CELLULAR NECROSIS MODEL IN TISSUE CULTURE: UPTAKE OF ^{99m}Tc-TETRACYCLINE AND THE PERTECHNETATE ION

Mrinal K. Dewanjee and Eileen W. Prince

Harvard Medical School, Boston, Massachusetts

The recent development of ^{99m}Tc-tetracycline in our laboratory and its successful use in delineating myocardial infarcts have encouraged further investigation into the mechanism of its uptake and its localization on a subcellular level. The affinity of ^{99m}Tc for cells and subcellular constituents when incubated with ^{99m}Tc-tetracycline was evaluated in our recently developed necrosis model of tissue culture. Live cells obtained from exponentially growing cultures or dead cells obtained from the media of nutritionally depleted plateau-phase cultures were incubated with ^{99m}Tc-tetracycline at 37°C for various times. After incubation, the cells were washed and the total cellular activity as well as the subcellular localization of ^{99m}Tc determined. About 60 times more ^{99m}Tc remained associated with the dead cells than with the live cells. This affinity was not seen when the dead cells were incubated with the pertechnetate ion. On the subcellular level, at least 50% of the ^{99m}Tc appeared to be associated with the DNA and protein fractions of the cell. This system may provide a rapid and economical means of screening radioactive compounds currently being prepared to detect necrotic tissue.

The fluorescence of tetracycline and its analogs in necrotic myocardial, pleural, and renal tissue, whether due to ischemia or neoplasia, has been observed by previous investigators (*1–6*). The mechanism of action of tetracycline in bacteria (*7–14*) as well as its localization within the bacterial cell (*15–17*) has been well documented also. Yet, little information is available concerning the action and localization of this compound in mammalian cells (*18*).

The recent development of ^{99m}Tc-labeled tetracycline in our laboratory (*19*) and its successful use in delineating myocardial infarcts in dogs (*20*) generated the development of an in vitro cellular model in culture to investigate the uptake and localization of ^{99m}Tc-tetracycline in both living and dead cells.

Because many pathological lesions (whether they are caused by infarction or carcinoma) often involve necrosis at some stage, this study has been designed to aid in understanding the mechanism of uptake of certain ^{99m}Tc-labeled compounds and to evaluate the potential of this model for the screening of other radiopharmaceuticals.

MATERIALS AND METHODS

Chang liver cells (Lich) were grown in monolayer culture at 37°C in an atmosphere of 5% CO_2 in 75 cm^2 plastic flasks using a modified Eagle's basal media (MEM) (*21*) and supplemented with 10% heat-inactivated calf serum. The cultures were divided into two groups: live cells, or those maintained in exponential growth, and dead cells, or those harvested from the media of plateau-phase cultures whose media had not been changed for 5 days prior to this experiment.

Cell labeling. The live cells were labeled by removing the growth media from exponentially growing cultures and replacing it with 10 ml of media, pH 7.3, containing 10 μCi of ^{99m}Tc-pertechnetate or ^{99m}Tc-tetracycline in isotonic saline. After incubation at 37°C for 15, 30, or 60 min with the radioactive media, the media was removed and a 3-ml aliquot was retained for later counting. The cells were then washed twice with calcium-free salts (CFS) and removed from the surface of the flask by exposure to a 0.25% trypsin solution for 2 min. Aliquots of the salt solution and trypsin were retained for counting. After trypsinization, the cells

Received Oct. 19, 1973; revision accepted Jan. 31, 1974.

For reprints contact: Eileen W. Prince, Joint Program in Nuclear Medicine, 50 Binney St., Boston, Mass. 02115.

were resuspended in CFS and counted; the activity per million cells was determined using a gamma scintillation counter with a NaI(Tl) crystal.

The dead cells were removed from the culture medium by centrifugation at 1,000 rpm for 5 min. They were then resuspended in 10 ml of MEM containing 10 μCi of ^{99m}Tc-pertechnetate or ^{99m}Tc-tetracycline in isotonic saline and incubated at 37°C for 15, 30, or 60 min. After incubation, the cells were removed from the radioactive media by centrifugation and a 3-ml aliquot of the supernatant was retained for counting. The cells were washed twice with CFS and 3-ml aliquots of the supernatant were retained for determination of the unbound activity. The cells were counted and the amount of radioactivity per million cells was calculated as described previously.

Determination of activity distribution at subcellular levels. A Schmidt-Thannhauser procedure (*22*) with the following modifications was used to separate the acid-soluble and acid-insoluble cell fractions. Fractions of labeled, washed live or dead cells were centrifuged and the supernatant was removed. Three milliliters of freshly prepared, cold 13.3% trichloroacetic acid (TCA) was added to the cell pellet, vortexed for 1 min, and incubated at 4°C for 15 min. The cells were then centrifuged for 3 min at 2,000 rpm and the procedure repeated twice with incubation periods of 3 min each. The supernatant contained the acid-soluble fraction. Four milliliters of 95% ethyl alcohol saturated with sodium acetate were added to the remaining precipitate. After vortexing for 1 min, the tube was incubated for 3 min at 4°C and centrifuged; the supernatant containing the lipid-soluble fraction was removed. To hydrolyze the RNA, 1 ml of 0.3 *N* KOH solution was added to the precipitate. The mixture was incubated at 37°C for 1 hr with vortexing every 15 min. After this incubation period, 1 ml of 20% cold TCA was added to the above mixture which was then centrifuged at 2,000 rpm for 3 min. The supernatant contained the activity associated with the RNA fraction. The remaining precipitate contained the activity associated with the DNA and protein fractions. The precipitate was suspended in 1 ml of 5% TCA. This was followed by incubation at 90–95°C for 30 min with vortexing every 10 min to bring the DNA into solution. The protein precipitate was removed by centrifugation and washed with 5% TCA solution to free it of any contaminating DNA solution. The activities in the different subcellular fractions were determined with gamma NaI(Tl) well detector.

TABLE 1. LABELING OF LIVE AND DEAD CELLS WITH ^{99m}Tc-TETRACYCLINE

Fractions	Total activity (cpm)	
	Dead cells	Live cells
Medium (MEM)	1,243,104	1,178,916
Washing 1 (CFS)	50,160	142,764
Washing 2 (CFS)	2,556	29,640
Trypsin	—	5,568
Washing 3 (CFS)	3,300	2,004
10^6 cells*	4,359	77

* 14.7×10^6 dead cells and 57.5×10^6 live cells used for determination.

RESULTS

On incubation with ^{99m}Tc-tetracycline about 60 times more activity is associated with the dead cells in comparison with the live cells (Table 1). This contrast can be seen throughout the washing procedure of the live cells with CFS: almost three times more activity was removed after the second washing in comparison with the dead cells.

When the cells were resuspended after labeling and allowed to remain for various periods of time, the activity remained bound to the dead cells. A typical experiment in which replicate tubes of labeled cells were suspended in CFS and centrifuged after various periods of incubation at 37°C indicated that most of the unbound activity was removed with the previous washings. Very little more was eluted during the subsequent 3 hr. About 60 times more activity remained with the dead cells than with the live cells.

This preferential uptake of ^{99m}Tc in dead cells incubated with labeled tetracycline was not seen on incubation with the pertechnetate ion. An experiment in which dead cells were exposed to ^{99m}Tc-tetracycline or ^{99m}Tc-pertechnetate showed consistently higher relative uptakes of ^{99m}Tc in the cells exposed to ^{99m}Tc-tetracycline at the three incubation times (Table 2). No preference for either compound was observed in live cells, however. In a typical experiment with ^{99m}Tc-pertechnetate in which the cells were exposed to pertechnetate for 30 min, all the activity remained either in the medium or was lost in the washing procedure (Table 3). Essentially no activity remained with either the dead or the live cells and there was no concentration of activity by the dead cells as was seen with tetracycline.

Although considerable work has been done to determine the site at which tetracycline is bound in bacterial cells, there appears to be some controversy over its localization in mammalian cells. In a typical experiment in which the cell fractions were separated to localize the ^{99m}Tc activity after labeled tetracycline exposure, very little of the activity was found in the

TABLE 2. THE UPTAKE OF ^{99m}Tc-TETRACYCLINE (Tc-T) AND THE PERTECHNETATE ION (TcO_4^-) IN DEAD CELLS (CPM/10^6 CELLS)

Cell type	Time of incubation (min)		
	15	30	60
Dead ^{99m}Tc-T	13,824	16,020	15,852
Dead $^{99m}TcO_4^-$	576	504	536
Ratio $\frac{^{99m}\text{Tc-T}}{^{99m}TcO_4^-}$	24.1	31.8	28.1

TABLE 3. LABELING OF LIVE AND DEAD CELLS WITH PERTECHNETATE ION

Fractions	Total activity (cpm)	
	Dead cells	Live cells
Medium (MEM)	103,728	97,904
Washing 1 (CFS)	4,158	16,861
Washing 2 (CFS)	147	3,379
Trypsin	—	950
Washing 3 (CFS)	65	50
10^6 cells*	42	78

* 15.7 × 10^6 dead cells and 14.7 × 10^6 live cells used for determination.

TABLE 4. ACTIVITY DISTRIBUTIONS OF LABELED LIVE AND DEAD CELLS (CPM/10^6 CELLS)

Fractions	Dead cells*		Live cells	
	Activity	Percent	Activity	Percent
Acid soluble (3)†	2,100	7	828	23
Lipid soluble	972	3	120	3
RNA (3)†	9,306	30	876	24
DNA + protein	18,635	60	1,788	50

* Five times the number of live cells were used for determinations.
† Number of combined fractions.

lipid and acid-soluble fractions of dead cells whereas about one-fourth of the activity was associated with the acid-soluble fraction in the live cells (Table 4). In both cases, however, at least 50% of the activity was associated with the DNA and protein fractions and 25% with the RNA fraction.

When the cell components were labeled *after* homogenization, more of the activity was associated with the components of dead cells than of live cells (Table 5). The majority of the activity appeared to be divided equally between the RNA and the DNA and protein fractions.

Dead cells were exposed to four other ^{99m}Tc-labeled compounds (^{99m}Tc-chlorotetracycline, ^{99m}Tc-oxytetracycline, ^{99m}Tc-demethylchlortetracycline, and

TABLE 5. ACTIVITY DISTRIBUTION IN HOMOGENIZED LIVE AND DEAD CELLS (CPM/10^6 CELLS)

Fractions	Dead cells		Live cells	
	Activity	Percent	Activity	Percent
Acid soluble (3)*	4,404	14	2,292	33
Lipid soluble	1,416	4	144	2
RNA (3)*	14,280	44	2,676	39
DNA + protein	14,292	38	1,824	26

* Number of combined fractions.

TABLE 6. ACTIVITY DISTRIBUTIONS OF DEAD CELLS LABELED WITH DIFFERENT ^{99m}Tc-LABELED RADIOPHARMACEUTICALS

Activity	Tetracycline	Chlorotetracycline	Oxytetracycline	Demethylchlorotetracycline	Ascorbate-DTPA
	Activity (cpm/10^6 cells)				
Total	4,319	5,876	3,772	4,807	1,303
	Activity (percent of total)				
Acid soluble (3)*	15	15	15	16	33
Lipid soluble	3	6	2	5	3
RNA (3)*	27	26	26	27	24
DNA	13	12	12	11	8
Protein	42	41	45	41	32

* Number of combined fractions.

^{99m}Tc-ascorbate-DTPA) (Table 6) and the results compared with tetracycline. After exposure to four tetracycline analogs the cellular activity was similar; whereas after exposure to ^{99m}Tc-ascorbate-DTPA much less ^{99m}Tc was taken up by the cells. Fractionation of these cells showed very similar patterns of uptake for the four tetracycline analogs but a significant proportion of the activity appeared in the acid-soluble fraction after ^{99m}Tc-ascorbate-DTPA incubation. When the DNA and protein fractions were separated, the majority of the activity was associated with the protein fraction.

DISCUSSION

Our data show that ^{99m}Tc is readily taken up by dead cells in tissue culture after exposure to ^{99m}Tc-tetracycline. These dead cells which in our model were produced by depletion of essential nutrients appeared to incorporate ^{99m}Tc into their macromolecular structure whereas live cells appeared to exclude or prevent this compound from becoming incorporated. Although a necrotic cell system could be obtained by other means (such as heat or irradiation), cell death due to depletion of essential nutrients may correspond to the situation seen with

myocardial infarction. The uptake of ^{99m}Tc in canine myocardial infarcts after ^{99m}Tc-tetracycline administration (*20*) seems to substantiate this but studies in different modes and stages of cell death, both reversible and irreversible, due to the aforementioned mechanisms have yet to be completed.

This study also sheds light on the localization of ^{99m}Tc-tetracycline in mammalian cells at the subcellular level. In bacteria, tetracyclines have been reported to bind to cellular RNA. Specifically, bacteriostatic concentrations of tetracycline have been found by some investigators to bind to 30S ribosomes while other investigators (*17,19*) have found the drug associated with the 50S ribosomal component also.

There appears to be less substantial data on the localization of ^{99m}Tc-tetracycline in mammalian cells. Our data suggest that at least 50% of the total ^{99m}Tc-tetracycline activity was associated with the DNA and protein fractions of the Lich cell and 25% with RNA. Separation of the DNA and protein fractions showed that over 75% of the activity in that fraction was associated with protein. Homogenization of the cells before labeling increased the amount of activity associated with the RNA fraction.

The uptake of ^{99m}Tc in dead cells may be due to structural changes associated with the degeneration of the subcellular components. These changes, including conformational alterations of the macromolecules, may be necessary to allow ^{99m}Tc to bind to DNA, RNA, or protein. This postulate is supported by certain chemical studies.

Zubay and Doty (*23*) have found increased binding of tetracycline to heat-denatured DNA, probably due to the availability of new groups such as purine rings. It has been reported that tetracyclines bind to DNA, proteins, soluble RNA, and ribosomes in the presence of cationic species. Kohn (*24*), studying the binding of native and heat-denatured DNA with tetracycline in the presence of Zn^{2+}, Ga^{2+}, Mg^{2+}, and Mn^{2+}, found that tetracycline binding was four to five times higher with denatured DNA in the presence of Zn^{2+} ions.

In our system the ^{99m}Tc-ion was chelated to tetracycline and was probably positively charged in the presence of excess Sn^{2+} ion. Under these conditions it is reasonable to assume that during the process of cell death, changes in the subcellular components occur which open up sites for the binding of ^{99m}Tc-labeled tetracycline potentiated by the stannous ion.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the helpful suggestions of S. J. Adelstein and M. A. Davis in the preparation of this manuscript. We express our appreciation to A. Kassis and E. C. Williams for their expert technical assistance. This work was supported by AEC contract AT(11-1)-3229 and USPHS Grant GM 18674.

REFERENCES

1. MALEK P, KOLC J, ZASTAVA V, et al: Fluorescence of tetracycline analogs fixed in myocardial infarction. *Cardiologica* 42: 303–318, 1963

2. RALL DP, LOU TL, LANE M, et al: Appearance and persistence of fluorescent material in tumor tissue after tetracycline administration. *J Natl Cancer Inst* 19: 79–85, 1957

3. PHILLIPS JW, COBB EG, RICHARDS V, et al: The deposition and retention of tetracycline in cancer. *Am J Surg* 100: 384–388, 1960

4. KANTOR SM: Preoperative differentiation of benign and malignant gastric lesions by tetracycline induced fluorescence. *Bull Sinai Hosp Detroit* 9: 66–70, 1961

5. MCLEAY JF: The use of systemic tetracyclines and ultraviolet in cancer detection. *Am J Surg* 96: 415–419, 1958

6. WHITMORE WF, BUSH IM, ESQUIVEL E: Tetracycline ultraviolet fluorescence in bladder carcinoma. *Cancer* 17: 1528–1532, 1964

7. NEWTON BA: Mechanisms of antibiotic action. *Ann Rev Microbiol* 19: 209–240, 1965

8. SAZ AK, MARTINEZ LM: Enzymatic basis of resistance to aureomycin. III. Inhibition by aureomycin of protein-stimulated electron transport in *Escherichia coli*. *J Bacteriol* 79: 527–531, 1960

9. GOLDMAR DS: The inhibition of alanine dehydrogenase by metal chelates of tetracycline. *J Biol Chem* 235: 616–619, 1960

10. GALE EF, FOLKES JP: The assimilation of amino acids by bacteria. 15. Actions of antibiotics of nucleic acid and protein synthesis in *Staphylococcus aureus*. *Biochem J* 53: 493–498, 1953

11. FRANKLIN TJ: The inhibition of incorporation of leucine into protein of cell-free systems from rat liver and *Escherichia coli* by chlortetracycline. *Biochem J* 87: 449–453, 1963

12. FRANKLIN TJ: The effect of chlortetracycline on the transfer of leucine and "transfer" ribonucleic acid to rat liver ribosomes *in vitro*. *Biochem J* 90: 624–628, 1964

13. CONNAMACHER RH, MANDEL HG: Binding of tetracycline to 30 S ribosomes and to polyonidylic acid. *Biochem Biophys Res Comm* 20: 98–103, 1965

14. HASH JH, WISHNICK M, MILLER PA: On the mode of action of the tetracycline antibiotics in *Staphylococcus aureus*. *J Biol Chem* 239: 2070–2078, 1964

15. DAY LE: Tetracycline inhibition of cell-free protein synthesis. II. Effect of the binding of tetracycline to the components of the system. *J Bacteriol* 92: 197–203, 1966

16. CONNAMACHER RH, MANDEL HG: Studies on the intracellular localization of tetracycline in bacteria. *Biochim Biophys Acta* 166: 475–486, 1968

17. MAXWELL IH: Studies of the binding of tetracycline to ribosomes *in vitro*. *Mol Pharmacol* 4: 25–37, 1968

18. DU BUY HG, SHOWACRE JL: Selective localization of tetracycline in mitochondria of living cells. *Science* 133: 196–197, 1962

19. DEWANJEE MK, FLEIGEL C, TREVES S, et al: ^{99m}Tc-labeled tetracycline: A new radiopharmaceutical for renal imaging. *J Nucl Med* 13: 427–428, 1972

20. HOLMAN BL, DEWANJEE MK, IDOINE J, et al: Detection and localization of experimental myocardial infarction with ^{99m}Tc-tetracycline. *J Nucl Med* 14: 595–599, 1973

21. CHANG RS: Metabolic alterations with senescence of human cells. Some observations in vitro. *Arch Intern Med* 110: 563–568, 1962

22. EDELMAN M, HIRSH CA, HIATT HH, et al: Apparent changes in mouse liver DNA content due to interference by non-DNA diphenylamine-reacting cytoplasmic material. *Biochim Biophys Acta* 179: 172–178, 1969

23. ZUBAY G, DOTY P: Nucleic acid interactions with metal ions and amino acids. *Biochim Biophys Acta* 29: 47–58, 1958

24. KOHN KW: Mediation of divalent metal ions in the binding of tetracycline to macromolecules. *Nature* 191: 1156–1159, 1961

Accepted Articles To Appear in Upcoming Issues

Protocol for Camera Deadtime Measurement (Letter to the Editor). Accepted 1/31/74.
R. Adams, C. Jansen, G. Grames, and D. Zimmerman

Use of Indium Chloride Scintigraphy in Patients with Myelofibrosis. Accepted 1/31/74.
B. J. McNeil, B. L. Holman, L. N. Button, and D. S. Rosenthal

Epidural Hematoma with Decreased Radionuclide Uptake (Case Report). Accepted 2/20/74.
E. B. Silberstein

Binding of ^{99m}Tc Ion to Hemoglobin (Concise Communication). Accepted 2/26/74.
M. K. Dewanjee

Use of ^{99m}Tc as a Radioisotopic Label to Study the Migratory Patterns of Normal and Neoplastic Cells. Accepted 2/26/74.
R. F. Barth, O. Singla, and G. Y. Gillespie

Analysis of the Relationship between ^{99m}Tc-Sn-Polyphosphate and ^{99m}Tc-Sn-Pyrophosphate. Accepted 2/26/74.
B. M. Bowen, and E. S. Garnett

Gel Chromatography as an Analytical Tool for ^{99m}Tc Radiopharmaceuticals (Letter to the Editor). Accepted 3/6/74.
M. W. Billinghurst and R. F. Palser

Improvement of Pancreatic Imaging (Concise Communication). Accepted 3/6/74.
A. G. Mattar and Y. Laor

Kinetics of Large Therapy Doses of ^{131}I in Patients with Thyroid Cancer. Accepted 3/6/74.
B. Singh, S. M. Sharma, M. C. Patel, K. V. Raghavendran, and M. Berman

Radiopharmaceutical Scientist (Letter to the Editor). Accepted 3/6/74.
C. T. Peng

^{59}Fe Whole Body Scanning. Accepted 3/6/74.
T. K. Chaudhuri, J. C. Ehrhardt, R. L. DeGowin, and J. H. Christie

Parallel Hole Collimator Design (Letter to the Editor). Accepted 3/6/74.
M. S. Gerber and D. W. Miller

An Automated Method for the Evaluation of Non-Focused Collimator Performance in Water Medium. Accepted 3/6/74.
L. R. Bennett

Use of the Delayed Brain Scan in Differentiating Calvarial from Cerebral Lesions. Accepted 3/6/74.
J. Bernstein and P. B. Hoffer

Radiochromate-Binding Capacity of Human Tonsillar Lymphocytes. Accepted 3/6/74.
M. T. Szabo, A. Hrabak, and F. Antoni

Focal Hyperfixation of Radiocolloid by the Liver (Letter to the Editor). Accepted 3/6/74.
J. Pasquier and T. Dorta

Renal Cortical Imaging in 35 Patients: Superior Quality with ^{99m}Tc-DMSA. Accepted 3/6/74.
D. Enlander, P. M. Weber, and L. V. dos Remedios

Radiozinc Uptake and Scintiscanning in Prostatic Disease. Accepted 3/6/74.
G. D. Chisholm, M. D. Short, R. Ghanadian, C. U. McRae, and H. I. Glass

Radioisotopes in Preliminary Screening of Cervical Cancer. Accepted 3/6/74.
J. H. Nodine, S. C. Chang, R. Holmes, G. Lewis, and M. Fletcher

Lipoma of the Corpus Callosum (Case Report). Accepted 3/6/74.
R. Addlestone and J. B. Workman

Enhancement of Pancreatic Concentration of ^{75}Se-Selenomethionine. Accepted 3/6/74.
M. A. Winston, P. Guth, J. S. Endow, and W. H. Blahd

Distribution of ^{14}C-Diphenylhydantoin in Pancreas Islet Cell and Other Tissues of Toadfish. Accepted 3/6/74.
U. Y. Ryo, M. J. Shaw, and W. H. Beierwaltes

Imaging Patterns of Subdural Hematoma—A Proposed Classification. Accepted 3/6/74.
W. M. Sy, G. Weinberger, N. Ngo, and J. P. Sackler

Practical Application of a Simplified Vitamin B_{12} Radioassay. Accepted 3/6/74.
R. B. Shafer, D. Ripley, J. McMahon, and A. Doscherholmen

False-Positive Scintigrams Due to Malposition of Liver (Case Report). Accepted 3/7/74.
A. Platthy and U. Shah

The Appearance of Peripheral, Postsurgical Activity on Cerebral Dynamic Studies (Concise Communication). Accepted 3/7/74.
L. R. Witherspoon, J. W. Tyson, and J. K. Goodrich

Expression of Tissue Isotope Distribution (Letter to the Editor). Accepted 3/14/74.
W. H. Oldendorf

Radionuclide Imaging of the Kidney in Tuberous Sclerosis. Accepted 3/14/74.
C. J. Blatt, D. B. Hayt, and L. M. Freeman

Acromegalic Cholecystomegaly (Case Report). Accepted 3/14/74.
G. A. Cohen, W. M. Smoak, and L. D. Goldberg

A Comment on the Possibility of ^{99m}Tc-Sulfur Colloid Retention in the Lungs (Letter to the Editor). Accepted 3/14/74.
P. Bruun

The Author's Reply. Accepted 3/14/74.
W. C. Klingensmith III

The Author's Reply. Accepted 3/14/74.
P. J. Gillespie

Deadtime Losses (Letter to the Editor). Accepted 3/15/74.
J. R. Tatarczuk

The Author's Reply. Accepted 3/15/74.
P. H. Cooper

An Efficient Method for the Fractional Labeling of Microspheres (Concise Communication). Accepted 3/23/74.
H. C. Wieland, G. M. Grames, C. Jansen, and T. Davidson

Computer-Assisted Scanning in the Evaluation of ^{67}Ga-Citrate Uptake in Thyroid Disease (Concise Communication). Accepted 4/6/74.
M. Erjavec, M. Auersperg, R. Golouh, J. Šnajder, and T. Turnšek

Radioimmunoassay of Parathyroid Hormone: Peripheral Plasma Immunoreactive Parathyroid Hormone Response to Ethylenediaminetetraacetate. Accepted 4/8/74.
I. W. Chen, H. M. Park, L. R. King, G. K. Bahr, and R. E. Goldsmith

Use of ^{125}I for Thyroid Uptake Measurements and Depression of ^{131}I-Thyroid Uptakes by Incomplete Dissolution of Capsule Filler. Accepted 4/8/74.
J. S. Robertson, M. Verhasselt, and H. W. Wahner

An Index to Compare the Performance of Scintigraphic Imaging Systems. Accepted 4/8/74.
F. D. Rollo

Visualization of the Splenic Blood Pool with ^{131}I-Rose Bengal (Letter to the Editor). Accepted 4/8/74.
J. V. Rivera

The Author's Reply. Accepted 4/8/74.
L. M. Freeman

Regional Intrarenal Perfusion in Man: An Assessment with the Scintillation Camera. Accepted 4/8/74.
M. Kinoshita, B. L. Holman, R. E. Zimmerman, D. F. Adams, S. J. Adelstein, and N. K. Hollenberg

A Quick Method for the Calculation of the Vascular Mean Transit Time (Concise Communication). Accepted 4/8/74.
M. E. Phelps and J. O. Eichling

^{169}Yb-DTPA Distribution and Dosimetry in Cisternography. Accepted 4/11/74.
R. L. Morin and V. A. Brookeman

^{67}Ga-Uptake in the Regenerating Rat Liver (Concise Communication). Accepted 4/11/74.
J. H. Hill and H. N. Wagner, Jr.

A Rapid Reliable Method of Labeling Sn-MAA with ^{113m}InCl$_3$ (Concise Communication). Accepted 4/11/74.
D. R. Allen, D. E. Hartnett, W. B. Nelp, and G. W. Hamilton

Necessity of Oblique Views in Evaluatjng the Functional Status of a Thyroid Nodule. Accepted 4/11/74.
J. R. Karelitz and J. B. Richards

Radionuclide Angiography in the Diagnosis of Carotid Cavernous Sinus Fistula. Accepted 4/11/74.
T. E. Tully, R. B. Shafer, D. B. Reinke, and M. P. Pliego

Cancer of the Colon Visualized by Strontium Scintigraphy (Letter to the Editor). Accepted 4/26/74.
C. Papavasiliou

The Author's Reply. Accepted 4/26/74.
T. K. Chaudhuri

Survival and Sequestration of ^{51}Cr- and ^{99m}TcO$_4$-Labeled Platelets. Accepted 4/26/74.
T. Uchida, K. Yasunaga, S. Kariyone, and G. Wakisaka