DILUTION CHARACTERISTICS OF COOMASSIE BLUE IN MAN

BY

E. H. O. PARRY and JOHN TUCKMAN*

From the Department of Medicine, Postgraduate Medical School of London

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The azo dye Coomassie Blue is relatively non-toxic, has a rapid disappearance rate, and does not cause any skin discoloration (Taylor and Shillingford, 1959). Because it has these properties the dye can be given repeatedly to man at short intervals of time, a particularly important quality for investigations based upon the Stewart-Hamilton indicator dilution curve. Indeed, the dye has already been successfully used in studies of cardiac output (Taylor and Shillingford, 1959) and of congenital heart defects (Oakley *et al.*, 1960). This paper presents further information about the dilution characteristics of the dye in man. The results reported include procedures in which radio-iodinated human serum albumin (I^{131} H.S.A.) and Coomassie Blue were injected simultaneously.

PATIENTS AND METHODS

Seventeen patients were investigated, eleven men and six women. Their diagnoses are listed in Table I. Each lay supine for at least one hour before, and throughout, the procedure, and all had been fasting for at least five hours before study.

TABLE I
SUMMARIZED DATA ON 15 PATIENTS INJECTED WITH COOMASSIE BLUE

No.	Patients		Diagnosis	Weight (kg.)	Hæma- tOCRIT (%)	Dye injected (mg.)	Plasma volume dye	Plasma volume I^{131} H.S.A.	Mixing time (minutes)	
	Age	Sex							Dye	I^{131} H.S.A.
1	56	M	Carpal tunnel syndrome	89.9	45	19.7	5330	4569	—	—
2	58	M	Essential hypertension	58	35	(twice)	6823	4738	—	—
						23.9	2690	2575	—	—
						(thrice)	2450	2328	—	—
3	33	M	Acute rheumatic fever	63.6	34.5	41.2	6336	2771	—	—
4	55	F	Constrictive pericarditis	44.5	41	(twice)	4604	3506	—	—
						38.3	4882	3709	—	—
5	69	F	Ischæmic heart disease	40	40	41.2	3600	2764	5	5
6	51	M	Splenomegaly (cause uncertain)	64.5	48	30.4	3007	2199	16	16
7	61	M	Cor pulmonale	54	65	42.5	4053	2780	6	7
8	38	M	Hypertensive heart failure	59	36	44.8	2417	2132	7	11
9	54	M	Megaloblastic anæmia	60	22	27.2	4240	3687	—	—
10	61	M	Ischæmic heart disease	86.3	46	27.6	3126	—	—	—
11	47	F	Drug eruption	61.4	42.8	21.1	3247	—	8.9	—
12	69	F	Ischæmic heart disease	41.1	37	22.4	3768	—	3	—
13	59	F	Iron deficiency anæmia	43.2	38	29.7	4148	—	4	—
14	32	M	Myocardial infarction	53.6	46.5	19.6	2884	—	6	—
15	49	M	Megaloblastic anæmia	54.5	25	21.3	2178	—	3	—
							4096	—	4	—

* Postdoctoral Fellow, National Heart Institute, United States Public Health Service, also in receipt of a grant from the Dazian Foundation for Medical Research.

Four different procedures were carried out.

(A) Seven patients received Coomassie Blue alone. Dilution curves were constructed from venous samples taken every one or two minutes during the first ten minutes, and at three-, five-, or ten-minute intervals, between ten and sixty minutes after injection.

(B) Four patients received an injection of a solution containing both Coomassie Blue and I^{131} H.S.A. Venous samples were taken at approximately the same time as in group A.

(C) Three patients received an injection of the dye-isotope mixture and venous samples were taken at four-minute intervals from twenty to forty minutes after injection. These patients were given a second injection forty minutes after the first and samples were again taken at the same intervals. A third injection was given to one patient eighty minutes after his first injection (forty minutes after the second) and venous samples were taken as after the first two injections.

(D) Two patients received a rapid intravenous injection of the dye isotope mixture and femoral arterial samples were obtained at three-second intervals during the first minute after injection.

In all procedures a syringe calibrated by weighing was used and the injection was given into a prominent antecubital vein. The duration of injection was two to four seconds in groups A, B, and C, and one to two seconds in group D. Group A was given undiluted 2 per cent Coomassie Blue. Groups B and C received injections from a dye-isotope mixture prepared as follows: approximately 20 microcuries of I^{131} H.S.A. were added to 2 ml. of the patient's plasma, 2.5 ml. of 2 or 4 per cent Coomassie Blue (50 or 100 mg.), and 18 ml. of isotonic saline. The volume injected was between 5 and 10 ml. The patient's plasma was used as a source of extra protein to prevent adsorption of the labelled albumin on to the glassware during injection and preparation of standard solutions (Reeve and Franks, 1956). The venous samples were taken without stasis from an antecubital vein in the opposite arm in groups A, B, and C.

Group D received 4 ml. of an injection solution prepared from 60 microcuries of I^{131} H.S.A., 2 ml. of the patient's plasma, 4 ml. of 2 per cent Coomassie Blue, and 14 ml. of isotonic saline. Samples were collected during three-second periods from a 5-cm. polythene tube connected by a three-way stopcock to a No. 17 thin-walled needle in a femoral artery.

The Coomassie Blue was extracted by the acetone method of Clausen and Lifson (1955). All standard solutions, blanks, and samples were read in a Unicam SP 600 spectrophotometer at 585 m μ ., or in a Unicam SP 300 G.P. colorimeter with an Ilford No. 626 filter.

The radioactivity of each injection solution was determined by preparing three 1:50 diluted standard solutions. One ml. of each standard, plasma blank, and plasma sample was counted in a well-type scintillation counter. For measurement of plasma volume the disappearance slope between twenty and forty minutes was extrapolated on semilogarithmic paper to the time of injection according to the method of Gibson and Evans (1937).

Free I^{131} in the I^{131} H.S.A. was determined by counting the supernatant fluid after twice precipitating protein with 20 per cent trichloroacetic acid. As it has been shown that most of the free I^{131} escapes from the intravascular compartment during the first ten minutes after injection (Berson *et al.*, 1952; Berson and Yalow, 1952), the plasma volume results reported in Table I have been corrected for this free I^{131} by multiplying the observed plasma volume by 0.95.

The hæmatocrit was determined after spinning a Wintrobe tube at 3000 r.p.m. for thirty minutes in a centrifuge with a radius of 15 c.m. to the far end of the tube.

RESULTS

Fig. 1 shows that the dilution curves of Coomassie Blue and I^{131} H.S.A. are very similar during the first transit through large vessels, lungs, and heart after simultaneous injection. The patient studied was a man aged 50 with Caplan's syndrome. The second patient, a woman aged 49 with symptomless mitral stenosis, had an almost identical dilution curve.

Fig. 2 shows typical dilution curves of Coomassie Blue and I^{131} H.S.A. obtained from venous samples after simultaneous injection.

FIG. 1.—Simultaneous injection of I^{131} H.S.A. and Coomassie Blue. Arterial sampling.

FIG. 2.—Simultaneous injection of I^{131} H.S.A. and Coomassie Blue. Venous sampling.

Fig. 3 shows the disappearance slopes after repeated and equal simultaneous injections of Coomassie Blue and I^{131} H.S.A. The first two disappearance slopes of Coomassie Blue are quite similar (as was found in two other patients), but the third Coomassie Blue disappearance slope is seen to be much less than the preceding two.

FIG. 3.—Patient 2. Repeated simultaneous injections of I^{131} H.S.A. and Coomassie Blue. Venous sampling.

The full details of every patient are given in Table I, and it can be seen that although the mixing time of Coomassie Blue and I^{131} H.S.A. are closely similar, the plasma volume results measured by Coomassie Blue are consistently higher than the volumes measured by I^{131} H.S.A.

DISCUSSION AND CONCLUSIONS

From the data presented it is seen that Coomassie Blue has the same distribution as I^{131} H.S.A., during its first transit through the large vessels, heart, and lungs. Since it has been shown that I^{131} H.S.A. remains intravascular during this period (Lilienfield *et al.*, 1956; Tuckman *et al.*, 1959), we conclude that this is also true for the dye. These findings are consistent with the work of Taylor and Shillingford (1959) who found that the cardiac output measured by both Coomassie Blue (indicator dilution method) and the direct Fick method were not significantly different. Moreover, as the dye can be repeatedly given during a short period without toxic reactions or skin discoloration, we conclude that this is an excellent dye for indicator dilution methods, based upon the initial periodic portion of the dye dilution curve.

Before an indicator can be considered for use in plasma volume determinations the characteristics of its dilution curve after the first lesser circulation must be known. From the results of mixing times reported above, with one exception, it can be seen that the plasma concentration of Coomassie Blue falls most rapidly between the second and tenth minutes after injection. The next phase, when the plasma concentration falls less rapidly, lasts until approximately sixty minutes after

injection. In eight of nine patients in whom samples were taken sixty minutes after injection the concentration of dye in the plasma was consistently greater than that predicted from the preceding disappearance slope.

The first phase of rapid fall in plasma Coomassie Blue concentration coincides with a similar phase in the dilution curve of simultaneously injected I^{131} H.S.A. Also when the dye is given alone this first phase falls within the time limits established for a similar phase in the dilution curve of T-1824 (Evans Blue) (Gregerson and Rawson, 1959), and for I^{131} H.S.A. injected alone (Benson, 1954; Tuckman and Finnerty, in preparation). The first phase for T-1824 and I^{131} H.S.A. is primarily due to intravascular mixing (Berson and Yalow, 1952; Tuckman *et al.*, 1959; Gregerson and Rawson, 1959). We interpret our results as indicating that this first phase of Coomassie Blue dilution is primarily, although not solely, due to intravascular mixing, but that there may also be additional and significant extravascular escape. The evidence for this early significant extravascular escape of Coomassie Blue is based on the observation that, if an exponential constructed from the twenty- to forty-minute samples is extrapolated to the time of injection, the plasma volume so calculated is found to be larger than the plasma volumes similarly determined from simultaneously injected I^{131} H.S.A. Also, similar estimates of plasma volume after injection of dye alone usually showed values much above the limits expected in subjects with the pathological conditions present (Berson, 1954; Gregerson and Rawson, 1959). Further evidence for this early extravascular dye escape, which cannot be corrected for by extrapolation, is the observation in eight of nine cases of unexpectedly high dye concentrations in plasma samples at sixty minutes after injection. This is the period when lymph that had taken up dye during the first phase of the dilution curve would be returning to the systemic circulation in sufficient quantity to change the previous disappearance slope (Krieger *et al.*, 1950; Wasserman and Mayerson, 1951; Schultz *et al.*, 1953). We feel therefore that the dye Coomassie Blue cannot be used in estimation of plasma volume by the usual method of Gibson and Evans (1937).

SUMMARY

The dye Coomassie Blue has been studied in seventeen patients, after injection alone, and together with radio-iodinated human serum albumin.

Evidence has been presented to indicate that Coomassie Blue remains intravascular during its first transit through the lesser circulation. There is, however, a substantial early extravascular escape of the dye and this invalidates its use for plasma volume measurement by the usual methods.

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