PURIFICATION OF RAT GAMMA GLOBULIN AND THE PRODUCTION OF A SPECIFIC ANTI-RAT GAMMA GLOBULIN SERUM

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Atomic Support Agency
Bethesda, Maryland

Distribution of this document is unlimited.
All aspects of investigative programs involving the use of laboratory animals sponsored by DOD components are conducted according to the principles enunciated in the "Guide for Laboratory Animal Facilities and Care", prepared by the National Academy of Sciences - National Research Council.
PURIFICATION OF RAT GAMMA GLOBULIN AND THE PRODUCTION OF A
SPECIFIC ANTI-RAT GAMMA GLOBULIN SERUM

E. D. EXUM
R. T. BOWSER

S. J. BAUM
Chairman
Experimental Pathology Department

HUGH B. MITCHELL
Colonel, USAF, MC
Director

ARMED FORCES RADIOLOGY RESEARCH INSTITUTE
Defense Atomic Support Agency
Bethesda, Maryland

Distribution of this document is unlimited
ACKNOWLEDGMENT

The authors wish to express their appreciation to J. S. Finlayson and A. M. Young, Laboratory of Blood and Blood Products, National Institutes of Health, Bethesda, Maryland, for performing the ultracentrifugal analysis.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreword (Nontechnical summary)</td>
<td>iii</td>
</tr>
<tr>
<td>Abstract</td>
<td>iv</td>
</tr>
<tr>
<td>I. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>II. Material and Methods</td>
<td>1</td>
</tr>
<tr>
<td>III. Results</td>
<td>3</td>
</tr>
<tr>
<td>IV. Discussion</td>
<td>9</td>
</tr>
<tr>
<td>V. Conclusions</td>
<td>10</td>
</tr>
<tr>
<td>References</td>
<td>11</td>
</tr>
</tbody>
</table>
**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.</td>
<td>Immunoelectrophoretic patterns of adsorbed rat serum against rabbit anti-rat serum. Successive adsorptions (A-E) on 10 g aliquots of DEAE-Sephadex A-50.</td>
<td>4</td>
</tr>
<tr>
<td>Figure 2.</td>
<td>Comparative immunoelectrophoretic pattern of DEAE-Sephadex column fractionation of a 5X adsorbed rat serum and normal rat serum against rabbit anti-rat serum</td>
<td>5</td>
</tr>
<tr>
<td>Figure 3.</td>
<td>Elution from DEAE-Sephadex A-50 column of a 5X adsorbed rat serum in 0.01 M phosphate buffer, pH 7.5.</td>
<td>6</td>
</tr>
<tr>
<td>Figure 4.</td>
<td>Ultracentrifugal pattern of adsorbed and column fractionated rat serum.</td>
<td>7</td>
</tr>
<tr>
<td>Figure 5.</td>
<td>Immunoelectrophoretic pattern of rat 7S globulin and normal rat serum against rabbit anti-rat 7S globulin.</td>
<td>8</td>
</tr>
<tr>
<td>Figure 6.</td>
<td>Radioimmunoelectrophoretic pattern of the 5X adsorbed fraction against rabbit anti-rat serum showing the presence of transferrin.</td>
<td>8</td>
</tr>
</tbody>
</table>
FOREWORD
(Nontechnical summary)

Postirradiation changes in serum proteins have been noted in several species. Accurate identification of changes in the serum proteins requires a precise knowledge of the normal serum protein composition. In the past, serum proteins have been separated and named mainly on the basis of electrophoretic mobility. Other methods for characterizing serum components have been physical-chemical procedures based on the size and shape of the individual protein components and immunological techniques based on antigen-antibody reactions.

Ideally, the identification of serum components depends on the individual isolation of these components. Electrophoretic and chromatographic as well as antigen-antibody techniques can be utilized for the isolation of the several serum proteins. Chromatographic methods (for separation) and electrophoretic techniques (for identification) have been utilized in the present study to separate and characterize 7S gamma globulin. Rat serum repeatedly adsorbed with DEAE-Sephadex (a cross-linked dextran polymer) finally yielded a single protein fraction. This fraction when subjected to electrophoresis, immunoelectrophoresis as well as ultracentrifugal analysis satisfies the criteria for 7S gamma globulin (immune globulin).

Single component or homogeneous rat 7S globulin, when used to immunize rabbits, should produce an antiserum specific only for 7S globulin. Antiserum thus produced in rabbits, when tested against whole rat serum, produced reactivity against a single component, 7S gamma globulin.
ABSTRACT

A method for the isolation of rat 7S gamma globulin based on multiple adsorptions with DEAE-Sephadex A-50 has been presented. The separation of rat 7S globulin requires a procedure modified from the one used for humans in terms of the buffer pH, and the number of adsorptions. Ultracentrifugal analysis indicates the resulting fraction is a single component. In addition, immunoelectrophoresis of the fraction when tested against anti-rat sera confirms the homogeneity of this 7S fraction. A monospecific antiserum to rat 7S gamma globulin has been produced. This antiserum is free of reaction with all other serum components when subjected to immunoelectrophoresis against normal rat serum.
I. INTRODUCTION

Several methods have been published for the purification of gamma globulin from human serum.\textsuperscript{1-3, 8, 9} However, these procedures were not satisfactory for the isolation of 7S gamma globulin from whole rat serum. No method for the isolation of rat 7S gamma globulin has been published previously.

The purpose of this paper is to present a laboratory procedure for obtaining an immunoelectrophoretically pure rat 7S gamma globulin and the preparation of a monospecific antiserum to rat 7S globulin. The procedure presented here requires modifications from that used for the separation of human 7S globulin by the DEAE-Sephadex A-50 batch method.\textsuperscript{1}

II. MATERIAL AND METHODS

**Serum.** The serum was obtained from fasting adult Sprague-Dawley rats. The rats were anesthetized with Fluothane-ether mixture (125 ml Fluothane + 59 ml ether), and bled by cardiac puncture. Serum was separated by centrifugation, and stored at \(-20^\circ\text{C}\) until ready to be used.

**Separation Procedure.** DEAE-Sephadex A-50 gel (3.5 ± 0.5 meq per gram, 40–120 μm) was conditioned as previously reported by Baumstark et al.,\textsuperscript{1} with the following slight modification. In attempts to determine the optimum buffer pH for separating 7S globulin from rat serum, .01 M phosphate buffers with pH ranges of 5.0–7.5 in increments of 0.5 pH units were tried. From this preliminary assay, a buffer pH of 7.5 was found to be effective for the elution of a fairly clean, though not completely pure rat gamma globulin when characterized by immunoelectrophoresis.
Fifty milliliters of normal rat serum (undialyzed) were added to a beaker containing 10 g of moist DEAE-Sephadex A-50. The mixture of serum and gel was allowed to adsorb in a refrigerated room at 3°C for 1.5 hours while constantly being stirred with a magnetic bar. Following the 1.5 hours adsorption period, the serum-gel mixture was transferred to two 40-ml cellulose nitrate tubes and centrifuged in a refrigerated centrifuge at 4,080 g for 15 minutes. The supernatant was decanted into a beaker containing another 10 g portion of moist gel, and again allowed to stand in the cold room while being stirred for 2.5 hours. After this adsorption (adsorption number 2) the serum-gel mixture was centrifuged once again as before, and the supernatant decanted into a flask. If necessary it can be stored under refrigeration at any point during the adsorption procedure. The procedure was continued with a third adsorption time of 1.5 hours, a fourth of 2.5 hours and a fifth of 1.5 hours. Even after five adsorptions the sample was not immunoelectrophoretically pure gamma globulin.

Further isolation involved the use of a DEAE-Sephadex A-50 freely packed column (20 cm x 11 mm) using a phosphate buffer pH 7.5 as eluant.

Before the adsorbed fraction was added to the top of the column, a small filter disc was carefully placed on top of the gel. To the properly prepared column 2.0 ml of the five-times adsorbed rat serum fraction were added. After the fraction had passed through the filter disc, buffer was added slowly with a pipette until it reached a point of about 5 cm above the top of the gel. The eluted fractions were collected in 2 ml portions at a flow rate of 1 ml per minute. The content of the tubes corresponding to each peak was pooled, and concentrated 8-fold in Carbowax. Protein determinations were made by the method of Lowry et al. 7
Characterization of Gamma Globulin. Immunoelectrophoresis for the characterization of the various preparations was conducted in 0.75 percent agarose media made in 0.05 M Tris buffer pH 8.6. The fractions were electrophoresed 1 hour at 300 volts using the LKB apparatus. The ultracentrifugal analyses were carried out in a Spinco analytical ultracentrifuge employing a standard 12 mm cell.

Radioimmunoelectroporesis Technique. After the immunoelectrophoretic pattern was developed and the slides washed in saline 48 hours, Fe diluted to 10 $\mu$Ci/ml was added to the trough and allowed to stand in a covered tray 24 hours. The slides were washed in saline 48 hours and then dried. The dried slides were placed in contact with x-ray film for 14 days, after which the film was processed.

Preparation of Specific Antisera. Antisera were prepared in adult male rabbits, New Zealand strain, by two injections with immunoelectrophoretically purified gamma globulin. The first injection containing approximately 2 mg of immunoelectrophoretically pure rat 7S globulin incorporated in complete Freund's adjuvant was made subcutaneously in the nuchal area and all foot pads. The second injection of antigen (1.5 mg) incorporated in incomplete Freund's adjuvant was administered in the nuchal area only. The total amount of antigen received by each rabbit was 3.5 mg.

III. RESULTS

The pH of the serum after the removal of the clot was 8.1. When adding 50 ml of normal rat serum to 10 g of moist gel that had been previously conditioned in phosphate buffer pH 7.5, the serum-gel mixture had a pH value of 8.0. The pH value of the serum-gel mixture remained at 8.0 during the five adsorptions while the final pH value of the extracted material, separated from the gel by centrifugation, was 7.6.
Figure 1 shows the immunoelectrophoretic pattern of the supernatant (consisting of gamma globulin) following each DEAE-Sephadex A-50 adsorption. The samples were tested against rabbit anti-rat serum. Note the gradual disappearance of various precipitin bands from one adsorption to the other, with the fifth and final adsorption having essentially two bands, one with a faster mobility than the other. It is suggested that the faster moving component is one of the beta globulins or transferrin.

Figure 1. Immunoelectrophoretic patterns of adsorbed rat serum against rabbit anti-rat serum. Successive adsorptions (A–E) on 10 g aliquots of DEAE-Sephadex A-50. (A) 1st adsorption; (B) 2nd adsorption; (C) 3rd adsorption; (D) 4th adsorption; (E) 5th adsorption; (NRS) normal rat serum.
Two milliliters of the supernatant consisting of the two components were put on a freely packed DEAE-Sephadex A-50 column. The eluted fractions were collected and pooled with their respective peaks. Figure 2A illustrates the immunoelectrophoretic pattern of the pooled fraction against rabbit anti-rat whole serum.

![Figure 2](image)

Figure 2. Comparative immunoelectrophoretic pattern of DEAE-Sephadex column fractionation of a 5X adsorbed rat serum and normal rat serum against rabbit anti-rat serum. (A) column fractionated serum; (B) normal rat serum.

A diagrammatic tracing of the eluted fractions from the column is demonstrated in Figure 3. The first peak in Figure 3 represents a component which does not react with an anti-rat whole serum when subjected to immunoelectrophoresis. The second peak and all of the trailing material contained rat gamma globulin, and is illustrated immunoelectrophoretically in Figure 2. The results obtained here indicate that the material is specific for rat gamma globulin only, and shows a high degree of homogeneity.
Upon examination of the purified gamma globulin at a concentration of 2.2 mg/ml in the analytical ultracentrifuge, a single homogeneous peak was observed. Figure 4 shows the sedimentation pattern. The sedimentation coefficient computed from this sample was 6.0 at 20°C.

To obtain a monospecific antiserum to rat gamma globulin, adult rabbits were injected initially with immunoelectrophoretically pure rat 7S globulin. Four weeks after the initial injection, the rabbits were given a second injection of the antigen previously used. Figure 5 shows the results obtained 7 weeks after the initial injection.

Attempts were made to characterize the faster moving component in the five-times adsorbed rat serum by employing the radioimmunoelectrophoresis technique. It was noted that ⁵⁹Fe would attach to the faster moving component. Since transferrin is the iron-containing constituent of the serum and will incorporate ⁵⁹Fe, this may indicate that the faster moving component is transferrin. Figure 6 shows the radioautograph along with the plain immunoelectrophoretic pattern.
Figure 4. Ultracentrifugal pattern of adsorbed and column fractionated cat serum. Protein concentration, 2.2 mg/ml in 0.01 M phosphate buffer, pH 7.5. Speed: 59,780 rpm at 20°C. Photograph taken 61 min after indicated rotor speed. $S_{20}$ value = 6.0.
Figure 5. Immunoelectrophoretic pattern of rat 7S globulin and normal rat serum against rabbit anti-rat 7S globulin.
(A) 7S preparation (5X adsorbed column fraction);
(B) normal rat serum.

Figure 6. Radioimmunoelectrophoretic pattern of the 5X adsorbed fraction against rabbit anti-rat serum showing the presence of transferrin.
Left -- radioautograph. Right -- immunoelectrophoretic pattern.
(A), (A¹) 5X adsorbed rat serum; (B), (B¹) normal rat serum.
IV. DISCUSSION

The results presented here show that rat gamma globulin can be separated by an adsorption method on DEAE-Sephadex A-50 pH 7.5 followed by elution from a freely packed column containing DEAE-Sephadex A-50, pH 7.5. When the eluted material trailing the first peak is pooled and concentrated in Carbowax and characterized by immunoelectrophoresis against normal rat serum, a single precipitin band occurs only in the gamma globulin region. This indicates that the preparation is monospecific for gamma globulin. That the eluted material is a single component is confirmed when the resulting antiserum gives a single band against normal rat serum (Figure 5).

Attempts were made to separate the 7S globulin from rat serum by using the batch two-times adsorption method as used in human 7S separation (Baumstark et al.\textsuperscript{1}). However, successful separation of 7S gamma globulin free of transferrin components could not be obtained despite modifications in: (1) buffer pH values; (2) adsorption time; and (3) gel to serum ratios. On most separations the fractions were composed of other serum components in addition to transferrin.

The transferrin was finally separated from the 7S globulin when a portion of the five-times adsorbed fraction was put on a DEAE-Sephadex A-50 freely packed column. During the 140 minutes elution time the transferrin component was apparently not eluted from the column, because it could not be detected immunoelectrophoretically, nor was it recognized in the antiserum.

From these methods, it is possible to obtain approximately 5 mg of immunoelectrophoretically pure rat gamma globulin from 2 ml of the five-times adsorbed rat serum fraction. Due to the number of adsorptions and passages through the
column it is evident that a great deal of loss occurs. Baumstark et al.\textsuperscript{1} reported that 600 mg of immunoelectrophoretically pure gamma globulin could be isolated within 3 hours from 50 ml undialyzed human serum. The difficulty in isolating greater yields from rat serum may be a function of species differences.

V. CONCLUSIONS

A method for the isolation of rat 7S gamma globulin based on multiple adsorptions with DEAE–Sephadex A–50 has been presented. The separation of rat 7S globulin requires a procedure modified from the one used for humans in terms of the buffer pH, and the number of adsorptions. Ultracentrifugal analysis indicates the resulting fraction is a single component. In addition, immunoelectrophoresis of the fraction when tested against anti-rat sera confirms the homogeneity of this 7S fraction. A monospecific antiserum to rat 7S gamma globulin has been produced. This antiserum is free of reaction with all other serum components when subjected to immunoelectrophoresis against normal rat serum.
REFERENCES


DISTRIBUTION LIST

AIR FORCE

Executive Officer, Director of Professional Services, Office of the Surgeon General, Hq. USAF (AFMSPA) T-8, Washington, D. C. 20333 (1)
Headquarters, U. S. Air Force (AFMSPAB), Washington, D. C. 20333 (1)
USAFSAM (SMRB), ATTN: Chief, Radiobiology Branch, Brooks AFB, Texas 78235 (1)
Air Force Weapons Laboratory, ATTN: WLIL (1), ATTN: WLRB-2 (1), Kirtland AFB, New Mexico 87117 (2)
Chief, Nuclear Medicine Department, P. O. Box 5088, USAF Hospital, Wright-Patterson AFB, Ohio 45433 (1)
Office of the Command Surgeon (ADCSG), Hq. ADC, USAF, Ent AFB, Colorado 80912 (1)
Commander, 6571st Aeromedical Research Laboratory, Holloman AFB, New Mexico 88330 (2)

ARMY

The Surgeon General, U. S. Department of the Army, Washington, D. C. 20315 (1)
USACDC CSSG, Doctrine Division, Fort Lee, Virginia 23801 (1)
CG, USCONARC, ATTN: ATUTR-TNG (NBC), Fort Monroe, Virginia 23651 (1)
Commanding Officer, U. S. Army Medical Research Laboratory, Fort Knox, Kentucky 40121 (1)
Commanding Officer, U. S. Army Environmental Hygiene Agency, ATTN: USAEHA-RP, Edgewood Arsenal, Maryland 21010 (1)
Commandant, U. S. Army Medical Field Service School, ATTN: MEDEW - ZNW, Fort Sam Houston, Texas 78234 (1)

NAVY

Chief, Bureau of Medicine and Surgery, U. S. Navy Department, Washington, D. C. 20390 (1)
Director, Biological Sciences Division, Office of Naval Research, Washington, D. C. 20360 (1)
Commanding Officer and Director (222A), U. S. Naval Radiological Defense Laboratory, San Francisco, California 94135 (2)
Head, Biological and Medical Sciences Division, U. S. Naval Radiological Defense Laboratory, San Francisco, California 94135, ATTN: Dr. E. L. Alpen (1)
Commanding Officer, Naval Aerospace Medical Institute, NAMC, ATTN: Research Director, Pensacola, Fla. 32512 (3)
Head, Animal Behavioral Sciences Branch, Naval Aerospace Medical Institute, Naval Aerospace Medical Center, Pensacola, Florida 32512, ATTN: Dr. John S. Thach, Jr. (1)
Commanding Officer, U. S. Naval Hospital, ATTN: Director, REEL, NNMC, Bethesda, Maryland 20014 (1)
Commanding Officer, Nuclear Weapons Training Center, Atlantic, Nuclear Warfare Department, Norfolk, Virginia 23511 (1)

D. O. D.

Director, Defense Atomic Support Agency, Washington, D. C. 20305 (1)
Director, Defense Atomic Support Agency, ATTN: DDST, Washington, D. C. 20305 (1)
Director, Defense Atomic Support Agency, ATTN: Chief, Medical Directorate, Washington, D. C. 20305 (4)
Director, Defense Atomic Support Agency, ATTN: Technical Library (APTL), Washington, D. C. 20305 (2)
Commander, Field Command, Defense Atomic Support Agency, ATTN: FC Technical Library, Sandia Base, Albuquerque, New Mexico 87115 (1)
Director, Armed Forces Institute of Pathology, Washington, D. C. 20305 (1)
Administrator, Defense Documentation Center, Cameron Station, Bldg. 5, Alexandria, Virginia 22314 (20)

OTHER GOVERNMENT

U. S. Atomic Energy Commission, Headquarters Library, Reports Section, Mail Station G-17, Washington, D. C. 20545 (1)
U. S. Atomic Energy Commission, Division of Biology and Medicine, Washington, D. C. 20545 (1)
OTHER GOVERNMENT (continued)

U. S. Atomic Energy Commission, Bethesda Technical Library, 7920 Norfolk Avenue, Bethesda, Maryland 20014 (1)
National Aeronautics and Space Administration, ATTN: Lt. Col. Charles M. Barnes, USAF, DB-3, MSC, Houston, Texas 77038 (1)
National Bureau of Standards, ATTN: Chief, Radiation Physics Division, Washington, D. C. 20234 (1)
U. S. Public Health Service, Deputy Chief, Division of Radiological Health, Washington, D. C. 20201 (1)
U. S. Public Health Service, Radiological Health Laboratory, ATTN: Library, 1901 Chapman Avenue, Rockville, Maryland 20852 (1)
U. S. Public Health Service, Northeastern Radiological Health Laboratory, 109 Holton Street, Winchester, Massachusetts 01890 (1)
U. S. Public Health Service, Southwestern Radiological Health Laboratory, P. O. Box 684, Las Vegas, Nevada 89101 (1)
U. S. Public Health Service, National Center for Radiological Health, Information Office, Room 3, Twinbrook Laboratory, RBE Program, 1901 Chapman Avenue, Rockville, Maryland 20852 (1)

OTHER

Argonne National Laboratory, Library Services Department, Report Section Bldg. 203, RM-CE-125, 9700 South Cass Avenue, Argonne, Illinois 60440 (1)
Dr. Donald G. Baker, Radiobiology Department, Zellerbach Saroni Tumor Institute, 1600 Divisadero Street, San Francisco, California 94115 (1)
Brookhaven National Laboratory, Information Division, ATTN: Research Library, Upton, Long Island, New York 11973 (2)
Dr. J. S. Burkic, Director of Nuclear Medicine, York Hospital, York, Pennsylvania 17403 (1)
Director, Radiobiology Laboratory, University of California, Davis, California 95616 (1)
University of California, Lawrence Radiation Laboratory, Library, Bldg. 50, Room 134, Berkeley, Calif. 94720 (1)
University of California, Lawrence Radiation Laboratory, Technical Information Division Library L-3, P. O. Box 808, Livermore, California 94551 (2)
University of California, Laboratory of Nuclear Medicine and Radiation Biology, Library, 900 Veteran Avenue, Los Angeles, California 90024 (1)
Director, Collaborative Radiological Health Laboratory, Colorado State University, Fort Collins, Colorado 80521 (1)
Dr. L. W. Davis, Radiology Department, University of Pennsylvania, 3400 Spruce Street, Philadelphia, Pa. 19104 (1)
Professor Merril Eisenbud, New York University, Tuxedo, New York 10987 (1)
Dr. T. C. Evans, Radiation Research Laboratory, College of Medicine, University of Iowa, Iowa City, Iowa 52240 (1)
Dr. Arnold Feldman, Institute of Radiology, School of Medicine, Washington University, 510 South Kingshighway, St. Louis, Missouri 63110 (1)
Mr. Orin Gekcilloo, Department of Biological Sciences, Northwestern University, Evanston, Illinois 60201 (1)
General Dynamics/Fort Worth, ATTN: Librarian, P. O. Box 748, Fort Worth, Texas 76101 (1)
Gulf General Atomic Incorporated, ATTN: Library, P. O. Box 608, San Diego, California 92112 (1)
Harleton Nuclear Science Corporation, ATTN: Library, 4062 Fabian Way, Palo Alto, California 94303 (1)
IIT Research Institute, ATTN: Document Library, 10 West 35th Street, Chicago, Illinois 60616 (1)
Dr. R. F. Kallman, Department of Radiology, Stanford University, Palo Alto, California 94305 (1)
Dr. L. S. Kelly, Donner Laboratory, University of California at Berkeley, Berkeley, California 94720 (1)
Los Alamos Scientific Laboratory, ATTN: Report Librarian, P. O. Box 1663, Los Alamos, New Mexico 87544 (1)
Director, Nuclear Science Center, Louisiana State University, Baton Rouge, Louisiana 70803 (2)
Lovelace Foundation for Medical Education & Research, Document Library, 5200 Gibson Boulevard, S. E. Albuquerque, New Mexico 87108 (1)
Dr. Ross A. McFarland, Guggenheim Professor of Aerospace Health & Safety, Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115 (1)
Dr. J. I. Marcum, Rand Corporation, 1700 Main Street, Santa Monica, California 90401 (1)
Massachusetts Institute of Technology, M.I.T. Libraries, Technical Reports, Room 14 E-210, Cambridge, Massachusetts 02139 (1)
Dr. Charles W. Mays, Physics Group Leader, Radiobiology Division, University of Utah, Salt Lake City, Utah 84112 (1)
Dr. B. D. Newsom, Colony Oaks, Apt. 32, 18100 Nassau Bay Drive, Nassau Bay, Texas 77058 (1)
Ohio State University, Nuclear Reactor Laboratory, 1298 Kinnear Road, Columbus, Ohio 43212 (1)
Dr. Harvey M. Patt, Laboratory of Radiobiology, University of California, San Francisco Medical Center, San Francisco, California 94122 (1)
Purdue University, Nuclear Engineering Library, Lafayette, Indiana 47907 (1)
Dr. S. M. Reichard, Director, Division of Radiobiology, Medical College of Georgia, Augusta, Georgia 30902 (1)
University of Rochester, Atomic Energy Project Library, P. O. Box 287, Station 3, Rochester, New York 14620 (1)
OTHER (continued)

Dr. H. H. Rossi, 630 West 168th Street, New York, New York 10032 (1)
Dr. Eugene L. Saenger, Director, Radioisotope Laboratory, Cincinnati General Hospital, Cincinnati, Ohio 45229 (1)
Sandia Corporation Library, P. O. Box 3800, Albuquerque, New Mexico 87115 (1)
Scientific Committee on the Effects of Atomic Radiation, ATTN: Library, United Nations Room 3267, United Nations Plaza, New York, New York 10017 (1)
Scope Publications, Franklin Station, P. O. Box 7407, Washington, D. C. 20004 (1)
Dr. Arthur R. Tamplin, Biophysicist, Information Integration Group, University of California, Lawrence Radiation Laboratory, L-612, Livermore, California 94550 (1)
Radiation Biology Laboratory, Texas Engineering Experiment Station, Texas A. & M. University, College Station, Texas 77840 (2)
Texas Nuclear Corporation, ATTN: Director of Research, Box 9267 Allandale Station, Austin, Texas 78756 (1)
Western Reserve University, Department of Radiology, Division of Radiation Biology, Cleveland, Ohio 44106 (1)
Mr. Lionel Zamore, 601 Brightwater Court, Brooklyn, New York 11235 (1)

FOREIGN

International Atomic Energy Agency, Kaerntnerring 11, Vienna I 1010, Austria (1)
European Atomic Energy Community, C. E. E. A., Library, 51 rue Belliard, Brussels 4, Belgium (1)
Dr. L. G. Lajtha, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, England (1)
Dr. L. F. Lamerton, Biophysics Department, Institute of Cancer Research, Surrey Branch, Belmont, Sutton, Surrey, England (1)
National Lending Library for Science and Technology, Boston Spa, Yorkshire, England (1)
Directorate of Medical and Health Services, FAF (Federal Armed Forces), Bonn, Ermekeilstr. 27, West Germany (1)
Abteilung fur Strahlenbiologie im Institut fur Biophysik der Universitat Bonn, 53 Bonn-Venusberg, Annaberger Weg 15, Federal Republic of Germany (2)
Prof. Dr. H. Langendorff, Direktor des Radiologischen Instituts der Universität, 78 Freiburg im Breisgau, Albertstrasse 23, Germany (1)
Dr. Helmut Mitschrich, Akademie des Sanitaets-und Gesundheits, Weseus BW, Spezialstab ATV, 8 Muenchen Schwere-Reiterstr. 4, Germany (2)
Prof. Dr. F. Wachsmann, Gesellschaft fur Strahlenforschung m.b.H., 8042 Neuherberg bei Muenchen, Institut fur Strahlenschutz, Ingolstader Landstrasse 1, Muenchen, Germany (1)
Joachim Emde, Col. Director ATV/Stab, ABC- und Selbstschutzschule, SpezStATV/R, 8972 Sonthofen 2/Allgaeu, Berghoferstrasse 17, West Germany (1)
Dr. M. Feldman, Section of Cell Biology, The Weizmann Institute of Science, Rehovoth, Israel (1)
Dr. G. W. Barendsen, Radiobiological Institute TNO, Rijswijk, Netherlands (1)
Puerto Rico Nuclear Center, ATTN: Reading Room, College Station, Mayaguez, Puerto Rico 00708 (2)
Dr. H. Cottier, Pathological Institut der Universitat, Bern, Switzerland (1)
A method for the isolation of rat 7S gamma globulin based on multiple adsorptions with DEAE-Sephadex A-50 has been presented. The separation of rat 7S globulin requires a procedure modified from the one used for humans in terms of the buffer pH, and the number of adsorptions. Ultracentrifugal analysis indicates the resulting fraction is a single component. In addition, immunoelectrophoresis of the fraction when tested against anti-rat sera confirms the homogeneity of this 7S fraction. A monospecific antiserum to rat 7S gamma globulin has been produced. This antiserum is free of reaction with all other serum components when subjected to immunoelectrophoresis against normal rat serum.
### 1. ORIGINATING ACTIVITY: Enter the name and address of the contractor, subcontractor, grantee, Department of Defense activity or other organization (corporate author) issuing the report.

### 2. REPORT SECURITY CLASSIFICATION: Enter the overall security classification of the report. Indicate whether "Restricted Data" is included. Marking is to be in accordance with appropriate security regulations.

### 3. REPORT TITLE: Enter the complete report title in all capital letters. Titles in all cases should be unclassified. If a meaningful title cannot be selected without classification, show title classification in all capitals in parenthesis immediately following the title.

### 4. DESCRIPTIVE NOTES: If appropriate, enter the type of report, e.g., interim, progress, summary, annual, or final. Give the inclusive dates when a specific reporting period is covered.

### 5. AUTHOR(S): Enter the name(s) of author(s) as shown on or in the report. Enter last name, first name, middle initial. If military, show rank and branch of service. The name of the principal author is an absolute minimum requirement.

### 6. REPORT DATE: Enter the date of the report as day, month, year, or month, year. If more than one date appears on the report, use date of publication.

### 7a. TOTAL NUMBER OF PAGES: The total page count should follow normal pagination procedures, i.e., enter the number of pages containing information.

### 7b. NUMBER OF REFERENCES: Enter the total number of references cited in the report.

### 8a. CONTRACT OR GRANT NUMBER: If appropriate, enter the applicable number of the contract or grant under which the report was written.

### 8b, 8c, & 8d. PROJECT NUMBER: Enter the appropriate military department identification, such as project number, subproject number, system numbers, task number, etc.

### 9a. ORIGINATOR'S REPORT NUMBER(S): Enter the official report number by which the document will be identified and controlled by the originating activity. This number must be unique to this report.

### 9b. OTHER REPORT NUMBER(S): If the report has been assigned any other report numbers (either by the originator or by the sponsor), also enter this number(s).

### 10. AVAILABILITY/LIMITATION NOTICES: Enter any limitations on further dissemination of the report, other than those imposed by security classification, using standard statements such as:

- (1) "Qualified requesters may obtain copies of this report from DDC."
- (2) "Foreign announcement and dissemination of this report by DDC is not authorized."
- (3) "U. S. Government agencies may obtain copies of this report directly from DDC. Other qualified DDC users shall request through DDC."
- (4) "U. S. military agencies may obtain copies of this report directly from DDC. Other qualified users shall request through DDC."
- (5) "All distribution of this report is controlled. Qualified DDC users shall request through DDC."

If the report has been furnished to the Office of Technical Services, Department of Commerce, for sale to the public, indicate this fact and enter the price, if known.

### 11. SUPPLEMENTARY NOTES: Use for additional explanatory notes.

### 12. SPONSORING MILITARY ACTIVITY: Enter the name of the departmental project office or laboratory sponsoring (paying for) the research and development. Include address.

### 13. ABSTRACT: Enter an abstract giving a brief and factual summary of the document indicative of the report, even though it may also appear elsewhere in the body of the technical report. If additional space is required, a continuation sheet shall be attached.

It is highly desirable that the abstract of classified reports be unclassified. Each paragraph of the abstract shall end with an indication of the military security classification of the information in the paragraph, represented as (TS). (S). (C), or (U).

There is no limitation on the length of the abstract. However, the suggested length is from 150 to 225 words.

### 14. KEY WORDS: Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report. Key words must be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context. The assignment of links, rules, and weights is optional.