Estimation of blood (plasma) volume in sheep with Evans Blue and Bromsulphalein

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Abstract. The reliability of two markers, Evans Blue (EB) and Bromsulphalein (BSP) for the estimation of blood volume and blood plasma volume, respectively, was studied in sheep. In vivo estimates were verified with in vitro determinations. Extinctions were read against standard solutions with and without plasma.
Key-words: blood volume, blood plasma volume, Evans Blue, Bromsulphalein, marker dilution, sheep.

Introduction. Blood plasma volume can be estimated with a marker (M) spreading over the plasma only and disappearing at a constant rate. Marker concentration declines then according the formula:

$$C_{M,t} = C_{M,0} \cdot e^{-kt}$$

with $C_{M,t}$ and $C_{M,0}$ representing marker concentrations at times t and 0, respectively, and k representing the fractional disappearance rate constant. After intravenous administration of the marker and subsequent collection of samples, k can be estimated after logarithmic transformation:

$$\ln C_{M,t} = \ln C_{M,0} - kt$$

From the intercept with the Y axis ($\ln C_{M,0}$) marker concentration at time 0 can be deduced and blood plasma volume can be calculated as the amount of marker injected divided by $C_{M,0}$.

If the marker applied spreads over both the plasma and the blood cells and marker concentration in the plasma equals that in the blood cells, from the amount of marker injected and $C_{M,0}$ the blood volume can be estimated.

In the present experiments, the reliability of Evans Blue (EB) and Bromsulphalein (BSP) for the estimation of blood volume and blood plasma volume, respectively, was investigated.

Material and methods. To a wether (ca. 70 kg live weight), provided with a silastic catheter in the jugular vein, 200 mg BSP (10 mg/ml 0.9 % NaCl) or 100 mg EB (10 mg/ml 0.9 % NaCl) were administered. Starting about 3 min after injection of the marker, blood samples were collected; after BSP administration with intervals of about 1 min, over a 10-min period, and after EB administration with intervals of about 5 min, over a 30-min period. These blood samples were centrifuged (2000 *g*, 10 min) and in the blood plasma marker concentrations were estimated in duplicate

as follows. For the estimation of EB, a blood plasma sample was diluted 5 times with 0.9 % NaCl and the extinction was read at 605 nm. BSP was estimated in a 1-ml blood plasma sample after addition of 4 ml 0.9 % NaCl and 0.5 ml 10 % (w/v) NaOH. Extinctions were read at 580 nm. For both EB and BSP extinctions were read against standard solutions with and without plasma.

In the in vitro experiments the influence of hematocrit value and of the amount of marker on the estimated blood (plasma) volume was studied. After addition of a known amount of marker, the samples were centrifuged and analysed as described before. Based on the marker concentration found and the amount of marker applied the volume was calculated and compared with the quantity of blood (plasma) actually used.

The parameters with their standard errors, as given in Tables 1 and 2, were calculated by regression analysis.

Results

In vitro experiments. The volume determined indirectly with EB approximated on an average blood volume (V_b) when standard solutions were used with plasma (I). Without plasma added to the standard solutions (II) the volume determined was approximately 20 % higher than V_b. With BSP, a volume was determined indirectly which was about equal to blood plasma volume (V_p) when no plasma was added to the standard solutions (II). With plasma present in the standard solutions (I) the volume determined approximated 95 % of V_p.

Table 1. Correction factors (F_M) for EB and BSP, as related to the analytical procedure (I, II) followed (SE in parenthesis); df = 15 (EB), df = 16 (BSP).

I	F_{EB}	$= -0.0125\ (0.0028)\ C_{EB,p} - 0.0123\ (0.0055)\ Ht + 1.588\ (0.153)$		$(r = 0.82)$
	F_{BSP}	$= 0.0028\ (0.0006)\ C_{BSP,p} + 0.869\ (0.017)$		$(r = 0.78)$
II	F_{EB}	$= -0.0181\ (0.0040)\ C_{EB,p} - 0.0147\ (0.0067)\ Ht + 1.904\ (0.185)$		$(r = 0.82)$
	F_{BSP}	$= 0.0033\ (0.0006)\ C_{BSP,p} + 0.921\ (0.018)$		$(r = 0.79)$

Table 2. Parameters of the in vivo experiments, as related to the analytical procedure (I, II) followed (SE in parenthesis), before and after application of correction factors (see Table 1). df = 4.[1]

	EB		BSP		Ht	
	I	II	I	II	I	II
$\ln C_{M,0}$	3.66 (0.02)	3.47 (0.02)	4.47 (0.06)	4.34 (0.07)		
k	0.97 (0.09)	0.95 (0.10)	39.5 (0.82)	37.3 (1.07)		
V	2.58 (0.04)	3.11 (0.06)	2.29 (0.13)	2.62 (0.20)	11.2	15.8
After application of correction factors						
$\ln C_{M,0}$	3.41 (0.01)	3.40 (0.01)	4.43 (0.07)	4.36 (0.09)		
k	0.36 (0.04)	0.36 (0.04)	40.6 (0.96)	38.4 (1.25)		
V	3.32 (0.03)	3.33 (0.03)	2.39 (0.16)	2.57 (0.21)	28.0	22.8

[1] $C_{M,0}$: blood plasma marker concentration at $t = 0$ (μg/ml); k: rate constant of disappearance (%/min); V: estimated volume (l); Ht: hematocrit value calculated as $(V_b - V_p)/V_b \times 100\%$.

Correction factors. The volume estimated with EB in the in vitro experiments depended on the hematocrit (Ht) and the quantity of EB added. Thus the theoretically expected blood plasma EB concentration, calculated as the amount of EB applied divided by the blood volume (V_b) used, deviated from the blood plasma concentration ($C_{EB,p}$) found. In order to correct for this deviation in the in vivo experiments, from the in vitro experiments correction factors (F_M) in relation to Ht and $C_{EB,p}$ were calculated by multiple regression analysis. For BSP the same procedure was followed. The correction factors found depended on $C_{BSP,p}$ only. In both cases the corrections depended on the analytical procedure (I, II) followed (Table 1).
In vivo experiments. The parameters found after injection of BSP and EB in both analytical procedures with (I) and without (II) plasma are summarized in Table 2. In the blood samples taken, the actual mean Ht amounted 25.5 % corresponding rather well with the values deduced from the estimated blood and blood plasma volumes, after application of correction factors, of 28.0 % (I) and 22.8 % (II) respectively.

Conclusions. In sheep, with EB and BSP as markers, it seems possible to obtain accurate estimates of blood and blood plasma volumes, respectively. After application of appropriate correction factors, determined in in vitro experiments, the estimated volumes are independent of the analytical procedures followed with respect to the use of standard solutions with and without plasma added.

Contrary to what is known from literature (Gregersen & Rawson, 1959; Swenson, 1977), the present results indicate unequivocally that with EB blood volume is estimated, and not blood plasma volume. This seems in accordance with Zizza & Reeve (1957) who suggested that EB overestimates plasma volume in the rabbit. In vitro, however, they found accurate estimates of plasma volume with EB.

References

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