemoglobin concentration	21 per cent
Mean corpuscular volume	115 c. microns
Mean corpuscular diameter	8 microns
Total plasma proteins	4.35 g. per cent

Blood volume before transfusion (congo red)

Absolute volume = 843 ml.

Unit volume = 74 ml. per kilo of body weight

= 8.9 ml. per cm. of height.

(This part of the work has been accepted for publication in the "Archives of Disease in Childhood").

Average values for healthy children of the same height

Absolute volume = 1,125 ml.

Unit volume = 11.8 ml. per cm. of height

Blood transfusion was given on Oct. 20th., 1945, using bank blood, 175 ml. of which had a haemoglobin value of 7.01 g. per cent, and 80 ml. a value of 9.83 g. per cent.

(2) Case 131 (Table 24) aet. 2 years and 3 months, was admitted on Nov. 6th., 1945, with a history of increasing pallor and cervical lymph gland enlargement, of three months' duration. The condition proved to be one of aleukaemic lymphatic leukaemia. He was a well-grown child, 109 per cent of expected weight, and 104 per cent of expected height.

Blood investigation (7.11.45) gave the following figures:-

Haemoglobin	3.7	g. per cent
Erythrocyte count	1.21	millions per c.mm.
Leucocyte count	8,000	per c.mm.
Haematocrit reading	9.4	per cent
Mean corpuscular volume	78	c. microns
Mean corpuscular haemoglobin concentration	36	per cent
Mean corpuscular diameter	7	microns
Total plasma proteins	7.35	g. per cent

Blood volume

(a) Before first transfusion (7.11.45)

Absolute volume = 1147 ml.

Unit volume = 84.3 ml. per kilo

= 13.2 ml. per cm.

(b) Before second transfusion (21.11.45)

Absolute volume = 1366 ml.

Unit volume = 103.5 ml. per kilo

= 15.7 ml. per cm.

Average values for healthy children of the same height.

Absolute volume = 950 ml.

Unit volume = 10.8 ml. per cm.

He was transfused on two occasions:

(1) 11.11.45 - 230 ml. of bank blood containing 11.6 g. per cent haemoglobin.

(2) 21.11.45 - 165 ml. of packed red cells, containing 15 g. per cent haemoglobin.

Haemoglobin Values in grams per cent.

	Case 141	Case 131 (1)	Case 131 (2)
Before transfusion	3.4	1.7	3.03
5 minutes after	4.42	3.74	6.02
12 hours after	3.51	-	-
24 hours after	3.58	4.19	5.7
48 hours after	4.34	-	-
Transfused blood	7.01 (175 ml.) + 9.83 (80ml.)	11.66 (230ml.)	15.0 (165ml.)

Using the above haemoglobin values, Hill's formula was applied to calculate the initial blood volume.

$$x = \frac{V(Hb_V - Hb_2)}{Hb_2 - Hb_1}$$ (Hill, 1941)

where x = initial blood volume

V = volume of blood transfused

Hb_V = haemoglobin concentration of the transfused blood

Hb_1 = haemoglobin concentration of the patient before transfusion.

Hb_2 = haemoglobin concentration of the patient after transfusion.

In neither of the children, did the initial blood volume calculated by Hill's formula tally with the results obtained by the congo red method.

	Case 141 ml.	Case 131 (1) ml.	(2)
Initial B.V. (congo red)	843	1147	1366
Initial B.V. (Hill)			
taking Hb_2 at five minutes	869	896	496
" " " 12 hours	10,167	-	-
" " " 24 "	5,447	690	575
" " " 48 "	964	-	-

As shown in the discussion of the anaemia section, fluid shifts form a frequent source of error, when using Hill's formula. From the results shown above, the following conclusions were drawn.

1. In Case 141, no significant fluid shifts took place during the transfusion. The results at 12, 24, and 48 hours, however, were obviously too high, that for 12 hours ridiculously so. Therefore it was assumed that fluid had been added to the circulation in considerable amount following the transfusion.

2. In Case 131, in both instances, fluid must have left the blood stream during the actual transfusion. After the transfusion, fluid continued to leave up till 24 hours, while after the second, a small amount of fluid was added to the circulation during the 24 hours ensuing.

The modified formula of McMichael, Sharpey-Schäfer, Mollison, and Vaughan (1943) discussed in the previous

section, was then applied to calculate y, the amount of fluid shift, by substituting for x, the observed initial blood volume estimated by the dye method.

$$x = \frac{V(Hb_V - Hb_2)}{Hb_2 - Hb_1} + \frac{yHb_2}{Hb_2 - Hb_1}$$ (McMichael et al., 1943)

The following results were obtained:-

	Case 141 ml.	Case 131 (1) ml.	(2)
Initial B.V.	843	1147	1366
Volume transfused	255	230	165
Fluid shift:-			
during transfusion	0	-137	-432
0-12 hours after	+ 292	-135	+61
12-24 hours after	- 61		
24-48 hours after	- 209	-	-

+ sign indicates addition of fluid to the circulation

- sign indicates withdrawal of fluid from the circulation.

It is understood that the figures calculated for the fluid shift should not be regarded as accurate, but they serve to give an estimate of the direction and magnitude of the volume changes concerned.

Discussion.

These two patients, who were alike in exhibiting a very severe degree of anaemia, showed an interesting contrast in their post-transfusion response. Two main points of difference existed, which may help to explain the dissimilarity in reaction.

1. The initial state of the blood volume differed in the two

children. Case 141, the coeliac dwarf with macrocytic anaemia, whose reaction during the first twelve hours was that of fluid addition to the blood, had a blood volume which was a low normal when related to weight, but when related to height, was definitely below average for children of his height. The latter forms a better basis for comparison, as the child was 53 per cent of his expected weight. Case 131, on the other hand, had an initial blood volume which was rather high, and the response to transfusion was that of fluid shift to the tissues, the exact opposite of what occurred in Case 141. It has been shown, that when the blood volume is at a normal level, transfused serum tends to leave the circulation within one or two hours (Sharpey-Schäfer and Wallace, 1942a). Similarly , with transfusion of whole blood in chronic anaemia, the added plasma may disapear even during the transfusion, particularly if the rate of giving is slow (Marriott and Kekwick, 1940).

2. The other factor which may explain these results is the different relationship existing in the two patients, between the plasma protein levels of the blood of donor and recipient. If a patient is transfused with blood of a higher protein content than his own, there is likely to be a transference of fluid from the tissues to equalise the osmotic pressures, and conversely, a transfused blood of lower protein content will tend to cause a fluid shift to the tissues (Metcalf, 1944). Taking an average of 6 g. per cent as the total protein level of bank blood, it will be seen that Case 141 had a plasma protein level well below this (4.85 g. per cent),

and Case 131 considerably above (7.35 g. per cent).

The failure in Case 141 to retain the additional fluid after forty-eight hours, may be due to the fact that the added protein was rapidly withdrawn from the circulation to make good the deficiencies in his protein stores, which considering his state of prolonged malnutrition, were probably low (Chang 1932; Madden and Whipple, 1940; Ebert, Stead and Gibson, 1941; Beattie and Collard, 1942b).

That some of the fluid removed from the plasma as a result of transfusion, may enter the red cells, is demonstrated in Case 131 (Dyson, Plaut and Vaughan, 1944).

Date		M.C.V. c.microns	M.C.Hb.C. per cent
7.11.45		78	38
10.11.45	Transfusion		
21.11.45		114	23
21.11.45	Transfusion		
28.11.45		109	24

In view of the recent work on the increased cardiac output, and poor cardiac reserve in chronic anaemia (Sharpey-Schäfer, 1944; Hunter, 1946) and the dangers of transfusion unless by slow drip (Altschule and Gilligan, 1938; Sharpey-Schäfer, 1945), it is interesting to note that in these two patients, blood was syringed into a vein at the rate of about 5 ml. per minute, and that neither showed any adverse reaction. Both cases before transfusion however, showed such extreme pallor, that it may be concluded that the compensatory vaso-constriction was well-marked. During the

transfusion, distinct flushing and heat of the skin were noted, but without venous engorgement, indicating that the dilatation of previously constricted vessels had enlarged the vascular bed sufficiently to accommodate the increased blood volume (Sharpey-Schäfer and Wallace, 1942b).

Summary.

The difference in the behaviour of the blood volume following transfusion in two severely anaemic children has been studied. In one case, fluid was added to the circulation; in the other it was withdrawn.

The suggestion is made that the results can be explained by the difference in the initial blood volume of the two patients, and in the plasma protein levels of the blood of the donor and recipient.

Conclusion.

For the individual groups of investigations, the results obtained and the conclusions drawn have been given at the end of each section, and need not be reiterated.

Throughout the studies, it has become more and more apparent, that a knowledge of the blood volume is essential for a complete understanding of many of the processes of metabolism. This is probably especially true of infants and young children, where the mechanisms of defence against disturbances of acid base balance are relatively inefficient. In blood diseases, too, it is evident that haemoglobin values can only be given their true significance if assessed along with the volume of the circulating blood.

The modern methods of estimating plasma volume, especially since the introduction of Evans blue, are relatively simple, and reasonably free from error. When these methods are used in conjunction with the recent advance of employing radio-active substances to estimate the red cell volume, it is to be expected that more information of a valuable character will be obtained in the future, concerning blood volume changes in health and disease.

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