A CRITICAL COMPARISON OF THE T-1824 DYE AND IODINATED ALBUMIN METHODS FOR PLASMA VOLUME MEASUREMENT [1]

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The T-1824 dye method for plasma volume measurement has been subjected to much criticism. The validity of its use as a tagged protein dilution method has been questioned. One of the major criticisms has been that part of the injected dye is rapidly removed from the plasma before binding with protein occurs. This objection does not apply to the use of human serum albumin tagged with radioactive iodine before injection. Comparison of the two methods has given conflicting results (1–3). The present study was undertaken to clarify the relationship between the dye and the iodinated albumin methods for the measurement of the plasma volume.

METHODS

A modification of the T-1824 dye method described by Gibson and Evelyn (4) was used in the present study. Two cc. of an aqueous solution containing 10 mg. of T-1824 dye [2] was measured in a calibrated syringe and injected intravenously. Blood samples were then drawn at 10-minute intervals for 1 hour by interrupting a continuous, slow, intravenous infusion of isotonic saline. The blood samples were collected in 4 cc. hematocrit tubes containing 1.25 mg. of heparin. The plasma was separated by centrifugation for 30 minutes at 3500 r.p.m. The plasma containing dye was compared with a blank plasma sample drawn just prior to the dye injection. An Evelyn colorimeter with a 620 millimicra filter was used. The optical densities were plotted against time on semilogarithmic paper and extrapolated to zero time (C_0). The optical density of a 1 : 1000 dilution of T-1824 dye in plasma was determined (K). The K factor of the dye was determined individually in the plasma of each of 21 normal subjects. The mean value for the individual K factors was 0.351 (S.D. 0.009). This mean K was then used in the calculation of the plasma volume in 13 patients in whom an individual K had not been determined. An individual K factor was determined in 15 patients and utilized in the calculation of the plasma volume. The K factor determined with plasma containing 1 gm. per cent of polyvinylpyrrolidone (PVP) did not differ significantly from that using plasma alone. A microadapter (5) for the Evelyn macrocolorimeter was used in all colorimetric measurements so that a 1 cc. plasma sample could be utilized. The calculation of the plasma volume was based on the following equation (6):

$$\text{Plasma volume (cc.)} = \frac{K \times 1000 \times \text{cc. of dye injected}}{C_0}$$

The I^{131} tagged human serum albumin used in this study was supplied by Abbott Laboratories, Chicago, Ill. The solution contained 5 mg. of albumin tagged with 250 to 500 microcuries of I^{131} per cc. The ionic (free) radioactive iodide was less than 1 per cent. The iodinated albumin was diluted with isotonic saline to yield a concentration of approximately 5 microcuries per cc. Ten cc. of this dilution was measured in a calibrated syringe and injected intravenously immediately following the injection of the T-1824 dye. The injected dose contained approximately 50 microcuries of I^{131} tagged albumin. Blood samples were obtained from the patient for determination of radioactivity simultaneously with the blood samples for determination of dye concentration. The plasma was separated by centrifugation. Five cc. aliquots of each plasma sample were counted with a Geiger counter and the counts per minute plotted against time. By extrapolation the counts per minute at zero time were determined (E). One cc. of the iodinated albumin solution containing approximately 5 microcuries per cc. was diluted volumetrically to 100 cc. with human plasma. A 5 cc. aliquot of this dilution was counted with a Geiger counter. This represented the standard (S) for the injected iodinated albumin. The plasma volume was then calculated from the following equation:

$$\text{Plasma volume (cc.)} = 100 \times \text{cc. of tagged albumin injected} \times \frac{S}{E}$$

All radioactivity measurements were made with a Tracer Lab. TGC-1 end window Geiger-Müller tube mounted in a lead chamber. The liquid plasma samples were counted in a cylindrical plastic cup with an internal diameter of 3 cm. The surface of the plasma sample was 1.5 cm.

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[2] The T-1824 dye (lot 027021) used in this study was supplied by William R. Warner, Division of Warner-Hudnut, Inc., New York, N. Y.

from the end window of the Geiger tube. Five cc. plasma samples were found to be an infinitely thick layer in this counting cup, and radioactivity counts could be duplicated with an error of less than 2 per cent.

The experimental group consisted of 10 normal men, 7 patients with carcinoma and 5 patients with lymphoblastoma, 2 patients with duodenal ulcer, 2 patients with recent small pneumothorax, 1 patient with hypertension, and 1 patient convalescing from infectious hepatitis.

RESULTS

Comparison of the two methods

Plasma volumes were determined simultaneously with dye and iodinated albumin in 28 subjects (Table I). The mean plasma volume with iodinated albumin was 3255 cc. and with the dye method it was 3314 cc. The mean difference of 59 cc. was not significant ($t = 1.49$, $p > 0.1$). The correlation coefficient was 0.927 (Figure 1). An individual K factor for the dye was determined in 15 of these patients. The mean difference between the plasma volumes in this group as measured by the two methods was only 12 cc. A comparison was made of 28 plasma volumes calculated from the initial 10-minute plasma sample and the plasma volumes calculated from an extrapolation of 6 samples taken at 10-minute intervals for 1 hour (Table II). No significant difference between the volumes calculated from a single sample and multiple samples was found with either iodinated albumin or dye (iodinated albumin: $t = 1.0$, $p > 0.3$; dye: $t = 0.7$, $p > 0.5$). The standard deviation of the difference in the plasma volumes as calculated with the 10-minute sample and the multiple sample method was 91 cc. for both iodinated albumin and T-1824 dye.

Measurement of plasma volume change

In 10 patients the plasma volume was determined with both methods simultaneously, before and immediately after the rapid intravenous administration of 1000 cc. of 3.5 per cent polyvinylpyrrolidone (PVP), a synthetic plasma volume expander (Table III). The increase in plasma volume as measured by the two methods was not significantly different ($t = 0.4$, $p > 0.6$).

T-1824 dye and iodinated albumin disappearance rates

The decrease in plasma concentration of iodinated albumin and T-1824 dye during a 1-hour period after injection was compared (Table IV).

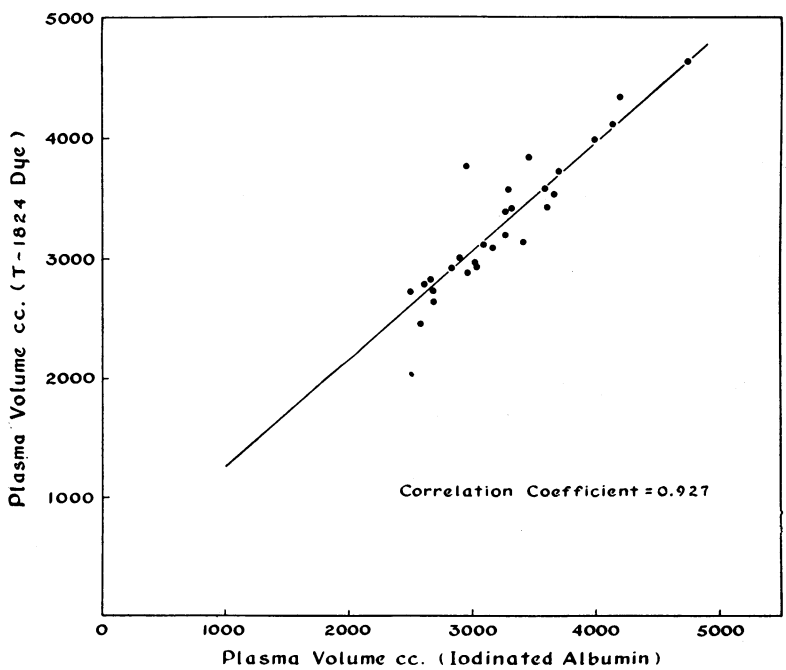

FIG. 1. CORRELATION BETWEEN PLASMA VOLUMES DETERMINED BY T-1824 DYE AND IODINATED ALBUMIN

TABLE I

Comparison of plasma volumes determined simultaneously with T-1824 dye and iodinated albumin

Patient	Plasma volume T-1824 dye	Plasma volume Iodinated albumin	Difference
	cc.	cc.	cc.
1	2937	2821	+116
2	3752	2940	+812
3	2647	2666	− 19
4	3114	3080	+ 34
5	3572	3287	+285
6	3423	3607	−184
7	3845	3450	+395
8	2724	2498	+226
9	3418	3312	+106
10	2971	3015	− 44
11	2935	3043	−108
12	4101	4137	− 36
13	4636	4747	−111
14*	3539	3651	−112
15*	3718	3701	+ 17
16*	3981	3982	− 1
17*	4343	4180	+163
18*	3395	3260	+135
19*	3144	3401	−257
20*	2836	2658	+178
21*	3091	3154	− 63
22*	3579	3580	− 1
23*	2895	2956	− 61
24*	3200	3260	− 60
25*	2791	2602	+189
26*	2455	2587	−132
27*	3007	2898	+109
28*	2743	2675	+ 68
Mean	3314	3255	+ 59

* Individual K factor determined.

TABLE II

Comparison of plasma volume calculated from 10-minute value and extrapolated value

Patient	T-1824 dye Extrapolated value	T-1824 dye 10-minute value	T-1824 dye Diff.	Iodinated albumin Extrapolated value	Iodinated albumin 10-minute value	Iodinated albumin Diff.
	cc.	cc.	cc.	cc.	cc.	cc.
1	2937	2894	− 43	2821	2747	− 74
2	3752	3627	−125	2940	3041	+101
3	2647	2741	+ 94	2666	2778	+112
4	3114	3154	+ 40	3080	3196	+116
5	3572	3481	− 91	3287	3542	+255
6	3423	3481	+ 58	3607	3698	+ 91
7	3845	3895	+ 50	3450	3607	+157
8	2724	2850	+126	2498	2538	+ 40
9	3418	3478	+ 60	3312	3259	− 53
10	2971	2891	− 80	3015	2928	− 87
11	2935	3029	+ 94	3043	3129	+ 86
12	4101	4186	+ 85	4137	4143	+ 6
13	4636	4745	+109	4747	4718	− 29
14	3539	3614	+ 75	3651	3592	− 59
15	3718	3687	− 31	3701	3655	− 46
16	3981	4067	+ 86	3982	4048	+ 66
17	4343	4356	+ 13	4180	4301	+121
18	3395	3171	−224	3260	3274	+ 14
19	3144	3196	+ 52	3401	3348	− 53
20	2836	2778	− 58	2658	2672	+ 14
21	3091	3057	− 34	3154	2983	−171
22	3579	3363	−216	3580	3473	−107
23	2895	2942	+ 47	2956	2892	− 64
24	3200	3263	+ 63	3260	3277	+ 17
25	2791	2850	+ 59	2602	2610	+ 8
26	2455	2502	+ 47	2587	2572	− 15
27	3007	3056	+ 49	2898	2940	+ 42
28	2743	2768	+ 25	2675	2676	+ 1
Mean	3314	3326	+ 13.5	3255	3273	+ 17.5

The mean per cent change in 1 hour was 11.5 for iodinated albumin and 11.0 for the dye. The difference between the means was not significant.

The urinary excretion of iodinated albumin was studied in 15 subjects. Over a period of two hours the urine contained 0.87 per cent of the injected radioactivity. In 4 patients 3.6 per cent of the injected radioactivity was excreted in the urine during an 8-hour period.

Thoracic duct studies

The cervical portion of the thoracic duct was cannulated in a 27 year old man undergoing a radical neck dissection for localized lymphoblastoma. The duct was not obstructed, and there was a free flow of lymph at the rate of approximately 1 cc. per minute. Samples of lymph and plasma were obtained at 10 to 20 minute intervals over a period of 140 minutes after the intravenous injection of 350 microcuries of I^{131} tagged albumin. The plasma and lymph concentrations of iodinated albumin, the ratios of these concentrations, and the per cent of the injected iodinated albumin remaining in the plasma at the various time intervals are shown in Table V. The radioactivity of cor-

TABLE III

Measurement of plasma volume with T-1824 dye and iodinated albumin before and after 1000 cc. polyvinylpyrrolidone (PVP)

Patient	T-1824 dye Before	T-1824 dye After	T-1824 dye Diff.	Iodinated albumin Before	Iodinated albumin After	Iodinated albumin Diff.
	cc.	cc.	cc.	cc.	cc.	cc.
6	3423	4224	801	3607	4371	764
7	3845	4403	558	3450	3872	422
8	2724	3421	697	2498	3480	982
9	3418	4067	649	3312	4085	773
10	2971	3695	724	3015	3737	722
11	2935	3686	751	3043	3809	766
12	4101	4853	752	4137	5068	931
13	4636	5432	796	4747	5604	857
15*	3718	4458	740	3701	4384	683
17*	4343	5067	724	4180	4722	542
Mean	3611	4330	719	3569	4313	744
S.D.	709	648	72	659	662	168

* Individual K factor determined.

TABLE IV

Per cent change in concentration of T-1824 dye and iodinated albumin in plasma in 1 hour

Patient	T-1824 dye	Iodinated albumin	Difference
	%	%	%
1	13.9	7.3	+ 6.6
2	2.6	20.0	−17.4
3	17.1	19.7	− 2.6
4	13.3	18.6	− 5.3
5	8.3	15.5	− 7.2
6	10.8	18.3	− 7.5
7	7.9	21.7	−13.8
8	24.1	19.5	+ 4.6
9	9.9	11.1	− 1.2
10	9.0	8.9	+ 0.1
11	12.3	12.7	− 0.4
12	13.2	11.7	+ 1.5
13	13.4	8.1	+ 5.3
14	11.3	6.1	+ 5.2
15	15.6	10.2	+ 5.4
16	12.9	8.5	+ 4.4
17	13.1	11.9	+ 1.2
18	5.4	13.4	− 8.0
19	12.4	8.4	+ 4.0
20	6.0	7.6	− 1.6
21	7.0	3.9	+ 3.1
22	5.8	5.5	+ 0.3
23	9.5	8.6	+ 0.9
24	11.4	7.7	+ 3.7
25	10.5	9.3	+ 1.2
26	13.6	8.6	+ 5.0
27	10.1	11.5	− 1.4
28	7.9	8.5	− 0.6
Mean	11.0	11.5	− 0.5

TABLE V

Studies on human thoracic duct lymph after the intravenous injection of iodinated human serum albumin

Minutes after injection	I^{131} dose per 100 cc. Plasma	Lymph	Plasma/ lymph ratio	Dose in plasma
	%	%		%
10	2.80	0.07	40.0	98.3
20	2.65	0.10	26.5	91.3
30	2.57	0.18	14.3	90.2
45	2.56	0.35	7.3	89.9
65	2.60	0.87	3.0	91.3
85	2.43	0.90	2.7	85.0
105	2.49	1.00	2.5	87.4
135	2.27	1.08	2.1	79.4

responding plasma and lymph samples is graphically illustrated in Figure 2. The plasma protein concentration was 8 gm. per 100 cc. with 5.3 gm. albumin; the lymph protein concentration was 5.7 gm. per 100 cc. with 4.2 gm. albumin. During the 6-hour period following the injection of the iodinated albumin, 4.1 per cent of the injected radioactivity was excreted in the urine. One hour after intravenous injection, approximately 10 per cent of the iodinated albumin had disappeared from the plasma. The plasma volume was 3512 cc. in this patient as measured by the iodinated albumin method.

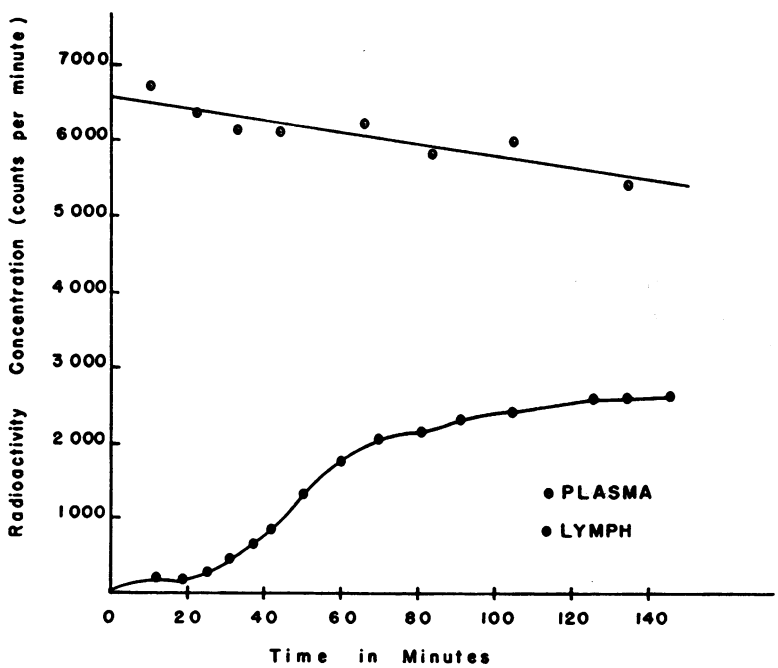

FIG. 2. RADIOACTIVITY CONCENTRATION IN 5 CC. SAMPLES OF PLASMA AND THORACIC DUCT LYMPH FOLLOWING INTRAVENOUS INJECTION OF I^{131} TAGGED SERUM ALBUMIN

DISCUSSION

Sterling (7), and Fine and Seligman (8) have established the reliability of the combination of I^{131} to albumin by *in vitro* and *in vivo* studies. Human serum albumin tagged with I^{131} would appear to fulfill all the desired requirements for plasma volume measurement based on the tagged protein dilution principle. Therefore, a careful comparison of the T-1824 dye and the iodinated albumin methods should be a logical and reliable experiment to study the validity of the dye method. Storaasli and his co-workers (1) and Aust and his co-workers (2) have reported that plasma volumes measured with T-1824 dye are greater than those determined simultaneously with iodinated albumin. These authors suggested that a certain amount of dye escapes from the vascular system during the first few minutes after injection. Crispell and his co-workers (3), however, found no significant difference in plasma volumes as measured by the two methods. In the present study, the T-1824 dye and the iodinated albumin methods of plasma volume measurement did not give significantly different results. This is to be expected since several investigators (9–11) have demonstrated that the intravenous injection of T-1824 dye is followed by a rapid combination with plasma albumin. It is very unlikely that any dye is lost before binding since the dye is bound to protein very rapidly. An error in the determination of the K factor in the dye method will introduce a systematic error in the calculation of the plasma volume. Such an error may account for the reported discrepancy between the two methods. From the results of the present study, the concept that there is a rapid loss of T-1824 dye from the circulation after injection and before binding to plasma protein appears untenable.

Studies on the rate of disappearance of iodinated albumin in the thoracic duct lymph of a human subject revealed that very little iodinated albumin appeared in the thoracic duct lymph during the first 30 minutes after intravenous injection. In experimental animals a somewhat larger amount of tagged protein appears in the thoracic duct lymph during the first hour after injection (12–17). From the present study it would seem that in the human the amount of tagged albumin appearing in thoracic duct lymph during the period of determination of the plasma volume is small and does not significantly influence the measurement of plasma volume.

Blood volume measurements with tagged erythrocytes have been reported to result in lower values when compared with blood volumes determined by tagged plasma protein methods (18–24). This discrepancy has been attributed to the fact that the total body hematocrit is lower than the venous hematocrit. Gibson and his associates (23) studied the hematocrit of the small blood vessels using I^{131} tagged albumin and erythrocytes tagged with Fe^{59}. The hematocrit of the blood in the small vessels was found to be much lower than the hematocrit of arterial blood in all organs except the spleen. These studies assume a rigid boundary to the vascular system. The tagged protein methods measure a rapidly exchangeable pool of albumin. The anatomic limits of this albumin pool may not be identical with the anatomic limits of distribution of the erythrocytes. In a strict sense the tagged protein methods should be used as a measure of this pool of albumin. Making the assumption that all the albumin in the pool is contained in the plasma volume and that the concentration of the albumin is constant throughout the plasma, one can calculate the plasma volume. Unfortunately these assumptions are not entirely correct. Determination of the plasma volume by methods employing tagged erythrocytes is based on the assumption that the ratio of plasma to erythrocytes is constant throughout the body. This assumption is also not entirely correct. Thus, the definition of plasma volume, like the definition of extracellular fluid, will always depend upon the method used in its measurement.

Plasma volume measurements made with T-1824 dye are as valid and reliable as those made with iodinated albumin. When carefully done the two methods give similar results. The criticisms leveled at the dye method can also be made of any tagged protein method for plasma volume measurement.

SUMMARY AND CONCLUSIONS

1. The T-1824 dye and the iodinated albumin methods for plasma volume measurement when performed simultaneously in human subjects gave values which did not differ significantly.

2. When the plasma volume was expanded by polyvinylpyrrolidone (PVP), the plasma volume change as measured by T-1824 dye and by iodinated albumin did not differ significantly.

3. Studies of human thoracic duct lymph with iodinated albumin revealed that very little tagged albumin appeared in the lymph during the first half hour after intravenous injection.

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