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Plasma Volume Estimation in Severely Ill Infants and Children Using a Simplified Evans Blue Method*

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Abstract. Plasma volume was measured using Evans blue dye and ^{125}iodinated human serum albumin (RIHSA) simultaneously in order to evaluate the accuracy of a simplified Evans blue method recommended by Nielsen and Nielsen (1962). 9 studies were performed in 8 newborn infants weighing 1.07 to 2.85 kg and 16 studies in 14 patients aged 6 months to 14 years suffering from severe circulatory disturbances. In 20 studies, plasma volumes measured by Evans blue and by RIHSA agreed within $\pm 5\%$, and in all the studies within $\pm 10\%$. The Evans blue method yielded higher plasma volumes than the RIHSA method in 19 instances. The mean difference (paired *t*-test) was only significant in newborn infants ($+4.0 \pm 3.6\%$; $P < 0.05$). The disappearance rates of Evans blue exceeded that of RIHSA in 16 studies, but this was only significant in the patients older than 6 months ($+2.3 \pm 4.2\%$/h; $P < 0.05$).

Key words: Evans blue – ^{125}Iodinated albumin – Plasma volume – Capillary permeability.

Introduction

The knowledge of blood volume may be of great importance in caring patients with considerable fluid imbalance, for example burned children and immature newborn infants. For clinical purposes the results of blood volume measurements have to be reliable and rapidly available. The technique should not be harmful to the patient. Tagging of autologous red cells with ^{51}chromium is commonly considered to be the most accurate method of measuring blood volume (Seifert and Messmer, 1971; Swan and Nelson, 1971). However, this method is time consuming and gives relatively high absorbed radiation doses to the whole body. The use of ^{125}iodinated human serum albumin is easy, rapid, and has been shown to give sufficiently accurate results, even in critically ill children (Linderkamp et

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al., 1977). Although our technique requires a dosage of only 0.025 μCi ^{125}iodine per kg of body weight, resulting in a radiation exposure to the whole body of 0.8 mrads (Gorten, 1965), radioactive substances should only be used for blood volume estimation in clearly indicated and approved situations.

However, the conventional Evans blue dye dilution methods require time-consuming extraction procedures and large amounts of blood, otherwise the results may be unreliable (Senn and Karlson, 1958). Since the errors of the dye dilution techniques are mainly due to variations in the blank density of the plasma, Nielsen and Nielsen (1962) introduced a method for determining the actual blank density in individual dyed plasma samples. The method is based on the finding of a rectilinear relationship between the negative logarithms of the extinctions of undyed plasma at the wave lengths 620 and 740 nm. Since Evans blue dye shows the highest extinction at 620 nm and no extinction at 740 nm, the blank value of dyed plasma at 620 nm can be calculated from the extinction at 740 nm.

The present work was undertaken in order to examine the practicability and accuracy of this method in severely ill newborn infants and children. Slight modifications are introduced.

Patients and Methods

The clinical data on the patients are summarized in Table 1. Blood volume was studied only when clinically indicated, each indication being approved by the ethical commitee of our hospital. Plasma volume was measured simultaneously by injection of 0.2 mg Evans blue dye and 0.025 μCi ^{125}iodinated human serum albumin (RIHSA) per kg of body weight via a peripheral vein. The perfusion sets were flushed with 2 ml saline. The thyroid gland was blocked by administration of one drop of Lugol's solution per kg of body weight before and for 5 days after injection of the tracers. 6 blood samples of 1.5 ml were taken at 5, 10, 20, 30, 45, and 60 minutes after injection of the tracers. In the newborn infants the blood was withdrawn from umbilical artery catheters prior to clinically indicated blood transfusion or exchange transfusion. In the children the blood samples were taken via perfusion sets. Plasma samples with visible hemolysis were discarded. Haematocrit was measured by the microhaematocrit method and corrected for 2% of trapped plasma (Linderkamp et al., 1974).

Radioactivity and extinction were measured in 0.5 ml plasma from each blood sample. The radioactivity was counted in a well-type scintillation counter (Frieseke and Hoepner, Erlangen-Bruck).

The optical density of the plasma samples was determined at 620 and 740 nm using a spectrophotometer (Zeiss, M 4, Q III). The actual blank density at 620 nm —the wave length with the highest light absorption of Evans blue dye—was calculated from the extinction at 740 nm, a wave length without absorption of Evans blue dye. For this purpose the extinction of 147 undyed plasma samples was measured at 620 and 740 nm and the regression equation between the results at both wave lengths calculated (Fig. 1). Nielsen and Nielsen (1962) recommended the use of the relationship between the negative logarithms of the optical

Table 1. Plasma volume and dissappearance rate of albumin measured with Evans blue and RIHSA[c]

No.	Sex	Age[a]	Weight (kg)	Haem-atocrit	Plasma volume (ml/kg)		Disappear-ance rate (%/h)		
					EB[b]	RIHSA[c]	EB[b]	RIHSA[c]	
Newborn infants									
1	f	32	1.07	40.2	53.4	51.8	13.6	17.3	Premature, RDS
2	f	10	1.37	44.1	46.5	47.9	23.7	19.5	Premature, RDS
3	f	50	1.45	54.6	48.8	47.7	13.3	15.9	Premature, RDS
4	f	12	1.57	51.7	40.5	39.2	35.1	36.1	Premature
5	m	48	1.65	49.1	48.8	46.8	33.5	26.2	Premature
6	f	55	2.47	37.4	49.0	45.6	30.9	29.0	Rh erythroblastosis
7	m	94	2.78	31.7	43.6	40.5	22.3	24.9	Hypoplastic left heart
8.1	m	14	2.75	38.9	54.8	53.7	24.3	21.1	Sepsis
8.2	m	35	2.85	52.6	40.6	37.3	29.1	15.6	After exchange trans-fusion
Infants and children									
9	m	1	9.5	36.1	52.6	54.4	10.3	9.7	Coeliac disease
10	f	8	14.0	37.8	57.7	56.4	16.2	20.7	Coeliac disease
11	f	12	23.0	38.7	60.4	58.0	22.7	17.2	Coeliac disease
12	m	15	80.3	35.9	43.7	43.3	18.7	14.0	Laurence-Moon-Biedl
13	m	1	12.2	29.3	58.5	61.0	11.7	14.1	Leukaemia
14	m	3	13.1	32.3	36.0	34.8	9.2	2.2	Sickle-cell anaemia
15	m	$^{6}/_{12}$	8.0	30.6	43.4	41.2	15.3	16.2	Sepsis
16	f	6	15.6	29.2	60.1	54.9	7.9	8.3	Sepsis
17	m	6	12.2	29.4	98.0	102.6	17.2	13.7	Sepsis
18	f	2	11.5	30.9	50.5	50.4	18.5	10.7	Cerebral oedema
19	m	4	14.4	25.8	39.0	37.6	9.0	7.2	Cerebral oedema
20.1	m	14	31.8	42.1	42.2	42.7	13.1	7.6	Cerebral haemorrhage
20.2	m	14	33.5	35.3	51.3	51.3	16.3	20.9	Anuria
21.1	m	3	14.2	39.2	46.8	45.5	32.1	23.3	Burns
21.2	m	3	15.0	36.3	50.6	49.1	19.6	16.3	
22	f	4	17.0	39.2	37.3	36.4	24.1	23.7	Burns

[a] Age in hours (newborn infants) and years (infants and children); [b] Evans blue; [c] ^{125}iodinated human serum albumin.

densities. However, our results indicate that there is a better correlation between the actual values ($r = 0.994$) than between the negative logarithms of the values ($r = 0.984$). The optical density of the Evans blue dye at 620 nm for each plasma sample was calculated by the following formula:

$$E_{620}\ (\text{Evans blue}) = E_{620}\ (\text{Plasma} + \text{Evans blue}) - (1.426\, E_{740}\ (\text{Plasma}) + 0.030)$$

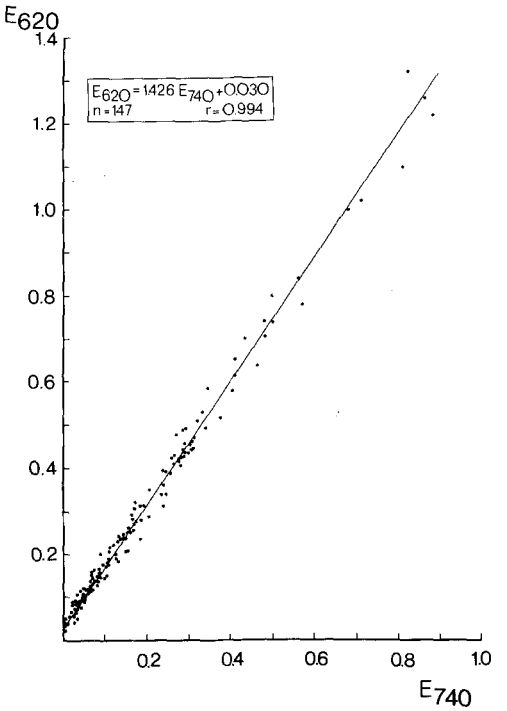

Fig. 1. Relationship between the blank densities at 620 and 740 nm for 147 samples of undyed plasma

As this method eliminates the influence of plasma on the optical density at 620 nm, no standards for plasma are necessary. Standards can be prepared using water or saline with known volumes of the original Evans blue solution.

Plasma volume is calculated by the following equation:

$$PV = \frac{Q \cdot St \cdot E_{St}}{E_0}$$

Q = volume of Evans blue dye solution injected
St = dilution of standard (volume of standard/volume of Evans blue dye solution)
E_{St} = Extinction of Evans blue of standard at 620 nm
E_0 = Extinction of Evans blue of plasma sample.

In this study the extinction of the plasma samples was extrapolated to zero time by calculating regression equations between extinction and time, after logarithmic transformation of extinction. The extinction at zero time was used as E_0 in the formula given above. The disappearance rate of the dye in per cent per hour was calculated from the extinctions at zero and 60 minute time, derived from the regression equations. Extrapolation to zero time and calculation of the disappearance of ^{125}iodinated human serum albumin was performed in the same way.

Pre-injection blood samples were only taken if the patient had been studied before. In these cases pre-injection extinction corrected for blank density and counts, respectively, were subtracted from the post-injection values.

Results

The results are summarized in Table 1. The Evans blue method gave higher plasma volumes than the RIHSA method in 8 of 9 studies in the newborn infants and in 11 of 16 studies in the patients aged 6 months to 15 years. The differences ranged from — 1.4 to + 3.4 ml/kg or — 2.9 to + 8.8% in the newborn infants and from — 4.6 to + 5.2 ml/kg or — 4.5 to + 9.5% in the older patients. The mean difference of + 4.0 ± 3.6% ($\bar{x} \pm$ SD) found in the newborn infants was significant different from zero ($P < 0.05$). In the older patients the mean difference (+ 1.6 ± 3.7%) was not significant. The disappearance rate of Evans blue exceeded that of RIHSA in 5 of 9 studies in the newborn infants and in 11 of 16 studies in the patients older than half a year. In the older patients this difference was significant ($P < 0.05$).

Discussion

Most reports on the simultaneous determination of plasma volume using Evans blue and radioactive iodinated albumin in adults have shown nearly identical mean distribution volumes (Crispell et al., 1950; Brady et al., 1953; Schultz et al., 1953; Freinkel et al., 1954; Inkley et al., 1955; Franks and Zizza, 1958; Davies and Topley, 1959; Andersen, 1962). However, the individual differences were higher than ± 10% in approximately one-quarter of the reported cases. Other authors have found significantly higher values for plasma volume with the Evans blue method (Aust et al., 1951; Zipf et al., 1955). Repeated plasma volume determinations in the same subject have shown the RIHSA method to be more accurate than the Evans blue method (Senn and Karlson, 1958).

The principle error in the Evans blue method is due to variation in the optical density of the plasma, even in the same subject (Senn and Karlson, 1958; Nielsen and Nielsen, 1962). i.e., the blank density of dyed plasma may be considerably different from the value of the control sample taken before injection of the dye. The extraction methods which eliminate the error of varying blank density for plasma are not much more accurate, since the procedures require pipetting and re-extraction of the dye (Senn and Karlson, 1958; Nielsen and Nielsen, 1962). The individual correction for blank density by measuring the optical density of the plasma at 620 and 740 nm suggested by Nielsen and Nielsen (1962) is apparently the most accurate method. In 20 of our 25 studies the results of both methods agreed to within ± 5%. In all the patients, the difference was less than 10%.

Simultaneous studies on the distribution volumes of Evans blue and radioactive iodinated human serum albumin have been performed in less disturbed newborn infants by two groups. Jegier et al. (1964), using the conventional Evans blue method, found significantly higher plasma volumes and transcapillary escape rates with iodinated albumin than with Evans blue. Parving et al. (1973), using the method described by Nielsen and Nielsen (1962), found identical mean plasma volumes with both methods. The differences ranged from — 5.6 to + 6.8%. Our data show a greater scatter, possibly because the patients were more

severely affected by their disease. In spite of the better agreement between the results of the RIHSA and the Evans blue methods obtained when individual corrections for blank density are applied, it has to be pointed out that the Evans blue method may overestimate plasma volume by up to 10% in patients with severe disease.

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