BLOOD VOLUME STUDIES IN HEALTHY CHILDREN*

BY

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Among the earliest recorded experiments on the estimation of the blood volume are those of Bischoff (1856, 1858) and Welcker (1858), who by exsanguination measured directly the amount of blood obtained from the bodies of executed criminals. Since that time much ingenuity has been exercised in devising methods of estimating blood volume which would combine accuracy with ease of application to the living human subject. The modern techniques involving the use of dyes owe their origin to the work of Keith et al. in 1915, and are in the writer's opinion the most practical of all the methods. These techniques have undergone several modifications, the two most important being the substitution of a blue for a red dye and the adoption of the photoelectric absorptiometer for comparison of colours. The blue dye (Evans blue, or T1824) now in common use, is more slowly excreted than the red dyes, such as vital red and congo red (Dawson et al., 1920), and, what is probably more important, does not mask the occurrence of haemolysis. Indeed the Evans blue method used in these studies not only permits the detection of haemolysis but allows for its correction. The photoelectric absorptiometer eliminates the personal factor involved in colour matching.

Of recent years much information has been gained regarding the changes in the volume of plasma and blood in such states as shock, dehydration, and anaemia. Most of the investigations, however, have been carried out in adults, and comparatively little work has been done in children, where the technical difficulty of venepuncture is greater. It is evident that any study of changes in the blood volume in children and their application to disease must depend on the establishment of a normal range of values. This range will possess wide limits, since the blood volume is constantly changing with growth. It was considered necessary, therefore, as a preliminary procedure, to define the average volumes and the range at various ages as accurately as possible.

The object of this paper is to report the results obtained from the plasma and blood volume studies of eighty apparently healthy children varying in age from three months to thirteen years, forty-two girls and thirty-eight boys, all in-patients of the Royal Hospital for Sick Children, Glasgow. In each instance the investigation was delayed until convalescence was well established and the child ready for dismissal. All the subjects were afebrile and on full diet, and none had diarrhoea or vomiting. The state of nutrition was also considered, and only those between 80 and 110 per cent. of their expected weight chosen (Holt's tables). The haemoglobin level was not less than 10 g. per cent., except in the group of infants, where two babies were included with haemoglobin values of 9·5 g. per cent. The studies have been grouped and averaged according to age; six subjects in each year were investigated (except in the first year, when ten cases were done), and the average recorded at three-monthly periods. For the first twenty-three cases the dye used was congo red, as at that time the advantages possessed by Evans blue were not fully appreciated. The plasma volume of the remaining fifty-seven cases was investigated with Evans blue. There is evidence to show, however, that the results are comparable.

Methods

Preparation of patient. On the morning of the investigation, in accordance with the technique used by most workers, the children were kept in bed and given nothing to eat or drink after 9 a.m. save sips of water. Estimations were carried out about five hours later between 2 and 3 p.m. Immediately before the start of the experiment the weight and height of each child were taken and recorded.

Procedure. For the dye injections a 5-ml. syringe accurately calibrated in fifths of a millilitre was used throughout. By venepuncture, 6 to 8 ml. of blood were removed, and through the same needle the dye solution (whether Evans blue or congo red) was injected. Blood was withdrawn into the syringe and the syringe washed out once. A mixing time of ten minutes was allowed. This interval was chosen after estimations on two of the older children from whom samples of blood were withdrawn every two minutes for twelve minutes and then every ten minutes for half an hour. The results of the dye

concentrations when graphed against time showed a ' mixing curve ' lasting ten minutes as described by Gibson and Evans (1937a) followed by a gradual ' disappearance slope ' (Fig. 1). Thus the mixing time allowed was ten minutes, which is in accordance with the reports of others (Davis, 1942; Gregersen, 1944; Noble and Gregersen, 1946). After this interval 6 to 8 ml. of blood were again withdrawn using a different vein. In each case, after the introduction of the needle into the vein, all constriction was released and a minute allowed to elapse before blood was withdrawn, thus avoiding errors attributable to venous stasis. To prevent haemolysis, the blood was withdrawn into dry syringes and then ejected without frothing down the sides of the receiving tubes. The latter were graduated centrifuge tubes containing 0·3 ml. of an anticoagulant which did not alter the osmotic pressure of the plasma. The mixture used was that advocated by Wintrobe and Landsberg (1935), a solution of 2 per cent. potassium oxalate and 3 per cent. ammonium oxalate. The tubes were then covered with light aluminium caps to prevent evaporation and centrifuged at 2,800 r.p.m. for thirty minutes. The haematocrit readings of the two tubes were taken and averaged, allowance being made for the dilution with the oxalate mixture. The difference in the two readings as a rule was
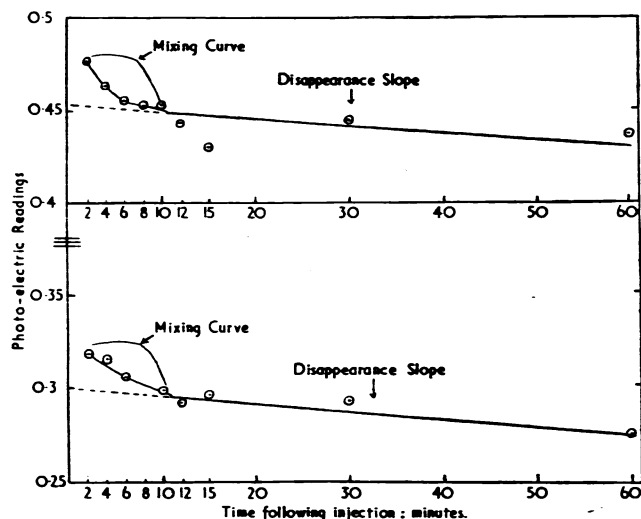
FIG. 1.—Time-excretion graphs for Evans blue.

insignificant. The plasma obtained after centrifuging was pipetted off and used in the cups of the photoelectric apparatus without further preparation, except in the congo red experiments where dilution to 1 in 2 of the specimens of plasma with normal saline was usually required.

The photoelectric technique was similar for both dye methods, apart from the use of different coloured filters which will be discussed later. After ' setting '

the colorimeter, the undyed plasma was placed in the specimen cup and a reading taken, to be followed by a reading for the dyed plasma. In both cases the ' blank ' cup was filled with distilled water. By subtracting the former from the latter result, the colour effect of natural plasma was removed, and the reading due to dye alone obtained. A graph plotted from the readings of the standard dilutions of the dye in use gave the concentration attained by the dye in the plasma. The final calculations were simple.

(1) $P.V. = \dfrac{D \times a}{y}$

where P.V. = plasma volume
D = dilution of dye in the plasma
a = amount of dye injected in grams
and y = diluting factor involved by the addition of anticoagulant.

(2) $B.V. = \dfrac{P.V. \times 100}{100 - b}$

where B.V. = blood volume
P.V. = plasma volume
and b = average haematocrit reading.

Description and comparison of methods. For the early experiments, in which congo red was used, the dye was prepared as a 1 per cent. solution made up from sterile ampoules of 0·1 g. and companion ampoules of 10 ml. of sterile doubly distilled water. The dye was injected in the following dosage:

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

Standards were prepared for each ampoule of the dye, 1 ml. of the 1 per cent. solution being used to prepare dilutions ranging from 1 in 30,000 to 1 in 100,000, and the photoelectric readings were graphed against these dilutions. A green filter (Ilford No. 3) was used in the absorptiometer for all congo red samples. Although it was realized that solutions of congo red in water and of congo red in plasma gave slightly different absorption curves (Heilmeyer, 1929), water was used in making up the congo red standards in all instances. In the later estimations using Evans blue, however, blood bank plasma was used for the standards.

For the Evans blue method the dye was obtained as a non-sterile powder and dissolved in distilled water to make 0·5, 0·2, and 0·1 per cent. solutions, which were thereafter sterilized by Seitz filtration. The amount injected varied between 0·5 and 0·8 mg. per kg. of body weight, as amounts within these limits were found to give suitable dilutions in

the plasma for reading on the photoelectric colori-meter. Fresh Evans blue solutions were prepared every four or five weeks, and for each batch of fresh solutions, standards were prepared in blood bank plasma and the photoelectric readings graphed. It was found that within that time interval no appreci-able change in the light absorption of the solutions took place. For the preparation of these standards, the technique was the same as that followed for the dye injections; the same syringe was used, and it was always rinsed out once. Thereafter the dilutions were prepared with a standard volumetric flask and an accurate burette. Plasma was used for dilution of the standards since it had been shown that the absorption curve of Evans blue dilutions in plasma differed from that of the same dilutions in normal saline (Kennedy and Millikan, 1938 ; Gregersen and Gibson, 1937). This finding was confirmed by the author. The small reading due to the plasma alone was, of course, deducted from the reading obtained for each dilution before con-struction of the graph. Readings were taken as a routine with an orange filter (Ilford No. 5), as this filter was found to give the maximum deviation of the galvanometer with Evans blue and the minimum with haemoglobin. When haemolysis was present in the dyed specimen, which fact was obvious to the naked eye from the purplish tinge of the plasma, a reading was also taken with a blue filter (Ilford No. 1), which gave maximum light absorption with haemoglobin and minimum with Evans blue. The photoelectric reading obtained with the orange filter could then be corrected for the small reading due to the contained haemoglobin by applying a formula similar to that described by Gibson and Evelyn (1938). The need for this adjustment actually occurred very seldom.

Excretion of dye. Excretion of both congo red and Evans blue takes place via the reticulo-endothelial system and the biliary tract (Gibson and Gregersen, 1935). The disappearance rate of Evans blue from the plasma was calculated from the data obtained for the two older children, who were investigated by the multiple sampling method, and was found to be 4 and 11 per cent. respectively in the first hour following injection. In adult subjects Noble and Gregersen (1946) found the average disappearance rate to be 7·8 per cent., while in dogs Gregersen and Rawson (1943) obtained the average figure of 8·8 per cent. for the loss of Evans blue.

The practice of basing the calculation of the plasma volume on the concentration of dye found in a single specimen of plasma has been much criticized for two reasons. First, there is the difficulty of judging the time required for thorough mixing of the dye solution with the blood stream; secondly, some of the dye may be removed from the circulation during the time allowed for mixing to take place. The choice of ten minutes as the mixing time has been justified by the result of the two experiments already mentioned (fig. 1). The

actual amount of dye eliminated from the circulation during this time is in fact negligible. The method of withdrawing several samples of blood at intervals following the dye injection has the advantage that in a time-concentration graph the line drawn through the values which constitute the ' disappear-ance slope ' can be extrapolated to the ordinate in order to obtain the theoretical dilution of the dye in the plasma provided mixing could be complete at the time of injection. When this ' zero ' concen-tration of the dye was calculated from the two multiple sampling experiments, and compared with the values observed after ten minutes, it was found that only 0·4 per cent. and 1 per cent. respectively of the dye had been excreted during the mixing time. It will thus be obvious that, by calculating the plasma volume from the single sample taken after ten minutes, the error involved will be not greater than 1 or 2 per cent. The alternative was to take from each child multiple specimens, extra-polate the ' disappearance slope,' and obtain the theoretical zero-time concentration of the dye. For technical and humane reasons this method was rejected when dealing with children.

Experimental accuracy. In order to test the reliability of the methods under similar conditions, plasma volume estimations were repeated in two children after an interval of three weeks in one and one week in the other, using congo red and Evans blue respectively. In both instances the duplicate estimations gave almost identical results, allowing for change of weight in the interval (table 1) (a).

TABLE 1
PLASMA VOLUME ESTIMATIONS

	Case No.	Date	Dye	Weight (kg.)	Plasma Volume (ml.)		Deviation (per cent.)	
					Total	per kg.	Total	per kg.
a.	66	5.10.45	C.R.	28·4	1.659	58·4		
		1.11.45	C.R.	27·68	1.618	58·4	−2·4	0
	52	31.1.47	E.B.	21·32	1.058	49·6		
		7.2.47	E.B.	21·52	1.066	49·6	−0·7	0
b.	139	8.3.46	E.B.	17·4	1.191	68·4		
		13.3.46	C.R.	17·5	1.256	71·7	·5	−4
	93	9.5.46	E.B.	23·5	1.288	54·8		
		23.5.46	C.R.	22·5	1.216	54·8	−5·5	0
	62	15.4.46	E.B.	22·4	1.149	51·3		
		23.5.46	C.R.	22·6	1.272	56·3	−10	−9

(a) = Duplicate plasma volume estimations in two children, in one case using congo red and in the other Evans blue.
(b) = Plasma volume estimations with congo red and with Evans blue in the same subject (three cases).

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

In spite of the advantages possessed by Evans blue, mainly because of its colour, and which have been discussed previously, it was found in practice that the results of plasma volume estimations using each dye, congo red and Evans blue, agreed within 10 per cent. (table 1) (b). When the individual results for plasma volume per kg. of the entire series were

compiled according to the dye used, no significant discrepancy was detected. Each group had a similar range of variation and the averages were very close (table 2). For these reasons the results

TABLE 2

COMPARISON OF CONGO RED RESULTS WITH EVANS BLUE RESULTS

	Congo red	Evans blue
Number of cases	23	57
Average plasma volume ml. per kg.	46·6	48·3
Range of plasma volume ml. per kg.	36·8–58·4	35·8–58
Standard deviation	6·7	5·57
Difference between averages ..	1·7	
Standard error of difference ..	1·58	

obtained by the use of congo red have been grouped together with those obtained with Evans blue, the series thereby gaining the advantage of having a greater number of estimations for consideration.

Results

The individual results are shown in detail in tables 3 to 9, but for ease of reference the average plasma and blood volumes per kg. for each age period have been collected in table 10.

TABLE 10

THE PLASMA AND BLOOD VOLUME OF HEALTHY CHILDREN

(Average values for each year of life up to 13 years)

Age	No. of Cases	Plasma volume (ml.)	Blood volume (ml.)
1 day* ..	1	180	418
Up to 3 mths.	1	213	359
4–6 mths. ..	3	273	487
7–9 „ ..	3	338	574
10–12 „ ..	3	379	623
1–2 yrs. ..	6	502	857
2–3 „ ..	6	547	956
3–4 „ ..	6	625	1,090
4–5 „ ..	6	752	1,316
5–6 „ ..	6	855	1,500
6–7 „ ..	6	913	1,535
7–8 „ ..	6	1,083	1,902
8–9 „ ..	6	1,092	1,898
9–10 „ ..	6	1,292	2,288
10–11 „ ..	6	1,297	2,317
11–12 „ ..	5	1,495	2,682
12–13 „ ..	5	1,304	2,397

* The one-day old baby included in the table was not a healthy child, but had haemolytic disease of the newborn. The infant weighed 3·79 kg. and measured 52 cm. The blood findings were as follows: Hb. 13·6 g. per cent.; red blood cells, 4,900,000 per c.mm. of blood; and haematocrit reading 57 per cent. It was therefore concluded that the degree of haemolysis was slight, and the blood volume result has been used for the above table to complete the series of normal children.

Influence of age. On the first day of life the plasma volume was found to be 180 ml. (one case only), while at the end of the first year it had increased to approximately double this level. Thereafter the rate of increase was more gradual, a figure of about 1½ litres being attained by 13 years. The total blood volume seemed to be relatively high at birth (418 ml. in the newborn baby investigated); it fell in the following three months to

about 360 ml., and by the end of the first year was approximately 150 per cent. of the birth value. In the following years it showed a steady increase with advancing age to about 2½ litres at 13 years. The difference ascertained in the behaviour of the plasma and blood volume in the first three months of life is of some interest. During this period the plasma volume increased, whereas the blood volume decreased. These facts indicate a considerable loss in the cell volume between birth and the third month, and can be correlated with the fall in haemoglobin values and red cell counts which are known to occur during the same period. The average figures quoted represent a gradual and steady increase in the plasma and blood volume from the first year onwards. The individual results, however, show that a wide variation existed between children of the same age group, due to considerable difference in body size within each group.

When expressed in relation to height and surface area (unit volume per cm. and per sq. m.), the volume of plasma and of blood increased gradually along with advancing age. (The calculation of the surface area from the weight and height of each child was based on the formula of Du Bois and Du Bois, 1916.) In contrast to these findings, unit volumes per kg. did not show any significant variation from age group to age group. Morse et al. (1947), in a comprehensive study of the blood volume in seventy-five children ranging from infancy to seventeen years, found that the values for plasma and blood volumes per kg. showed a very slight tendency to increase with growth during these years, but they regarded this increase as of questionable significance.
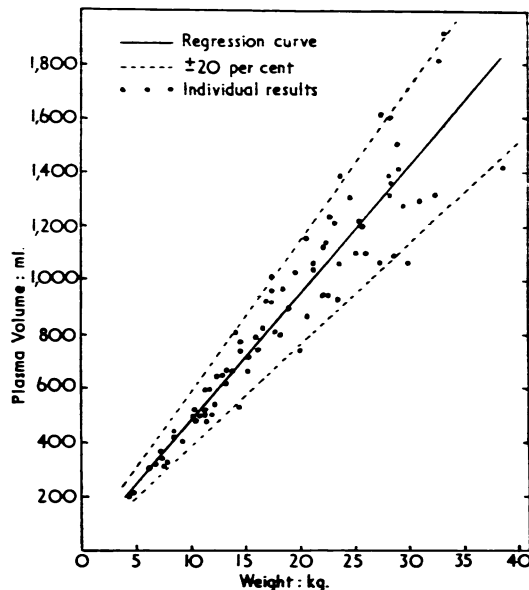

FIG. 2.—Showing the increase in plasma volume with increase in weight.

TABLE 3

HEALTHY INFANTS: 3 TO 11 MONTHS OLD

Case	Sex	Age (mths.)	Weight		Height		Hb	RBC	PCV	Plasma volume (ml.)				Blood volume (ml.)			
			kg.	% Exp. wt.	cm.	% Exp. ht.				Total	per kg.	per cm.	per sq. m.	Total	per kg.	per cm.	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	1,433
*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	1,454
*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	1,811
4	F.	6	7·675	110	64	101	10·9	4·30	40	306	39·8	4·78	880	510	66·4	7·97	1,467
AVERAGE		4-6								273	47·8	4·30	879	487	79·9	7·87	1,577
5	M.	7	6·73	92	64	99	11·0	4·30	39	316	47·0	4·94	961	518	77·0	8·09	1,574
6	M.	7	7·16	98	66	100	10·1	4·40	41	362	50·6	5·49	1,047	614	85·7	9·30	1,776
7	M.	9	7·16	86	73	106	10·7	4·50	43	337	47·1	4·62	906	591	82·6	8·10	1,588
AVERAGE		7-9								338	48·2	5·02	971	574	81·8	8·50	1,646
8	M.	10	9·095	109	75	108	9·4	3·80	41	402	44·2	5·36	958	681	74·9	9·08	1,623
9	F.	10	8·635	103	75	108	10·4	4·20	36	419	48·6	5·59	1,021	655	76·4	8·73	1,596
10	M.	11	7·91	87	70	95	10·0	4·50	40	317	40·1	4·53	842	533	67·4	7·62	1,416
AVERAGE		10-12								379	44·3	5·16	940	623	72·7	8·47	1,545

* in tables 3-9 indicates congo red.
Hb.=haemoglobin in g. per cent.   RBC=red blood count, millions per c.mm.   PCV=haematocrit reading, per cent.

TABLE 4

HEALTHY INFANTS: 1 TO 3 YEARS OF AGE

Case	Sex	Age (yrs.)	Weight		Height		Hb	RBC	PCV	Plasma volume (ml.)				Blood volume (ml.)			
			kg.	% Exp. wt.	cm.	% Exp. ht.				Total	per kg.	per cm.	per sq. m.	Total	per kg.	per cm.	per sq. m.
*11	F.	1	11·49	102	87	107	12·8	4·06	46	479	41·7	5·51	970	880	76·6	10·11	1,775
12	F.	1	8·61	93	76	103	11·0	4·2	38	435	50·5	5·72	1,051	701	81·4	9·23	1,694
13	F.	1	10·26	102	79	103	10·9	4·2	39	483	47·1	6·12	1,052	793	77·3	10·03	1,728
14	F.	1	11·08	110	82	105	10·0	4·2	39	516	46·6	6·29	1,058	846	76·4	10·31	1,735
15	F.	1	10·75	95	86	105	12·5	4·6	39	499	46·4	5·80	1,001	817	76·0	9·50	1,639
16	M.	1	11·31	100	87·5	110	12·2	4·65	46	598	52·9	6·84	1,161	1,108	98·0	12·66	2,150
AVERAGE		1-2					11·9	4·32	41	502	47·5	6·05	1,049	857	80·9	10·31	1,787
17	F.	2	11·92	94	89	103	11·2	4·54	43	599	50·3	6·73	1,124	1,052	88·2	11·81	1,969
18	F.	2	10·24	84	87	103	11·8	4·40	41	520	50·7	5·97	1,057	880	86·0	10·12	1,791
19	F.	2	10·12	87	88	105	13·4	4·95	44	500	49·4	5·68	1,015	892	88·1	10·14	1,811
20	M.	2	11·2	86	87	100	13·3	4·85	47	506	45·2	5·82	991	954	85·2	10·97	1,868
21	M.	2	12·52	95	95	105	12·1	4·60	41	653	52·2	5·88	1,143	1,108	88·4	11·65	1,937
22	M.	2	11·8	95	90·5	107	12·9	4·70	41	503	42·6	5·56	935	852	72·2	9·42	1,584
AVERAGE		2-3					12·4	4·67	43	547	48·4	6·17	1,044	956	84·7	10·68	1,827

TABLE 5

HEALTHY CHILDREN: 3 TO 5 YEARS OF AGE

Case	Sex	Age (yrs.)	Weight		Height		Hb	RBC	PCV	Plasma volume (ml.)				Blood volume (ml.)			
			kg.	% Exp. wt.	cm.	% Exp. ht.				Total	per kg.	per cm.	per sq. m.	Total	per kg.	per cm.	per sq. m.
*23	M.	3	15·24	102	100	108	10·3	4·2	44	723	47·4	7·20	1,120	1,289	84·6	12·90	2,001
*24	F.	3	13·08	96	94	106	12·1	4·6	45	623	47·7	6·63	1,079	1,141	87·3	12·14	1,976
*25	M.	3	13·9	98	93	105	10·4	4·14	46	673	48·4	7·20	1,140	1,127	81·1	12·06	1,909
26	M.	3	13·06	85	100	107	12·5	4·88	42	671	51·4	6·71	1,113	1,156	88·6	11·56	1,916
27	M.	3	12·32	84	99	108	10·1	4·2	42·5	535	43·4	5·41	918	931	75·5	9·40	1,596
28	F.	3	14·42	96	97	102	10·2	4·5	41	527	36·6	5·44	855	894	62·0	9·21	1,450
AVERAGE		3-4					10·9	4·42	42·4	625	45·8	6·43	1,037	1,090	79·8	11·21	1,808
29	F.	4	12·98	80	93	92	12·1	4·7	39	662	51·0	7·12	1,157	1,085	83·6	11·67	1,897
30	M.	4	14·64	84	100	99	12·8	4·9	44	777	53·0	7·77	1,226	1,388	94·7	13·88	2,191
31	F.	4	14·24	85	101	100	13·4	4·3	41	806	56·6	7·95	1,277	1,367	96·0	13·47	2,163
32	F.	4	16·24	104	105	107	13·1	4·8	45	735	45·3	6·97	1,068	1,312	80·8	12·44	1,905
33	F.	4	14·62	95	96	97	13·0	4·7	45	737	50·4	7·68	1,202	1,341	91·7	13·96	2,187
34	M.	4	15·96	100	103	103	13·0	4·98	44	793	49·7	7·69	1,182	1,403	87·9	13·62	2,091
AVERAGE		4-5					12·9	4·73	43	752	51·0	7·53	1,185	1,316	89·1	13·17	2,072

Table 6—HEALTHY CHILDREN, 5 TO 7 YEARS OF AGE

Case	Sex	Age (yrs.)	Weight kg.	% Exp. wt.	Height cm.	% Exp. ht.	Hb.	RBC	PCV	Plasma volume (ml.) Total	per kg.	per cm.	per sq. m.	Blood volume (ml.) Total	per kg.	per cm.	per sq. m.
*35	M.	5	18·4	90	111·5	100	10·1	3·9	38	797	43·3	7·15	1,055	1,288	70·0	11·55	1,706
*36	M.	5	16·74	90	108	102	13·0	4·96	44	818	48·9	7·57	1,153	1,466	87·6	13·57	2,068
37	M.	5	16·98	84	107	99	11·6	4·75	42	920	54·2	8·64	1,299	1,587	93·4	14·83	2,242
38	F.	5	15·17	85	108	103	13·0	4·6	42	672	44·3	6·22	989	1,158	76·4	10·73	1,704
39	F.	5	18·54	100	120	110	12·6	4·85	43	971	52·4	8·09	1,213	1,703	91·9	14·19	2,127
40	F.	5	22·12	110	116	105	14·9	4·98	47	954	43·1	8·22	1,133	1,799	81·3	15·51	2,139
AVERAGE		5–6					12·5	4·67	42·7	855	47·7	7·65	1,140	1,500	83·4	13·40	1,998
*41	M.	6	17·54	82	140	122	10·0	4·54	43	960	54·7	6·86	1,100	1,678	95·7	11·99	1,923
*42	F.	6	19·96	92	114·5	98	11·4	4·23	44	739	37·0	6·45	926	1,316	66·0	11·50	1,650
*43	F.	6	17·2	83	109·5	96	11·9	4·4	45	921	53·6	8·41	1,271	1,406	81·8	12·84	1,939
44	F.	6	17·52	88	117	105	10·5	4·1	42	1,016	58·0	8·69	1,328	1,666	95·1	14·24	2,178
45	M.	6	19·74	93	114·5	100	11·1	4·45	42	1,027	52·0	8·97	1,293	1,770	89·7	15·46	2,230
46	F.	6	17·88	89	113	101	10·3	4·1	41	812	45·4	7·18	1,079	1,376	76·9	12·17	1,828
AVERAGE		6–7					10·9	4·3	42·3	913	50·1	7·76	1,166	1,535	84·2	13·03	1,958

Table 7—HEALTHY CHILDREN, 7 TO 9 YEARS OF AGE

Case	Sex	Age (yrs.)	Weight kg.	% Exp. wt.	Height cm.	% Exp. ht.	Hb	RBC	PCV	Plasma volume (ml.) Total	per kg.	per cm.	per sq. m.	Blood volume (ml.) Total	per kg.	per cm.	per sq. m.
*47	M.	7	20·68	90	118·5	100	11·2	4·0	40	1,161	56·1	9·80	1,315	1,935	93·5	16·32	2,192
48	F.	7	25·06	108	125	104	12·7	4·7	46	1,105	44·0	8·84	1,181	2,046	82·0	16·37	2,189
49	F.	7	18·96	85	112	95	11·9	4·6	41	904	47·7	8·07	1,177	1,532	80·8	13·68	1,994
50	F.	7	21·4	95	125	105	13·6	4·9	46	1,050	49·1	8·40	1,201	1,945	90·9	15·55	2,225
51	M.	7	23·32	102	124	103	13·6	4·75	41	1,222	52·4	9·86	1,357	2,071	88·8	16·70	2,299
52	M.	7	21·32	91	118	99	13·6	4·85	45	1,058	49·6	8·97	1,263	1,923	90·2	16·30	2,296
AVERAGE		7–8					13·3	4·63	43	1,083	49·8	8·99	1,249	1,902	87·7	15·82	2,365
*53	F.	8	20·8	81	124·5	99	10·7	4·43	45	869	41·8	6·98	1,008	1,576	75·8	12·66	1,828
54	M.	8	26·0	105	127	104	12·5	4·85	43	1,101	42·4	8·67	1,147	1,932	74·3	15·21	2,010
55	F.	8	23·64	98	126·5	103	11·6	4·8	42	933	39·5	7·38	1,014	1,609	68·1	12·72	1,747
56	F.	8	25·36	100	131	105	13·6	4·94	42·5	1,211	47·7	9·24	1,245	2,105	83·0	16·07	2,164
57	F.	8	22·28	87	123	98	12·6	4·61	42	1,132	50·8	9·20	1,287	1,952	87·6	15·87	2,219
58	M.	8	24·56	99	129	102	14·2	4·8	41	1,307	53·2	10·14	1,378	2,216	90·2	17·18	2,336
AVERAGE		8–9					12·5	4·74	42·6	1,092	45·9	8·60	1,180	1,898	79·8	14·95	2,051

Table 8—HEALTHY CHILDREN, 9 TO 11 YEARS OF AGE

Case	Sex	Age (yrs.)	Weight kg.	% Exp. wt.	Height cm.	% Exp. ht.	Hb	RBC	PCV	Plasma volume (ml.) Total	per kg.	per cm.	per sq. m.	Blood volume (ml.) Total	per kg.	per cm.	per sq. m.
*59	M.	9	25·8	88	133·5	102	10·2	4·24	44	1,204	46·6	9·02	1,210	2,156	83·6	16·14	2,158
*60	M.	9	23·76	89	126	99	12·3	4·27	48	1,388	58·4	11·02	1,509	2,695	113·0	21·39	2,930
*61	F.	9	22·56	82	132	103	10·6	4·62	44	940	41·7	7·12	1,009	1,681	74·5	12·74	1,804
62	F.	9	22·4	81	124·5	96	10·0	4·1	40	1,149	51·3	9·23	1,293	1,900	84·8	15·26	2,136
63	M.	9	32·84	110	142·5	110	10·8	4·7	43	1,822	55·5	12·78	1,577	3,196	97·3	22·43	2,766
64	M.	9	22·92	82	128	100	13·4	4·8	41	1,239	54·0	9·68	1,354	2,099	91·6	16·40	2,295
AVERAGE		9–10					11·2	4·45	43	1,290	51·2	9·81	1,325	2,288	90·8	17·39	2,348
*65	F.	10	28·6	92	135	100	12·0	4·75	42	1,088	38·1	8·06	1,040	1,890	66·1	14·01	1,804
*66	F.	10	27·68	87	144	106	11·6	4·68	41	1,618	58·4	11·24	1,520	2,737	99·0	19·01	2,570
*67	F.	10	30·96	106	138	104	10·2	4·62	43	1,304	42·1	9·45	1,242	2,296	74·1	16·64	2,184
68	M.	10	29·1	96	127	96	12·7	4·63	45	1,416	48·6	11·15	1,404	2,575	88·5	20·28*	2,554
69	M.	10	23·68	80	130	98	12·5	4·7	47	1,074	45·4	8·27	1,145	2,027	85·6	15·60	2,160
70	F.	10	29·64	94	140	100	13·8	4·8	46	1,283	43·3	9·17	1,180	2,378	80·2	16·98	2,184
AVERAGE		10–11					12·1	4·69	44	1,297	45·9	9·55	1,255	2,317	82·2	17·09	2,243

Table 9—HEALTHY CHILDREN, 11 TO 13 YEARS OF AGE

| Case	Sex	Age (yrs.)	Weight kg.	% Exp. wt.	Height cm.	% Exp. ht.	Hb,	RBC	PCV	Plasma volume (ml.) Total	per kg.	per cm.	per sq. m	Blood volume (ml.) Total	per kg.	per cm.	per sq. m.
*71	F.	11	27·32	83	137	99	11·8	4·2	45	1,068	39·1	7·81	1,032	1,945	71·2	14·23	1,879
72	M.	11	33·08	98	146	105	11·7	4·6	42	1,918	57·9	13·15	1,629	3,296	99·6	22·57	2,800
73	F.	11	28·28	83	138	98	13·8	4·8	46	1,606	56·8	11·63	1,516	2,974	105·2	21·55	2,806
74	F.	11	29·0	87	135	97	14·6	4·9	45	1,514	52·2	11·21	1,460	2,752	94·9	20·39	2,641
75	M.	11	28·46	84	133	97	13·1	4·85	44	1,367	48·0	10·28	1,325	2,441	85·8	18·35	2,367
AVERAGE		11–12					13·0	4·67	44	1,495	50·8	10·82	1,392	2,682	91·3	19·42	2,499
*76	M.	12	28·4	80	139	98	12·0	4·70	46	1,322	47·5	9·51	1,250	2,441	87·7	17·56	2,308
*77	M.	13	38·75	102	151	104	12·2	4·2	47	1,424	36·8	9·43	1,103	2,683	69·2	17·77	2,076
78	M.	12	32·46	90	147	100	13·5	4·86	46	1,320	40·7	8·98	1,125	2,445	75·3	16·63	2,083
79	F.	12	29·8	80	145	100	14·0	4·9	48	1,066	35·8	7·35	950	2,049	68·8	14·14	1,828
80	F.	12	28·28	80	141	98	12·9	4·6	39	1,390	49·2	9·86	1,293	2,278	80·6	16·15	2,120
AVERAGE		12–13					12·9	4·65	45	1,304	42·0	9·05	1,210	2,379	76·3	16·45	2,207
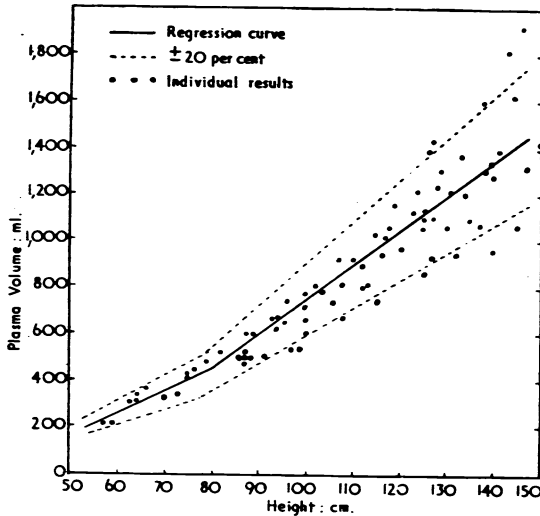
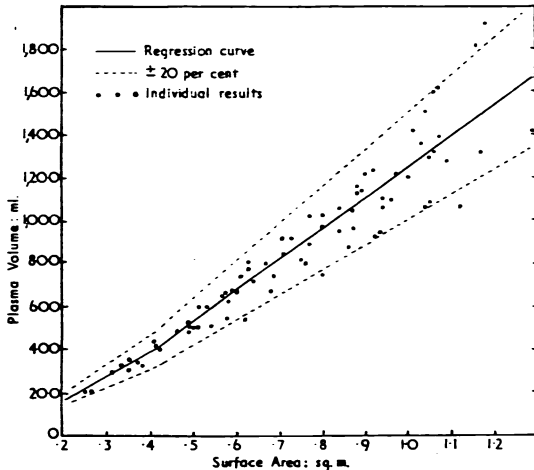FIG. 3.—Showing the increase in plasma volume with increase in height.

FIG. 4.—Showing the increase in plasma volume with increase in body surface.

Influence of body size. Weight, height, and surface area bear a closer relationship to plasma and blood volume than does age. It was noted in the scatter diagrams (figs. 2 to 7) that, while the plasma and blood volumes in general showed an increase with growth in these measurements, the individual

TABLE 11
COEFFICIENT OF CORRELATION

		Plasma		Blood	
		Correlation coefficient	Standard error	Correlation coefficient	Standard error
Weight	..	+0·9513	0·1125	+0·9563	0·1125
Surface area	..	−0·9344	„	+0·9506	„
Height	..	−0·9266	„	+0·9305	„

results occupied a fairly wide range round the average. During analysis of the eighty cases, calculation of the coefficient of correlation gave the results in table 11.

Weight gave the highest degree of correlation for both plasma and blood volumes but was followed closely by surface area and height in that order. The regression equations based on these results were used to form the average lines in figs. 2 to 7 (table 12).

TABLE 12
REGRESSION EQUATIONS

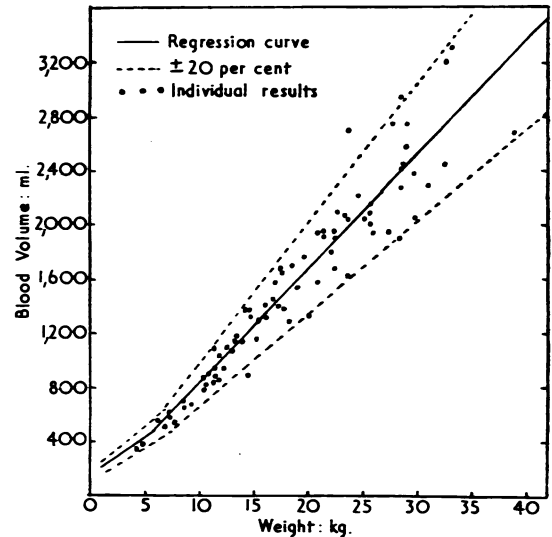|	Plasma volume			Blood volume		
Weight	P.V.=46·87	Wt.	−15·5	B.V.=85·27	Wt.	−25
Surface area	P.V.=1,433	S.A.	−186·5	B.V.=2,638	S.A.	−415
Height	P.V.=14·71	Ht.	−725·5	B.V.=26·74	Ht.	−1,369

FIG. 5.—Showing the increase in blood volume with increase in weight.
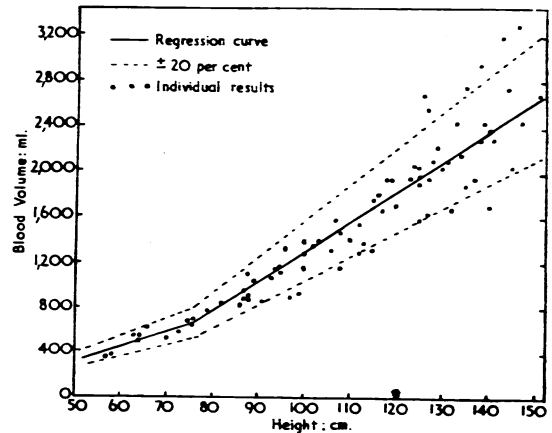

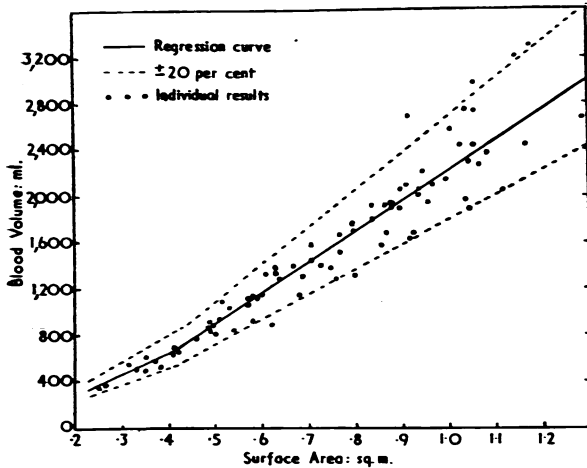FIG. 6.—Showing the increase in blood volume with increase in height.

FIG. 7.—Showing the increase in blood volume with increase in surface area.

It was discovered, in comparing the plasma volume figures obtained by using the three regression graphs with the values found by actual experiment, that those obtained from the weight graph gave a better or as good an approximation as the other two in thirty-eight of the eighty cases. Similarly, using the height graph this figure was twenty-eight, while for the surface area graph it was twenty-two. From the above data it was concluded that within the scope of the present series, that is, in children up to 13 years of age, weight formed the most reliable basis on which to calculate the blood volume.

Discussion

In the analysis of these results, certain important findings emerged and it will be helpful to discuss these in greater detail. Certain differences were also evident when comparing the studies with those of other workers.

Infants. Earlier reports on the blood volume in children under one year have recorded the use of

TABLE 13
GROUP REGRESSION EQUATIONS

	Plasma		Blood	
	Volume	Coefficient of correlation	Volume	Coefficient of correlation
WEIGHT (kg.)				
3–10	P.V. = 40·22 Wt. + 43	+0·8424	B.V. = 58·2 Wt. + 142	+0·8037
10–20	P.V. = 49·62 Wt. −14	+0·881	B.V. = 82·7 Wt. + 17	−0·8732
20–30	P.V. = 54·25 Wt. −167	+0·7698	B.V. = 95·7 Wt. −274	−0·861
SURFACE AREA (sq.m.):				
0·2–0·5	P.V. = 1,147 S.A. − 67	−0·913	B.V. = 1,907 S.A. −99	+0·9101
0·5–0·9	P.V. = 1,362 S.A. −150	+0·870	B.V. = 2,489 S.A. − 346	+0·9189
0·9–1·3	P.V. = 1,157 S.A. +114	+0·464	B.V. = 2,605 S.A. − 350	+0·665
HEIGHT (cm.):				
50–75	P.V. = 8·44 Ht. − 243	+0·8115	B.V. = 13·33 Ht. − 345	+0·8
75–100	P.V. = 10·77 Ht. −398	+0·730	B.V. = 21·81 Ht. − 969	−0·7661
100–125	P.V. = 16·58 Ht. −965	+0·704	B.V. = 33·13 Ht. −2,182	−0·7691

In order to ascertain if these equations calculated from the series as a whole could be relied upon to give a reasonable estimate of the plasma or blood volume for the individual age groups of children studied, the regression equations were formed for small groups of the results (table 13).

As the correlation coefficients for the group 125-150 cm. were not significant, regression equations were not calculated.

When plotted on the same graphs as the general equations, it was found that these sectional regression equations corresponded very well with the average trends, except in the case of the youngest children, for whom plasma and blood volumes calculated from the general equations were considerably lower than those found by experiment. To rectify this error, the regression lines for the groups up to 10 kg., 75 cm., and 0·5 sq. m., have been substituted for the lowest parts of the general regression graphs drawn for weight, height, and surface area respectively (except in the graph for plasma volume and weight, where correction of the general equation was not required).

the dye brilliant vital red with the following results. Lucas and Dearing (1921) found the average figure for blood volume per kg. to be 109 ml., Bakwin and Rivkin (1924) found it to be 101 ml., and Seckel (1936) 83 ml. McIntosh (1929), using the carbon monoxide method, found the blood volume in children up to two years to be on the average, 77 ml. per kg., a result which agrees closely with the mean of the present series for the group of ten infants, namely 78·7 ml. per kg. The only other publication found, which included infants under one year, investigated by the Evans blue method, gave the figure of 73·6 ml. per kg. (Brines et al. 1941).

The average figure of the author's studies for plasma volume in infants was shown to be 46·1 ml. per kg. Darrow et al. (1928) found that during the first year the figures for plasma volume per kg. rose from 50 to 62 ml. and slowly returned to 50 by the fourth year, after which the level remained constant. The results for the infants studied in the present series, however, did not conform to this trend, the values for plasma volume when related to weight

occupying the same range of variation as was found throughout the entire series. The idea that fluctuations in body weight in infants were often due to changes in the water content of the tissues and not to real tissue growth or loss was adduced by Bakwin and Rivkin (1924) to explain their finding that wide variations existed in the plasma volume in normal infants (range 38 to 72 ml. per kg., average 60·5 ml. per kg.). For the infants of the present investigation the average plasma volume calculated was 46·1 ml. per kg. with a range of 39·8 to 50·6 ml., results which are similar to those for this series as a whole. In a healthy infant 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.

Older children. To facilitate comparison with the present studies the results published by other authors of blood volume investigations in older children have been collected and tabulated (table 14). No

TABLE 14
PUBLISHED RESULTS FOR PLASMA AND BLOOD VOLUME

Plasma volume	Age in Years	No. of Cases	Range	Plasma volume (ml. per kg.)	
				Mean	Standard Deviation
Brines et al., 1941†	0–17	50	32–55·4	41·8	5
Schlutz et al., 1940					
(1)	12–15	7	39·5–56·8	45	6·2
(2)	16–17	9	40·1–60·2	47·2	6·7
Morse et al., 1947*†	1–13	40	42–66·8	50·1	5·74
Author's series ..	0–13	80	35–58·4	47·8	5·83
BLOOD VOLUME Brines et al., 1941†	0–17	50	46·5–95·9	69·8	9·64
Schlutz et al., 1940					
(1)	12–15	7	64·9–103·1	78·8	12·0
(2)	16–17	9	72·4–104·5	85·3	11·7
Morse et al., 1947*†	1–13	40	68·7–111·5	84·1	9·16
Author's series ..	0–13	80	62–113	83·5	10·39

* Only the first forty children of this series of seventy-five have been quoted as they corresponded in age to the present series.
† The spontaneous deviations supplied for the series of Brines et al. and Morse et al. have been calculated from the authors' figures.

significant differences were found, with the exception that those of Brines et al. (1941) were lower than all the others.

The steady increase of plasma and blood volume with advancing age in childhood has already been discussed. Periods of excessive rise in blood volume have been described by Seckel (1936), who thought that relatively high values were obtained at two periods during childhood, namely between 3 and 6 years, and between 11 and 13. There was no indication of this phenomenon in the present studies.

Sex differences. It has been shown that adult women tend to have both a lower absolute plasma and unit plasma volume than adult men of the

same weight due to their greater proportion of fat over tissues rich in blood (Gibson and Evans, 1937b). A greater difference was observed between the sexes as regards absolute blood volume, due in part to the discrepancy in plasma volume, but mainly to the greater values found in men for the red cell volume. The present series was therefore divided into its component groups of thirty-eight boys and forty-two girls, and analysed in search of any differences attributable to sex (table 15).

TABLE 15
THE PLASMA AND BLOOD VOLUME ACCORDING TO SEX

	Plasma volume (ml. per kg.)			Blood volume (ml. per kg.)		
	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		
S.E. of difference	1·32			2·17		

S.D. = standard deviation.
S.E. = standard error.

The girls had lower average plasma and blood volumes per kg. than the boys, the difference being greater for blood volume. Although the difference was just more than twice the standard error of the difference, it was considered that it was not sufficiently great to be judged significant. The unit volumes related to height, however, also showed a tendency to run at lower levels in the group of girls than in the group of boys (fig. 8). It is therefore tentatively suggested that a sex difference in blood volume may exist in children as well as in adults,
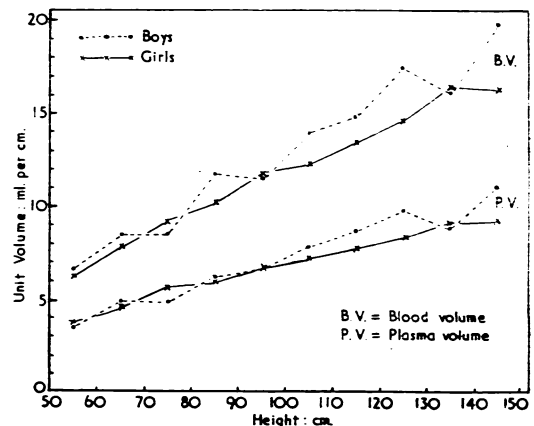

FIG. 8.—Comparison of the plasma and blood volume in boys and girls of the same height. (Each plotted figure represents the average for each group of 10 cm.)

but a much larger number of estimations would be necessary before any definite conclusion could be drawn. Brines et al. (1941), on the other hand, held the opposite opinion, namely, that there was no difference between the sexes until puberty, when boys began to have larger blood volumes than girls of the same measurements. No significant difference was found in this series, in haematocrit and haemoglobin readings, nor in red cell counts, during comparison of the two sexes.

General considerations. When the results were examined, a striking feature was noticeable, namely, the wide range within which the blood volumes of individuals of the same body size could be distributed. A reference to the diagrams (figs. 2 to 7) will show that all have several values lying outwith 20 per cent. of average. The height basis yielded the greatest number of aberrant results, as twelve of the eighty, or 15 per cent., lay outwith the 20 per cent. variation. All except one, however, were included within ± 30 per cent. This wide range of normality is in accord with the findings of other investigators (Gibson and Evans, 1937b; Morse et al., 1947).

No definite conclusions could be drawn from the present results regarding the influence of muscularity and obesity on the blood volume in individuals of comparable size. Gibson and Evans, however, concluded that in adults the absolute total blood volume was high in muscular and obese persons, and low in thin subjects. Morse and his colleagues considered that thin children tended to have a large blood volume and that both obese and undernourished children tended to have a low blood volume.

The Correlative Factor of Choice

Previous workers have held diverse opinions regarding the most suitable basis for the prediction of the blood volume of a given individual. McIntosh (1929) and Darrow et al. (1928), advocated weight as a basis of correlation, while recent investigations by Morse et al. (1947) would seem to establish surface area as giving the closest approximations to the values found by experiment. As stated previously, analysis of the present studies showed that weight provided the best correlation for both plasma and blood volume in healthy children. The soft tissues of the body, however, can undergo considerable changes in weight in such conditions as dehydration, marasmus, and oedema. As a consequence any concurrent change in plasma volume may be masked, or at least minimized, if plasma volume is expressed in relation to kg. of weight, and also to sq. m. of surface area, since any change in weight is reflected in the surface area measurement. For example, a child weighing 25 kg. and having a plasma volume of 1,250 ml. begins to show signs of congestive cardiac failure. His plasma volume may then increase by 20 per cent.,

or 250 ml. to become 1,500 ml. Meanwhile, his weight may increase to 26 kg. Before cardiac failure, therefore, his plasma volume was 50 ml. per kg., and after failure, 57 ml. per kg. Thus he has apparently gained 7 ml. of plasma per kg. or a total of either 175 or 182 ml. depending on whether the first or the second weight is used for the calculation. Neither of these figures approaches the extent of the true plasma increase. This type of error could, of course, be avoided by keeping the results in terms of absolute plasma or blood volume, but it is more convenient for purposes of comparison to reduce the figures to some sort of unit. It is, therefore, suggested that height should be adopted as the basis for prediction of the normal blood volume when investigating conditions of disease. For this opinion, some support has been found in the publications of other workers (Gibson and Evans, 1937b; Brines et al., 1941; Perera, 1946).

Summary

1. Plasma and blood volume investigations have been carried out in eighty healthy children. Although congo red was used in the early investigations and Evans blue in the later ones, evidence is produced to show that the results are comparable.

2. 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.

3. Of the three measurements, weight, height, and surface area, weight bore the closest relationship to both plasma and blood volumes, judged by the correlation coefficients and the scatter of the results round the regression equations.

4. Because of the tendency of body weight to undergo rapid change, especially in children, height has been suggested as the most suitable correlation factor when comparing the plasma and blood volumes in disease with those found in health.

5. In boys, both plasma and blood volumes showed a tendency to be higher than in girls of the same size.

REFERENCES.

Bakwin, H., and Rivkin, H. (1924). *Amer. J. Dis. Child.,* 27, 340.
Bischoff, T. L. W. (1856). *Z. wiss. Zool.,* 7, 331.
—— (1858). *Ibid.,* 9, 65.
Brines, J. K., Gibson, J. G., 2nd, and Kunkel, P. (1941). *J. Pediat.,* 18, 447.

Darrow, D. C., Soule, H. C., and Buckman, T. E. (1928). *J. clin. invest.*, **5,** 243.

Davis, L. J. (1942). *Edinb. med. J.*, **49,** 465.

Dawson, A. B., Evans, H. M., and Whipple, G. H. (1920). *Amer. J. Physiol.*, **51,** 232.

Du Bois, D., and Du Bois, E. F. (1916). *Arch. intern. Med.*, **17,** 863.

Gibson, J. G., 2nd, and Evans, W. A., Jr. (1937a). *J. clin. Invest.*, **16,** 301.

——, —— (1937b). *Ibid.*, **16,** 317.

——, 2nd, and Evelyn, K. A. (1938). *Ibid.*, **17,** 153.

——, 2nd, and Gregersen, M. I. (1935). *Amer. J. Physiol.* (Proc.), **113,** 50.

Gregersen, M. I. (1944). *J. Lab. clin. Med.*, **29,** 1266.

——, and Gibson, J. G., 2nd (1937). *Amer. J. Physiol.*, **120,** 494.

——, and Rawson, R. A. (1943). *Ibid.*, **138,** 698.

Heilmeyer, L. (1929). *Biochem. Z.*, **212,** 430.

Keith, N. M., Rowntree, L. G., and Geraghty, J. T. (1915). *Arch. intern. Med.*, **16,** 547.

Kennedy, J. A., and Millikan, G. A. (1938). *J. Physiol.*, **93,** 276.

Lucas, W. P., and Dearing, B. F. (1921). *Amer. J. Dis. Child.*, **21,** 96.

McIntosh, R. (1929). *J. clin. Invest.*, **7,** 203.

Morse, M., Cassels, D. E., and Schultz, F. W. (1947). *Amer. J. Physiol.*, **151,** 448.

Noble, R. P., and Gregersen, M. I. (1946). *J. clin. Invest.*, **25,** 158.

Perera, G. A. (1946). *Ibid.*, **25,** 401.

Schlutz, F. W., Morse, M., Cassels, D. E., and Iob, L. V. (1940). *J. Pediat.*, **17,** 466.

Seckel, H. (1936). *Arch. Dis. Childh.*, **11,** 21.

Welcker, H. (1858). *Z. rat. Med.*, **4,** 145.

Wintrobe, M. M., and Landsberg, J. W. (1935). *Amer. J. med. Sci.*, **189,** 102.