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The Fate of Macroaggregated Albumin Used in Lung Scanning

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Macroaggregated albumin labeled with radioactive tracers was selected for diagnostic purposes because of the rapid metabolism of the material once it had been introduced into the vascular system. A number of reports are now available which evaluate the clinical usefulness of this procedure [1-9]. Several investigations of the toxicity, distribution, physiological response and metabolism of the aggregated albumin have been published [1-3, 5-8, 10]. Further studies of the fate of the injected particle are reported in this paper.

MATERIALS AND METHODS

Two groups of subjects were selected for this study. The first group was composed of twenty adults admitted to the hospital for a variety of reasons. There were eleven females and nine males ranging in age from 33 to 78 years. [Eighteen of these patients had pulmonary lesions including carcinoma, inflammatory processes, and emphysema]. Two of these received 150 microcuries of ^{131}I macro aggregated albumin[2] and the remainder 300 microcuries. Six different lots of material were used. The aggregate was not examined microscopically for size; however, three of these were from the same batches reported by Taplin, *et al* [11] who found that about 70-75% were in the 10-20 mC range. At 8:00 a.m. on the morning of the procedure the patient received the MAA intravenously in a supine position. Anterior scan of the chest using a commercial type scanner with a 3″ × 2″ crystal was begun within minutes. Information was recorded on film and color print display. The direction of scanning was from top to bottom, beginning at the apices and extending generally far enough to include the liver, spleen, and upper portions of the kidneys. The highest concentration of radio-

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[2]Kindly furnished by E. R. Squibb Co. Specific activity 1000-1100 mC/cc and protein content 1.0 mg/ml.

activity over the lung field was recorded and the chest so marked. Subsequent scans at 4, 8 and 24 hours post injection were done and the radioactivity over this point of the chest recorded. Blood samples were obtained at each examination for radioactivity level. On the original and 24-hour samples serum bilirubin, lactic dehydrogenase, and serum glutamic oxaloacetic acid were determined. Twenty-four hour urine specimens were collected for three days. Thyroid uptake was measured at 24 hours. The thyroid gland was specifically not blocked in this group of patients.

The second group studied were four healthy males between the ages of 23 and 44 years. Variation in height was 69 to 71 inches and weight 145 to 155 pounds. Each subject received Lugol's iodine for two days before examination. Dosage of 300 microcuries of ^{131}I MAA was used from four additional lots. The average size of the aggregate was larger than that used in the first group of patients. The particles varied from 5 to 100 microns in diameter with about 60% above the 20 micron size. Each subject was scanned once, immediately after injection. Point surface counting was done over the thyroid, lungs, liver, spleen, left kidney, and left mid-thigh. These measurements were made with a 2″ × 2″ sodium iodide crystal and a custom-built, heavily-shielded flat field collimator. When measuring thyroid uptakes, the upper lung fields were shielded with ¾″ sheet lead. [This thickness was found to be as effective as 2″ of lead]. These measurements were made immediately after injection, every two hours for twelve hours, and every 24 hours for 7 days. In one subject, the daily measurements were

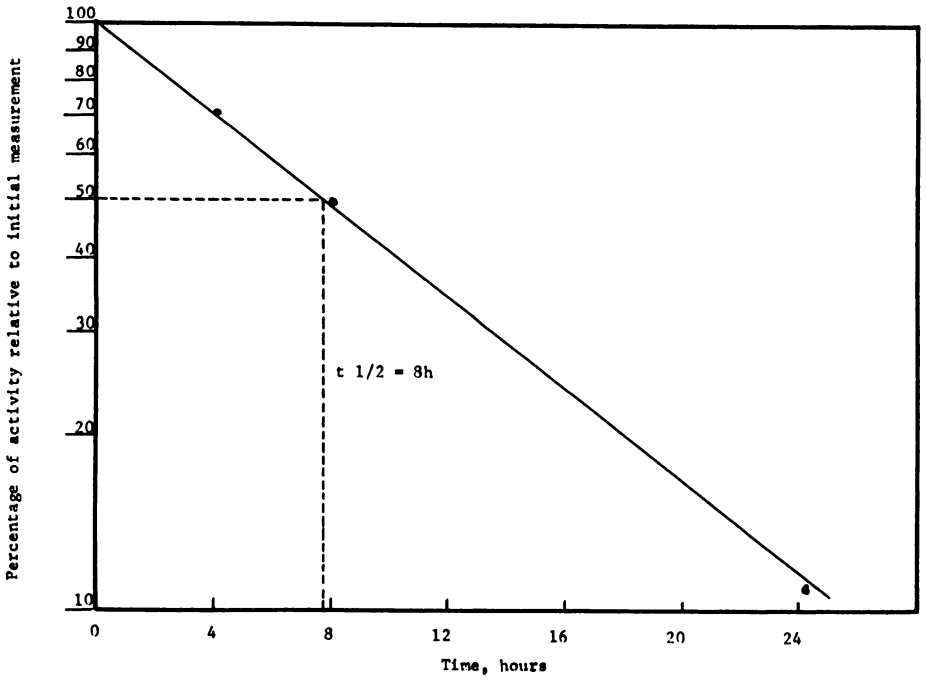

Fig. 1. Level of radioactivity in the lungs.

made for 21 days. Blood specimens were obtained immediately and every four hours for 12 hours, and daily for seven days. In one case, specimens were obtained for 21 days. Free and protein bound ^{131}I were determined by protein precipitation and protein separation by cellulose acetate electrophoresis. The individual protein bands were counted in a 2″ × 2″ crystal well type counter. Twenty-four hour urine specimens were collected for seven days and twenty-one days in one person.

RESULTS AND DISCUSSION

In the group of 20 hospitalized patients, the point of highest radioactivity in the pulmonary field following injection was used as the reference level and

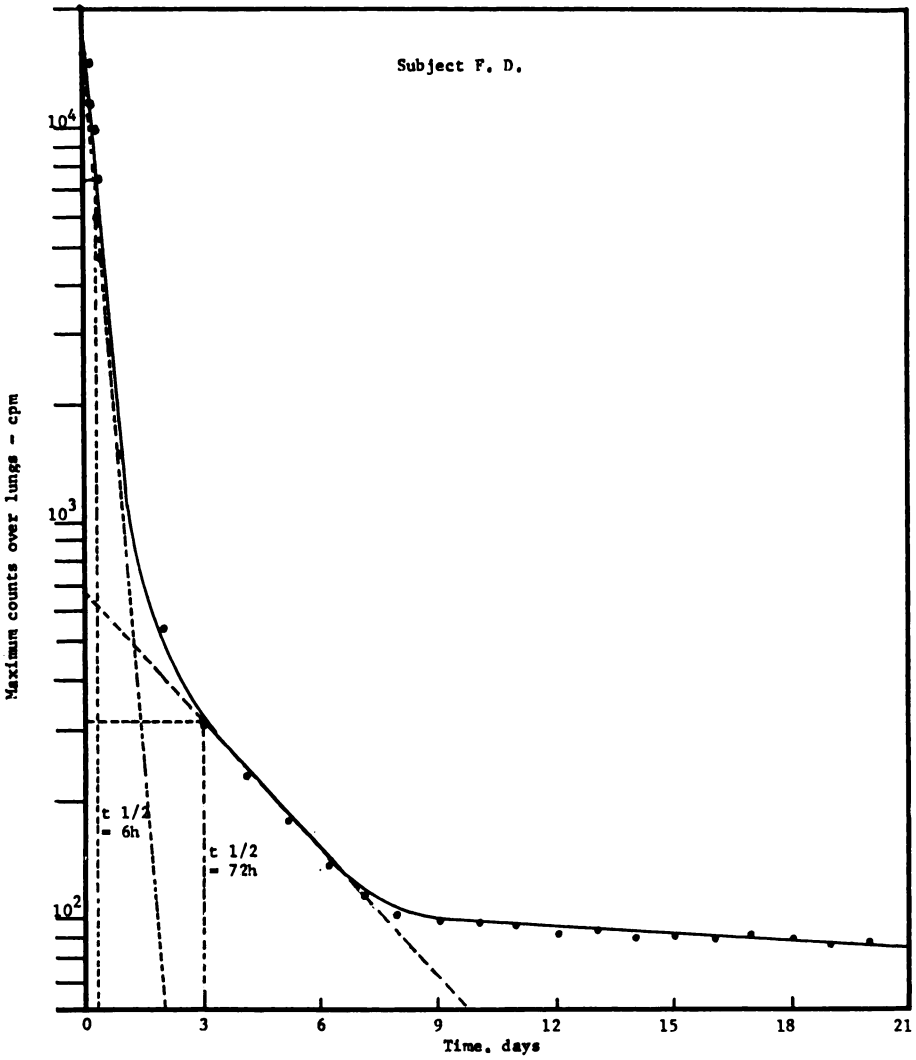

Fig. 2. Decrease of pulmonary radioactivity by surface counting. Measurements corrected for physical decay.

subsequent counts were related as percentages of this value. There was a wide range of residual pulmonary activity for each subsequent scanning interval. Four hours after injection the level varied from 50% to 100% with a mean of 74% [1 SD = 16%], 33% to 70% at 8 hours with a mean of 41% [1 SD = 11%], and 8% to 31% at 24 hours with a mean of 18% [1 SD = 5.9%]. The data obtained from the

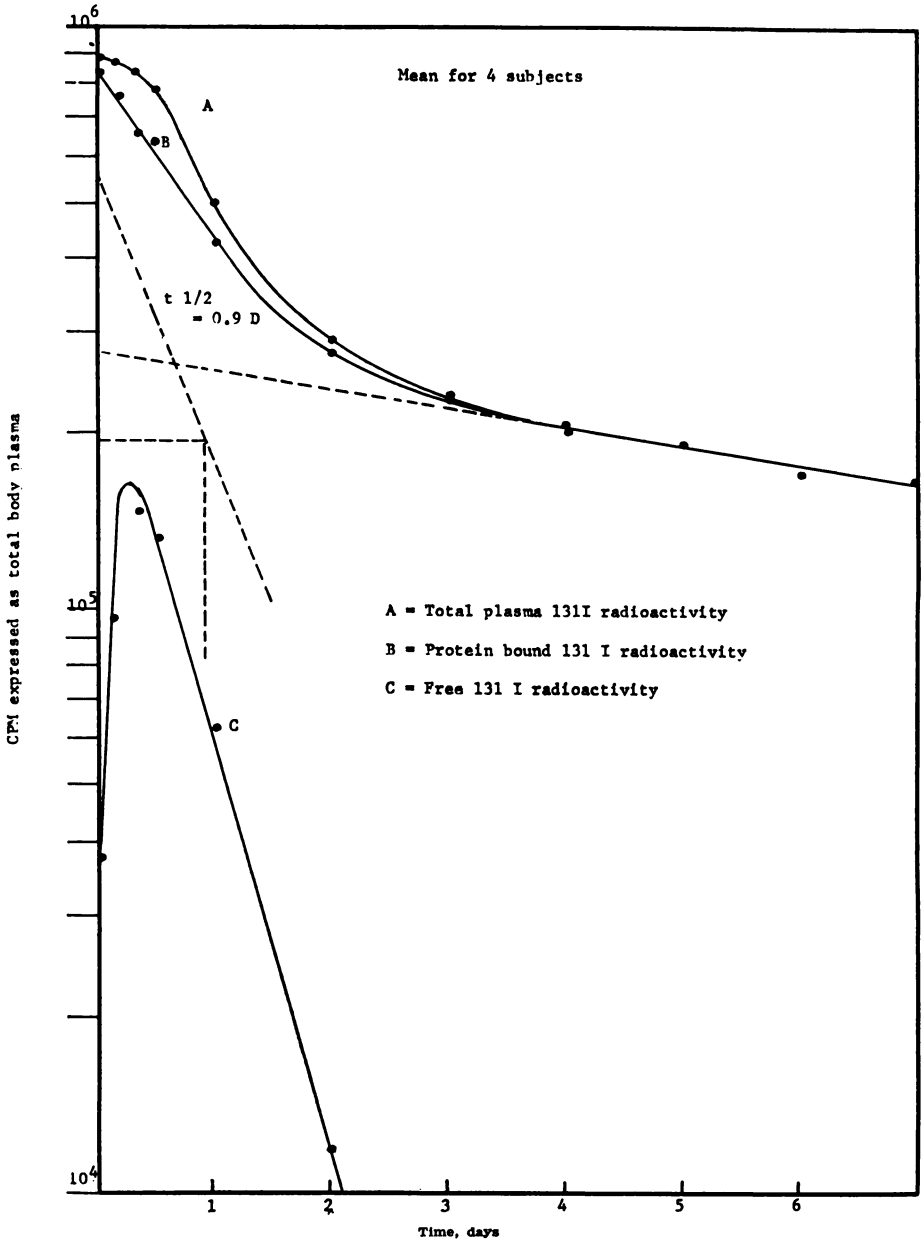

Fig. 3. Disappearance of radioactivity from plasma; total, PBI, and free Iodine-131.

immediate, the 4-hour, and the 8-hour examination were plotted and the distribution formed Gaussian curves, which would be expected with biological parameters, The t ½ pulmonary clearance time varied from 4 to 12 hours with a mean of approximately 8 hours [Fig. 1]. This was slightly longer than reported by Furth et al [10] of 6 hours and Sabiston and Wagner [7] of 3 to 7 hours. If a second component had been available for graphic construction purposes, the time would have been slightly less. In the group of normal males the t ½ time was 6 hours [Fig. 2]. The number of subjects involved was inadequate for statistical analysis and none was attempted. Nevertheless, this half time was essentially the same in each of the four males. A second component of the curve had a half time of 72 hours, agreeing closely with that of Furth [7]. In the one subject who was measured over a period of 21 days, a third slope was noted beginning approximately the 9th to 10th day and extended as a single function for the entire period of examination. When this portion of the curve was extrapolated, the t ½ was about 55 days. During the 2 hours after injection the level of radioactivity over the pulmonary field was only slightly less than the immediate post injection level in the normal subject. The rapid washout appeared to have begun immediately thereafter.

In the first group of patients, the blood specimens were obtained during the first 24 hours only. Expressed as the percentage of the immediate post-injection level at 4 hours, 86% of the activity remained in the blood [1 SD = 4.5%], 78% at 8 hours [1 SD = 3.6%], and 48% at 24 hours [1 SD = 4.6%]. In the group of 4 normal patients, specimens were obtained for 7 days in all and 21 days in one. The results presented a fairly complex curve [See Fig. 3]. Measurements are expressed as total body plasma radioactivity. During the first 12 hours, the curve was convex and then became concave. In order to simplify this function, the plasma free ^{131}I was subtracted from the total plasma activity. This procedure was carried out until the free iodine level approached zero by the 6th to 7th day. The free iodine level at five minutes post injection was an average of 6.8 per cent of the total count. The level increased very rapidly to an average peak of about 37% at approximately 6-7 hours. Thereafter, the free iodine washed out rapidly with a t ½ of about 0.45 days. The trace can be resolved into a more simple expression when only the protein-bound iodine is considered [Fig. 3]. The early portion of the curve presented an exponential function with a t ½ of 0.9 days, using standard graphic analysis [12]. This was followed by a second curve with a t ½ time of 7.5 days. This figure approaches the half time of iodinated human serum albumin as reported by Sterling [13]. After 10-11 days the plasma level decreases very slowly [Fig. 4]. This finding concurs with the findings in surface counting over the lungs.

In viewing the curve of free iodine-131 one might be able to make several interpretations. One of these is that at the peak of the curve [6 or 7 hours] the rate of release of iodine from degraded protein must equal the rate of excretion and therefore, prior to this peak, the rate of release exceeds excretion and afterwards the rate of release is less than excretion. Since the decreasing slope was a single exponential function to the measurable end, this condition suggests that degradation of the obstructed aggregate is a similar function.

Serum proteins were separated by cellulose acetate electrophoresis and the strips were stained with Ponceau S in order to separate the individual protein bands, which were measured in a scintillation well counter. Separation of the MAA was as follows: point of application, 65%; albumin and pre-albumin 27%; remainder [8%] distributed almost equally in the four globulin bands. In specimens obtained immediately after injection and at 24 hours, radioactivity in the globulin fraction was about 20% above background and in the albumin fraction 50%-100% above background. After the first day there was no measurable activity in any band except albumin and this varied from 20% to 40% per cent above background.

Activity in the spleen, liver, kidney and thigh presented several interesting characteristics [Fig. 5]. Radioactive levels in the thigh and kidney increased for the first 10 hours, coinciding to some extent with the total plasma curve A [See Fig. 3]. The relative increase, however, was much greater than that of plasma. Extravascular concentration of free and albumin bound ^{131}I appears to be the most logical explanation. The spleen and liver present a curve configuration somewhat similar to that of total plasma but with a sharper washout, from 2 -12 hours. Intra-organ degradation of iodinated aggregates is responsi-

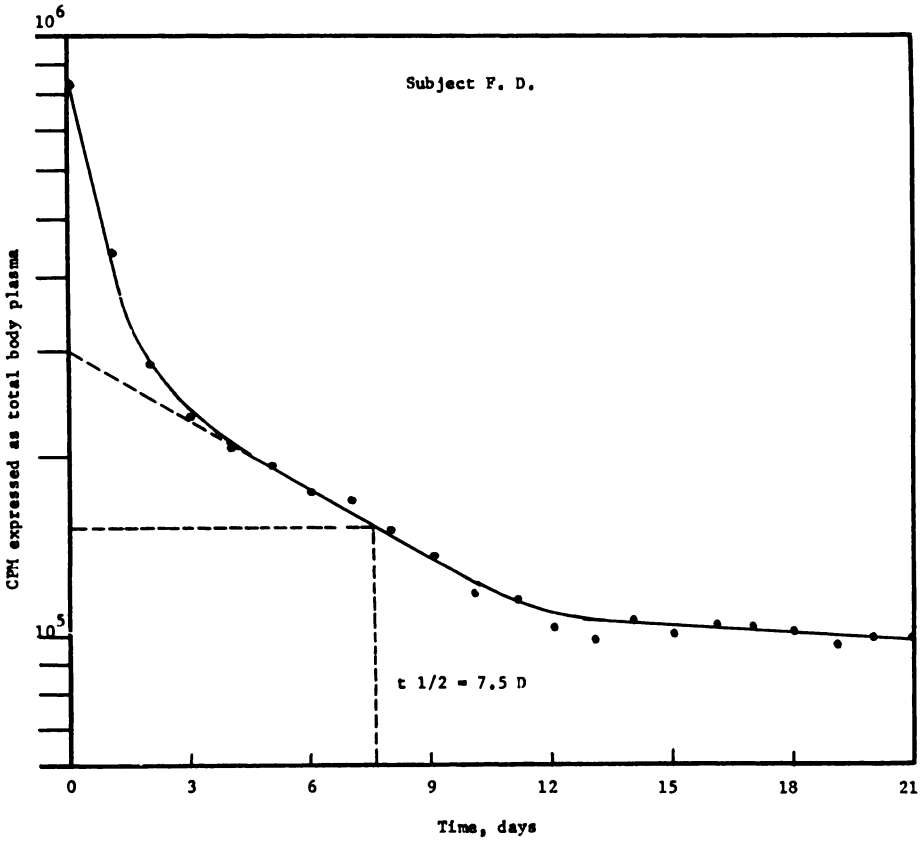

Fig. 4. Disappearance of radioactivity from plasma in one subject.

ble for at least part of this complexity. From 12 to 24 hours, all four of these sites have nearly parallel slopes and half times of about 72 hours, similar to the lung. The third components also show similar parallelism. It is possible that after 12 hours all body and organ areas have the same factors affecting activity level and that little, if any, aggregate remains in the lung.

In the hospitalized patients the 24 hour thyroid uptakes were quite variable, ranging from 2.5 to 22.4 per cent of the injected dose. Review of the patient's history revealed that three had been on medications containing iodine. These patients were eliminated from this portion of the study. The mean uptake was 13.5% with a standard deviation of 4.5%. In a number of these patients, previous measurements of various thyroid parameters were available for comparison and, in all instances, the uptakes were well below the results obtained prior to this study. Evaluation of this data suggested that the blood level of iodine-131 presented to the thyroid was roughly that which would have been obtained with a

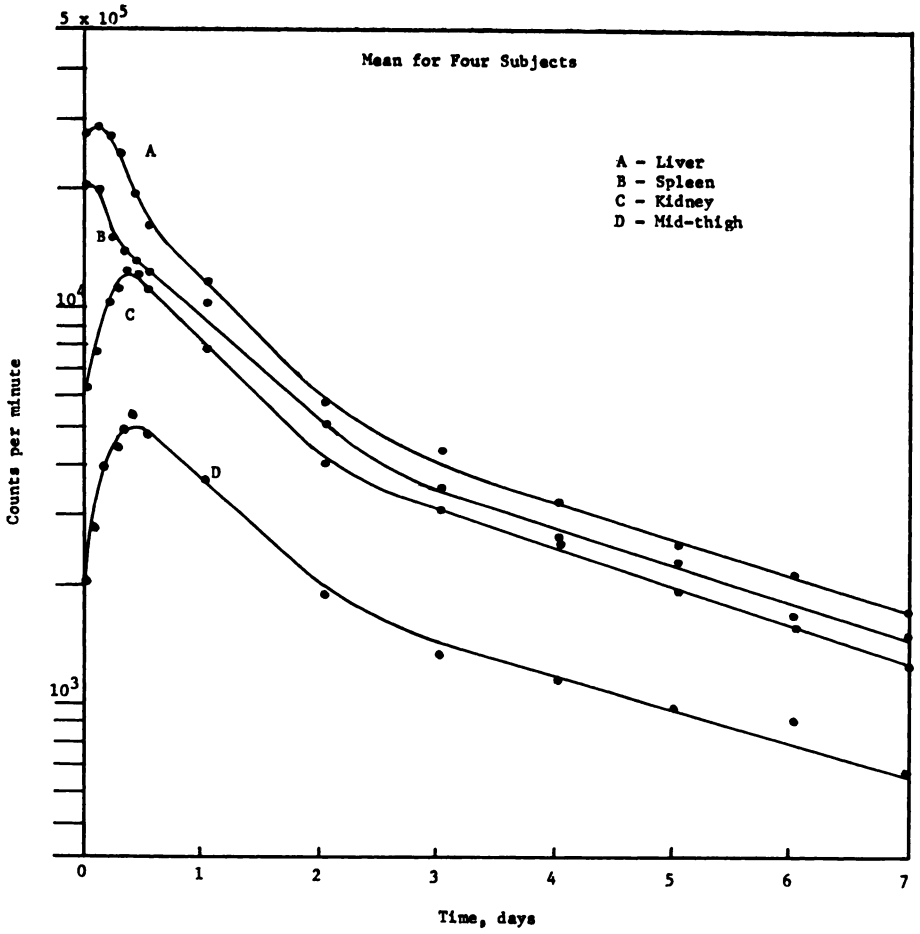

Fig. 5. Disappearance of radioactivity from several organs as determined by external measurement.

dosage of sodium iodine equal to 40% of the macroaggregate dose. In the group of four normal subjects, thyroid uptakes were measured for the first seven days and in one subject for 21 days. The radioactivity level when corrected for physical decay was unexpectedly quite constant and varied from 3.2% to 4.3% of the administered dose. In the one subject that was extended to 21 days, this consistent level continued through the 11th day and then gradually diminished to 1.8% by the 21st day. The biological half life was about 15 days and effective half life 5.3 days. This finding agreed closely with results from other thyroid studies [14]. Blockage of the thyroid with iodine for two days prior to examination was adequate.

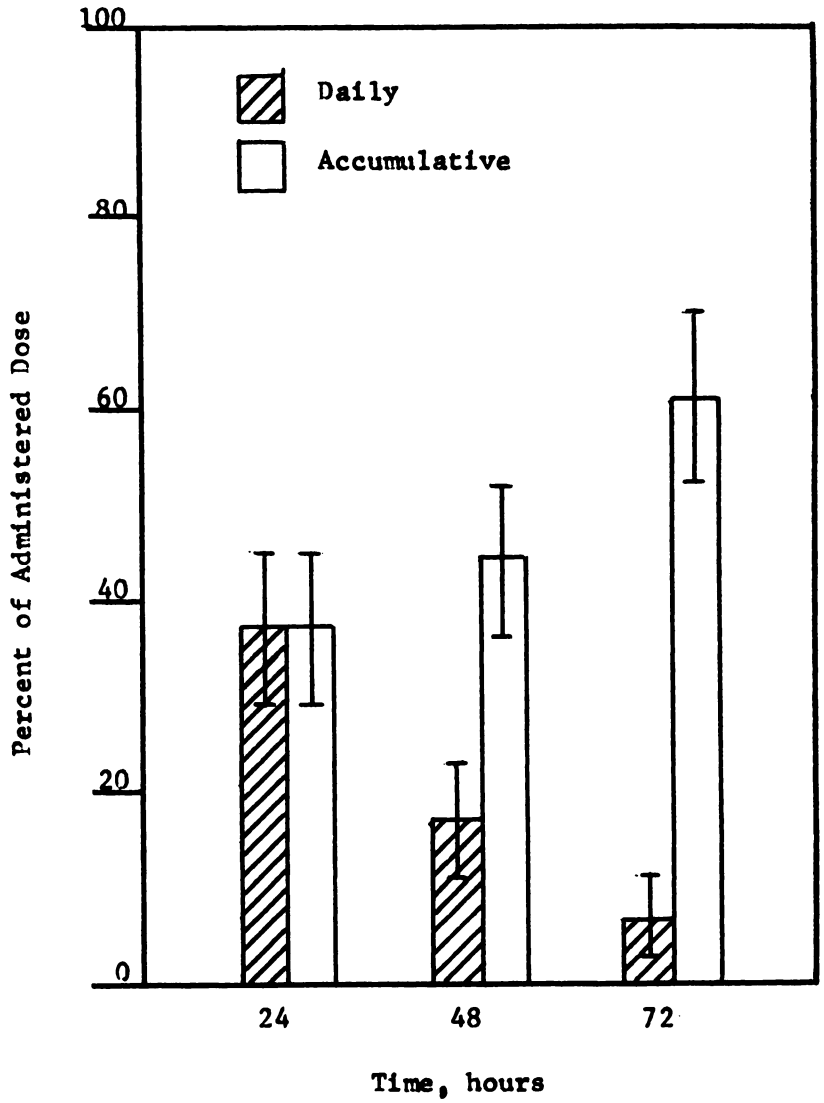

Fig. 6. Urinary excretion of Iodine-131 following intravenous macroaggregate, including 1 standard deviation (20 hospitalized patients).

Twenty-four hour urine specimens were collected for three days in the group of 20 hospitalized patients. The excretion rates were quite variable as follows: 25.1% to 54.3% at 24 hours [mean 37.4%, 1 SD of 8.6%], 24-48 hour urine excretion was 8.1% to 32.5% [mean 17%, 1 SD of 6.1%], and 48-72 hour urine excretion varied from 0.8% to 18.8% [mean 6.8%, 1 SD of 4.4%]. The mean total 48 hour excretion was 54.4% [1 SD = 8.6%] and 72 hour excretion 61.2% [1 SD = 8.8%] [Fig. 6]. The body biological half life was 1.7 days, similar to that reported by Dworkin, et al [2]. When the 24-hour urinary output and the 24-hour thyroid uptake in each patient were added, the variation became much less marked, 42.2% to 62.5%, a mean of 51.1% [1 SD = 8.0%]. The mean of this combined utilization agreed well with Biozzi's [15] finding of 50% urinary secretion of ^{131}I combined with denatured serum albumin in 24 hours, when the thyroid gland had been previously blocked by iodine. The daily accumulated urinary output in the group of four normal males was shown in Fig. 7, [means and ranges]. The daily excretion in the one subject with specimens from the eighth to the 21st day was extended as part of the mean curve because the absolute numbers were in accord with the previous mean numbers. The mean output in 24 hours was 55.2% [± 1.0 per cent]; 48 hours, 73.3% [± 0.6 per cent]; and 7 days, 82.1% [± 1.0%]. The total excretion at seven days compared quite favorably with the upper limits as noted by Furth *et al* [7]. The half life was in the order of 21-22 hours. Although the volumes varied widely from 850 to 1800 ml/day, the daily output of radioactive material varied much less in this group than in the hospitalized patients. The variation between the two groups was undoubtedly a combination of factors, but probably the most important was the opportunity to maintain very close controls on the latter group.

Evaluation of residual activity remaining in the body demonstrated one exponential function [Fig. 8], from seven to 21 days. When this curve was extrapolated the $t_{\frac{1}{2}}$ was in the order of 50 days and at this time, although there were 13 half lives of physical decay, 9% of the original dose was still present. This very prolonged terminal function noted both in the retained material vascularly and in excreted material agreed with Sterling's findings that this type curve represented the rate of degradation of albumin [13]. If such was the case, then extrapolation indicated that tagged undenatured albumin represented at least 18 per cent of the original injected material. This figure, however, represented only the values obtained from the second exponential function of ^{131}I albumin. If the first function was also considered, [tissue distribution], it was speculated that this could approach 30% or 40% [13]. Since in this study 91.1% of the dose was lodged within the pulmonary capillaries and only 8.9% was in the peripheral vascular system immediately after injection, degradation of the macroaggregate would produce undenatured albumin or a very similar product.

Since the intravenous injection of macroaggregated albumin produced obstruction of a certain number of pulmonary capillaries, possible parenchymal damage was investigated. In 1961 Wacker, *et al* [16], demonstrated the efficacy of a chemical triad for the diagnosis of pulmonary embolism and infarction. This diagnostic profile included serum glutamic oxaloacetic transaminase, lactic dehydrogenase, and serum bilirubin. After pulmonary embolism had occurred,

either with or without infarction, LDH was promptly elevated [16,17], SGOT remained essentially unchanged, [16], and the serum bilirubin elevated [16,18]. In 13 of these patients, analysis of this pulmonary triad was performed. These results are shown in Table I. In no instance was there any significant change of these parameters. From the standpoint of clinical chemistry, no tissue damage was demonstrable.

In seven cases, specimens of human lungs were obtained and examined histologically by autoradiography. These specimens were obtained at 1, 3, 4 hours and 3, 4, 7 days after injection. None of the microscopic sections showed evidence of tissue damage or reaction. Autoradiography demonstrated the occluded capillaries with radioactive material in those specimens obtained during the first four hours. Activity was also seen in a malignant neoplasm of the lung. It was not possible to differentiate quantitatively between one and four hours. In those specimens obtained from 3-7 days, occasional areas of radioactivity were noted, but were very sparse. These were found in larger vessels, unobstructed, and appeared to be within mononuclear macrophages.

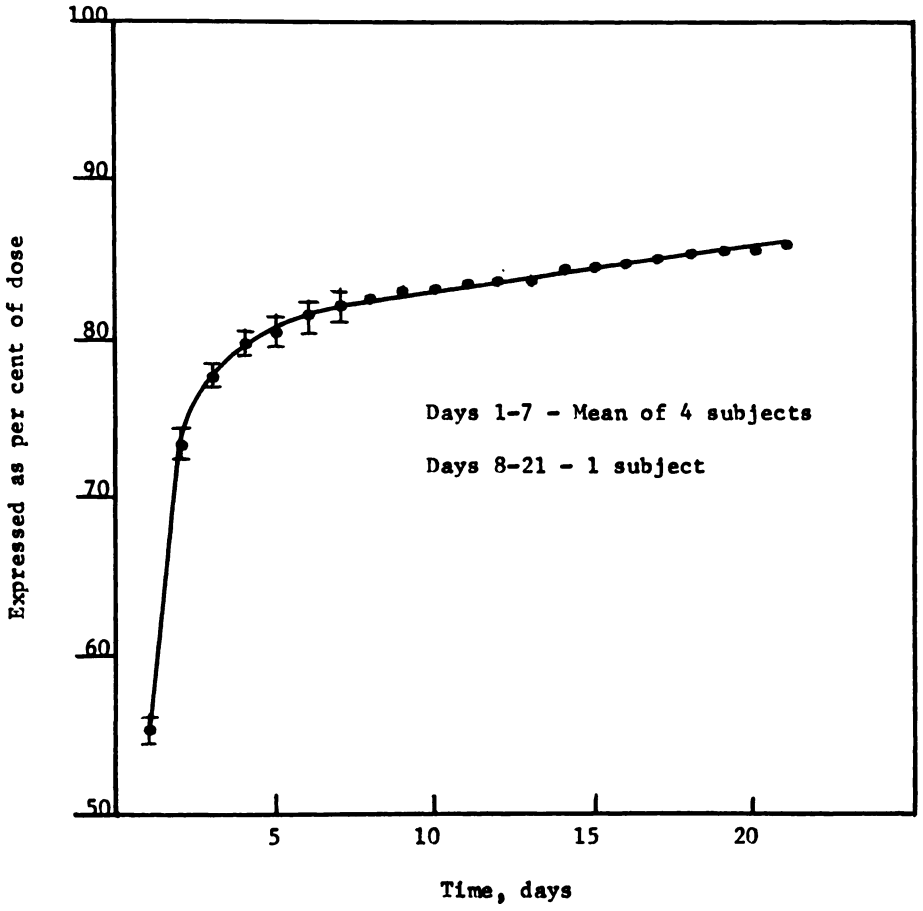

Fig. 7. Accumulative excretion of Iodine-131, corrected for decay.

CONCLUSIONS

Total radioactivity from ^{131}I MAA disappeared from the lung with at least three exponential functions with biological half times of 0.25, 3.0, and 55 days. Protein bound ^{131}I similarly showed three exponential functions with half times of 0.9, 7.5, and 55 days.

Free ^{131}I composed as much as 35%-40% of total plasma radioactivity at 6-7 hours post injection and then rapidly decreased with a t ½ of 0.45 days.

The second and third exponential functions for disappearance of activity from the liver, spleen, kidney and thigh were found to be essentially identical with respect to rate and very similar to those for the lung.

Urinary excretion indicated a body biological half time of 1.7 days existing in hospitalized patients without prior iodides and 21-22 hours in healthy males with prior iodides.

In this study, at least 20% of the original MAA behaved in a manner similar to ^{131}I albumin.

No pulmonary parenchymal damage could be demonstrated, either by the microscopic examination of pulmonary surgical specimens or by analysis of serum LDH, SGOT, or bilirubin.

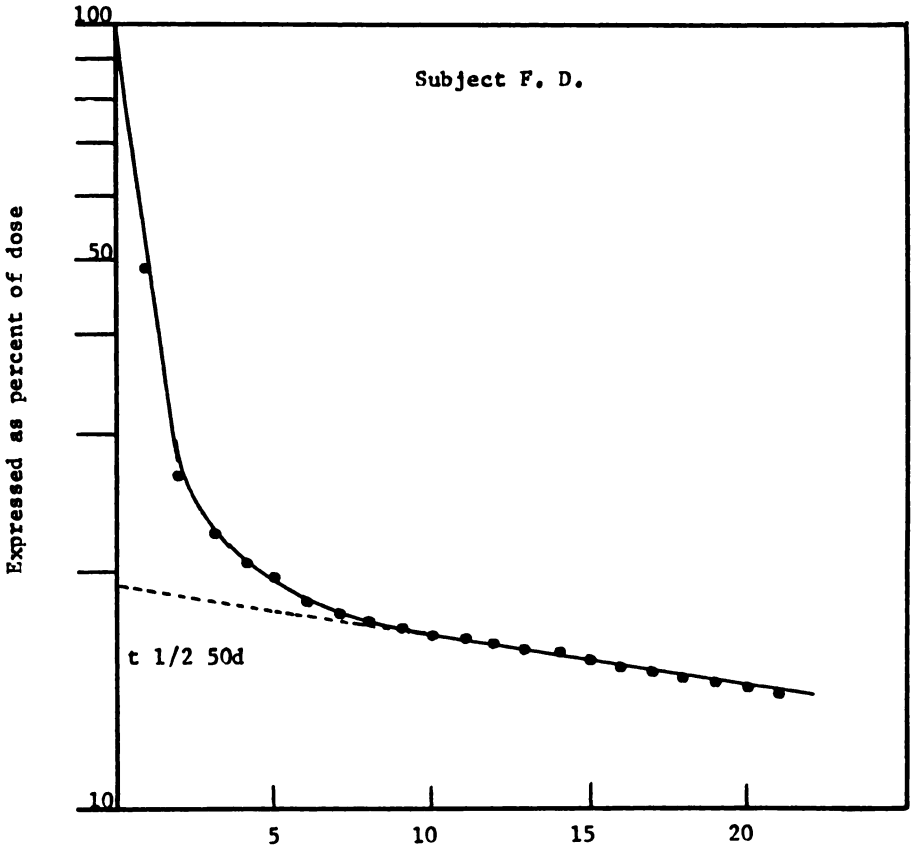

Fig. 8. Daily residual body radioactivity, corrected for decay.

TABLE I

PULMONARY PROFILE FOR EMBOLISM AND INFARCTION

Case number	*Lactic dehydrogenase B B units*		*SGOT R F units*		*Bilirubin mg%*	
	Initial	*24 hour post injection*	*Initial*	*24 hour post injection*	*Initial*	*24 hour post injection*
65–6569	840	790	24	26	0.25	0.21
65–7392	310	290	25	26	0.21	0.25
65–7323	205	195	25	26	0.39	0.39
65–7204	595	495	36	29	0.32	0.23
65–7931	450	285	32	21	0.22	0.22
65–8160	265	265	18	17	0.40	0.22
65–7356	400	308	16	15	0.25	0.45
65–8654	300	285	15	33	0.21	0.22
65–8896	605	280	29	26	0.32	0.32
65–9295	380	280	19	26	0.25	0.32
65–9935	295	270	20	20	0.39	0.45
65-10489	282	302	16	16	0.25	0.28
65-11677	157	152	12	14	0.25	0.28

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