Measurement of plasma volume by means of ^{59}Fe -labelled dextran and Evans blue compared

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SYNOPSIS The apparent volumes of distribution of Evans blue and ^{59}Fe -dextran were determined in 18 studies.

^{59}Fe -dextran volume was 5% lower than Evans blue volume, a highly significant difference.

The rate of loss of ^{59}Fe dextran from the circulation was significantly less than that of Evans blue.

Many substances have been used for the measurement of plasma volume. All depend on a dilution principle and measure the apparent volume of distribution of the particular substance used.

Evans blue and ^{131}I -labelled human albumin have been widely used (Schultz, Hammarsten, Heller, and Ebert, 1953; Anderson, 1962) and more recently ^{125}I -labelled human albumin has become available. Evans blue is bound to albumin after intravenous injection and so these substances all measure the volume of distribution of albumin, which may not correspond to the true plasma volume. Because of this, use has been made of various macropoteins, including gamma globulin (Anderson, 1962), fibrinogen (Baker and Wycoff, 1961), and alkaline phosphatase (Posen, Clubb, Neale, and Hotchkis, 1965). The polysaccharide macromolecule dextran has also been used (Jaenike, Schreiner, and Waterhouse, 1957; Jacobsson, 1959), but the chemical assay of dextran is laborious, and other workers have used iron-dextran and have estimated the iron (Mackenzie and Tindle, 1959).

The demonstration that iso-oncotic dextran is more efficient than iso-oncotic human albumin in expanding plasma volume (Mills, DeWardener, Hayter, and Clapham, 1961) suggested that dextran might give a more accurate estimate of plasma volume than labelled albumin, especially

in conditions of oedema, ascites, and proteinuria.

This paper describes the use of ^{59}Fe -labelled dextran of high molecular weight (approx. 200,000), and compares the results so obtained with those obtained using Evans blue.

Materials and Methods

The patients used in this study suffered from a variety of diseases (see Table I) and were purposely chosen to include cases of heart failure, liver disease, and nephrosis. Some had been treated with diuretics before the time of the study. The nature of the study was carefully explained to each and their consent obtained.

All patients were recumbent or semi-recumbent during the study, had fasted overnight, and fasted for a further four hours during the study. After taking a control blood sample to provide a plasma blank the ^{59}Fe -dextran and Evans blue¹ were injected within 45 seconds of one another into one antecubital vein. Blood samples were drawn, without stasis, into lithium heparin tubes from the opposite arm before and at intervals up to 120 hours after the injection, the first four or five samples being taken at 10-minute intervals. Supernatant plasma was obtained by centrifugation. The volume of the ^{59}Fe -dextran injected was known from the use of a calibrated syringe shown accurate to 0.01

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ml. Evans blue volumes injected were calculated by weighing syringes before and after operation. For ⁵⁹Fe-dextran, volumes injected were 0.5 to 2.0 ml, and for Evans blue 3 to 5 ml. The needle was washed through after the second injection. The radio-activity of the ⁵⁹Fe-dextran injected ranged from 0.5 to 2 μ c.

The ⁵⁹Fe-dextran was kindly supplied by Fisons Pharmaceuticals Ltd, Holmes Chapel, Cheshire, England, and had an initial specific activity of approximately 8.6 μ c/ml.

Radioactivity was measured in a well-type scintillation counter using 5 ml aliquots. The counts were plotted against time, and extrapolations made to zero time. In most cases, counting error was less than 1%. Standards were prepared with added ferric chloride to obviate the possibility of absorption of ⁵⁹Fe onto glass.

Evans blue concentrations were measured using a Unicam spectrophotometer set at 620 μ , and using a plasma blank. A reference curve using known dilutions of Evans blue in fresh normal plasma was constructed.

Results

COMPARISON OF PLASMA VOLUME DETERMINATIONS BY THE TWO METHODS
Simultaneous plasma volume measurements for Evans blue and for ⁵⁹Fe-dextran, obtained by extrapolation to zero time, were obtained for 18 studies in 16 patients (Table I). The mean

Fig. 1 Comparison of plasma volumes determined by Evans blue and ⁵⁹Fe-dextran. Plasma volumes obtained by extrapolation to zero time. Line of identity shown.

Correlation coefficient, $r = 0.97$
The regression line of plasma volume by Evans blue (y) in ml on plasma volume by ⁵⁹Fe-dextran (x) in ml is $y = 1.11x - 145$.

(and SE) of the plasma volume using Evans blue was 2,845 ml (± 194 ml) and using ⁵⁹Fe-dextran was 2,693 ml (± 170 ml). The difference between the values for the two techniques (based on an analysis of the paired estimations) was statistically highly significant ($P < 0.01$).

In Fig. 1 the individual plasma volume determinations for Evans blue are plotted against the corresponding plasma volumes by ⁵⁹Fe-dextran. There is good correlation, with a correlation coefficient of 0.97.

Patient No.	Diagnosis	Plasma Volume			Percentage Fall in Concentration during First and Second Hours				T½ (hr) (4-80 hr)
		Eo (ml)	Fo (ml)	Difference (Eo - Fo) ml	Evans Blue		⁵⁹Fe Dextran		
					First Hour	Second Hour	First Hour	Second Hour	
1	Polycythaemia	1,710	1,560	+150	6	6	5	5	
2(a)	Normal	3,150	2,670	+480	9	9	4	3	
2(b)	Normal¹	3,000	2,830	+170	9	9	9	4	
3	Anaemia	4,050	3,550	+500	9	9	8	8	
4	Portal cirrhosis	1,430	1,430	0	8	4	5	4	
5	Infective hepatitis	2,750	2,830	- 80	14	6	10	3	
6	Alcoholic cirrhosis	3,160	3,010	+150	7	7	5	5	
7	Cirrhosis + ascites²	2,110	2,410	-300	23	6	23	7	20
8	Cirrhosis + ascites³	2,110	2,080	+ 30	15	4	4	4	26
9	Cardiac failure	3,070	2,610	+460	7	7	11	4	
10	Cardiac failure	2,900	2,750	+150	12	6	11	5	27
11	Cardiac failure	3,270	3,010	+260	5	4	5	4	33
12	Uraemia with oedema	4,820	4,640	+180	13	5	5	3	
13	Malignant ascites	2,080	2,260	-180	8	8	7	5	29
14a	Primary amyloid (nephrosis)	3,630	3,210	+420	26	18	8	6	
14b	Primary amyloid (nephrosis)⁴	1,960	1,890	+ 70	32	15	10	6	
15	Nephrosis	3,040	2,980	+ 60	14	9	16	4	18
16	Nephrosis	2,970	2,740	+230	19	7	16	6	23

Table I Plasma volume measurements with Evans blue and ⁵⁹Fe dextran¹

¹Abbreviations Eo and Fo are the plasma volumes, obtained by extrapolation to zero time, for Evans Blue and ⁵⁹Fe Eextran respectively. $T_{\frac{1}{2}}$ is the half time for the decline in plasma concentration of ⁵⁹Fe dextran in the period four to 80 hours.
²Diuretic treated.

If plasma volumes are calculated using only a single 10-minute sample slightly higher mean plasma volumes are obtained, with a difference between means of 165 ml. The difference between the two sets of values (by analysis of paired results) is highly significant ($P < 0.01$).

Table II shows the mean plasma volumes, calculated by extrapolation, for the various groups of subjects. It will be seen that the Evans blue volume is higher than the ^{59}Fe -dextran volume in all but the cases of liver disease. The numbers of cases in each group are small, however, and no firm statistical conclusions can be reached for the liver disease group as compared with the other groups.

LOSS FROM THE CIRCULATION OF ^{59}Fe -DEXTRAN AND EVANS BLUE

The mean falls in concentration during the first hour, and during the second hour after injection, for ^{59}Fe -dextran and for Evans blue are shown in Table III.

The falls in concentration are expressed as a percentage of the initial value at the beginning of the corresponding time period. It will be seen that both in the first hour and in the second hour, the fall in plasma concentration of ^{59}Fe -dextran is significantly less than that of Evans blue. This difference is particularly marked in the case of primary amyloid. Jarnum (1960) showed that Evans blue may be taken up by amyloid tissue, thus invalidating its use as a plasma volume indicator in such cases. Table III also shows that

Group	No. of Studies	Plasma Volume (ml)		Difference E - F
		Evans Blue (E)	^{59}Fe -Dextran (F)	
Cardiac failure	4	3510	3250	+260
Miscellaneous	5	2780	2570	+210
Nephrosis	4	2900	2710	+190
Liver disease	5	2310	2350	- 40
Liver disease Without ascites	3	2450	2420	+ 30
With ascites ¹	2	2110	2240	-130

Table II Mean plasma volumes in various conditions

¹Treated with diuretics.

	Evans Blue	^{59}Fe -Dextran	Difference	t	p
Mean fall in concentration during first hour (%) ($\pm$ SE)	13.1 $\pm$ 1.7	9.2 $\pm$ 2.3	3.97	2.52	<0.01
Mean fall in concentration during second hour (%) ($\pm$ SE)	7.6 $\pm$ 2.0	4.9 $\pm$ 1.2	2.71	3.31	<0.0025
Difference t	5.5	4.2			
p	3.96	4.64			
	<0.0025	<0.0005			

Table III Comparison of mean falls in concentration

Fig. 2 Decline in plasma activity ^{59}Fe -dextran over 120 hours. Study 15, nephrosis $T_{1/2}$ (4-80 hours) = 19 hours.

the fall in plasma concentration for ^{59}Fe -dextran during the first hour is significantly higher than during the second hour.

In eight patients, the decline in plasma concentration of ^{59}Fe -dextran was followed for up to 120 hours, and was a series of exponential functions. An example is shown in Fig. 2, and the $T_{1/2}$ for the period four hours to 80 hours is shown in Table I. The rate of decline in plasma concentration of ^{59}Fe -dextran over the period four hours to 80 hours was less than for the period 1 to 4 hours.

In one case, peritoneal fluid was sampled at intervals for six hours, and in a second case pleural fluid was sampled at intervals for 20 hours following intravenous injection of ^{59}Fe -dextran. In neither case was ^{59}Fe detectable in the sample fluids.

In one man with nephrosis, and a proteinuria of 10 to 15 g per 24 hours, about 1% of injected radioactivity was detected in the urine over seven days, and whole body counting showed an 8% loss of radioactivity from the body.

In two cases of congestive cardiac failure and one case of cirrhosis with ascites, no loss of radioactivity was detected on the whole body counting, and no radioactivity was detected in stool or urine over seven days.

Apart from one instance of transient nausea no side effects due to injection of ^{59}Fe -dextran or Evans blue were encountered.

Discussion

Various workers (Schultz *et al*, 1953; Anderson 1962) have shown that plasma volume determinations with Evans blue and ^{131}I -labelled human albumin give identical results in men. Substances relying on binding to human albumin have distinct advantages (Anderson, 1962), and there is

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need for materials which approach more closely to the ideal volume indicator. Various substances have been tried, including ^{131}I -labelled fibrinogen (Baker and Wycoff, 1961), ^{131}I -gamma-globulin (Anderson, 1962), human placental alkaline phosphatase (Posen *et al.*, 1965) and high-molecular-weight dextran (Jaenick, Schreiner, and Waterhouse, 1957; Jacobsson, 1959).

Anderson and Jarnum (1966) refer briefly to the use of ^{59}Fe -dextran in the measurement of plasma volume.

In this study, plasma volume determinations using ^{59}Fe -dextran of high molecular weight are shown to be significantly lower by about 5% than plasma volume measured by Evans blue.

The rate of disappearance of ^{59}Fe -dextran is also shown to be less than that of Evans blue, indicating a slower rate of escape from the vascular compartment.

Other workers have used high-molecular-weight dextran and have obtained rather divergent results. Semple, Thomsen, and Ball (1958) found no difference between dextran and Evans blue plasma volumes, and Verel (1958) considered the dextran volume lower only in patients with increased capillary permeability. Jacobsson (1959) found the dextran volume 6-10% lower than the Evans blue volume. Jaenicke *et al.* (1957) also found the dextran volume significantly lower than ^{131}I -albumin volume. Other high molecular weight substances, namely, ^{131}I -gamma-globulin (Anderson, 1962), ^{131}I -fibrinogen (Baker and Wycoff, 1961), alkaline phosphatase (Posen *et al.*, 1965), ^{131}I -paraprotein (Anderson and Gabuzda, 1964), also give volumes of distribution 3-12% lower than ^{131}I -albumin values: the subjects used included normal people and normal dogs, and people with cirrhosis, paraproteinaemias, and a variety of chronic diseases.

The chemical estimation of dextran is laborious. Mackenzie and Tindle (1959) used an iron-dextran preparation and measured serum iron levels. However, the labelling with ^{59}Fe offers the simplicity and accuracy of radioactive counting, and the amount of radioactivity injected, 0.5 to 2 μC , compares with that of 10-20 μC ^{59}Fe used in iron-turnover studies. Because the half-life of ^{59}Fe is 46.3 days, the ^{59}Fe -dextran preparation may be stored and used over a period of several weeks.

The lower apparent volume of distribution of ^{59}Fe -dextran compared with Evans blue should not be due to differing rates of loss from the circulation after mixing, for extrapolation to zero time should allow for this factor. The implication is that there is a greater differential loss of Evans blue during the mixing period. Commercial preparations of Evans blue do contain 2-4% of a red impurity (Leeson and Reeve, 1949) disappearing from the blood stream within one minute (Cooley, 1954) and this may

account for some of the difference in apparent plasma volumes. The fact that radioiodine-labelled human albumin gives volumes of distribution identical with those of Evans blue may merely be due to a coincidence of errors. Anderson and Gabuzda (1964) found that Evans blue gave a volume of distribution some 5% higher than ^{131}I -paraprotein, and this is in close agreement with our findings for ^{59}Fe -dextran.

The presence of free iron, or of low-molecular-weight iron-dextran in the preparation would lead to a falsely high estimation of plasma volume. This source of error is not thought to be significant in view of the finding of no radioactivity in the urine in two cases of cardiac failure and one case of cirrhosis, and of only 1% in the first 24 hours after injection in one case of the nephrotic syndrome. The molecular weight of ^{59}Fe -dextran is uncertain, but is thought to be of the order of 200,000. Ricketts, Cox, Fitzmaurice, and Moss (1965) showed that the Fe-dextran complex of the type used in the present study had a molecular or particle size much greater than that of gamma globulin, and that there was no evidence of a wide distribution of molecular weight. As supplied, about 1% (61 mg/100 ml) of the iron was in the free ionic form, or in a weak ferrous dextran complex, the remainder being in a stable ferric dextran complex. The metabolism of the iron-dextran complex has been studied by Golberg (1958) who has shown that iron-dextran is removed from the circulation by cells of the reticuloendothelial system, and split into iron and dextran.

Anderson and Gabuzda (1964) found a 24-hour elimination from the circulation for ^{131}I -paraprotein of 22%, and for ^{131}I -albumin of 39%, while for ^{59}Fe -dextran we obtained a figure of 24.7%. Although we encountered no serious reactions in patients receiving repeated intravenous injections of iron dextran, severe anaphylactoid reactions have rarely been reported (Mackenzie and Lawson, 1959).

The present study suggests that ^{59}Fe -dextran is a useful addition to the substances available for the measurement of plasma volume.

^{59}Fe may be counted accurately in the presence of ^{51}Cr but the converse is not true, and so if combined determinations of red cell mass and plasma volume are required, the red cell mass, using ^{51}Cr -tagged red cells, should be determined before injection of ^{59}Fe .

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