Measurement of the Early Disappearance of Iodinated (I$^{131}$) Serum Albumin from Circulating Blood by a Continuous Recording Method

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A method for continuous recording of flowing arterial blood in humans has demonstrated that injected iodinated (I$^{131}$) human serum albumin disappears at variable rates from the circulation when losses are calculated from a two hour extrapolation to zero time. Losses from the circulation during the ten minute interval following injection were minimal. The recorded disappearance curve inscribed during the two hour period subsequent to mixing apparently declines as a single exponential function. In the absence of disturbed circulatory states, blood volume calculated from a single ten minute sample agreed with values obtained by extrapolation within the limits of error of counting methods.

RECENT reports$^{1-2-3-4-5}$ have indicated that although human serum albumin iodinated with I$^{131}$ is lost slowly at variable rates from the circulation, it appears to be a satisfactory substance for use in blood volume determinations. By the use of a method capable of frequent and accurate recording of the concentration in the circulating blood of labeled albumin without withdrawal of blood specimens, we sought to determine (1) the character of the disappearance curve of injected iodinated albumin for the two hour period after injection, (2) the possible existence of varying rates of dilution during this period which might be indicative of delayed mixing in different albumin “pools” ordinarily undetected by the usual method of serial sampling, and (3) the magnitude of the loss of albumin from the intravascular compartment following “mixing”. By slightly modifying the procedure that we have previously used to determine cardiac output$, a method was available which would appear to offer a solution to this problem. This is a report of these results during the immediate two hour period of this study and emphasizes (1) the apparent single exponential character of the disappearance slope of tagged albumin, (2) the variable losses of tagged albumin from the circulation and (3) the failure to demonstrate more than one basic dilution mechanism in normals. By continuous sampling, more definite mixing times, and effects of this variable on blood volume determinations were sought. We have designated “complete mixing” as that point at which there is no measurable departure from the normal disappearance slope.

PROCEDURE

In an accompanying paper*, MacIntyre and Leonards describe a continuous recirculation procedure which has been used in animals to study the disappearance from the circulation of several different isotopes. The technique employed in this study was somewhat similar to that described in previous reports$. A small external arterio-venous fistula was established by connecting polyvinyl tubing from a Courand $18$ needle inserted in the femoral artery to a $18 x 13$” needle inserted into the ipsilateral antecubital vein. Care was taken that the arterial needle was securely placed and that

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Fig. 1. Portable, shielded counter head in place with flow established.

free, pulsatile flow existed. The polyvinyl tubing connecting the needle was approximately 18" in length and this was led through a thin steel tube held in close approximation to the face of the crystal to insure constant geometry in counting. A volume of .2 ml. was presented to the crystal face. The counting head was well shielded with lead and mounted on a sturdy mobile stand which permitted it to be placed near the femoral artery (fig. 1).

After both needles had been inserted, the tubing was connected to the arterial needle with a small metal adapter and the venous end inserted after blood had begun to flow freely through the tubing. Continuous flow during the two hour period through the tube was checked by inserting a small air bubble, 0.1 ml. or less, into the tubing with a #27 needle and tuberculin syringes. The rate of flow through the tubing when disconnected at the venous end was under 40 ml./min.

A four ml. dilution of iodinated human serum albumin (Abbott)* containing approximately 100 mc of I\textsuperscript{131} was injected rapidly into the antecubital vein on the side opposite the fistula. Fifty to seventy-five mcgar. of heparin were given intravenously at the start of the procedure to inhibit clotting in the needles and tubing. The anticoagulant was neutralized with protamine at the completion of the study if necessary. Venous blood samples were drawn at variable intervals, counted as "whole blood" in a scintillation well counter, and compared with the continuously recording arterial sample. Following the mixing period, no difference in concentration between arterial and peripheral venous blood was demonstrated.

The subjects were chosen from hospitalized patients who were edema free and were suffering from conditions other than heart, liver or renal disease. The two alcoholics showed no evidence of liver disease. Prior to, and for 48 hours thereafter, each patient was given .64 ml. of Lugol's solution or potassium iodide three times daily to block thyroid uptake of dissociated I\textsuperscript{131}.

Counting Method: Continuous measurement of the dilution of the injected material by the intravascular blood volume was accomplished by recording the concentration of radioactivity flowing past the detector head as in the previously described method for recording cardiac output. The primary interest was not in the change in dilution immediately following injection but rather in changes in dilution occurring more slowly over a longer period after injection. Therefore a large number of counts was collected and recorded while still maintaining recording speed sufficiently fast to insure the identification of rapid changes if such should occur. A standard deviation of ±3 per cent or less on each point was present and as the normal counting rate was in the range of 3,000 to 6,000 counts per minute, a point was recorded at approximately every 20 seconds. For example, in the center range of a typical curve (fig. 2), each point plotted from the original graphic meter tape represents an average of four points totaling 12,000 collected counts replotted semilogarithmically from the graphic meter tape. Counts per minute were converted into mc/ml. counting of whole blood drawn for the determination of blood volume.

RESULTS

The procedure was not uncomfortable for the patient and there were no demonstrable changes in circulatory dynamics incident to the creation of the small arterio-venous fistula. Blood pressure, pulse rate and electrocardiograms showed no change during the time the small external A-V fistula was in operation.

A. Mixing Time: The time taken for "com-
plete mixing" of an injected tracer substance has been in the past calculated from the immediate "mixing slope" and the subsequent "disappearance slope" (fig. 3). The interception of these two slopes on a time axis affords a reasonable estimation as the best guess of "complete mixing" if gross mechanical sources of error can be excluded. The average time of "complete mixing" in these ten patients was 9 minutes with a range of from 4 minutes to 15 minutes. Mixing times were discrete and definite in eight of the patients studied. In two, mixing times were indefinite. For example, in fig. 4, the mixing slope merges gradually with the disappearance slope and the exact point of "complete mixing" is difficult to estimate. In calculating the blood volume in this patient, if the extrapolation were made only through the points from 10 to 30 minutes after injection, a value 6.5 per cent too low as compared with the two hour extrapolated value is obtained. When the mixing time is discrete and rapid as shown in patient number 10 (fig. 2), the blood volume determined by the ten minute sample, the extrapolated line of ten to 30 minute samples, and the two-hour sampling slope will be approximately the same.

B. Disappearance Rates: Figs. 2 and 4 show data on patients number 9 and number 10 in which the recorded counts are replotted against time in minutes through the two-hour period following injection of the tagged albumin. These are representative of the types of curves recorded in all ten patients studied. It was found that following the initial mixing period and within the errors of this method, the slope of disappearance of the tracer substance from the vascular compartment was exponential during this period in all cases irrespective of the inclination of the curve. The curves, following the mixing period, were extrapolated from two hours back to zero time to obtain each value from which the percentage loss could be calculated. Table 1 shows data from all patients. The loss of tagged albumin from the circulation varied greatly by the end of two hours averaging 15.5 per cent with a range from 5 per cent to 23 per cent. In spite of this variable and occasionally large loss by the end of two hours, the average percentile loss at 10 minutes after injection is less than 2 per cent with a range from 1 per cent to 3 per cent in all subjects. This is consistent with the findings of Schultz, et al. 4

The mechanism of the difference of rate of loss of the injected tagged albumin among the patients over a two-hour period has not been studied in detail. In two patients (number 1 and number 12) with total two hour blood losses of 14 per cent and 0.6 per cent, urinary loss of the tracer during this time did not exceed 2.0 per cent. If these are representative of the group, it is apparent that metabolism of the albumin accounts for only a minor portion of the loss of the injected material during the period of study. From acute experiments in dogs, Krieger, et al. 9 and Wasserman and Mayerson 10 found that a negligible amount of tagged albumin appeared in the thoracic
**Table 1.**—Loss of Tagged Iodinated (¹²⁵I) Serum Albumin from the Intravascular Space over a Two-Hour Period

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Dose (µc)</th>
<th>Mixing Time (min.)</th>
<th>Percent Disappearance (100% at 0 time)</th>
<th>Blood Volume</th>
<th>Per cent Deviation (%)</th>
<th>Weight</th>
<th>Arterial Hb (%)</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>102</td>
<td>5</td>
<td>10²  14.2  7.7  23.0</td>
<td>5410</td>
<td>5460</td>
<td>-0.9</td>
<td>76.5</td>
<td>Cardiac Output 5.6 L/M. Urine showed 1.4% of injected radio activity at 2 hrs. Thyroid uptake 2% in 24 hrs.</td>
</tr>
<tr>
<td></td>
<td>Paget’s disease of bone</td>
<td>101</td>
<td>10</td>
<td>1.0  1.72.3  3.5  5.1</td>
<td>4750</td>
<td>4750</td>
<td>0.0</td>
<td>88</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>CNS leu (paresis)</td>
<td>96</td>
<td>8-8</td>
<td>1.0  1.72.3  3.5  5.1</td>
<td>4750</td>
<td>4750</td>
<td>0.0</td>
<td>88</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Chronic alcoholism</td>
<td>121</td>
<td>8</td>
<td>1.3  2.53.2  4.5  6.3</td>
<td>5750</td>
<td>5740</td>
<td>0.2</td>
<td>91</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Lymphosarcoma</td>
<td>100</td>
<td>10-15</td>
<td>1.3  2.53.2  5.5  9.6</td>
<td>5000</td>
<td>5100</td>
<td>-3.3</td>
<td>66</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>CA lung with metastasis to brain</td>
<td>101</td>
<td>8-10</td>
<td>1.4  2.84.0  7.7  14.2</td>
<td>3760</td>
<td>3760</td>
<td>0.0</td>
<td>58</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Lymphosarcoma</td>
<td>104</td>
<td>10-12</td>
<td>1.4  2.84.0  7.7  14.2</td>
<td>5630</td>
<td>5700</td>
<td>-0.9</td>
<td>43</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Pneumonia, LLL</td>
<td>100</td>
<td>6-8</td>
<td>1.9  3.34.7  8.5  15.2</td>
<td>5535</td>
<td>5460</td>
<td>+2.3</td>
<td>61</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Chronic alcoholism</td>
<td>120</td>
<td>13-16</td>
<td>2.0  3.55.5  10.4  19.4</td>
<td>3970</td>
<td>4100</td>
<td>-3.2</td>
<td>60</td>
<td>36</td>
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<tr>
<td></td>
<td>Convalescent meningitis</td>
<td>110</td>
<td>3-5</td>
<td>1.9  3.55.1  9.3  17.2</td>
<td>5300</td>
<td>5240</td>
<td>+1.1</td>
<td>68</td>
<td>39</td>
</tr>
<tr>
<td>Average values</td>
<td></td>
<td></td>
<td>8.8</td>
<td>1.63 4.3  7.6  13.5</td>
<td>58</td>
<td>±1.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

lymph in the first ten minutes following injection but, at the end of two hours, widely variable amounts had traversed the thoracic duct in all animals. The rate of change of the concentration of tagged albumin in the plasma will therefore be influenced by the way equilibrium is reached with all extravascular albumin spaces, of which the lymphatic system is one.

**Discussion**

In all methods currently in use, the assumption has been made that following a variable mixing interval, either a single sample drawn at 10 minutes or several samples drawn over approximately 30 minutes and extrapolated to zero time may give a reasonable estimate of the volume of the circulating blood in patients without diseases causing disturbances of the cardio-vascular system. Gregersen concluded\(^1\) that a single venous sample drawn ten minutes following injection of T 1824 dye is entirely satisfactory for clinical determination of blood volume. Our data on comparison of ten minutes to extrapolated values, show an average agreement of ±1.3 per cent with a span from -3.3 per cent to +2.3 per cent. It is evident from table 1, that at 10 minutes, loss from the vascular bed was minimal and mixing was usually complete. In our own data of cardiac output determinations on dogs and man, the ten minute single sample gave sufficient accuracy for the determination of blood volume from which the cardiac output could be calculated; extrapolation of the disappearance curve is unnecessary in most instances. Wherever mixing time is slow and the disappearance is rapid,
falsely low blood volume determinations may result from extrapolation of the early disappearance slope. The single ten minute sample blood volume, obviously, will also be in error in those patients whose mixing is incomplete at this time, but to a lesser degree.

It would seem reasonable, therefore, that whenever slow mixing time is anticipated, enough samples should be drawn over a sufficient time interval to allow the injected substance to reach a steady state of decline, particularly, if the slope of disappearance is fairly steep, indicating more rapid losses.

The counting of whole blood without necessity of plasma separation enables one to eliminate errors in "trapped" plasma of the hematocrit but the value obtained is obviously a calculation for arterial blood and does not obviate the possible source of error inherent in large vessel hematocrit as contrasted to "whole body hematocrit." For most clinical studies, however, the simplicity of "whole blood" counting has some technical and theoretical advantages over blood volume calculations based on plasma counts and venous hematocrits.

**SUMMARY AND CONCLUSIONS**

The disappearance slope of iodinated (I\(^{131}\)) human serum albumin has been followed in man over a two-hour period by continuous in vivo counting of gamma rays and has been found exponential in the ten cases studied.

Despite a variable loss of tagged albumin from the intravascular space at the end of the two-hour period, the average loss at ten minutes ranged from 1.0 per cent to 3.0 per cent.

In 10 subjects the total blood volume calculated from the single 10 minute blood sample agreed within a range from −3.3 per cent to +2.3 per cent with the value obtained from the two-hour extrapolation of continuously recorded points. The data support the thesis that a single ten minute sample in subjects with normal circulation usually gives a high degree of accuracy from which to calculate blood volume.

The sources of error in the calculation of blood volume by the extrapolation to zero time of samples drawn in the usual 10 to 30 minute period after injection are discussed.

**BIBLIOGRAPHY**

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