^{99m}Tc-Labelled serum albumin in cardiac output and blood volume studies

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The use of human serum albumin labelled with iodine-131 (RIHSA) as the radioactive indicator in the measurement of cardiac output by the external counting technique is not ideal since the emission characteristics, physical half-life, and physiological fate restrict the amount that can be administered. A more suitable material, especially because of the short physical half-life of the isotope involved, is albumin labelled with technetium-99m.

This communication describes a simple three-stage process for the preparation of such a complex which was then tested in subjects with no impairment of cardiac performance for its suitability in assays of cardiac output by the external counting method. Values were within the same range as those obtained with RIHSA in comparable subjects. Blood volumes estimated with ^{99m}Tc-albumin were within the limits of physiological variation of values derived with RIHSA in the same subjects.

Serial blood samplings and urine collection during a period of 24 hours after administration showed that the preparation was lost continuously from the circulation at a more rapid rate than RIHSA. The greatest loss was in the first few hours when most of the urinary excretion of the isotope occurred. Comparison of the present data with published results using an alternative preparation suggests that our product may be more stable.

Of the various methods available for the measurement of cardiac output, the external counting technique using a gamma-emitting isotope (Huff, Feller, Judd, and Bogardus, 1955; Veall and Vetter, 1958) is probably the least traumatic as far as the patient is concerned. Disturbance is minimal and, in particular, there is no requirement for catheterization of the heart or great vessels as is the case with the Fick and dye dilution methods. In general, ¹³¹I-labelled human serum albumin (RIHSA) is used as the indicator but this material is not ideal since the emission characteristics restrict the amount that can be administered and the physical half-life and physiological fate leave a high circulating residuum in serial assays.

Technetium-99m is potentially a better isotope as the emission characteristics permit more activity to be given and the short physical half-life contributes to a lower residuum. However, a prerequisite of the external counting method is the need for the indicator to remain intact in the circulation for at least 10 to 15 minutes after administration so that samples may be withdrawn for blood volume assay. To meet this condition the isotope must be combined to a relatively stable circulating entity such as albumin.

Previous methods for the preparation of ^{99m}Tc-albumin are generally somewhat involved (Stern, Zolle, and McAfee, 1965; Harper, Lathrop, and Gottschalk, 1966). This paper describes a simple method for the preparation of the conjugate, of sufficient stability for use in cardiac output and blood volume assays, and also reports investigations into its physiological fate.

METHOD OF PREPARATION OF ^{99m}Tc-ALBUMIN

The labelling process consisted of three steps. Firstly sodium pertechnetate (the heptavalent state of the isotope) was eluted with 0.15 N sodium chloride from a ⁹⁹Mo generator column.¹ The anions were converted to pertechnetate acid, with simultaneous removal of sodium cations and of any alumina fines from the generator column, by passage through a 5 g Dowex-50 ion exchange column (10 cm long × 10 mm diameter) in the H⁺-ion form. Evaporation of the acid solution to dryness in vacuo converted the isotope into the pentavalent state (Williams and Deegan, 1971), which has been shown to be the necessary condition for combination with protein (Harper, Lathrop, McCordle and Andros, 1964).

¹Supplied by the Radiochemical Centre, Amersham

In the second step, a solution of 3% human serum albumin¹ containing 0.2% ascorbic acid was added to the deposit of pentavalent ^{99m}Tc.

Immediately afterwards the pH of the solution was adjusted to 2.5 by addition of N HCl and maintained at this value for 20 to 30 minutes. Sodium bicarbonate (1 M) was then added to readjust the pH to 5.0–6.0, a range suitable for intravenous administration. Unbound isotope was removed by passage through a 5 g column of Amberlite IRA-400 anion exchange resin (10 cm long × 10 mm diameter) in the Cl⁻-ion form and sterilization was effected by membrane filtration (Millipore Swinnex-13 filter, pore size 0.45 μ). The yield of bound material was 90 ± 3% (mean of 20 preparations).

PHYSIOLOGICAL INVESTIGATIONS

Investigations covered two aspects:

(1) the application of the material to the measurement of blood volume and cardiac output; and

(2) the breakdown and excretion rates of the material during the 24-hour period after administration.

In addition, direct comparison of the behaviour of ^{99m}Tc-albumin and RIHSA was obtained by similar studies in the same subjects using 5 μCi amounts of the iodinated protein.

TECHNICAL DETAILS Four normal subjects and six ambulant patients convalescing after a myocardial infarction participated in the tests. None of the patients had any impairment of cardiac performance as a result of the acute episode. All were rested in the supine position for at least 15 minutes before the start of an investigation.

The detector head used for the measurement of cardiac output comprised a thallium-activated sodium iodide crystal (2 in long × 2 in diameter (50 × 50 mm)) integrated with a photomultiplier and shielded with lead (1 in (25 mm) thickness). The area monitored was defined with a simple cylindrical lead collimator (2 in long × 2 in diameter (50 × 50 mm)).

The detector was positioned over the third left intercostal space, 1 in (25 mm) from the mid-line, and the output was fed simultaneously into a linear pen recorder and a pulse tape-recorder (Herbert and Hyde, 1963). The latter device allowed for retrospective appraisal of the dilution curves in serial assays.

^{99m}Tc-albumin, in 1 ml volumes containing 50 to 150 μCi of isotope², was injected into the antecubital vein and the response curve was followed for 1 to 2 minutes; 10 to 15 minutes later a further recording was made and at the same time a blood sample was withdrawn from an alternative site to that used for the injection.

From all the normal subjects and three of the patients further samples were withdrawn at intervals up to 24 hours to follow the fall in activity in the circulation. Urine was collected as convenient during that period. Aliquots of blood were taken for haematocrit estimation and for plasma separation.

ASSAY OF ACTIVITIES The activities of 1 ml plasma or urine samples were determined in a well-type scintillation counter (type 6006) linked to a series 7000 system, consisting of a scaler and an amplifier/analyser³. All counts for ^{99m}Tc were made with a channel of 0.1–0.2 MeV, resulting in a counting efficiency of 74%. At least 5,000 pulses were recorded for each sample.

CALCULATION OF RESULTS Cardiac output in blood volumes/minute was calculated on the basis of the Stewart-Hamilton formula, as described by Veall and Vetter (1958). The initial descending portion of each dilution curve was re-plotted on semilogarithmic paper, yielding in each case the straight line characteristic of an exponential decay. The line was extrapolated beyond the time at which recirculation began to give the values theoretically applicable to the original dilution curve and these were then inserted in the linear representation (see Fig. 1).

Plasma volumes were calculated by simple dilution principles and blood volumes were derived therefrom by application of the haematocrit, suitably corrected for trapped plasma and the difference between the peripheral and central circulations (Veall and Vetter, 1958).

RESULTS

CARDIAC OUTPUT AND BLOOD VOLUME ASSAYS ^{99m}Tc-albumin dilution curves were obtained from 7 of the 10 subjects; a typical example is shown in Figure 1. Both the original curve and the extension of the descending portion following semi-logarithmic replotting are depicted.

The individual values of cardiac output, in blood volumes/minute, were within the range 0.91–1.47, with a mean value of 1.12 ± 0.19 (Table I). The latter value did not differ significantly from 1.18 ± 0.19, the mean of outputs in 37 comparable subjects, using RIHSA as the indicator (Clarke, 1968).

Blood volumes with ^{99m}Tc-albumin, obtained for three normal subjects and three patients, were compared with volumes assayed later with RIHSA (Table II). The results showed a tendency for the values by the first method to be higher, the mean difference being of the order of 6%. Differences of 4 to 6% have been reported between duplicate determinations using RIHSA (Clarke, 1968; Kloster *et al.*, 1966), suggesting that the difference noted was within the limits of physiological variation. Collectively the results indicate that ^{99m}Tc-albumin, prepared by the described method, can be used as an alternative to RIHSA to provide

¹Blood Products Laboratory, Lister Institute, Elstree

²The radiation dose to the whole body was less than 2.5 mrem

³Nuclear Enterprises Ltd., Beenham, Reading

FIG. 1. Isotope dilution curve by external counting method with ^{99m}Tc -albumin. (The broken line represents the theoretical exponential fall-off of the injected material. This portion was derived from extension of the semilogarithmic replot of the descending portion of the curve beyond the time at which recirculation began. The values so obtained were then inserted into the linear curve above.)

acceptable determinations of cardiac output and blood volume.

PHYSIOLOGICAL FATE OF ^{99m}Tc -ALBUMIN The circulating levels during the 24 hours after injection were expressed as the percentage of the total activity remaining in the blood at the various times of sampling (Table III). Where possible the blood volume determined with RIHSA was used for the definition of the space occupied by the isotope at zero time. When this factor was not available, the blood volume was derived from a nomogram, using the subject's height and weight (Allen *et al.*, 1956). The applicability of the nomogram was tested in a series of 13 normal subjects by direct comparison of the predicted value with that obtained with RIHSA. The mean isotope

TABLE I
CARDIAC OUTPUT ASSAYS USING ^{99m}Tc -ALBUMIN

Subject	Cardiac Output (blood volumes/min)
D	1.04
E	1.11
F	0.91
G	1.02
H	1.27
I	1.47
J	0.99
Mean	1.12 ± 0.19

Mean with RIHSA (N=37) 1.18 ± 0.19^1 .

¹Data of Clarke (1968)

TABLE II
COMPARISON OF BLOOD VOLUMES ESTIMATED WITH
 ^{99m}Tc -ALBUMIN AND RIHSA

Subject	^{99m}Tc -Albumin Blood Volume (l.) V(Tc)	RIHSA Blood Volume (l.) V(I)	% Mean Difference $\frac{2(V(\text{Tc}) - V(\text{I}))}{(V(\text{Tc}) + V(\text{I}))}$
A	5.1	4.8	6
C	5.7	5.4	5
D	6.9	6.3	9
H	4.0	3.9	3
I	4.7	4.3	9
J	6.4	6.1	5
		Mean	6

TABLE III
RELATION OF CIRCULATING ^{99m}Tc LEVEL TO TIME AFTER
INJECTION
(All values refer to % dose remaining in the blood)

Subject	15 min	1 hr	3 hr	24 hr
<i>Plasma volumes derived from RIHSA dilution</i>				
A	94	85	70	35
C	95		70	41
D	91	84		42
H	98			
I	92			
J	95			
<i>Plasma volumes derived from nomogram and haematocrit</i>				
B	94	89	70	
E	100		69	38
F	95		67	35
G	98		69	37
Mean	95 ± 3	86 ± 3	69 ± 1	38 ± 3

blood volume was $98 \pm 5\%$ of the nomogram value.

A continuous fall in circulating activity was observed throughout the period studied. Of special interest was the marked similarity in the levels at the main times of sampling, 15 minutes, 3 hours, and 24 hours. A similar closeness was also seen in the values for the sampling at 1 hour in three of the subjects. Such a constant rate of fall-off in the isotope levels in the various individuals with the various preparations of the conjugate injected, five in all, must reflect the reproducibility of the labelling process.

The physiological fates of ^{99m}Tc-albumin and RIHSA were compared in subjects A, C, and D. A common basis was provided by using the nomogram blood volume to define the initial dilution space of the injected material in both instances. After the first 10 to 15 minutes, the ^{99m}Tc activity

FIG. 2. Comparison of fall-off in circulating activity with time after administration of ^{99m}Tc-albumin and RIHSA.

FIG. 3. Urine excretion following injection of ^{99m}Tc-albumin. (The values in each block represent the percentage of the dose in each period of collection.)

fell more rapidly than that of ¹³¹I, resulting after 1 hour in plasma levels between 81 and 93%, compared with levels between 92 and 96%. After 24 hours, the levels lay between 37 and 41%, and 55 and 63% respectively for the two isotopes. Although at all times the ^{99m}Tc activity fell more rapidly than that of ¹³¹I, the greatest decrease occurred between 15 minutes and 1 hour after injection (Fig. 2).

The initial loss of ^{99m}Tc from the blood was reflected in the high urinary output of isotope during the first few hours (Fig. 3). The excretion levels in subjects A and C after 90 minutes were 5 and 8% of the injected dose, values equivalent to 25 and 28% of the total 24-hour excretions. After 4 hours, the urine levels in subjects A, C, and D were respectively 12, 20, and 19% of the dose. These values corresponded to 60, 69, and 61% of the 24-hour totals.

In the group of four normal subjects and three patients, as a whole, the 24-hour excretions were within the range of 16 to 31% of the injected material, with a mean of $23 \pm 6\%$.

DISCUSSION

The cardiac output and blood volume data show that the present preparation of ^{99m}Tc-albumin is adequate for measuring these factors. The more rapid fall-off in the circulating activity compared with RIHSA, however, emphasizes two points.

Firstly, sensible blood volumes will be obtained only by adhering to a strict criterion of blood sampling at 10 minutes after administering the indicator; and, secondly, the combination of the short physical half-life of the isotope and the greatly decreased physiological half-life of the conjugate will result in a much lower circulating residuum in the course of serial assays.

Previous information on the physiological fate of ^{99m}Tc -albumin has been confined to studies (Beazley, Matthews, Leaver, and Smith, 1968; Walker and Kohorn, 1968; Herbert, Hibbard, and Sheppard, 1969) using the material prepared by the method of Stern *et al.* (1965). The most comprehensive was that of Herbert *et al.* (1969), in which the blood levels, urine excretions, and other factors were measured in a series of pregnant women after placentography. The findings showed a similar type of fall-off in the circulation, but at a slightly faster rate compared with the present preparation (Fig. 4).

FIG. 4. Comparison of fall-off in circulating activity with time after administration of the present preparation and that of Stern *et al.* (1965). (The latter values are from Herbert *et al.* (1969).)

The presentation in Fig. 4 follows that of Herbert *et al.* (1969). The residual levels in the blood at various times are expressed as the percentage of the dose administered per litre of plasma. The slower fall-off with the present preparation compared with that of Stern *et al.* (1965) is evident at all times. A corollary arising

from this difference was the higher mean 24-hour urine excretion with the Stern material, 32%, compared with the corresponding value of 23% with the present conjugate.

The associated interplay between the rates of fall-off in the blood and excretion in the urine is at variance with the findings of McAfee *et al.* (1964). After giving the Stern preparation to pregnant women for placentography, these authors reported that less than 0.5% of the dose was excreted in the urine or faeces, although between 25 and 30% of the isotope had left the blood stream within 30 minutes of injection. Further investigations have described high levels of activity in the bladder within 30 minutes of injection of the Stern preparation (Beazley *et al.* 1968; Walker and Kohorn, 1968; Herbert *et al.* 1969), followed by excretions of 25 to 46% in the urine the first day and subsequently 6% in the faeces.

The differences in the physiological fate of ^{99m}Tc -albumin and RIHSA reflect the different types of molecular binding in the two conjugates. In RIHSA the isotope is attached to the tyrosine residues of the protein chains by covalent bonds, whereas in ^{99m}Tc -albumin the binding is more associative than chemical in nature and akin to that between albumin and entities such as fatty acids and hormones. As such, a more rapid breakdown of the combination would be expected.

The fate of the present material seems to be biphasic in nature. The initial rapid fall-off in the blood in the period following administration is followed by a slower decrease during the interval between 3 and 24 hours. These findings suggest that the isotope may be attached to two broad classes of binding site, one of which has a higher affinity for pentavalent ^{99m}Tc than the other. Rapid losses from the weaker sites would realize eventually a circulating entity largely composed of isotope bound on stronger sites, which would be lost less readily from the blood.

The released ^{99m}Tc must represent an extraneous factor as far as the body is concerned, a fact reflected by its subsequent rapid extraction via the bladder. Not all the isotope was traceable to the circulation or the urinary output during the first 24 hours. From 20 to 30% was unlocated in this period. Even if an allowance of as much as 10% is applied for faecal losses, a measurable quantity remains elusive.

The most probable site for the breakdown of the conjugate is the liver as the process is essentially one of detoxication. Such sequestered activity would not be detected by scanning because of the

blood content of the organ. Examples of the retention of ^{99m}Tc by the liver in circumstances of its liberation in the free state in that organ, rather than its transportation by the blood into the organ, have been reported (Sorensen and Archambault, 1963). It appears likely that the undetermined 10 to 20% may be located in the liver following liberation therein by breakdown of the preparation. The final solution of this problem, however, must await further studies.

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