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Research Article

STUDIES OF PLASMA VOLUME USING HUMAN SERUM ALBUMIN TAGGED WITH RADIOACTIVE IODINE^{131}

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The purpose of this paper is to report a method of determining plasma volume in man, using human serum albumin tagged with radioactive iodine, I^{131}. The preparation of the tagged material and a check of its stability and physiological behavior will also be discussed. We will also give a preliminary report of our results using this method for the determination of plasma volume in man as compared with the plasma dye method using Evans Blue Dye, T-1824.

The determination of plasma volume, using the Evans Blue Dye, T-1824 (1, 2), has become an accepted laboratory procedure in clinical investigation. In the course of an investigation of changes in blood volume it became apparent that repeated determinations in the same individual over a short period of time were impossible using this method. A method combining a radioactive isotope with a non-toxic vehicle seemed worthy of trial. This vehicle must of necessity be macromolecular, to prevent rapid diffusion from the vascular space, freely miscible with plasma, and of a chemical nature to combine with a radioactive isotope. The radioactive isotope must also be non-toxic and possess a relatively short half-life.

Lerman (3) has shown that serum albumin is a suitable agent for iodination because it contains approximately 4.5 per cent tyrosine. Fine and Seligman (4) have reported on the iodination of bovine albumin, and its use in determining plasma volume in dogs. In this study in man salt-poor human serum albumin (Cohn's Fraction V) has been chosen as the tracer vehicle, and radioactive iodine, I^{131}, with a half-life of eight days as the isotope.

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METHODS AND MATERIALS

Preparation of human serum albumin labelled with I^{131}

The isotope of iodine I^{131} can be obtained from the Isotopes Division of the Atomic Energy Commission as carrier-free I^{131} dissolved in $NaHSO_3$. The iodine when received is present mainly as iodide. In order to insure chemical combination with the tyrosine of the albumin, it is necessary to make the iodine available in a free state. A small amount of potassium iodide (5 mg.) is added to 1 ml. of the original I^{131} solution; this mixture is acidified with 1 ml. of 0.08 N sulphuric acid, and 1 ml. of 4.0 per cent hydrogen peroxide is added. This is allowed to stand one hour to insure a maximum liberation of the iodine from the iodide. Twenty ml. of 25 per cent human serum albumin is added to the above mixture together with 5 ml. of 7 per cent sodium bicarbonate solution. This final mixture is allowed to stand 12 hours at room temperature with occasional agitation. The iodo-albumin mixture is then dialyzed against ice-cold, running water which is freshly distilled. The dialysis is continued for approximately 48 hours. This procedure removes any inorganic iodide which may be present. The complete removal of inorganic iodide is assured by frequent sampling of the dialysate for the presence of radioactivity. Five ml. samples of the dialysate are analyzed and the dialysis is considered complete when duplicate samples give only a background count. The proportion of the original activity remaining with the albumin is approximately 25 per cent. Aseptic precautions are observed throughout the entire procedure. Samples of the dialyzed iodo-albumin are taken for bacteriological culture.

Determination of stability and physiological behavior

A check was made for the presence of free iodine or inorganic iodide in dialyzed iodo-albumin after it had been standing at room temperature for one week. On careful inspection no precipitate was visible. An aliquot was redialyzed in running water, and samples of the dialysate were taken to determine the presence of radioactivity. Carbon tetrachloride extractions of the same iodo-albumin mixture were also made.

Sedimentation constants were determined with the ultracentrifuge by direct observation of the serum albumin before and after iodination. The technique described by

Rawson (5) was used. Preliminary observations of the electrophoretic pattern of the serum albumin before and after iodination were obtained.

After injection of the iodo-albumin the surface radiation over the area of the thyroid, liver, and lower end of the femur was followed by means of an externally placed Geiger-Müller tube attached to a scaling circuit. Standard aluminum filters were used to screen out beta rays. The urinary excretion of radioactive iodine was followed over a 24 hour period. The radioactivity of the separated red cells was also determined.

Determination of plasma volume

Calibrated syringes are used to measure the iodo-albumin for injection. A known volume with a radioactivity per milliliter is injected intravenously. An amount of iodo-albumin equivalent to 12 microcuries is usually injected. This amount is well below the usual 100 microcurie tracer dose of I^{131}. The blood to be used for sampling is withdrawn in heparinized syringes from a vein other than the one used for injection. To obtain a disappearance curve, frequent samples are obtained beginning five minutes after injection and extending over a 24 hour period. The blood samples are centrifuged for one-half hour at 3000 rpm to separate the serum from the cells. One ml. of the serum is pipetted into standard sample pans. The pans are quarter ounce ointment tins, 3.3 cm. in diameter and 0.8 cm. in depth. A filter paper of the same diameter is cemented to the bottom of the pan with rubber cement. The samples are dried for counting in a closed oven at a constant temperature of 70 degrees. Duplicate sampling is used throughout to check for geometric and volumetric errors. An empirically determined correction is made for self-absorption of the I^{131} in the sample. This correction amounts to approximately 12 per cent.

The plasma volume is calculated by dividing the counts per milliliter of the injected material by the counts per milliliter of subsequently extracted plasma; this quotient, multiplied by the volume injected, yields the plasma volume.

$$\text{Plasma Volume} = \frac{\text{Counts per ml. iodo-albumin injected}}{\text{Counts per ml. of plasma extracted}} \times \text{Volume of iodo-albumin injected}$$

The familiar "background" technique is used in carrying out repeated determinations on the same patient. A blood sample is always drawn before injecting the next dose of iodo-albumin. The counts per second per milliliter of this sample are always subtracted from the counts per second per milliliter of the 10 minute sample.

The plasma volume is determined simultaneously using Evans Blue Dye, T-1824. Blood samples taken 10 minutes after injection of the tracer are used for comparison.

RESULTS

The tests described for the presence of inorganic iodide and free iodine in the iodo-albumin were entirely negative. Bacteriological cultures of eight separate lots of the dialyzed iodo-albumin showed no growth in 48 hours. The separated red cells showed no detectable radioactivity.

The sedimentation constant of the albumin before and after iodination are shown in Table I. Preliminary observations of the electrophoretic pattern of the albumin after iodination showed no marked alteration. These studies are being continued.

TABLE I

Sedimentation Constants

Sample	S_{20}
Serum albumin with salt	3.95×10^{-13}
Serum albumin without salt	3.80×10^{-13}
Iodinated albumin dialyzed	4.38×10^{-13}

The surface radiation over the area of the thyroid, liver, and lower end of femur was followed over a 24 hour period. These areas were chosen as sites to determine differential uptake for the following reasons: *1*) inorganic iodide is rapidly taken up by the thyroid, *2*) organic iodides are metabolized in the liver, and *3*) the femur is an area not directly concerned with iodine metabolism. Cognizance is made of the fact that surface radiation will not detect small amounts of radiation within an organ. However, it offers a means of determining any marked differential uptake. As shown in Table II there was no detectable differential uptake for at least two hours.

TABLE II

Surface radiation after injection of iodo-albumin containing 12 microcuries of I^{131}

Hours after injection	Thyroid area	Liver area	Femur—lower 1/3 RD
	mr.	*mr.*	*mr.*
1/2	.04	.05	.015
1	.04	.05	.015
2	.03	.06	.03
3	.05	.10	.03
6	.12	.15	.08
24	.03	.01	.00

Studies of the disappearance rate of iodo-albumin from the plasma show a definite plateau. This plateau lasting from 10–25 minutes compares favorably with that of the Evans Blue Dye, T-1824. These curves are shown in Figure 1.

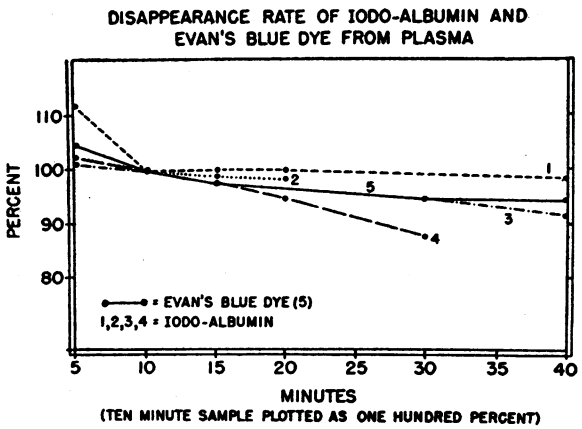

FIG. 1.

The duration of time that iodo-albumin remains in the body cannot be definitely determined. This is due to the fact that organic iodides are metabolized in the liver and the iodine released is reutilized by the thyroid for the manufacture of thyroid hormone. Therefore very little iodine is excreted by a person in the euthyroid state. Figure 2 illustrates the percentage of administered radioactivity present in the urine 24 hours after the injection of radioactive iodine as an inorganic iodide (A) and as iodo-albumin (B, C, D.) The amount of radioactivity in the urine 72 hours after injecting 12 microcuries of radioactive iodine as iodo-albumin was too small to be detected. A common dosage level employed in human studies is 100 microcuries of I^{131}. It is felt that significant risk of injury to the patient can be avoided by using a 12 microcurie dose.

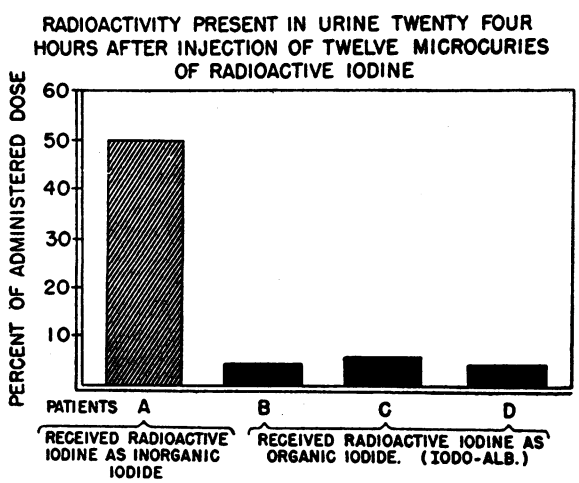

FIG. 2.

As a pilot experiment, the plasma volume of dogs was determined using iodo-albumin and Evans Blue Dye, T-1824, simultaneously. The results are given in Table III.

TABLE III

Plasma volume of dogs using iodo-albumin

Dog No.	Volume injected	Total counts per second injected	Counts per second per ml. of plasma	Iodo-albumin	Evans Blue Dye
	ml.			*ml.*	*ml.*
1	2	6,290	6.22	1,010	925
2	5	12,700	13.70	927	*
3	6	13,800	13.50	1,020	940
4	8	11,500	13.80	832	830

* Blood sample was hemolyzed.

Determinations of plasma volume have been made in man using iodo-albumin and Evans Blue Dye, T-1824, simultaneously. The results of single determinations are shown in Table IV. Repeated determinations have been carried out in the same patient and compared with the Evans Blue Dye, T-1824, method. The results are shown in Table V.

The values obtained by the two methods were subjected to statistical analysis. The significant

TABLE IV

A comparison of plasma volume in man using iodo-albumin and Evans Blue Dye simultaneously

Subject	Plasma volume	
	Iodo-albumin	Evans Blue Dye
	ml.	*ml.*
1	2313	2268
2	3128	3465
3	3587	3430
4 (*1*)	3260	3290
4 (*2*)	3292	3030
5 (*1*)	3370	3470
5 (*2*)	3440	3000
6	2664	3040
7	1687	2050
8	3228	3100
9	3860	3800
10	2900	2750
11 (*1*)	2729	2800
11 (*2*)	2690	2750
11 (*3*)	2716	2890
12	3277	3300
13	2321	2120
14 (child)	792	750
15	2232	2220
16	3194	3050
17	2830	2720
18	3750	3594
19	3214	3120
20	2377	2220

TABLE V

Repeated determinations of plasma volume using iodo-albumin

Patient	Time	Plasma volume	
		Iodo-albumin	Evans Blue Dye
		ml.	*ml.*
I	original	3260	3290
	24 hrs. later	3292	3030
II	original	3370	3470
	24 hrs. later	3440	3000
III	original	2729	2800
	1 hr. later	2690	2750
	24 hrs. later	2716	2890

figure (t) was found to be 0.11. This is not a statistically significant figure for 24 cases.

Early studies were carried out in patients having advanced malignancy. Only one minor reaction occurred, so studies were extended to normals and routine hospital admissions. The reaction was febrile in nature and was thought to be due to contamination with *B. subtilis* after repeated sampling. Patients receiving repeated injections have been followed for at least a month and no anaphylactic reactions have been observed. The usual precaution of discarding cloudy albumin solutions should be followed.

SUMMARY

Human serum albumin (Cohn's Fraction V) has been iodinated with radioactive iodine, I^{131}.

In vitro studies of the iodo-albumin indicate that it is chemically stable. The physical properties of the albumin have not been materially altered by iodination.

In vivo studies of the disappearance rate of iodo-albumin from the plasma indicate a definite plateau. This plateau is present after sufficient time has been allowed for complete mixing of the tracer vehicle with the plasma.

The pattern of surface radiation obtained indicates that there is no gross differential uptake by the thyroid or liver of the injected iodo-albumin. This aids in establishing the fact that the dilution of the tracer vehicle is by the plasma. Any other dilution mechanism would invalidate the method.

It is pointed out that the duration of time that the iodo-albumin remains in the body cannot be definitely determined. However, repeated determinations may be made without exceeding an accepted safe level of radiation.

Plasma volume determinations made simultaneously using iodo-albumin and Evans Blue Dye, T-1824, give comparable results. Repeated serial determinations in the same patients give values which check within a range of plus or minus 2 per cent.

The determination of red cell volume using red cells tagged with radioactive phosphorus has been reported by this group (6). The combination of this method of determining red cell mass with iodo-albumin method of determining plasma volume allows one to make a direct measurement of total blood volume (7).

CONCLUSIONS

1. Human serum albumin (Cohn's Fraction V) has been iodinated with radioactive iodine, I^{131}.
2. Plasma volume in man has been determined using this substance.
3. This method of determining plasma volume offers the advantage of repeated serial determinations.

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