Pathologic Changes in the Lungs of Mice Following Injection of Human Albumin Microspheres

J. Szymendera, Olga Mioduszewska, Iwona Licinska, Alina Czarnomska, and Barbara Lucka

Curie Memorial Institute of Oncology and Institute of Drugs, Warsaw, Poland

Mice injected with ^{131}I-human albumin microspheres equivalent to 10–20 or 200 human doses were sequentially killed over a 12-day period. About 90% of the microspheres initially lodged in the precapillary arterioles and capillaries of the lungs. Their pulmonary clearance was essentially complete after 3 days. Occlusion of the vessels always led to focal hyperemia of the surrounding tissue and to slight hemorrhage into the alveoli. This was followed, though less frequently, by perivascular nodular inflammation. Hemorrhagic infarcts were quite uncommon and occurred only after the massive doses. Some emboli underwent organization, but most were resolved. Circulatory disturbances and perivascular inflammation receded in about 1 week and seldom led to obliteration of the involved vessels. Hemorrhagic infarcts were converted into minute scars. Twelve days after injection of microspheres in massive doses, the only findings were post-infarct scars and obliterated vessels, which were sparse and difficult to detect. The lower doses of microspheres did not leave any detectable residues.

J Nucl Med 18: 478–482, 1977

Pulmonary perfusion agents can be divided into two categories: the hydrated iron hydroxides and the albumin-based agents (*1*). In the former category, aggregates containing ferrous hydroxide do not show long-term toxicity (*2,3*) whereas those containing ferric hydroxide produce significant toxic changes (*4,5*).

We still lack a more detailed sequential histopathologic examination of lung tissue following injection of human albumin microspheres. Only two cursory microscopic studies showed moderate-to-severe lung changes: either infarction and hemorrhage shortly after the injection (*4*), or subsequent granulomatous inflammation (*6*). Serial microscopic studies of tissue samples after microsphere injection have not been published (*7*).

An investigation using mice was begun, aimed at following the fate of albumin microspheres in the lungs by serial histologic studies and characterizing the ensuing pulmonary sequelae.

MATERIALS AND METHODS

Sterile microspheres were made from human serum ^{131}I-albumin by a method similar to Zolle et al. (*8*).* After hydration the diameter ranged from 20 to 50 μm, with a mean of 35 μm, and numbered about 50,000/mg. The microspheres were suspended by ultrasonification before injection.

Lung retention and histologic studies in mice were performed according to two experimental groups on 23–26-gm RIII/W females, a strain known to be resistant to chronic respiratory disease. In the first group, each mouse was injected through the tail vein with 0.01–0.02 mg of microspheres in 0.1 ml of solution, and the animals were killed in groups of two at 5 hr and 1, 2, 3, 7, and 12 days after injection. In the second group, each mouse was injected with 0.20–0.22 mg of microspheres in 0.2 ml of solution and the whole-body radioactivity counted immediately; this activity was taken to represent the dose. The animals were killed in groups of four at 15 min, 2, 5, 21, and 24 hr, and 2, 3, 6,

Received Aug. 3, 1976; revision accepted Dec. 7, 1976.

For reprints contact: J. Szymendera, Institute of Oncology, P.O. Box 47, 00-973 Warsaw, Poland.

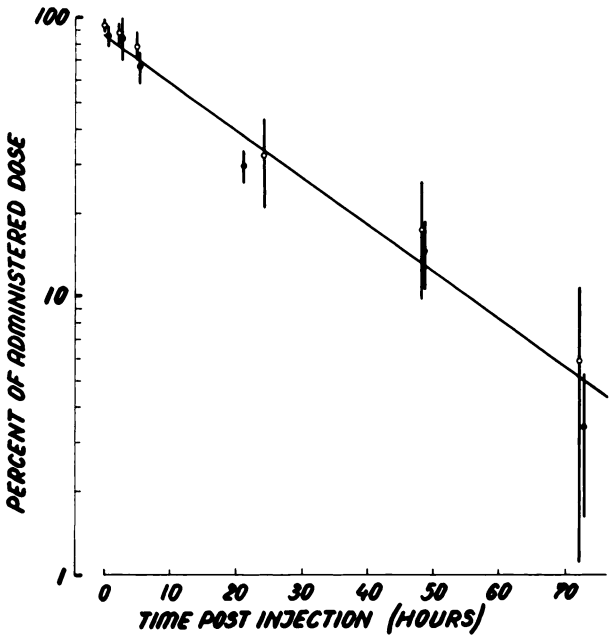

FIG. 1. Time course of ^{131}I retention in lungs after injection of microspheres in massive doses. Open circles, OPiDI preparation; solid circles, Sorin preparation. Bars represent 95% confidence limits for means.

7, and 12 days after injection. They were bled by cardiac puncture under light ether anesthesia, a procedure that did not bring about any pathologic changes in the lungs. A lung–heart block specimen was removed and fixed in 10% formalin. The activities of the preceding preparation and residual carcass were measured with a 3 × 3-in. NaI(Tl) crystal connected to a single-channel analyzer and scaler. All data, corrected for decay, were expressed as percentages of the injected dose of ^{131}I. Four mice acting as controls for histologic studies were killed on Day 1.

For histologic examination the lung–heart preparation fixed in formalin was processed and embedded in paraffin. Sections were cut across the largest frontal diameter of the lungs at thicknesses of 6 μm and were stained (A) with hematoxylin and eosin; (B) with orcein-D for the elastic fibers of the pulmonary arteries; (C) with Gram–Weigert's stain for fibrin; and (D) with Gomori's stain for reticulum fibers (9). The diseased area of the lung was also measured with a planimeter and expressed as a percentage of the corresponding cross-sectional area of the lung.

RESULTS

Mice were given either 19,000 to 43,000 microspheres (0.38–0.87 mg) per kilogram body weight, or 380,000–480,000 (7.7–9.6 mg)/kg. Figure 1 shows the time course of the ^{131}I activity retained in the lungs after massive doses of microspheres. Fifteen minutes after injection of the Sorin and OPiDI preparations, the activities in the lungs were, respectively, 86.4 ± 1.69% (mean ± s.e.) and 93.1 ± 1.27% of the dose. The subsequent clearing of the activity was exponential and essentially complete after 3 days. Activity retention of both preparations could be fitted with an expression $A_t = 87.3\ e^{-0.0394t}$.

Histologic sections of the lungs showed abnormalities attributable to the microspheres: emboli lodged in precapillary arterioles and capillaries, accompanied by circulatory disturbances and inflammatory reactions. With the lower doses, the lung area involved increased to approximately 10% of the total section at Day 2, declined to an essentially negligible 2% at Day 7, and was unnoticeable at Day 12 after injection. With the massive doses, the involved area increased significantly during the first 2 days, reaching 46% at Day 2; it then declined to under half of that value by the end of a week and became essentially negligible at Day 12 (Fig. 2). These data, based on measurements of the areas at the largest diameter of both lungs, can be treated only as rough estimates.

The microspheres filled lumina of capillaries or precapillary arterioles, occluding them totally and sometimes even distending their walls (Fig. 3.1). Larger vessels were not affected. A few hours after injection, the occluded vessels responded with an inflammatory reaction. Its early stage reached a maximum of 3 days (Fig. 3.2), characterized by exudation of fibrin and infiltration of lymphocytes around the plugged vessels. At Day 6 the inflammatory reaction entered either (A) the proliferative stage, characterized by development of connective tissue rich in reticular fibers and leading to obliteration of some vessels (Fig. 3.3), or (B) a stage in which macrophages invaded, leading to complete reabsorption of the inflammatory nodules (Fig. 3.4). At the same time the microspheres were partially dissolved and the plugged vessels retrieved patency (Fig. 3.5). Reabsorption of the inflammatory nodules and dissolution of the emboli reached their maxima 6–7 days after injection, leading to almost complete clearing of the lungs.

Complete occlusion of the vessels was followed by focal hyperemia of the surrounding tissue, hemorrhage into the alveoli, infarction, and edema (Figs. 3.6–3.9). Hyperemia (Fig. 3.6) and slight hemorrhage could be found in all mice and lasted approximately 1 week. Subpleural hemorrhagic infarcts appeared in few animals and only after the massive doses (Fig. 3.7). Six to seven days after injection,

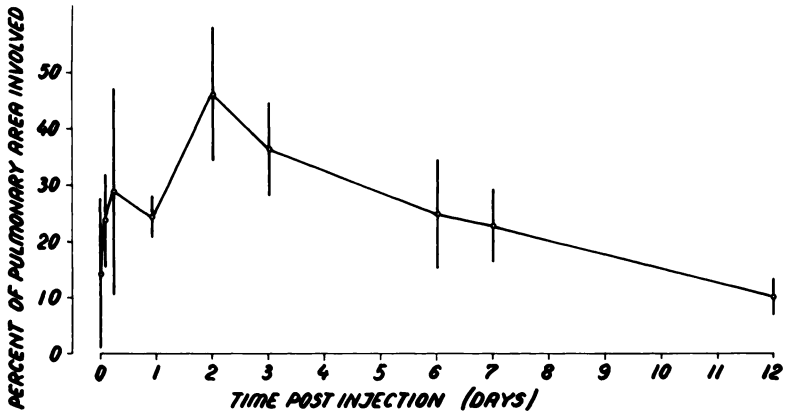

FIG. 2. Time course for lung area involved by disease processses after injection of microspheres in massive doses. Bars represent 95% confidence limits for means.

connective tissue abundant in reticular fibers appeared in the infarcts (Fig. 3.8).

During the first 3 days, interstitial edema could be observed. It was characterized by exudate in interalveolar septa accompanied by slight atelectasis and infiltration of leucocytes. By approximately Day 3, this process was replaced by intra-aveolar edema: exudation into the lumina of the alveoli lasting through 6 to 7 days after injection. Edema was not a separate process but accompanied only the more massive hemorrhages (Fig. 3.9).

At 3 to 7 days bronchogenic pneumonia appeared adjacent to the areas involved by the hemorrhage and edema (Fig. 3.10) and, if the primary lesions were larger, secondary emphysema could occasionally be found (Fig. 3.11).

At Day 12 only faint postinfarct scars and obliteration of precapillary arterioles could be seen. Such sequelae were extremely rare and difficult to detect even after the more massive doses of microspheres. The pulmonary embolism never produced thrombotic reactions.

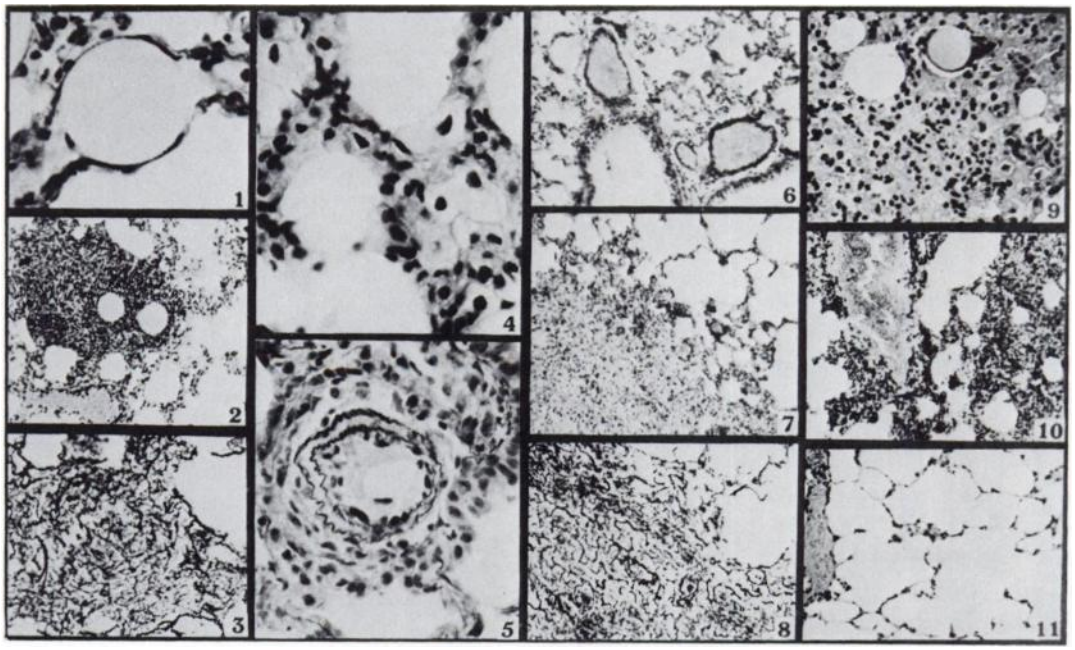

FIG. 3. Photomicrographs of lung sections from mice after microsphere injection. (1) Microsphere distending walls of precapillary arteriole, ×243. (2) Early stage of inflammation: lymphocytic infiltrates surround occluded arterioles, ×54. (3) Late stage of inflammation: nodular proliferation of reticular fibers, ×92. (4) Agglomeration of macrophages in nodule, ×243. (5) Central dissolution of microsphere within artery and proliferation of intima cells, ×243. (6) Distension of arterioles and thickening of interalveolar septa following capillary hyperemia, ×108. (7) Hemorrhagic infarct, ×108. (8) Same preparation showing proliferation of reticular fibers, ×108. (9) Intra-alveolar hemorrhage and edema, ×162. (10) Bronchogenic pneumonia, ×108; (11) Emphysema, ×108. Stains: orcein-D for elastic fibers (1,5), hematoxylin and eosin (2,4,6,7,9–11), and Gomori's stain for reticulum (3,8). (All magnifications photographically reduced by 50%.)

DISCUSSION

The rate of pulmonary clearance of albumin microspheres is related mainly to their "hardness," which depends on the production procedure (*4,8,10,11*). Although the rate of disappearance of the radiolabel from the lung cannot be treated as a measure of biodegradation of the microspheres (*6,7,12,13*), it can at least be assumed to be an indication of it. In this study, deposition of the ^{131}I-microspheres in the lungs of mice was within the range reported previously (*4,6*) and their pulmonary clearance, determined by whole-organ radioactivity analysis, was within the time frame permissible for human studies (*13*).

The doses administered to the mice ranged from 0.38–0.87 to 7.7–9.6 mg/kg of body weight [i.e., 10–20 or 180–220 times the equivalent doses for patients (*14,15*)]. The number of particles in a dose was approximately 500–1,000 or 10,000 per 23–26-gm mouse [i.e., at most one-third of that previously considered as the minimum toxic amount (*4,10*)]. Despite the relatively small number of microspheres, we found a series of pathologic changes similar to those found by Rhodes et al. (*4*) and Bolles et al. (*6*).

Depending on size, the microspheres plugged pulmonary capillaries and precapillary arterioles, leaving larger vessels (distribution arteries and distribution artery connectors) (*1*) unaffected. The plugged vessels responded to occlusion with an inflammatory nodular reaction: perivascular exudation of fibrin and lymphocytic infiltration, similar to previous reports (*6*). The majority of the inflammatory nodules were reabsorbed by macrophages, and only a few led to obliteration of the vessels. The microspheres, moreover, were dissolved and the occluded vessels retrieved patency. These pathologic changes were conspicuously milder than those observed after embolism by ferric hydroxide macroaggregate (*5*).

Complete occlusion of the vessels always led to focal hyperemia of the surrounding tissue and slight alveolar hemorrhage. Hemorrhagic infarcts, accompanied by edema (initially interstitial and after 3 days intra-alveolar) were rare and only after massive doses and underwent rapid healing that resulted in faint scars. These lesions were different from those following injection of ferric hydroxide macroaggregate, where hemorrhagic infarcts were numerous and more severe (*5*).

Thus, pathologic changes in the lungs following microsphere injection were in general similar, irrespective of the number of the particles, i.e., the difference was predominantly one of degree rather than of a different type of pathologic process. Though changes after the massive doses were more extensive, they healed in about 1 week and seldom left obliteration of a few of the precapillary arterioles and capillaries involved. The only qualitatively different changes, observed solely after massive doses, were the subpleural hemorrhagic infarcts, occasionally accompanied by edema and secondary emphysema. These infarcts healed relatively quickly, leaving minute postinfarct scars and such lesions probably do little, if any, harm to the patients examined. Hence, human albumin microspheres can be considered safe for pulmonary scanning.

The radiopharmaceutical results in some slight toxicity, but it is of short duration and small extension. This conclusion agrees with the results reported by Burdine et al. (*11*) who found only occasional blood constituents within pulmonary alveoli in about 11% of 28 dogs killed at 4 hr, 1 day, and 1 week after injection of microspheres in an amount not exceeding 15 times the equivalent doses for patients.

ACKNOWLEDGMENTS

This work was supported by research grant PR-6/0209 from the Polish National Cancer Program. The authors thank Jan Steffen and Raymond L. Hayes for critical review of the manuscript.

FOOTNOTE

* Available from Sorin, Italy, or the Radioisotope Production and Distribution Center (OPiDI), Poland.

REFERENCES

1. DAVIS MA: Particulate radiopharmaceuticals for pulmonary studies. In *Radiopharmaceuticals*, Subramanian G, Rhodes BA, Cooper JF, Sodd VJ, eds. New York, Society of Nuclear Medicine, 1975, pp 267–281

2. WATTS RS, SOPHER RL, PENA HG: Iron hydroxide particle retention in primate lungs. *J Nucl Med* 15: 616–619, 1974

3. DAVIS MA, KING MF: Effects of capillary blockade with ^{99m}Tc-iron hydroxide: A histopathologic study. *J Nucl Med* 15: 436–438, 1974

4. RHODES BA, ZOLLE I, BUCHANAN JW, et al.: Radioactive albumin microspheres for studies of the pulmonary circulation. *Radiology* 92: 1453–1460, 1969

5. SZYMENDERA J, MIODUSZEWSKA O, LICINSKA I, et al.: Fate of ferric hydroxide macroaggregate in the lungs of mice. *J Nucl Med* 15: 17–21, 1974

6. BOLLES TF, KUBIATOWICZ DO, EVANS RL, et al.: ^{99m}Tc-labelled albumin (human) microspheres (15–30 μm). Their preparation, properties and uses. In *Radiopharmaceuticals and Labelled Compounds*, vol 1. Vienna, IAEA, 1973, pp 151–167

7. RHODES BA, BOLLES TF: Albumin microspheres: Current methods of preparation and use. In *Radiopharmaceuticals*, Subramanian G, Rhodes BA, Cooper JF, Sodd VJ, eds. New York, Society of Nuclear Medicine, 1975, pp 282–291

8. ZOLLE I, RHODES BA, WAGNER HN: Preparation of metabolizable radioactive human serum albumin microspheres for studies of the circulation. *Int J Appl Radiat Isot* 21: 155–167, 1970

9. Manual of Histologic and Special Staining Technics, 2nd ed. New York, McGraw-Hill, 1960

10. WAGNER HN, STERN HS, RHODES BA, et al.: Design and development of new radiopharmaceuticals. In *Medical Radioisotope Scintigraphy*, vol 2. Vienna, IAEA, 1969, pp 3–24

11. BURDINE JA, SONNEMAKER RE, RYDER LA, et al.: Perfusion studies with technetium-99m human albumin microspheres (HAM). *Radiology* 95: 101–107, 1970

12. BURDINE JA, RYDER LA, SONNEMAKER RE, et al.: ^{99m}Tc-human albumin microspheres (HAM) for lung imaging. *J Nucl Med* 12: 127–130, 1971

13. RHODES BA, STERN HS, BUCHANAN JA, et al.: Lung scanning with ^{99m}Tc-microspheres. *Radiology* 99: 613–621, 1971

14. BUCHANAN JW, RHODES BA, WAGNER HN: Labeling albumin microspheres with ^{113m}In. *J Nucl Med* 10: 487–490, 1969

15. HECK LL, DULEY JW: Statistical considerations in lung imaging with ^{99m}Tc-albumin particles. *Radiology* 113: 675–679, 1974

Accepted Articles To Appear in Upcoming Issues

Rapid, Rigorous Computation of Modulation Transfer Function on a Pocket Calculator. Accepted 11/11/76.
Peter M. Ronai and Dennis L. Kirch

The Scintigraphic Investigation of Sacroiliac Disease. Accepted 12/23/76.
B. C. Lentle, A. S. Russell, J. S. Percy, and F. I. Jackson

Technetium-99m-Phytate as a Bone-Marrow Imaging Agent: Biodistribution Studies in Animals (Concise Communication). Accepted 12/23/76.
Robert G. Hamilton, Philip O. Alderson, and Patricia A. McIntyre

The Scintigraphic Findings in Ankylosing Spondylitis. Accepted 12/23/76.
B. C. Lentle, A. S. Russell, J. S. Percy, and F. I. Jackson

Comparison of Different Noninvasive Methods of Infarct Sizing During Experimental Myocardial Infarction. Accepted 12/28/76.
Lawrence R. Poliner, L. Maximilian Buja, Robert W. Parkey, Ernest M. Stokely, Marvin J. Stone, Robert Harris, Shelly W. Saffer, Gordon H. Templeton, Frederick J. Bonte, and James T. Willerson

Gallium-68-Labeled Red Cells and Platelets: New Agents for Position Tomography. Accepted 12/28/76.
Michael J. Welch, Mathew L. Thakur, R. Edward Coleman, Mohan Patel, Barry A. Siegel, and Michel M. Ter-Pogossian

Gastroesophageal Reflux: A Potential Source of Confusion in Technetium Thyroid Scanning (Case Report). Accepted 1/5/77.
Michael Grossman

Comparison of Ga-67-Citrate Images Obtained with Rectilinear Scanner and Large-Field Anger Camera. Accepted 1/6/77.
Paul B. Hoffer, Robert Schor, Dillu Ashby, Charles Metz, Robert Hattner, Hirsch Handmaker, David C. Price, David M. Shames, David Lilien, and Chun B. Lim

The Role of Ga-67-Citrate Imaging and Diagnostic Ultrasound in Patients with Suspected Abdominal Abscesses. Accepted 1/13/77.
Bharath Kumar, Philip O. Alderson, and Guillermo Geisse

Segmental Analysis of Tl-201 Stress Myocardial Scintigraphy. Accepted 1/21/77.
Andre Lenaers, Pierre Block, Eddy van Thiel, Monique Lebedelle, Paul Becquevort, François Erbsman, and Andre M. Ermans

Quantification of Flow in a Dynamic Phantom Using ^{81}Rb-^{81m}Kr, and a NaI Detector. Accepted 1/21/77.
John D. Idoine, B. Leonard Holman, Alun G. Jones, Robert J. Schneider, Kathleen L. Schroeder, and Robert E. Zimmerman

Location of the Site of Bacterial Bile-Salt Deconjugation by Combining Abdominal Scintigraphy with Expired C-14. Accepted 1/21/77.
B. I. Hirschowitz, R. Beschi, H. Bondi, J. Goel, and W. N. Tauxe

Localization of ^{99m}Tc-Pyrophosphate in the Liver Due to Massive Liver Necrosis. Accepted 2/1/77.
Kenneth P. Lyons, John Kuperus, and Hilliard W. Green

Hepatobiliary Radiopharmaceuticals: Defining Their Clinical Role Will Be a Galling Experience (Editorial). Accepted 2/4/77.
Peter M. Ronai

Radionuclide Angiography, Brain and Bone Imaging in Craniofacial Fibrous Dysplasia (CFD) (Case Report). Accepted 1/13/77.
P. M. Fitzer

Bone Scanning in Osteolytic Paget's Disease. (Case Report). Accepted 1/28/77.
James M. Rausch, Donald Resnick, Thomas G. Goergen, and Andrew Taylor

Myocardial Imaging with Tc-99m-Pyrophosphate in Patients on Adriamycin Treatment for Neoplasia. Accepted 1/28/77.
Anna K. Chacko, David H. Gordon, John M. Bennett, Robert E. O'Mara, and George A. Wilson

Thyroid Hormones and Glucose (C-14) Metabolism in Bacteria. Accepted 2/1/77.
K. T. Singh, R. D. Ganatra, M. S. Shanta, Y. S. Nimbkar, and B. V. Gaitonde

Pancreatic Scanning Using Retrograde Injection of Technetium-99m-Labeled Compounds. Accepted 2/4/77.
Paul F. Varley, Stephen E. Silvis, and Rex B. Shafer

A Qualitative Method for Determining the Level of Oxidant in a Solution of Pertechnetate (Tc-99m). (Letter to the Editor). Accepted 2/4/77.
M. W. Billinghurst and S. Rempel

An Automated Cerebral Blood-Flow Analyzer (Concise Communication). Accepted 2/5/77.
Robert E. Anderson and Thoralf M. Sundt, Jr.

Phantom Kidney in Technetium-99m-DTPA Studies of Renal Blood Flow (Case Report). Accepted 2/9/77.
Edwin R. Holmes, III, William C. Klingensmith, III, Peter T. Kirchner, and Henry N. Wagner, Jr.

Comparison of 19-Iodocholesterol and 6-Iodomethylnorcholesterol as Adrenal-Scanning Agents. Accepted 2/10/77.
Margaret W. Couch and Clyde M. Williams

Detection of Prethrombotic or Thrombotic States (Letter to the Editor). Accepted 2/11/77.
Kenneth Alonso

Uptake of 99mTc-Diphosphonate in a Massively Calcified Mitral Annulus (Case Report). Accepted 2/14/77.
David A. Epstein

The Diagnosis of Splenic Injury Using 99mTc-Red Blood Cells (Letter to the Editor). Accepted 2/14/77.
John Selby and Nihal S. Gooneratne