Recipes for Human Albumin Macroaggregates

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The search for new diagnostic approaches is important, especially when they hold promise for solving an unmet clinical need. Yet, no less important is the simplification of methodologic aspects of new techniques. Only then can they be readily, easily, and successfully used in the clinical setting. Taplin and his coworkers had fully recognized such a need for streamlining and simplifying an emerging diagnostic approach by publishing their work, ''Suspensions of Radioalbumin Aggregates for Photoscanning the Liver, Spleen, Lung and Other Organs,'' in *The Journal of Nuclear Medicine* in 1964 (*1*). The nuclear medicine community welcomed Taplin's contribution, as the high number of citations the article received would suggest, because it set a standard for how to prepare radiolabeled albumin microspheres, how to control their quality and size, how to obtain images of optimum diagnostic quality, and how to avoid possible adverse effects.

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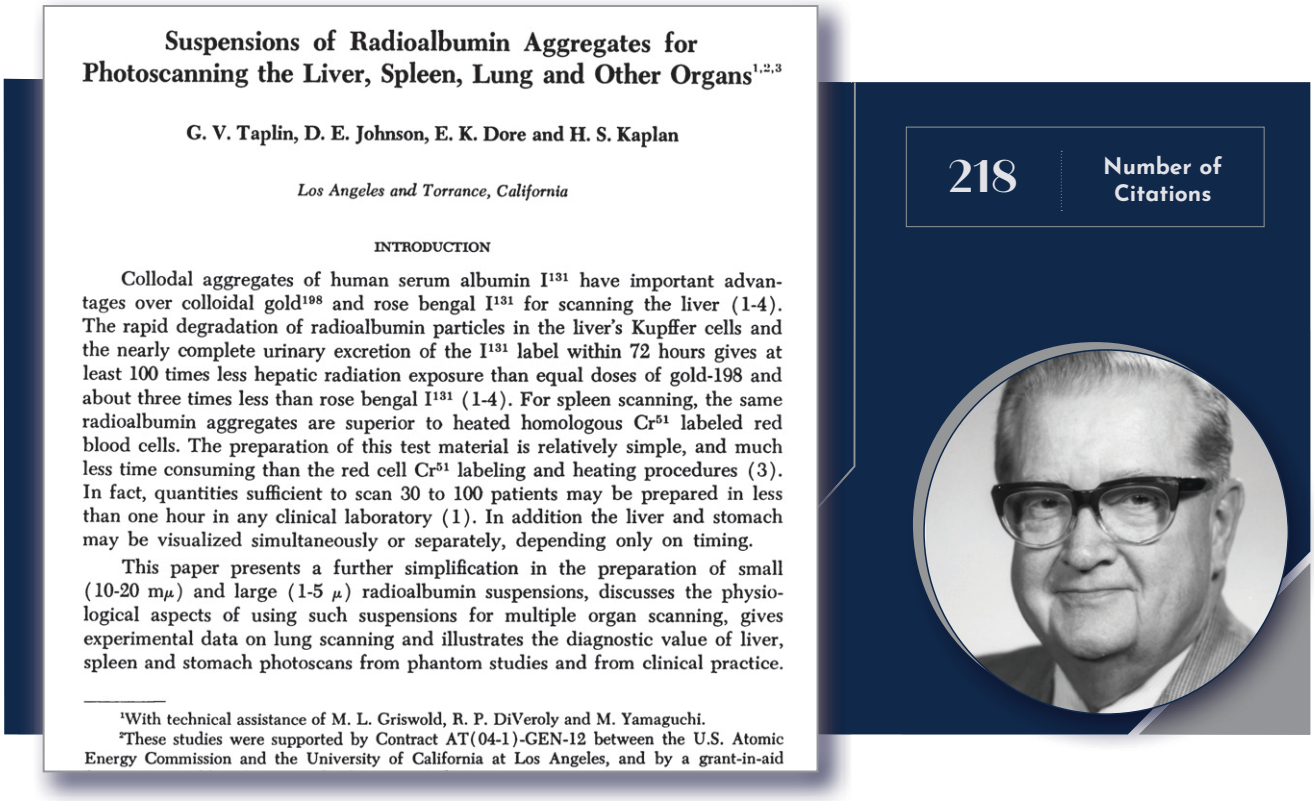
Suspensions of sterile and pyrogen-free human serum albumin labeled with ^{125}I or ^{131}I had in fact been in use in clinical investigations and had become commercially available (Albumatope; E.R. Squibb and Sons) (*2*,*3*). The emergence of nuclear imaging devices at that time, such as Cassen's rectilinear scanner or Anger's γ-camera, accelerated the interest in radioiodinated human serum albumin or albumin derivatives because the ^{131}I label was suitable for radionuclide imaging. Initial studies with human serum albumin aggregates of colloid size had indeed confirmed the feasibility of this radionuclide for imaging (*3*). The 1964 Taplin publication reads like a cookbook recipe for how to consistently and reproducibly prepare macroaggregates of human serum albumin of different sizes ranging from small-particle colloidal suspensions (10–20 nm) to larger-particle suspensions (1–5 μm). A short section of the article defines physiologic aspects of colloid-sized particles as an underpinning for understanding their pharmacokinetics. After being phagocytosed by Kupffer cells in the liver, macroaggregates are retained in the cells sufficiently long for radionuclide imaging and then are degraded by proteolytic processes, with the radioiodine released into blood in the form of ^{131}I-tyrosine or small proteins or even in free form, with



Suspensions of Radioalbumin Aggregates for Photoscanning the Liver, Spleen, Lung and Other Organs[1,2,3]

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INTRODUCTION

Collodal aggregates of human serum albumin I^{131} have important advantages over colloidal gold^{198} and rose bengal I^{131} for scanning the liver (1-4). The rapid degradation of radioalbumin particles in the liver's Kupffer cells and the nearly complete urinary excretion of the I^{131} label within 72 hours gives at least 100 times less hepatic radiation exposure than equal doses of gold-198 and about three times less than rose bengal I^{131} (1-4). For spleen scanning, the same radioalbumin aggregates are superior to heated homologous Cr^{51} labeled red blood cells. The preparation of this test material is relatively simple, and much less time consuming than the red cell Cr^{51} labeling and heating procedures (3). In fact, quantities sufficient to scan 30 to 100 patients may be prepared in less than one hour in any clinical laboratory (1). In addition the liver and stomach may be visualized simultaneously or separately, depending only on timing.

This paper presents a further simplification in the preparation of small (10-20 mμ) and large (1-5 μ) radioalbumin suspensions, discusses the physiological aspects of using such suspensions for multiple organ scanning, gives experimental data on lung scanning and illustrates the diagnostic value of liver, spleen and stomach photoscans from phantom studies and from clinical practice.

[1]With technical assistance of M. L. Griswold, R. P. DiVeroly and M. Yamaguchi.

[2]These studies were supported by Contract AT(04-1)-GEN-12 between the U.S. Atomic Energy Commission and the University of California at Los Angeles, and by a grant-in-aid

accumulation of ^{131}I in the gastric mucosa as noted on late imaging.

Referring to findings in animals, the text confirms a wide safety margin for albumin aggregates in doses needed for imaging but warns of potentially serious adverse effects from excessive doses of large macroaggregates. Scan doses of the radioligand, even when injected repeatedly, remained without detectable antigenic effects, most likely because human serum albumin had been converted to a particulate form by heat treatment and pH adjustment during its preparation. A series of rectilinear photoscans illustrates the high-quality liver, spleen, and salivary gland images that could be obtained with the new, simplified preparation method. Of note, the series of images includes one of lung perfusion in an experimental animal after intravenous administration of larger (≈15 μm) albumin aggregates. Curiously, a recipe for preparing macroaggregates of the particular size is missing from the paper, even though this size of albumin microspheres became fundamental to imaging of tissue perfusion. Unlike smaller albumin aggregates, they were mechanically trapped in the microvasculature in strict proportion to blood flow. With the observation of microsphere trapping, Taplin laid the foundation for a new imaging approach suitable for studying multiple organs and having a substantial and lasting impact on nuclear medicine imaging, as highlighted by the subsequent pulmonary perfusion imaging for the diagnosis of pulmonary embolism (*4*).

NOTE

I would like to mention that I had the privilege of personally knowing Dr. George V. Taplin, the author of the publication this commentary addresses. Taplin, or "Tappy," as we called him, was Professor Emeritus when I joined the Laboratory of Nuclear Medicine and Radiation Biology and the Department of Radiological Sciences at UCLA. In fact, because my office was located next to his, we often talked together or exchanged new ideas. His keen interest in advancing nuclear medicine through high-quality research, and his inquisitive mind, were truly impressive, as was his absolute honesty in judging research accomplishments. Even more so was his encouragement and support of young investigators. Yet, there was never any doubt about Tappy's pioneering contributions to today's nuclear medicine.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

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This paper presents a further simplification in the preparation of small (10-20 mμ) and large (1-5 μ) radioalbumin suspensions, discusses the physiological aspects of using such suspensions for multiple organ scanning, gives experimental data on lung scanning and illustrates the diagnostic value of liver, spleen and stomach photoscans from phantom studies and from clinical practice.

MATERIALS

Solutions of sterile, pyrogen free, human serum albumin I^{131} (Albumatope)[1], normal human serum albumin (Albumisol)[2], 0.2 normal hydrochloric acid, 0.2 normal sodium hydroxide and normal physiological saline are employed. The latter, preserved with 0.9 per cent decyl alcohol is obtained from the pharmacy at UCLA in multiple injection type rubber stoppered bottles.

EQUIPMENT

The main items of equipment are:

1. A precision water bath with a suitable attachment for continuous gentle agitation of 30-100 ml samples in multiple injection type bottles at a constant temperature of 79° C (Fig. 1).
2. A well type scintillation counter facility for measuring radioactivity levels in albumin I^{131} samples.
3. An electron microscope with magnificant to ~70,000.
4. An electrophoresis apparatus.
5. Photoscanning equipment with 19 and 31 hole focusing collimators.
6. One scintillation detector-ratemeter-recorder system.

Current Methods of Preparing Radioalbumin Suspensions

The present procedures originate from Benacerraf (*2*) and from an abbreviated method reported from this laboratory in 1961 (*3*).

(a) Small Size Colloidal Suspensions (10-20 mμ)

1. A 1 per cent radioalbumin solution is prepared by dilution of 5 per cent Albumatope with physiological saline in a sterile multiple injection type rubber stoppered bottle.
2. The pH is adjusted to 10 ± 0.5 by adding 0.8 ml sterile 0.2 normal NAOH per 10 ml of solution.
3. The solution is heated in a water bath at 79° C for 20 minutes while being agitated continuously. It is then cooled below room temperature by immersion in cold water.
4. The pH is reduced to 7.5 ± 0.5 by adding 0.2 normal Hcl (0.8 ml/10 ml of solution).
5. Test for sterility and store at 5° C.

(b) Large Size Suspensions (1-5 μ)

Steps 1, 2 and 3 are the same as for the small size suspensions.

4. The pH is reduced from 10.0 to 6.0 by adding 0.2 normal Hcl (1.2 ml/10 ml of solution). It is further reduced to 5.0 ± 0.3 by adding another 0.1 ml/10 ml slowly, drop by drop while shaking as the pH approaches the isoelectric point of albumin during which the clear opalescent colloid is converted to micron size particles and takes on a milky appearance.
5. Centrifuge for 10 minutes at 1500 rpm—remove supernatant (colloidal particles < 1.0 μ) and re-suspend sediment in normal saline by vigorous shaking.
6. Test for sterility and store at 5° C.

[1]With technical assistance of M. L. Griswold, R. P. DiVeroly and M. Yamaguchi.

[2]These studies were supported by Contract AT(04-1)-GEN-12 between the U.S. Atomic Energy Commission and the University of California at Los Angeles, and by a grant-in-aid from E. R. Squibb and Sons, Radiopharmaceutical Division.

[3]From the Laboratory of Nuclear Medicine and Radiation Biology of the Department of Biophysics–Nuclear Medicine, and the Department of Radiology, School of Medicine, University of California at Los Angeles; and from the Nuclear Medicine Division of the Department of Radiology, the Los Angeles County Harbor General Hospital, Torrance, California.

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[1]The radioiodinated human serum albumin (Albumatope) is obtained from E. R. Squibb and Sons, Radiopharmaceutical Division.

[2]The 25 per cent human albumin solution (Albumisol) containing 0.02 molar sodium caprylate and 0.02 molar sodium acetyl tryptophanate are obtained from Merck-Sharpe and Dohme and Co.

FIGURE 1. Motor driven sample shaker and precision water bath.

Precautions

1. Sterile equipment, supplies, techniques, and pyrogen free solutions are employed throughout.
2. Slow reduction of pH in the large size preparation (step 4) is necessary to *prevent* the formation of very large aggregates (10-100 μ) which form when strong acid is added *rapidly* to an albumin solution as it approaches the isoelectric point ($\sim$ pH 5.0)[1].

Particle Size Measurement by Electron Microscopy

Samples of small albumin aggregates and colloidal gold-198 are diluted 100,000 times in pure distilled water and then examined by electronmicroscopy, employing shadow casting techniques (*5*). Figure 2, a composite permits size comparison of colloidal albumin I^{131} and gold-198 aggregates. Single aggregates of albumin measure 10-20 mμ and clumps of the individual particles rarely exceed 200 mμ. The gold suspension is composed of two main size particles. The larger measure 10-20 mμ and the smaller are probably in the range of 1-3 mμ or less (sizes below the maximum resolving power of the scope).

Stability of the I^{131} Label During Storage of the Suspension at 5° C

Repeated tests for free I^{131} in the heated suspensions show that I^{131} remains firmly bound to the protein aggregates and less than 3.0 per cent is released after six weeks (Table I).

PHYSIOLOGICAL FACTORS

Particle Size and Organ Distribution

Dobson and Jones in their studies to determine the optimal particle size of colloidal agents for measuring liver blood flow (*6*) discovered that particle size was also a determining factor in organ distribution. Suspensions having a mean size of approximately 1 μ were optimal because at least 90 per cent of the injected dose is deposited in the liver and spleen and only a few per cent in other organs. They found that colloidal suspensions of extremely small particles gave relatively high bone marrow deposition whereas suspensions of particles larger than 1 μ gave appreciable lung deposition. Several studies of particle size in relation to the measurement of liver blood flow by the single injection colloid blood disappearance technique have indicated that liver extraction efficiency *and* the rate of blood disappearance are both related to particle size distribution even in the submicron range of 25-1000 mμ (*4,7,8,9*). The optimal size is $\sim$ 1000 mμ according to Dobson (*6*), however, other workers have obtained similar results with much smaller size suspensions (*10,11,12,13–16*). From a clinical view point which stresses the need for reproducible results in serial tests of the same patient, optimal size is not as important as *uniformity of size* within successive samples of the same test material (*4*). By contrast, for liver and spleen scanning, variations in particle size of the colloid *within the submicron range*, cause no apparent differences in liver-spleen visualization.

Recent studies in this laboratory were conducted to determine the optimal particle size for scanning the lungs. Radioalbumin suspensions ranging from submicron to 50 μ size were investigated. The results of these studies are to be reported separately in greater detail. However, it may be stated that colloidal suspensions of human serum radioalbumin composed of submicron sized particles are deposited almost entirely in the liver and spleen regardless of size within the range of 10-1000 mμ. However, as the mean size is increased above 1 μ the fraction temporarily retained in the lungs becomes progressively greater. Nearly all is so retained following I.V. injection of large size suspensions (mean $>$10 μ) and about half when the mean size is 2.5 μ. Finally, the pulmonary deposition and temporary retention of intravenously injected large size radioalbumin suspensions is caused mainly by mechanical factors and not by phagocytosis.

Liver Turnover of I^{131} Albumin Aggregates

Following I.V. injection of tracer doses ($<$ 0.2 mg/kg) of small radioalbumin aggregates, the particles disappear from the blood and accumulate in the liver's Kupffer cells at rapid rates. The half time blood disappearance and liver uptake rates are approximately 3 minutes. Peak activity is reached in the liver at about 15 minutes. It then diminished at a much slower rate, while total activity in the blood begins to rise as "free I^{131}" is released from the liver's Kupffer cells.[1] When the levels of radioactivity are monitored over the liver, the thyroid, and the side of the head following *tracer* doses of radioalbumin particles (with the thyroid previously blocked) the liver radioactivity reaches a peak in 15 minutes and descends to approxiamtely half this value by 40-60 minutes. Concurrently the blood and thyroid curves fall in parallel with

[1]Step 4 was added after the Montreal exhibit to eliminate particles $>$10 μ and to reduce the probability of capillary embolization.

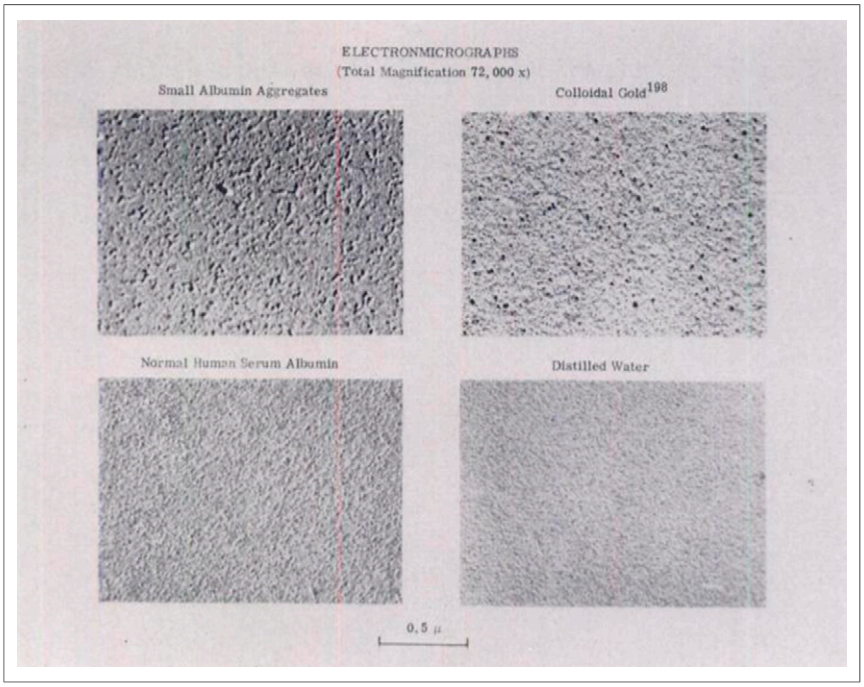

FIGURE 2.

each other, reach minimum values at about 15 minutes and rise to about half of their initial values in 40-60 minutes. Subsequently all three curves descend much more slowly in parallel fashion as determined by the rate of I^{131} urine excretion ($T_{1/2}$ = ~l0 hrs).

The half time of I^{131} discharge from the liver (corrected for "free I^{131}" re-entry into the blood) is approximately 20 minutes in normal subjects (*4*). The mechanism of I^{131} liver release is probably by proteolytic enzymatic digestion of the albumin particles in the Kupffer cells (*12*). Evidence for this hypothesis includes the detection of albumin degradation products such as I^{131} labeled tyrosine plus large and small I^{131} labeled peptides both in the plasma and the urine during the first few hours following injection (*12*) and of I^{131} in the bile. When the thyroid gland is blocked by prior ingestion of excess iodide nearly all of the injected I^{131} may be recovered in the urine within 72 hours, 50-75 per cent during the first 24 hours and little accumulates in the thyroid gland (*~2%)* (*4*).

Photoscanning Extrathyroidal Sites of Iodine Metabolism

The salivary glands and stomach are known to concentrate ionic iodide to levels 20-40 times greater than those in the plasma almost immediately following I.V. injection (*17*). The highest gastric concentrations of I^{131} are found (by radioautography) in the epithelial cells lining the surface of the gastric mucosa and in the crypts of the gastric glands and *not* in the chief or parietal cells (*17*). The cells of the salivary glands have the same capacity for concentrating iodide. The mechanism of the I^{131} concentrating capacity in the cells of the stomach and salivary glands is different from that in the thyroid because excess iodide ingestion largely prevents further thyroid uptake but does not interfere with this function in either the stomach or the salivary glands (*17*). In respect to gastric and salivary gland photoscans, the physical basis on which these organs may be visualized is the high I^{131} organ to plasma ratios attained. The high I^{131} level also present in the gastric juice may be a contributing factor. However, in our experience, the stomach is visualized equally as well during continuous gastric suction. Therefore, the controlling factor is the I^{131} concentrating power of the epithelial cells lining the stomach mucosa.

PHOTOSCANNING TECHNIQUES

The heart, liver and spleen, stomach and salivary glands may all be photoscanned in this sequence after I.V. injection of 200-300 μc doses of colloidal radioalbumin aggregates. During the first 10-20 minutes, tracer remains largely within the vascular system and the heart is readily visualized. After 20 minutes, high levels are attained in the liver and spleen. However, because of the rapid liver release of I^{131}, a loading dose (4 mg/kg) of unlabeled albumin aggregates is required to slow the I^{131} turnover in the liver. With such a loading dose given immediately prior to tracer injection, liver radioactivity levels usually remain sufficiently constant from 15-75 minutes post injection to permit accurate scanning. If

TABLE I
Dialyzable I^{131} in Heated vs. Unheated Radioalbumin Samples During Storage at 5° C

Storage Time When Tested	*Batch Number*	*Percent Dialyzable I^{131}*	
		*Unheated Samples**	*Heated Samples***
1 day	147	1.85	2.20
2 weeks	146	1.50	1.60
4 weeks	145	1.49	2.10
6 weeks	144	9.40	2.97
10 weeks	142	—	2.96

*Normal human serum albumin I^{131}, obtained from E. R. Squibb and Sons.
**Heated samples of same batches (20 minutes, 79° C) at pH 10.0.

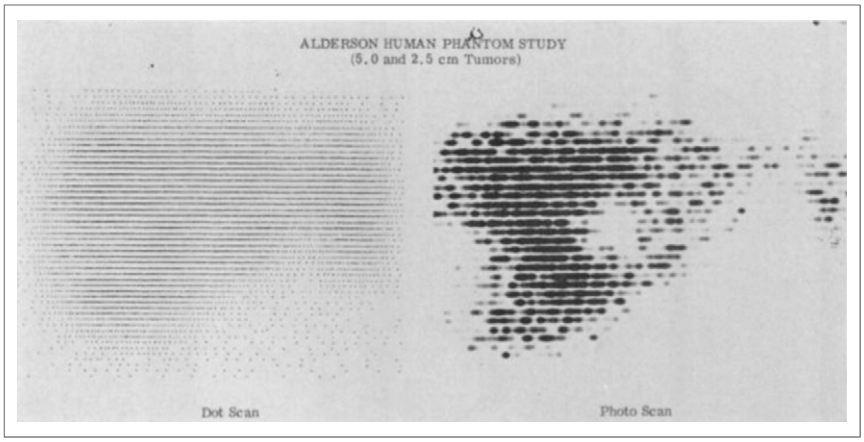

FIGURE 3.

these organs are re-scanned 3-5 hours later the entire stomach becomes visible because sufficient I^{131} re-enters the circulation and concentrates in the stomach. A continuing I^{131} cycling process takes place with maintenance of relatively high gastric I^{131} levels for at least 10 hours or until the I^{131} tracer is largely excreted in the urine. The stomach I^{131} levels remain high because of the concentrating capacity of the gastric epithelial cells and the inability of the stomach to absorb iodide. The cycling process is maintained by rapid I^{131} absorption from the duodenum.

In our experience the stomach is better visualized 3-5 hours after albumin I^{131} aggregates than after equal doses of inorganic iodide. A possible explanation is that albumin I^{131} is known to be concentrated in the stomach and slowly excreted into the gastric juice (*18*). The I^{131} label is then released rapidly by digestive action. Thus, with the colloidal radioalbumin test agent, I^{131} concentrates in gastric mucosa both in the free and protein bound forms. However, the free form is dominant. Only about 4 per cent of normal albumin is catabolized per day in the stomach (*18*).

RESULTS

Photoscans of Human Phantoms

The advantage of photo *vs* dot scanning of large organs is demonstrated with studies using the Alderson human abdominal phantom. The liver and spleen are filled with I^{131} solutions containing 150 μc in the liver and 7.5 μc in the spleen. Sixteen μc are added to the body of the phantom to simulate the background activity of surrounding organs and tissues. Radionegative tumors are much more difficult to localize than radiopositive ones unless they exceed 2.5 cm in diameter and are located close to the liver surface. Liver photo and dot scans presented in Figure 3 demonstrate that radionegative tumors located directly beneath the surface, measuring 5.0 and 2.5 cms diameter are readily visualized by photoscanning, but may be difficult to identify by dot scanning. The photoscan was made with high contrast to accent differences between maximum and minimum liver radioactivity levels, the dot scan was made to show the liver outline accurately plus surrounding tissue background. Simultaneous use of photo and dot scanning techniques in this manner, provides optimal data for diagnostic interpretation

Photoscanning Results in Man

The normal position, shape and size of the liver, all of which vary considerably from person to person are illustrated in Figure 4. The heart outline also may be demonstrated during the first 20 minutes post injection because sufficient tracer remains in the vascular system during this period.

Identification of Upper Abdominal Masses

The photoscan in Figure 5 is from a patient with acute severe upper intestinal bleeding. It was performed just after he was relieved of shock. A palpable epigastric mass was believed to be spleen by most examiners. However, no radioactivity accumulated in the region of the palpable tumor. Both the spleen[1] and liver are shown to be displaced laterally. At operation a pseudocyst of the pancreas was found to have eroded the stomach and caused the gastric bleeding. This is an example of how a photoscan provides useful diagnostic information in a clinical condition wherein routine radiographic examination is hazardous.[2]

Demonstration of Metastatic Liver Lesions

The composite of three photoscans shown in Figure 6 demonstrates the capacity of photoscans to identify areas of abnormal tissue in the liver. The first is from a patient with proved liver abscesses. The other two are from individuals with metastatic liver malignancy. The scan is useful to indicate the site where liver biopsy should be taken for histological examination and exact diagnosis.

Serial Photoscans in Hodgkins Disease

The three photoscans in Figure 7 are from the same patient, a 14 year old boy with Hodgkins disease. The initial scan performed 15-75 minutes following injection of albumin I^{131} aggregates, shows an enlarged liver and spleen. In the second scan, four hours later, the stomach appears superimposed upon the liver and spleen. The third scan after 7 hours demonstrates more radioactivity in the stomach and less in liver and spleen. These serial scans display the transposition of "free I^{131}" from the liver and spleen to the stomach, and demonstrate the proteolytic capacity of the RE cells.

Stomach and Salivary Glands

Stomach scans of nearly equal quality with the same dose (250 μc) of inorganic radioiodide and radioalbumin are demonstrated in Figure 8. In addition, the salivary glands may be visualized either immediately after sodium I^{131} injection or three to four hours following the same dose of colloidal radioalbumin aggregates. The parotid, the sublinguals and the thyroid glands are shown in Figure 9. The thyroid was previously blocked with excess iodide but is still visible. The salivary glands can and must be distinguished from thyroid metastases by their location, size and shape.

Experimental Lung Scans in Dogs

The lung scans illustrated in Figure 10 demonstrate that the dog lung fields are readily visualized immediately following I.V. injection of 95 μc of large radioalbumin suspensions (about 15 μ mean size).

[1]The term "free I^{131}" is used to indicate radioiodide no longer bound to albumin.

[2]The outline of the spleen was drawn from the dot scan made simultaneously. It was faintly recorded in the photoscan but lost during reproduction.

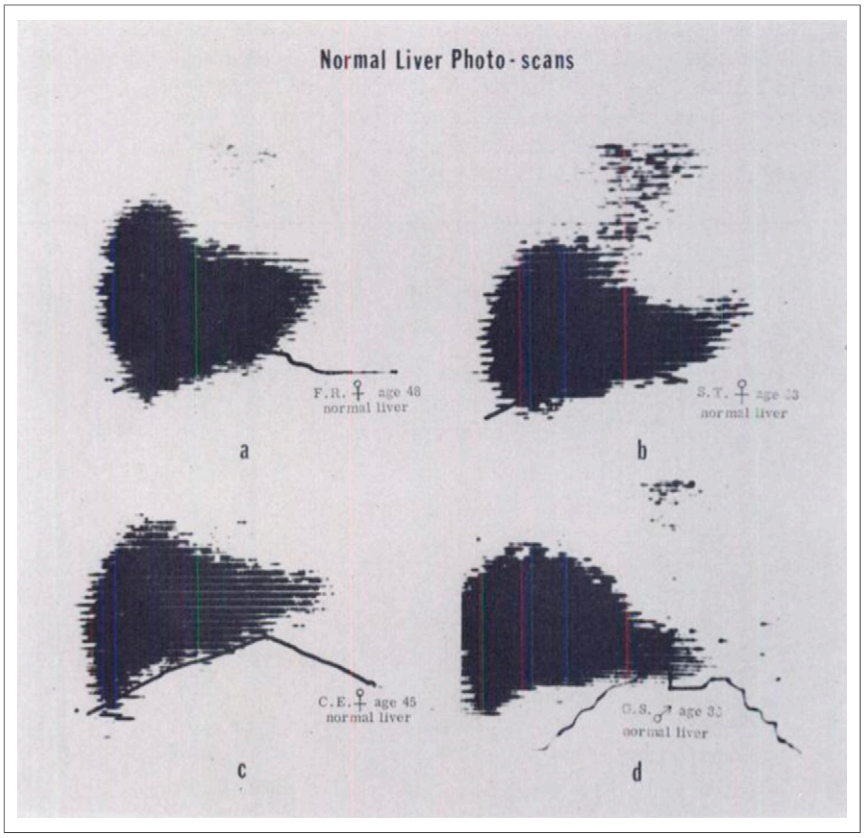

FIGURE 4.

Furthermore since the half time in the lungs is approximately one hour the scanning procedure may be repeated and in the second scan the liver area becomes visible.

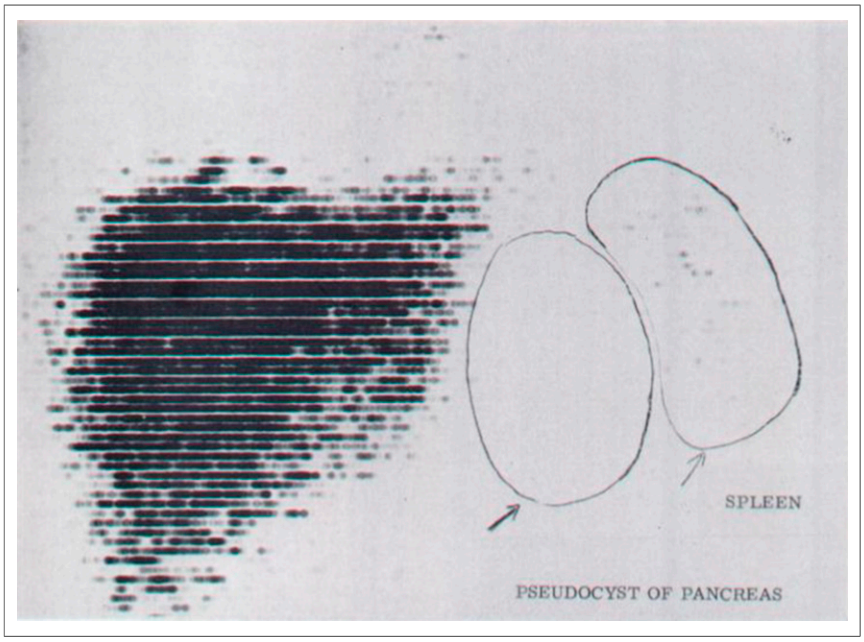

FIGURE 5.

Potential Hazard from Large Particle Injections

Preliminary toxicity experiments were conducted in 24 rabbits and an equal number of dogs to determine the doses in mg/kg of large particle albumin suspensions (mean size ~ 15 μ) which produce death or severe immediate reactions as opposed to those needed to perform lung scans in man (0.1-0.3 mg/kg. Repeated injections at doses below the toxic level were made to evaluate early cumulative effects and subsequent antigenic reactions. Similar studies in dogs were made using suspensions of the same material but of smaller size (~ 2.5 μ). Results indicate a relatively wide margin of safety in both animal species and for both particle size preparations. Scan doses are not *detectably* antigenic, whereas repeated large doses induce antibody formation. Single and repeated injections of both preparations in the dose range of 0.1-5.0 mg/kg produce no observable reactions in either species. Single injections of 5-20 mg/kg are well tolerated but repeated doses of 20 mg/kg produce immediate reactions in some animals. The LD_{50} dose probably exceeds 40 mg/kg for a single rapid injection and 20 mg/kg for repeated injections given every other day. The immediate reactions from massive doses (20-50 mg/kg) include sudden death with respiratory failure, laboured breathing, vomiting of mucus and/or blood, mucus and/or bloody diarrhea, and various central nervous system symptoms. Gross findings at autopsy after such reactions include multiple petechial hemorrhages and small infarcts in the lungs and similar but less extensive lesions in the brain and intestines. Most animals apparently make a complete recovery from their reactions to large doses within 24 hours and present no residual manifestations thereafter.

Twelve additional dogs have tolerated single and repeated doses of the smaller size suspensions (~2.5 μ) in the dose range of 15-30 mg/kg with far less frequency and severity of reaction. Thus the margin of safety is increased as the particle size is reduced. By comparison with other test agents, such as radiopaque media used for diagnostic purposes, the 2.5 μ size albumin suspensions appear to be practically innocuous. The dose which produces only occasional mild reactions (15 mg/kg) in dogs is at least 100 times greater than that required for lung scans in man (0.15 mg/kg).

Antigenicity of Human Serum Albumin Aggregates

Since the introduction of this material in 1956 for measurements of liver blood flow

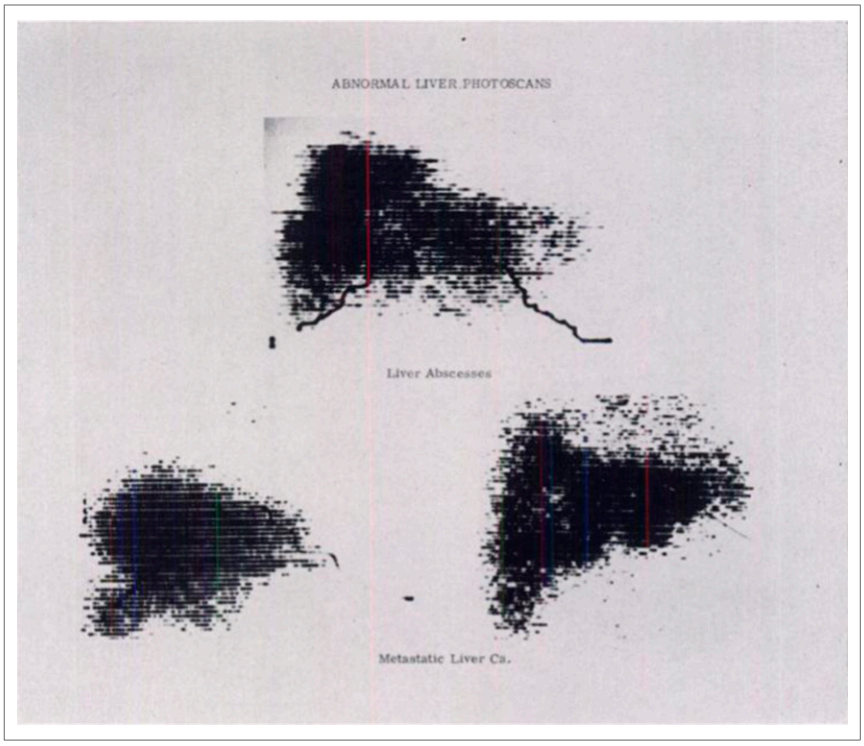

FIGURE 6.

and phagocytic function in man (*2,14*), and later for liver-spleen scanning (*1*) no serious antigenic reactions have been recorded (*1,4,9–11,14–17*). Our experience during the past three years with the same material (prepared by simplified methods of heat treatment and pH adjustment) has included single injections of tracer doses (< 0.2 mg/kg) in several hundred patients and single injections of tracer plus loading doses (4 mg/kg) in an additional 300 patients for scanning purposes without antigenic reactions. Furthermore no reactions were encountered in 50 patients receiving multiple injections (*3–10*) of tracer plus loading doses (*4*). None of these 50 patients developed detectable serum antibodies or positive skin reactions to the test agent (*4*). Therefore, human serum albumin when converted to particulate form by heat treatment and pH adjustment is not made antigenic to man. This lack of *detectable* antigenicity and absence of observable allergic reactions may be tentatively explained as follows: The albumin may be only physically changed into aggregates of *normal* albumin molecules which remain chemically unaltered (*4*). The amount of denaturation either chemical or physical may be insufficient to render the protein antigenic. Furthermore, both the normal molecular and heat aggregated forms of human serum albumin are equally weak antigens as compared with egg albumin, when injected repeatedly into *foreign* species such as the dog and rabbit.

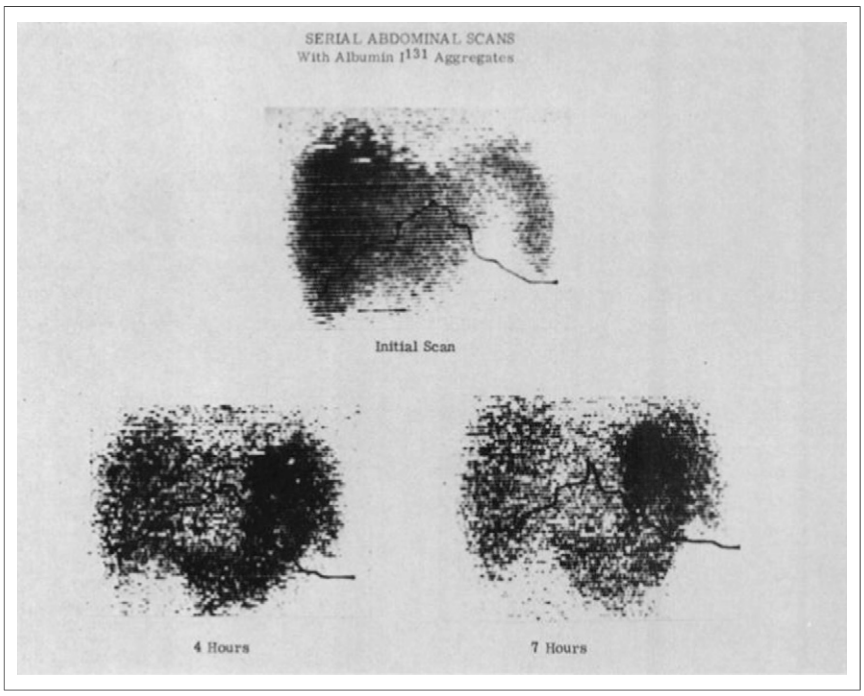

FIGURE 7.

DISCUSSION

Simplified Preparation of Small Size Albumin Suspensions

During recent studies with various techniques for preparing large size (1-5 μ) radioalbumin suspensions by heat treatment and pH adjustment, further simplification in the preparation of small size suspensions (10-20 mμ) was found feasible. The first heating period at 70° C is unnecessary. A single 20 minute heat treatment at 79° C gives an identical suspension in respect to particle size distribution, biological turnover rate and firmness of the I^{131} label. Furthermore the pH values during the heating process (10.0) and prior to use (7.5) *are not* as critical as previously indicated (*3*). In fact, 1 per cent human serum albumin solutions may be heated at pH values ranging from 9.0-11.0 and then readjusted to the 7.0-8.0 pH range. Measurements with a pH meter are unnecessary. Use of a pH paper color indicator is adequate. The end product retains the same characteristics just described.

Significance of Gastric and Salivary Gland Scans

The capability of scanning the stomach and salivary glands for several hours after intravenous injection of as little as 250 μc of sodium I^{131} or colloidal albumin I^{131} strongly suggests that the radiation exposure of these organs should be considered, especially following millicurie doses of radioiodide for therapeutic purposes. If excess iodide is ingested to block the thyroid, the relative radiation exposure to the stomach and salivary glands is increased

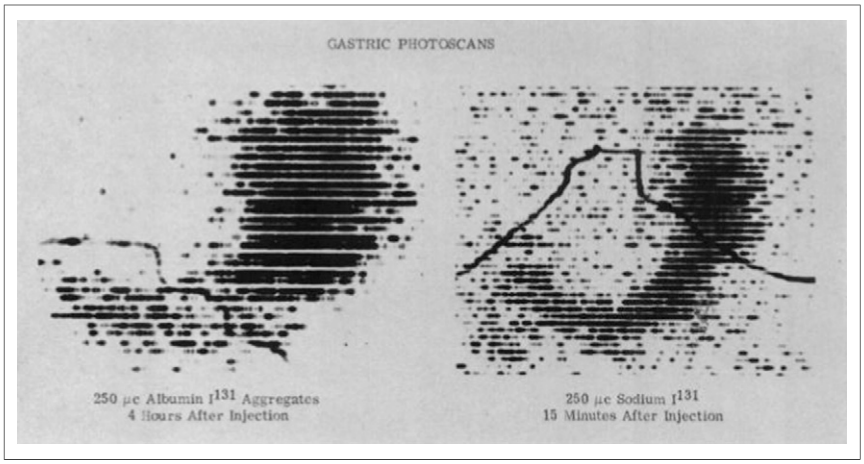

FIGURE 8.

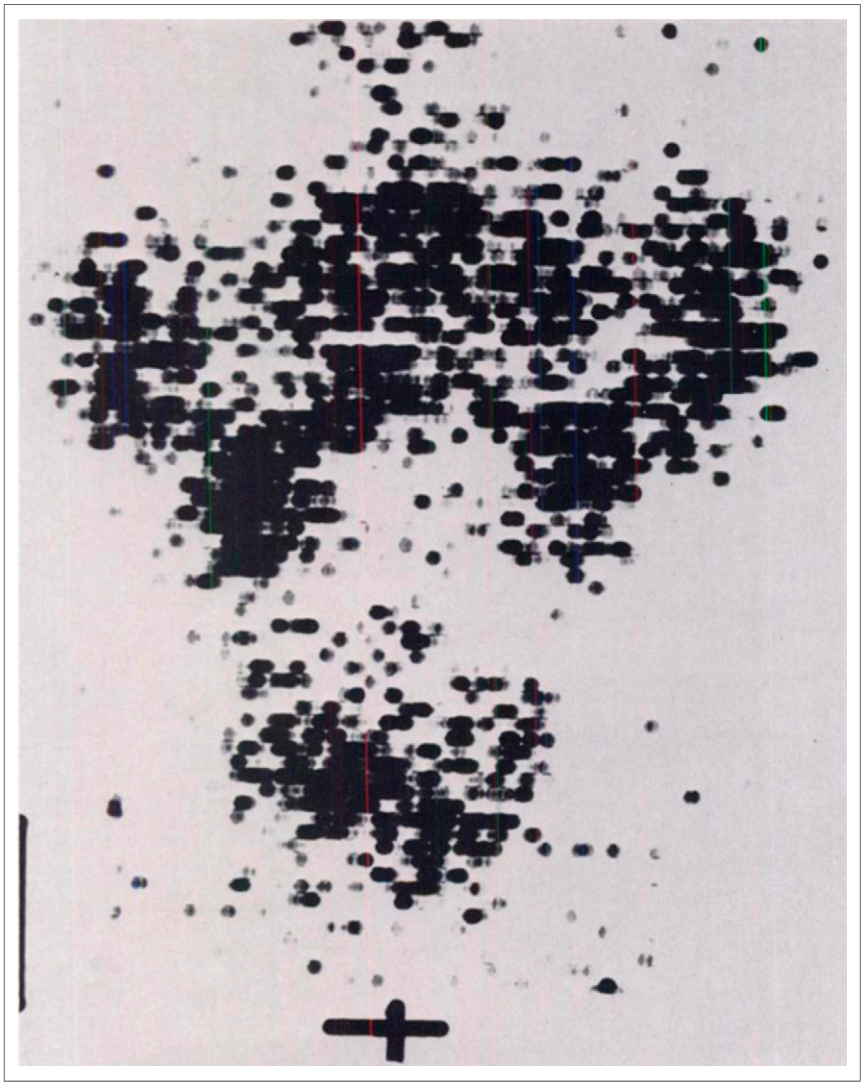

FIGURE 9. Photoscan of salivary glands and thyroid 3 hours after 250 μc NaI^{131}, I.V. (Thyroid blocked previously with excess iodide)

because their I^{131} concentrating capacity is not suppressed.

Liver and gastric photoscans were made in 12 gastric carcinoma patients in the hope of confirming the initial observations of Baptista (*19*) that some gastric neoplasms concentrate radioiodine far more than normal stomach mucosa. Should such tumors metastasize to the liver, the highly radioactive lesions would be more easily detectable by photoscanning than the usual radionegative metastatic liver tumors. No such cases were encountered. All twelve patients showed little or no radioactivity in the primary tumor site in comparison with the highly radioactive normal surounding gastric mucosa.

The liver turnover and gastric cycling of I^{131} following intravenous injection of colloidal albumin I^{131} (as demonstrated by serial abdominal scans) has considerable physiological significance. The rate of release of the I^{131} label from the albumin particles by the Kupffer cells and its subsequent appearance in the blood and stomach are indicators of the proteolytic function of these cells. It may be possible to determine disturbances of this function of the Kupffer cells in disease by measuring the rates of liver release, appearance in the blood, stomach and urine of free I^{131}.

Lung Scans with Large Albumin Aggregates (1-5 μ)

The intra arterial injection of radioactive ceramic microspheres (50-100 μ) is reported recently to have therapeutic value by confining the radiation within the tumor site (*20,21*). Dobson has indicated that particles larger than 1 μ are initially deposited in the lungs (*6*). Studies in this laboratory *(unpublished)* on the blood disappearance rates of submicron size colloids as measured with external detectors over the head and heart, demonstrate that a small fraction is retained in the lungs during the first few minutes. Current work in animals with suspensions of large albumin particles (2.5 and 15 μ) shows initial pulmonary retention, subsequent clearance from the lungs and transposition to the liver and spleen. The smaller the mean size of the suspension the faster the clearance from the lungs and subsequent appearance in the liver-spleen. Likewise, the incidence of capillary embolization in the lungs and other organs is greatly diminished as the mean size is reduced from 15 μ to about 2.5 μ and also as the number of large particles (> 25 μ) is decreased. In fact, individual particles larger than 5 μ are not required for lung scanning.

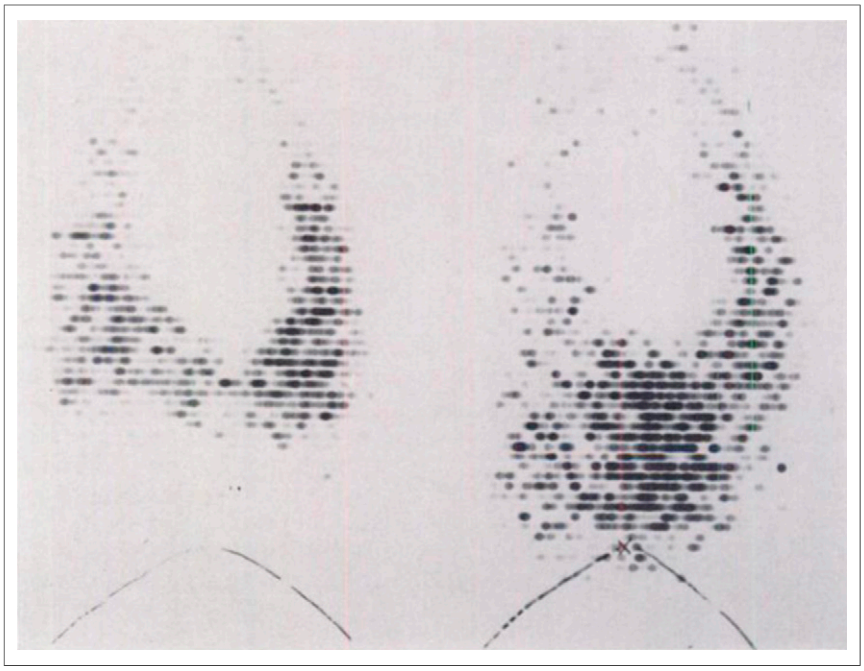

FIGURE 10. 25-55 Minutes Post Injection. 85-115 Minutes Post Injection. Dog Lung Photoscans (15 μ Mean Size Albumin I^{131} Aggregates).

The ideal preparation should have particles no larger than 10 μ or smaller than 1.0 μ and a mean size of 2.5 μ. Even with this material, clinical trials must await further animal investigation to ensure a wide margin of safety. Preliminary findings in this respect are most promising for the early initiation of clinical work with lung cancer patients.

SUMMARY

Current methods of preparing small and large size suspensions of radioalbumin aggregates by heat treatment and pH adjustment are presented. The small size colloidal suspensions (10-20 mμ) are shown to be superior to other agents for liver-spleen scanning. Radiation exposure to these organs is minimized and the albumin suspensions can be prepared with relative ease and simplicity. Also the heart, stomach and salivary glands may be clearly visualized by photoscanning with this material. The techniques and physiological basis for performing and interpretating scans of these organs are described. Their diagnostic value is illustrated.

The preparation and physiological basis for using large size suspensions of the same material for visualization of the lungs is presented along with scans typical of those produced with suspensions of each of two size ranges (2.5 and 15 μ). The smaller size is preferable because of far less probability of capillary embolization. Trials in patients with lung cancer will be initiated after additional animal studies ensure the complete safety of the test procedure.

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ADDENDUM

Since this paper was submitted for publication, 50 patients with a variety of lung diseases have been scanned. The optimal mean size of radioalbumin aggregates for lung scanning in man is $\sim$ 5.0 μ with a range of 1-20 μ. The main value of lung scans in clinical practice is in the early detection of pulmonary emboli as localized areas of impaired pulmonary arterial blood supply. Similar findings are observed in the pneumonias, lung abscesses, cysts and tumors wherein the arterial component of the pulmonary circulation is affected. No reactions to the test material have been encountered.

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