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February 6, 1956

H/7/4
James

L. D. McKay, Director
Finance Division

Herman M. Roth, Director
Research and Development Division

714708

CONTRACT NO. AT-(40-1)-1964 - BAYLOR UNIVERSITY

SYMBOL: OBS:EMM

This is to advise you that Contract No. AT-(40-1)-1964 with Baylor University has expired and the work is now covered under a new contract with the Wally-Bohannon Research Foundation. Inasmuch as the annual progress report covering the work performed during the period of the contract has been submitted, and since the work is still being conducted under a new contract, we have accepted the annual progress report as the complete scientific report as required in Appendix "C" of the contract. Therefore the obligations of the University have been concluded, and the contract should be closed out.

ORIGINAL SIGNED BY
HERMAN M. ROTH

Herman M. Roth

CC: J. R. Moore ✓
F. E. McPherson

REPOSITORY Oak Ridge Operations
Records Holding Area
COLLECTION Documents 1944-94
Contracts AT-(40-1)-1962-1965
BOX No. 1 of 1 Bldg 2714-14
FOLDER Contract Correspondence
Contract AT-(40-1)-1964

Res. Serv. Br. Res.&Dev.Div.
Mason:vhs

1097743

Contract AT-(40-1)-1964

Baylor University, College of Medicine

Distributed January 12, 1955

Washington

Contract files

Finance (2)

Research & Medicine (2)

Washington (B&M-2)

extra copies

1097744

In Reply Refer To:
AEC:JN

Oak Ridge, Tennessee
January 12, 1955

Baylor University College of Medicine
Department of Medicine
Houston, Texas

Attention: Dr. Jack M. Rose

Subject: CONTRACT NO. AT-(10-1)-1964

Gentlemen:

Enclosed, for your retention, you will find one duly signed
copy of Contract No. AT-(10-1)-1964.

Very truly yours,

John R. Moore
Director
Contract Division
Oak Ridge Operations


Enclosure:
Contract

Nicholson: jn

OFFICE ▶					
SURNAME ▶	Nicholson	Moore			
DATE ▶	1-22-55	255			

Office Memorandum • UNITED STATES GOVERNMENT

TO : J. W. Ould, Jr., Assistant General Counsel

DATE: November 15, 1954

FROM : R. G. Humphries, Acting Director, Contract Division

SUBJECT: REQUEST FOR PREPARATION OF A RESEARCH CONTRACT WITH BAYLOR UNIVERSITY COLLEGE OF MEDICINE, DR. JACK M. ROSE, PROJECT LEADER

SYMBOL: ADA:ARB

Enclosed is an approved proposal from Baylor University College of Medicine for a research project entitled "Radio-Isotope Studies in Hodgkin's Disease", with Dr. Jack M. Rose as Project Leader. This action is covered by Activity No. 6210, Allotment No. 06-51-91(24) (F.Y. 1955 Funds), Contract Authorization No. BM-55-53, dated October 1, 1954.

Please prepare an appropriate research contract to cover this program for a period of one year, beginning November 1, 1954, with Commission funds in the amount of \$12,000.00.



R. G. Humphries

Enclosures:

1. Request for Contract Action
2. Budget for New Contract
3. Objectives of Proposed Research
4. Cy Memo fm Geckler dtd 11/3/54
5. Ltr fm Rose dtd 10/26/54
6. Contract Authorization BM-55-53
7. Application for Research Grant

CC: C. S. Shoup
L. D. MacKay
J. Nicholson, w/Encls. 1-3

Brown:arb

1097746

BUDGET FOR NEW CONTRACT - DR. J. M. ROSE
FOR PERIOD 11-1-54 - 10-31-55

Salaries & Wages:		\$12,800
Dr. J. M. Rose (50% of time)	0*	
Research Associate	7,500	
Technician, Laboratory Helper, Social Security	5,300	
(2) Animals & Supplies		3,000
(3) Travel		1,000
(4) Overhead (45% of Salaries)		5,800
		<u> </u>
		\$22,000

The Commission's contribution to the above budget will be \$12,000.

Recognize support from other agencies as outlined in the contractor's propo

*Dr. Rose is [redacted]; he has an appointment at the University as Assistant Professor of Medicine at no salary. No contribution on part of the University.

monetary

* It is recognized that Dr. Rose receives no salary from the Contractor for work under this contract. It is further noted that additional support for the general project may be received from other sources as reported in the contractor's proposal.

The objectives of the proposed research are as follows:

1. To determine whether a rabbit anti-serum against Hodgkin's diseased tissue will combine with tissue involved by this disease upon intravenous administration to the living Hodgkin's patient.
2. To determine the cellular localization of any combination that takes place in (1).
3. To tag with a radio-isotope rabbit antibodies against Hodgkin's diseased tissue.
4. To determine the effects of tagged antibodies on specific cells that may have exhibited localization of the radio-isotope.
5. To compare specificity and strength of anti-sera against various protein fractions and ultra-centrifugal fractions of Hodgkin's diseased tissue with antibodies against the whole homogenate by means of localization of tagged antibodies "in vivo".
6. To evaluate anti-sera tagged with Iodine¹³¹, Sulfur³⁵, and Carbon¹⁴ in the diagnosis and treatment of Hodgkin's disease.
7. To determine cross reactivity of the rabbit anti-Hodgkin's serum with other abnormal cells such as carcinoma and lymphosarcoma cells.

*... the results will indicate the purpose
of the study in a series of lines;*

1. Chairman
TO: J. R. Moore Contract Board. From: Research & Medicine Div.

It is requested that the Contract Board take the necessary action to process the following described contract action in accordance with the provisions of Bulletin OR-O&M-19:

2. Nature of Action Requested

Selection of New Contractor and Negotiation of Contract.
Baylor University College of Medicine
~~Waco~~, Texas
Houston, Review and approval of Contract, Sub-contract or Purchase Order.
Number: _____
Name: _____

Modification of Contract
No. _____
Contractor: _____

Other (Explain) _____

3. Nature of Services to be Covered by Contract

Construction Architect-Engineer Other (Explain) Research

4. Funding Amount to be Obligated by this Contract Action \$ 12,000.00

Source of Funds

Approved ORO Financial Plan, _____ Quarter, Fiscal Year 19____
Project No. _____ or, Activity No. 6210
Funds to be Obligated: Allotment No. 66-57-91(24) Y. 1955 Funds)
Procurement Directive No. BM-55-53 Dated 10-1-54
Issuing Office Biology & Medicine Div.

Concurrence in Funding Statement: (signed) _____
Chief, Funds Control
Budget Branch

5. Project or Activity to be Covered by Contract Action:

Location of Work: _____ Construction Directive No. _____
Estimated Cost of Work to be Covered by this Contract Action \$ _____
Schedule: Date Work to Start _____ Estimated Completion Date _____
Description of Project or Activity: _____

(If more space is required use separate sheets and attach hereto:)

6. Contract Board Docket
No. _____
(To be assigned by
Board Secretary)

7. Request Submitted By: (signed) _____
Date: 11/12/54 Title: _____

Herman de Roth

8. Complete Description of Services to be Furnished by Contractor:

Washington designated research contract
Title: Radio-Isotope Studies in Hodgkin's Disease

(If more space is required use separate sheets and attach hereto:)

9. Description of other changes to be covered by Modification:

New contract for a period of one year beginning November 1, 1954, with
Commission funds in the amount of \$12,000.00.

(If more space is required use separate sheets and attach hereto:)

10. Negotiated Contracts. (Show why it appears desirable to negotiate new contract or to negotiate
modification to existing contract)

Memo J. C. Bugher to S. R. Sapirie dated October 1, 1954

(If more space is required use separate sheets and attach hereto:)

11. Contracts, Subcontracts, or Purchase Orders Submitted for Review and Approval: (Furnish brief descrip-
tion of action in this space and attach pertinent documents)

None

12. Disputes:

Attach a statement summarizing the dispute together with pertinent documents and Background
Material.

None

12609

1097750

James Rounsaville

November 3, 1954

Robert P. Geckler

BAYLOR UNIVERSITY CONTRACT AUTHORIZATION NO. BM-55-53

I talked with Dr. J. M. Rose regarding the budget for his research contract and also had a conversation with the Business Office regarding overhead.

Dr. Rose has a University appointment as Assistant Professor of Medicine at no salary. He is [REDACTED]. Half of his time will be spent on this research and he places a value of \$5000 for half of his time. He was not familiar with the University's regulations regarding overhead and referred me to the Business Office. The Business Manager is Mr. J. L. Johnson. My conversation was with an unidentified subordinate. At the present time the Army is determining the overhead rate for the Institution but this figure will not be available for sometime. The business office, however, made an unofficial determination of overhead and found it to be approximately 30% of the total funds of the grant or 45% of salaries. In view of the Government reluctance to provide full cost of overhead a minimum acceptable to the University has been decided to be 30%. In the unofficial determination maintenance was prorated among the various department. Usage on equipment was taken to be 6 2/3%. Buildings and grounds were allowed 2% and 20% of the department chairman's salary was included in overhead. Other details on the determination were not available.

The University has recently instigated social security payments and would like to have these included in the budget.

The contract should be dated Nov. 1, 1954.

The business office would like any literature available on regulations, etc., regarding contract funds. If none is available, we should write them to this effect.

Robert P. Geckler

Geckler:ef

1097751

Baylor University
College of Medicine
Texas Medical Center
Houston, Texas
October 26, 1954

Division of Research Grants
Atomic Energy Commission
Oakridge, Tenn.

Dear Sir:

Inclosed is a copy of the budget drawn up in a form similar to the one suggested in your letter.

Dr. James A. Green, chief of the Department of Medicine, apparently did not understand that the application required his full signature. He has merely marked it O.K. with his initials. If this is not satisfactory, please let me know and I will have him sign an additional copy when he returns to town.

Sincerely yours,

J. M. ROSE, M. D.

Jack M. Rose, M.D.

JER:rk

1097752

CCY 2-185A
D-5430

Baylor University
 College of Medicine
 Texas Medical Center
 Houston, Texas

TOTAL COST BUDGET FOR RESEARCH PROPOSAL COVERING WORK ON

"RADIO-ISOTOPE STUDIES IN HODGKIN'S DISEASE"

FOR PERIOD 11-1-54 through 10-31-55

	<u>TOTAL</u>	<u>BAYLOR</u>	<u>AEC</u>	<u>USPHS</u>	<u>OTHER</u>
Salaries & Wages:					
Immuno-chemist & Radio-biologist	\$ 7,500	\$5,000	\$2,500	0	0
Immunologic Technician	3,500	0	0	\$3,500	0
Radio-biological Technician	3,500	0	3,500	0	0
Chemical Technician	3,500	0	0	0	*
Photographic Technician	1,800	0	0	1,800	0
Laboratory Helper	1,800	0	1,800	0	0
Animals & Supplies	4,800	600	2,400	1,800	0
Equipment	450	0	0	450	0
Travel	1,350	0	1,000	350	0
Overhead-(8% of the total grant)	<u>1,432</u>	<u>0</u>	<u>800</u>	<u>632</u>	<u>0</u>
	\$29,632	\$5,600	\$12,000	\$8,532	\$3,500

*Funds from one of several sources pending

J.A. Coe

1097753

UNITED STATES ATOMIC ENERGY COMMISSION
WASHINGTON, D. C.

Contract Authorization No. EM-55-53

OCT 1 1954

TO : S. R. Sapirie, Manager
Oak Ridge Operations Office

FROM : Dr. John C. Bugher, Director, Division
of Biology and Medicine, Washington, D. C. *J. C. B.*

SUBJECT : FUND AUTHORIZATION AND TRANSMITTAL OF RESEARCH PROPOSAL FOR
CONTRACT NEGOTIATION

REFERENCE : AEC 102/16 APPROVED OCTOBER 7, 1953, AS IMPLEMENTED BY MEMO
TO MANAGERS, OPERATIONS OFFICES, DATED 10/23/53, JOINTLY
SIGNED BY THE DIRECTORS OF THE DIVISIONS OF RESEARCH AND
BIOLOGY AND MEDICINE.

SYMBOL : BMM:FGL
The research proposal described below has been approved by
the Division of Biology and Medicine, funds are available, and
you are authorized and requested to negotiate a contract in
accordance with the following terms and conditions:

1. Institution: Baylor University College of Medicine
2. Investigator (s): Dr. Jack M. Rose
3. Title: "Radio-Isotope Studies in Hodgkin's Disease"

4. (x) New Contract or () Renewal of Contract No. _____

5. Duration - From: One year To:

6. AEC Technical Representative: Paul G. LeFevre *LeF*

7. Contract Fund Authorization:

Funds are authorized for the obligation of this contract
as follows:

<u>Allotment No.</u>	<u>Budget Category</u>	<u>Previous</u>	<u>Amount This Action</u>	<u>Total</u>
06-51-91 (24)	6210	_____	\$12,000	\$12,000
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____

1097754

OCT 4 1954

D-499

UNITED STATES ATOMIC ENERGY COMMISSION
WASHINGTON, D. C.

Contract Authorization No. EM-55-53

TO : S. R. Sapiro, Manager
Oak Ridge Operations Office

FROM : Dr. John C. Bugher, Director, Division
of Biology and Medicine, Washington, D. C.

SUBJECT : FUND AUTHORIZATION AND TRANSMITTAL OF RESEARCH PROPOSAL FOR
CONTRACT NEGOTIATION

REFERENCE : AEC 102/16 APPROVED OCTOBER 7, 1953, AS IMPLEMENTED BY MEMO
TO MANAGERS, OPERATIONS OFFICES, DATED 10/23/53, JOINTLY
SIGNED BY THE DIRECTORS OF THE DIVISIONS OF RESEARCH AND
BIOLOGY AND MEDICINE.

SYMBOL : **EMM:FGL**
The research proposal described below has been approved by
the Division of Biology and Medicine, funds are available, and
you are authorized and requested to negotiate a contract in
accordance with the following terms and conditions:

1. Institution: Baylor University College of Medicine
2. Investigator (s): Dr. Jack M. Rose
3. Title: "Radio-Isotope Studies in Hodgkin's Disease"

4. New Contract or () Renewal of Contract No. _____

5. Duration - From: **One year** To:

6. AEC Technical Representative: **Paul G. LaFevre**

7. Contract Fund Authorization:

Funds are authorized for the obligation of this contract
as follows:

<u>Allotment No.</u>	<u>Budget Category</u>	<u>Previous</u>	<u>Amount This Action</u>	<u>Total</u>
<u>06-51-91 (24)</u>	<u>6210</u>	_____	<u>\$12,000</u>	<u>\$12,000</u>
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____

1097756

1-475

APPLICATION FOR RESEARCH GRANT
FROM
ATOMIC ENERGY COMMISSION

- I. Title of the project--Radio-Isotope Studies in Hodgkin's Disease.
- II. The institution and department in which the work will be done--
Baylor University College of Medicine, Department of Medicine.
- III. Scientific background-- Immunologic studies in Hodgkin's Disease have been in progress in our laboratory for the past two years. The basic studies are supported by the United States Public Health Service (Grant No. C 10707--\$8900/annum). As a result of this work an anti-serum has been developed which shows evidence of specificity against an antigen or antigens contained in Hodgkin's diseased tissues.

Pilot studies have been conducted employing the absorbed globulin fraction of the rabbit anti-Hodgkin's serum tagged with I¹³¹. No support is available for these studies at the present time.

A. Literature relevant to the proposal:

1. Rose, J.M., Immunologic Studies in Hodgkin's Disease. A preliminary Report presented at the American Academy of Allergists in February of 1954, now in press, American Journal of Allergy.
2. Pressman, David, The zone of localization of anti-tissue antibodies as determined by the use of radioactive tracers. J. Allergy, 22 (5) 387-96. 1951.
3. Pressman, David, The zone of localization of antibodies. III. The specific localization of antibodies to rat kidney. Cancer, 2 (4). 1949.
4. Talmadge, D. V., Dixon, F. J., et al, Antigen elimination from the blood as an early manifestation of the immune response. J. Immunol. 67, 243. 1951
5. Kidd, John G., Suppression of growth of Brown-Pearce tumor cells by a specific antibody with a consideration of the nature of the reacting cell constituent. J. Exp. Med., 83, 227-50. 1946
6. Marshal, A. H. E. and White, R. G., Reaction of reticular tissue to antigen. Brit. J., of Exp. Path., 31 (2), 157-74, 1950.

- B. Significance of this work--If tumor antibodies or antibodies against any abnormal cell can be shown to be specific for such cells without having damaging effects on normal cells in the body, such antibodies may be of value in diagnosis and treatment of an important group of diseases in man. This research is directed toward demonstration of the specificity of any antibody against Hodgkin's diseased cells in the living patient and "in vitro". Results of this type of investigation do not have the objections of the applicability to human disease as in experiments performed entirely on animals.

IV. Scientific scope of the proposed research

A. Objectives

1. To determine whether a rabbit anti-serum against Hodgkin's diseased tissue will combine with tissue involved by this disease upon intravenous administration to the living Hodgkin's patient.
2. To determine the cellular localization of any combination that takes place in (1).
3. To tag with a radio-isotope rabbit antibodies against Hodgkin's diseased tissue.
4. To determine the effects of tagged antibodies on specific cells that may have exhibited localization of the radio-isotope.
5. To compare specificity and strength of anti-sera against various protein Tselius and ultra-centrifugal fractions of Hodgkin's diseased tissue with antibodies against the whole homogenate by means of localization of tagged antibodies "in vivo". Preparations prepared by physical methods such as use of a fine mesh screen, approximately 37 micra diameter, for differential cellular separation will be employed.
6. To evaluate anti-sera tagged with Iodine¹³¹, Sulfur³⁵, and Carbon¹⁴ in the diagnosis and treatment of Hodgkin's disease.
7. To determine cross reactivity of the rabbit anti-Hodgkin's serum with other abnormal cells such as carcinoma and lymphosarcoma cells.

B. Its relation to present knowledge and comparable work in progress elsewhere--Tagged antibodies have not been reported to have been used in human subjects. Numerous studies have been done with anti-tissue antibodies tagged with radio-iodine and injected into animals. Work is in progress at the present time on anti-tumor antibodies in transplantable animal tumors. The globulin fraction of anti-sera have been successfully tagged by several experimenters.

C. Plan of accomplishments for the first Year's work:

1. General methods

a. Preparation of Rabbit Anti-Hodgkin's Serum

The same method will be used that is being employed in the Immunologic Studies in Hodgkin's Disease.* In these experiments tissue homogenates were prepared from material removed from Hodgkin's patients at autopsy within four hours of death and made up immediately for injection, or else stored at approximately -63°C. in an atmosphere of carbon dioxide ice. Homogenization of tissue was performed in an ice bath using an ordinary glass tissue homogenizer. An anti-serum was produced by the injection of the Hodgkin's homogenate (0.4 ml.) with falba (0.2 ml), paraffin oil (0.4 ml), and Myco-bacterium phlei (0.16 mgm) Injections with this material were made in three to four sites subcutaneously, at monthly intervals, using 1 cc.

* Research supported by a U.S. Public Health Service Grant

in each of the depots. Preceding each immunisation, tests were made on the rabbit serum for determination of antibody titers. In addition to this preparation various ultra-centrifugal and protein fractions will be employed for immunization.

b. Absorption of Non-Specific Antibodies.

The procedure followed in the earlier study will also be followed here. Experiments conducted in previous studies indicated that the arbitrary use of a final dilution of 1:5 suspension of normal lymph node antigen was effective in absorbing out antibodies against normal lymph node components. Generally two to three such absorptions carried out in the cold overnight with continuous gentle rotation, satisfactorily removed non-specific antibodies while permitting to remain intact antibodies against the Hodgkin's disease components. Removal of other non-specific antibody components was accomplished by absorption. Absorption with heterophile antigen was also performed. Antigen antibody testing procedures were carried out with a normal lymph node antigen and a Hodgkin's antigen prepared by homogenization of the respective tissues followed by suspension in normal saline.

c. Antibody Testing Procedures.

Routine complement fixation reactions have been used. Use of trypsin-treated red cells and colloidal coated antigen particles will also be employed as a method of antibody determination. Testing anti-sera against Hodgkin's antigen and normal lymph node antigen and using a serum and antigen control have consistently shown the presence of an antibody specific for Hodgkin's tissue after a sufficiently long period of immunization.* In addition to testing anti-sera against the anti-serum against the whole homogenate with the comparable homogenate, tiselius, ultra-centrifugal and physical fractions will also be employed as test antigens.

d. Preparation of Globulin Fraction

The globulin fraction of the absorbed rabbit anti-serum will be removed by dialysis against 1.4 volumes of 3 M ammonium sulfate in the cold (1). At present modifications of ethanol fractionation techniques are being investigated to arrive at a method suitable for this laboratory. Comparisons of the globulin fractions recovered by this method will be made with the one previously referred to. The precipitated globulin fraction will then be washed several times with 1.75 M ammonium sulfate and redissolved in one-third to one-half

* As part of a continuation study, under a U.S. Public Health Grant on immunologic studies in Hodgkin's disease, other methods of determining accomplishment of complete absorption are in progress. These include Schultze-Dale and Gross anaphylaxis in the passively sensitized Guinea pig.

of the original volume. An additional dialysis will be used to remove the ammonium sulfate remaining behind until negative reaction to Hessler's solution is obtained.

- e. **Tagging of Antibody Globulin with Radio-Isotopes.** Various isotopes will be considered for use in tagging the rabbit anti-Hodgkin's serum, including Iodine¹³¹, Sulfur³⁵, and Carbon¹⁴. "In vitro" labeling of the antibody will be attempted using I¹³¹ after the method of Pressman and Sternberger (2).

Antibody globulin will be labeled with carrier-free radio-active I¹³¹ by the addition of free radio-active iodine to 6.25 mg of globulin at a pH of 9.7. Uncombined radio-active iodine will be removed by ion exchange using Amberlite IR4B anion exchange resin. Yields as high as 80% have been obtained without undue denaturation of the antibody fraction, confirmed in this lab by tagging an antibody globulin fraction of known titer and detecting any change in titer after labeling procedure.

In addition to the "in vitro" labeling of Hodgkin's antibody globulin, an attempt to biologically label the Hodgkin's antibody will be made. Rabbits will be injected with S³⁵Methionine available commercially and after an indefinite period of time the animals will be bled and the rate of formation of normal globulin and Hodgkin's antibody globulin will be determined in addition to determining the yield and specific activity of the labeled globulin. Procedures outlined by Tarber and Reinhardt (3) will be followed in which 0.023% of the injected S³⁵ appeared in the globulin fraction. Should the purchase of S³⁵ Methionine, commercially available, not be found economical, the biological synthesis of S³⁵ Methionine will be done, utilizing cultures of Escherichia coli after the method of Dean Coyne (4). Cultures of E. coli will be incubated with S³⁵ Sulfate. S³⁵ Sulfate is reduced and utilized by the bacteria for synthesis of protein and by this method 20% of the sulfur is incorporated in methionine and 80% cysteine. The protein is then hydrolyzed and with either paper chromatography, electrophoresis or by ion exchange the two amino acids will be separated. By this method 0.7 mgm. of methionine per cc. of E. coli can be obtained. Should this method of biological synthesis of antibody prove successful, as well as economical, it is planned to attempt the biological synthesis of methionine with Carbon 14.

- f. **Autoradiographic Techniques.**

These will be performed on tissue removed from the Hodgkin's patient following injection of a tagged anti-serum. Gross autoradiographs will be made from the excised node. Tissues removed from other patients who have not been injected with an anti-serum will also be studied using this technique. Microscopic autoradiographs will be prepared on tissues as follows: A portion of the involved node will be dissected from the frozen state in an atmosphere of liquid nitrogen and then embedded in paraffin. Sections will then be cut with

the microtome and mounted on specially prepared emulsion on glass microscope slides (VWR plates). Hodgkin's tissue slices will also be incubated with tagged anti-serum in small beakers. These beakers will then be placed in a Dubnoff metabolic apparatus and allowed to remain for several hours. Paraffin blocks will then be prepared from this tissue in the same manner as in the "in vivo" studies.

By microscopic examination of the histologic sections and superimposed autoradiographs, attempts will be made to determine exact cellular localization of the antibodies. Particular note will be made of any localization that might indicate differential pick-up by either the cell membrane, the cytoplasm, or the nucleus of involved cells, as well as to determine whether normal components of the node show evidence of localization of the tagged antibody or whether it is confined to abnormal components such as the Sternberg-Reed type cell.

2. Specific Experiments

a. In vitro Studies

Frozen Hodgkin's tissue will be incubated in the Dubnoff metabolic apparatus with the tagged globulin fraction of rabbit anti-Hodgkin's serum and followed by autoradiographic studies to determine uptake and localization.

Chromatographic studies have been initiated on Hodgkin's tissue and controls on suitable normal human tissue. Chromatograms will be prepared of the minced tissue slice following incubation and of tissue removed from the patient following injection of the tagged anti-serum. Counts will then be made on the various separated fractions to determine areas of localization of radio-activity. Comparisons will be made with Tiselius and ultra-centrifugal fractions of the antigen containing localized radio-activity. Subsequently comparison of complement fixation studies will be performed using all types of fractions described above.

b. Intravenous injection of a patient with Hodgkin's disease with the globulin fraction of a rabbit anti-Hodgkin's serum tagged with radio-isotopes.

(1). Use of Antibody Tagged with Iodine-131

One milligram of antibody tagged with 5 millicuries of Iodine-131 will be injected intravenously in the early studies. The thyroid gland will have been previously blocked with an anti-thyroid compound such as Tapazole. Determination of uptake over various parts of the body will be conducted by use of a directional scintillation counter using calcium tungstate as the phosphor. Counts will be made over involved lymph nodes and compared with counts over corresponding and symmetrical areas on the contralateral side of the body. Uptake by lymph node groups such as the neck, groin, or axillae, as well as counts over the thyroid, the rate liver, spleen and knee will be performed.

of disappearance of radio-iodine from the blood stream will be checked by determinations on the blood and urine with collection periods corresponding to the times at which counts are made over the body. Persistent differences of 10% or more between involved lymph nodes and counts in a symmetrical area on the contralateral side of the body will be considered significant. If sufficient activity is found, the involved areas will be scanned with an automatic scintillation scanning detector similar to that described in the literature for scanning the thyroid, liver, prostate, etc.

General studies on the patient will be made at the same time that the radio-isotopic study is being carried out, i.e., blood counts, sedimentation rates, etc.

As more patients are studied the amount of tagged globulin injected will be increased to a maximum of 20-30 mc. of radio-iodine attached to 4 - 5 mgm. of antibody nitrogen unless specific uptakes can be demonstrated with smaller quantities. A sufficient number of patients will be studied to determine such levels. If positive evidence is obtained in pursuing this line of approach, at least fifteen patients will be injected with the amount of tagged antibody protein determined to be adequate for demonstrating specific uptake.

- (a) Similar studies to that in (1) using antibodies tagged biologically with Sulfur³⁵ and Carbon¹⁴.
 - (b) Similar studies to that in (1) using double tagged antibodies (I¹³¹ and C¹⁴ or I¹³¹ and C¹⁴).
- c. Autoradiographic Studies.
Diseased lymph nodes will be removed from patients in whom in vivo uptake studies following injection of the anti-serum have been completed. Half of the node will be used for autoradiographic studies. These will be made according to the technique described under General Methods.
- d. A portion of the node removed in the previous study will be minced and placed in the Texas Well Geiger counter to determine the specific activity from the total number of counts and thus the amount of radioactivity per gram of tissue.
- e. Controls on studies in (b), (c), and (d) will include the following:
- (1) Uptake of the radio-active antibody on one side of the body will be compared with measurements over symmetrical areas on the contralateral side of the body. Where superficial nodes are involved, the lack of involvement on one side of the body or the other can be determined without difficulty.
 - (2) Determination of the amount of radioactivity present in excised tissue involved by the Hodgkin's process will be compared with similar studies on uninvolved tissue from the same patient, such as normal lymph node or normal portions from nodes that have some areas of involvement, muscle tissue and skin.
 - (3) Comparison of counts over node bearing areas in

patients who have node involvement in that area with patients who do not have involvement in that area.

- (1) Uptake studies with tagged normal rabbit globulin in patients who are later to be studied following injection of the tagged anti-serum.
 - (2) Uptake studies in normal patients.
 - (3) Comparison of autoradiographs of normal lymph nodes treated 'in vitro' with tagged anti-serum as compared with Hodgkin's lymph nodes similarly treated 'in vitro'.
- f. Studies similar to those described in (a) will be made with anti-sera prepared against the albumin, globulin, ribonucleic acid and deoxy-ribonucleic acid fractions of the Hodgkin's tissue proteins (5).
- g. Comparison of areas of development in autoradiograph with the corresponding microscopic sections will be made to determine localization in cellular elements with particular note as to whether localization takes place in all cells or only those which appear to be abnormal such as Sternberg-Reed cells, atypical reticular cells, or multi-nucleated giant cell. Examination will also be made to determine whether localization in any particular cell is confined to certain portions of that cell such as the cell membrane, the cytoplasm, or the nucleus. Anti-sera against various protein fractions of Hodgkin's tissue such as the globulin fraction, the albumin fraction, the deoxy-ribonucleic acid fraction and the ribonucleic acid fraction will be tagged in a fashion similar to that with the anti-serum against whole homogenate. Studies will then be conducted to determine whether there is any quantitative or qualitative difference between the various anti-sera against the protein fractions and the anti-serum against the whole homogenate insofar as localization in Hodgkin's diseased tissue in the living patient or in Hodgkin's tissue treated 'in vitro' with the tagged anti-serum. Experiments similar to those used with the anti-serum against the whole homogenate will be employed here.
- h. If studies on specificity in localization indicate that the tagged anti-serum is specifically carried to Hodgkin's diseased cells, this anti-serum will be evaluated in attempting to detect areas of involvement in patients with Hodgkin's disease. Larger amounts of serum will then be employed to determine the effects of ionizing radiation on a focus of Hodgkin's disease in order to determine whether such a procedure is of possible therapeutic worth. The clinical course of the disease, X-ray studies, sedimentation rates, and other general laboratory tests, will be used as criteria to determine the efficacy of therapy. These studies

on diagnostic and therapeutic worth will be carried out if previous investigations, already outlined, have shown definite promise.

- i. "In vitro" studies will be conducted on other types of cells such as carcinoma and lymphomas. Autoradiographs will be made on sections of such tissue after incubating with tagged Hodgkin's anti serum in a Dubnoff metabolic flask. Microscopic autoradiographs will be superimposed on the histologic sections to determine uptake by various types of cells. If indicated, "in vivo" studies will also be made in other type of malignancies employing the tagged anti-Hodgkin's serum.
- j. Special staining techniques will be employed as well as microscopic autoradiographs to ascertain the portion of the cell affected by the absorbed anti-serum. If specific localization is demonstrated to take place in the abnormal cellular components of the Hodgkin's diseased nodes, additional information from this technique may be of extreme importance since the antibody may be against antigens contained in the cell membrane or in the cytoplasm or nucleus or perhaps all three. However, there is no definite information available to indicate whether an antibody against cytoplasmic components would be able to penetrate the cell membrane of the living. Thus presence of a specific antibody may remain undemonstrated because of its inability to reach the antigen against which it is directed. Differential staining of abnormal cells before and after treatment with anti-serum will be used to demonstrate possible effects on different portions of the cell.

V. Scientific Personnel

- (1) Jack M. Rose, M.S., M.D. - [REDACTED]
Assistant Professor of Medicine, Baylor University College of Medicine
Basic Allergic Research--Northwestern University 1946-1948
Tissue culture research--University of Texas 1948-1950
Tissue Culture Laboratory
Immunologic Research in Hodgkin's Disease 1951 to present
Certified American Board of Internal Medicine, 1948
Certified American Board of Allergy, 1949
Half time will be spent by principal investigator on this project.

Publications:

1. Antigen Studies in tissue cultures of human allergic tissues were carried out at University of Texas Medical School in the laboratory of Dr. Charles Pomerat for 2 1/2 years. A summary of this work was presented at the American Academy of Allergy annual meeting, February, 1952.
2. Rose, J.M., Pomerat, C.M., Danes, B.: Tissue Culture Studies in Citrused Nasal Mucosa in Man. Anat. Rec. 104 (4) 4092-520, 1949.

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3. Rose, Jack M., Feinberg, Allen R., Friedlander, Sidney, and Feinberg, Sam M.: Histamine Antagonists. VII Comparative Anti-anaphylactic activity of Some New Antihistamine Drugs. J. Allergy 11 (3) 140-55, 1947.
 4. Immunologic Studies in Hodgkin's Disease. In press, Journal of Allergy.
- (2) [REDACTED] Ph.D. DONALD A. RAPPAPORT
 --4 years
 [REDACTED] of Carbohydrates Employing C14
 --1 year
 [REDACTED] Nucleic Acid Metabolism Employing P32 and C14
 --1 1/2 years
 [REDACTED] Use of P32 and I-131 for Tagging Red Cells
 Metabolic Studies in the Warburg Apparatus with C14
- (3) Scientific personnel to be newly employed
 B.A. Rubin, Ph.D., Immuno-chemist and radio-biologist

VI. Other Personnel

- A. Graduate Students (Graduate studies are planned starting in September, 1954).
- B. Micro-Biologist--Full-time at present.
- C. Chemist--Full time at present
- D. Two additional individuals of technician grade will be required for this project.

- VII. Other Financial Assistance--Support of the two graduate students and of the program of immunization of animals is committed by the U.S. Public Health Service until March 31, 1956. A supplementary grant for the study of immuno-genetic relationships of the Hodgkin's antigen has been submitted to the U.S. Public Health Service. If awarded, these funds will be used for enlarging the animal immunization program and for technical help necessary in collecting post-mortem specimens and preparation of tissue sections for pathologic examination. In addition, technical help will be required for the large additional amount of serologic studies required.

Technical help proposed in the present application will be employed in the preparation of isotopic compounds and the uptake studies on tissue slices preparation of autoradiographs from "in vivo" studies, "in vitro" tissue slices, and tissue cultures treated with radio-active anti-serum.

VIII. Facilities Available

- A. General laboratory equipment
- B. Completely equipped tissue culture laboratory
- C. "Hot" laboratory
- D. Animal quarters
- E. Temporary use of Spinco ultra-centrifuge
- F. Radio-Isotope Equipment
 1. Major items of equipment include wide angle scintillation counter, utilizing calcium tungstate as the detecting phosphor. Model No. LAI 12u.
 2. Directional Scintillation Counter utilizing calcium tungstate as the detecting phosphor with tungsten columnating head, Ser. No. T-241, Model TAX21A.

3. Scintiscaler, Ser. No. C 240, Model No CX14WC.
 - Scintiscaler, Ser. No. C 241, Model No CX14WC.
 4. Esterline Angus Recording Milliammeter, Style No. 90H, Ser. No. 36817.
 5. Texas We.. Geiger Mueller Counter.
 6. Scintiscanner Model RZXB, Ser. No. -242.
 7. Tracer Lab-Monitor, Ser. No. 835, Model No. SU3B.
 8. Victoreen Minometer, Model 287, Ser. No. 2872527.
 9. Cutiepie, Model No. SU7F, Serial No. 549.
 10. 6 - Dosimeters, Model 362
- G. Minor Items: Refrigerator, Centrifuge, Water Bath, Lab Glassware, Lead Bricks, Animal Room, Animals, Phase Contrast Microscope.

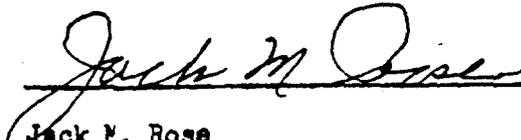
IX. Travel and Other Items-Travel to and from appropriate meetings including transportation to and from attendance at courses at Oak Ridge for some of the personnel to be employed.

- X. Budget- Listed below is a composite of finances required for the support of the three divisions of this program:
1. Basic immunologic and tissue culture studies.
 2. Immuno-genetic studies and extended immunization program (Application for support of this portion has been made to the U.S. Public Health Service)
 3. Radio-isotope studies (Application for support of this portion of the program is herewith submitted in #XI

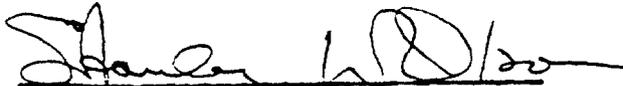
(1.) Present support from the U.S. Public Health Service		
Photographic technician	\$1800	
Immunologic technician	3500	
Animals and consumable supplies	1800	
Travel	350	
Permanent equipment	450	(sub-totals)
Overhead	632	\$8532.00
(2.) Pending support from the U.S. Public Health Service		
Chemical Research Assistant	3500	
Contribution toward immuno-chemist's and radio-biologist's salary for immuno-chemical portion of his work,	5000	
(In the event that U.S. Public Health funds are not made available, this amount can be obtained by means of local support)		
Animals and consumable supplies	1260	
Travel	300	
Overhead	804.80	\$10,864.60
(3.) Radio-biologist	2500	
Radio-Isotope technician	3500	
Chemistry technician	2400	
Laboratory helper	1800	
Consumable supplies (including radio-isotopes plus minor equipment)	2900	
Travel	1000	
Overhead	1128	
Total Budget	34,624.80	* \$15,228.00

* The salaries for the efforts of principal investigator and budgetant pathologist, Dr. Harvey Rosenberg, are not included in total

XI. Amount Requested: \$15,228.00 This corresponds to #X(3), the portion of the program submitted for possible support by The United States Atomic Energy Commission. The laboratory, animal rooms, service of the principal investigator and of Dr. Lappaport and Dr. Rosenberg are furnished by the institution.



Jack F. Rose
Principal Investigator



Stanley W. Olson
Dean, Baylor University College of Medicine