

UNITED STATES GOVERNMENT

Memorandum

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TO : Ralph Elson, Director
Contract Division

FROM : Herman M. Roth, Director
Laboratory and University Division

SUBJECT: REQUEST FOR CONTRACT ACTION

DATE: Oct 11 1967

OLE:JDB

It is requested that you take the necessary steps to process the following described contract action:

1. Nature of Action Requested:

Selection of New Contractor and/or
Negotiation of Contract
Number:
Contractor:

Modification of Contract
Number: AT-(40-1)-2459
Contractor: Medical College of Virginia
Richmond, Virginia

2. Nature of Services to be Covered by Contract: Research

Title: "The Effect of Radiation on Transplantation Immunity"

3. Type of Contract: Support Agreement Cost-Type Other

4. Amount of AEC Funds to be Obligated by this Contract Action: \$52,000.00

5. AEC Percentage of Estimated Total Cost to be Shown by this C/A: 49%

6. Description of Other Changes to be Covered by Contract Action:

Modify contract to provide for the performance of additional research to be completed during the period October 1, 1967 through September 30, 1968. The approved AEC support ceiling is \$52,000.00 which is 49% of the estimated total cost of the project (\$103,101). Title to equipment, if any, shall vest in the Contractor under Authority of Atomic Energy Act of 1954 since the Contractor's contribution is expected to equal or exceed the value of the equipment.

7. Authority:

Form AEC-481 (Contract Authorization)
from John R. Totter to S. R. Sapirie
dated August 30, 1967.

CONTRACTS-2459(VA) OLA

Herman M. Roth
Herman M. Roth

*CD Shoup
9-12-67*

S 5820

REPOSITORY: *Cell Ridge Operations*
COLLECTION: *Records of Cell Ridge Operations 1944-1994*
BOX#s: *H-75-4 Bldg. 2714-H*
No. 2 cont 2439
FOLDER: *Med College of VA*

*OKS
Buckner
9-12-67*

APPENDIX "A"

For the Contract Period October 1, 1967 through September 30, 1968.

A-I RESEARCH TO BE PERFORMED BY CONTRACTOR

The Contractor will continue studies of the action of radiation on transplantation immunity in the laboratory animal to include (1) radiation of the circulating blood with dosimetry, strontium-90 beta irradiation and movement, renal transplant, perfusion and extracorporeal irradiation, (2) local graft irradiation, (3) total-body irradiation and thymectomy, (4) histocompatibility and in vitro immunity studies as related to cytotoxicity, antibody determinations, antigenic stimulation, use of perfusion, etc.

A-II WAYS AND MEANS OF PERFORMANCE(a) Items Included in Total Estimated Cost:

(1) <u>Salaries and Wages</u>	\$52,467.00
Dr. D. M. Hume, Principal Investigator (25% of Time)	
3 Research Associates (30% of Time)	
8 Laboratory Technicians & Specialists (55% of Time)	
Animal Caretaker (50% of Time)	
(2) <u>Social Security</u>	2,307.00
(3) <u>Supplies and Equipment</u>	8,028.00
Photo Microscope	
Isotopes	
Laboratory Glassware	
Drugs	
Shunt Materials & Other Miscellaneous Items	
(4) <u>Reprints and Publication Costs</u>	300.00
(5) <u>Animals and Care</u>	16,280.00
(6) <u>Travel</u>	800.00
(7) <u>Maintenance of Research Facilities</u>	12,000.00
(8) <u>Overhead (30.34% of Salaries & Wages)</u>	15,919.00

(b) Items Significant to the Performance of this Contract,
but Excluded from Computation of Total Cost and from
Consideration in Proportioning Costs:

None

A-III The total estimated project cost of A-II (a) above for the contract period stated above is \$108,101.00.

1039049

U. S. ATOMIC ENERGY COMMISSION
CONTRACT AUTHORIZATION

1. DATE AUG 30 1967	2. AUTHORIZATION NO. BM-68-102	
S.A. TO S. R. Sapirie, Manager Oak Ridge Operations Office	S.B. FROM John R. Totter, Director Division of Biology and Medicine	
CONTRACTOR (Name, Address, Department, etc.) MEDICAL COLLEGE OF VIRGINIA Richmond, Virginia	4.B. PRINCIPAL INVESTIGATOR(S) DAVID M. HUME, M.D.	
5. <input type="checkbox"/> NEW CONTRACT <input checked="" type="checkbox"/> RENEWAL <input type="checkbox"/> OTHER	6. TERM OF CONTRACT 10-1-67 thru 9-30-68	7. CONTRACT NUMBER AT(40-1)2459
8. RECOMMENDED TYPE OF CONTRACT: <input checked="" type="checkbox"/> FIXED PRICE <input type="checkbox"/> COST REIMBURSEMENT <input type="checkbox"/>	9. PROPERTY TITLE TO VEST IN: <input type="checkbox"/> AEC <input checked="" type="checkbox"/> CONTRACTOR	10. SECURITY CLASSIFICATION: Work to be performed is under category I as defined by AEC Manual Appendix 3401.

11. PROJECT TITLE
THE EFFECT OF RADIATION ON TRANSPLANTATION IMMUNITY

12. HEADQUARTERS TECHNICAL CONTACT
William R. Bibb

13. FINANCING (New AEC Funds, Not To Exceed Amounts Indicated):

A. OPERATING EXPENSES **\$52,000**
 Budget and Reporting Classification: **06 03**
 Allotment Transfer: **06-61-91(24)**

B. PLANT AND CAPITAL EQUIPMENT \$
 Budget and Reporting Classification:
 Allotment Transfer:

14. SPECIAL PROVISIONS AND INSTRUCTIONS:
 The technical aspects of the proposed work have been reviewed and are approved. A need currently exists for the results of the research or other work that is to be undertaken. None of the AEC funds shall be used to confer a fellowship.
 Please keep us informed as to any problems encountered in your negotiations, as well as the date of execution of this contract and the amount of funds obligated. If the budget as negotiated differs substantially from that in the proposal, please forward a copy of the revised budget to Headquarters.
 If not already submitted, a 200-word summary of the proposed work should be forwarded by the contractor as soon as possible after negotiation of the contract.

52% off
 Initial AEC Support Ceiling (new AEC funds) \$52,000; no unexpended funds are anticipated. Total AEC funding envisioned: \$52,000, or ~~52%~~ of total project cost, whichever is less. Should contractor volunteer to contribute the full amount originally offered, or more, please adjust the cost-sharing percentage accordingly.

15. SCOPE OF WORK

Studies of the feasibility and mechanisms of extra-corporeal irradiation on organ-graft survival.

*\$52,000 AEC approved support
 56,101. Contractor support
 108,101. total
 = 49% AEC support level*

400

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 CONTRACTS-2459(VA)
 SEP 1 1967

RECEIVED
ORO-AEC
TELETYPE SECTION

SEP 6 4 16 PM 1967

BY JH-WV

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CKP 405P EDT SEP 6 67 CTA225

SSA553 CT RA312 PD RICHMOND VIR 6 350P EDT

C S SHPUP, ANS DT SEPT 5 CTA580 CTA587

USAEC OAK RIDGE TENN

*B.5592
JH*

RETEL WILL ACCEPT AEC SUPPORT NOT TO EXCEED 52,000.00

D M HUME L DANIEL CROOKS MEDICAL COLLEGE OF VIRGINIA

(55).

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CONTRACTS-2459(VA)

SEP 6 1967

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USAEC
C. S. SHOUP, CHIEF, BIOLOGY BRANCH
RESEARCH AND DEVELOPMENT DIVISION
OAK RIDGE, TENNESSEE

ORIGINAL SIGNED BY
C. S. SHOUP

C. S. SHOUP

SEP 5 1967

DR. DAVID M. HUME, DEPARTMENT OF SURGERY, MEDICAL COLLEGE OF VIRGINIA,
RICHMOND, VIRGINIA

INFO: MR. L. DANIEL CROOKS, COMPTROLLER, MEDICAL COLLEGE OF VIRGINIA,
RICHMOND, VIRGINIA

RECEIVED AUTHORIZATION TO EXTEND PERIOD UNDER CONTRACT NO. AT-(40-1)-2459
TO SEPTEMBER 30, 1968. HOWEVER, AEC'S SUPPORT CEILING WAS APPROVED IN AN
AMOUNT NOT TO EXCEED \$52,000. PLEASE ADVISE WHETHER MEDICAL COLLEGE WILL
ACCEPT ABOVE SUPPORT LEVEL TO A TOTAL PROJECT COST OF \$108,101. IF NOT,
PLEASE SUBMIT A REVISED TOTAL-COST BUDGET BASED ON APPROVED AEC SUPPORT.

ORS:JDB / 8

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BY _____

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CONTRACTS-2459(VA)

OFFICE ▶	Res. Sec. Br.	Biology Br.			
SURNAME ▶	Burleson:dac	Rm 472 3-4388	<i>C. S. Shoup</i>		
DATE ▶	9/5/67	9-5-67			

AUG 3 0 1967

David M. Hume, M.D.
Department of Surgery
Medical College of Virginia
Strauss Surgical Research
Laboratories
Richmond, Virginia 23219

Dear Dr. Hume:

I am pleased to inform you that Atomic Energy Commission contribution toward support of your project, "The Effect of Radiation on Transplantation Immunity," has been approved but at less than the requested level.

The negotiation of the contract, including financial detail, will be handled by our Oak Ridge Operations Office and you may expect to hear from a representative of that office shortly. Responsibility for the technical and scientific administration will, of course, remain with the Division of Biology and Medicine in Washington.

We wish you every success in your work.

Sincerely yours,

William R. Bibb
Medical Research Branch
Division of Biology and Medicine

cc: Oak Ridge Operations Office ←

1039053

ORS:JDB

JUL 18 1967

Dr. David M. Hume
Department of Surgery
Medical College of Virginia
Richmond, Virginia 23219

Subject: CONTRACT NO. AT-(40-1)-2459

Dear Dr. Hume:

The renewal proposal, progress report and other related documents submitted under Contract No. AT-(40-1)-2459 have been forwarded to AEC Headquarters for review. We will notify you of their decision relative to renewal of the contract as soon as it is received.

In accordance with our letter of April 25, 1966, it is necessary that all technical documents (except reprints of publications) prepared under the contract be assigned appropriate report numbers and a Form AEC-427 be completed and submitted on each. Therefore, please furnish us with an appropriate report number and a Form AEC-427 for each of the following listed documents:

- a. Progress Report, "Effect of Radiation on Transplantation Immunity".
- b. "The Preparation of Specific, Potent, ALS in the Horse Using Dog Thymocytes".
- c. "Quantitation of Lymphocyte Production in Normal and Stimulated Lymph Nodes".
- d. "Antigens in Immunity XIII".
- e. "Serotyping for Homotransplantation XII Occurrence of Cytotoxic Antibodies Following Kidney Transplantation in Man".

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CONTRACTS-2459(VA)

1039054

Dr. David M. Hume

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JUL 18 1967

f. "Serological Studies in Transplant Recipients with the Nephrotic Syndrome".

A supply of Forms AEC-427 is enclosed.

Very truly yours,

ORIGINAL SIGNED BY
C. S. SHOUP
C. S. Shoup
Chief, Biology Branch
Research and Development Division

NFF

Enclosure:
Forms AEC-427 (15cys)

1039055

OAK RIDGE OPERATIONS
NS REC

JUL 18 1967

DISPATCHED

OFFICE ▶	Res. Ser. Br.	Biology Br.			
SURNAME ▶	Burleson:dac	<i>C. S. Shoup</i>			
DATE ▶	7/17/67	7-18-67			

II. EXPENDITURE STATEMENT

A. Cost of Project to Date.

1. Expenditure on AEC Grant 10-1-65 to 6-30-66.

PERSONNEL

S. Aikens		\$ 494.22
C.S. Stanley		388.50
F.O. Winkler		210.52
M.L. Greene		3,112.00
E.H. Borum		5,433.30
J.J. Dwyer		4,500.00
		<u>14,138.54</u>
Social Security @ 4.4%		563.62
Overhead @ 30.34%		4,382.24
		<u>19,084.40</u>
Consumable Supplies, Animal Care		13,374.24
Travel		745.79
		<u>33,204.43</u>
Total Expenses AEC		
		<u>49,526.00</u>
Renewal of Grant 10-1-65		16,321.57
Balance 6-30-66		

2. Institutional Contribution, Expenses.

D.M. Hume, M.D.	35%	8,750.00
H.M. Lee, M.D.	30%	5,800.00
G.M. Williams, M.D.	50%	7,550.00
J.S. Wolf, M.D.	75%	4,150.00
B. Semb, M.D.	90%	4,300.00
A. Newhoff, M.D.	60%	2,600.00
J.C. Pierce, M.D.	40%	4,400.00
T.C. Moore, M.D.	30%	4,800.00
W. Smellie, M.D.	30%	2,234.00
		<u>44,584.00</u>
Social Security @ 4.4%		1,961.70
Overhead @ 30.34%		13,526.79
		<u>60,072.49</u>
Maintenance of Research Facilities		9,000.00
		<u>69,072.49</u>
Total Institutional Expenses		33,204.43
Total AEC Expenses		
		<u>\$102,276.92</u>
Total Cost of Project to 6-30-66		

B. Anticipated Expenses of Remainder of Contract Period-(7-1-66 to 9-30-66).

1. AEC Grant.

PERSONNEL

S. Aikens	\$ 216.00
C. Stanley	210.00
M. Greene	1,112.00
E. Borum	1,920.00
J. Dwyer	1,500.00
C. Sung	600.00
P. Fens	1,410.00
Lab. Mechanic	750.00

7,718.00

Social Security @ 4.4% 339.59

Overhead @ 30.34% 2,341.64

10,399.23

Consumable Supplies and Animal Care 5,868.13

Travel 54.21

Total Anticipated Expenses, AEC 16,321.57

2. Anticipated Expenses of Institutional Contribution 7-1-66 to 9-30-67.

D.M. Hume, M.D.	35%	2,910.00
H.M. Lee, M.D.	30%	1,800.00
G.M. Williams, M.D.	50%	2,500.00
J.S. Wolf, M.D.	75%	1,050.00
B. Semb, M.D.	90%	1,600.00
A. Newhoff, M.D.	50%	800.00
J.C. Pierce, M.D.	40%	1,450.00
T.C. Moore, M.D.	30%	1,530.00
W. Smellie, M.D.	30%	680.00

14,320.00

Social Security @ 4.4% 630.08

Overhead @ 30.34% 4,344.69

Maintenance of Research Facilities 3,000.00

Total Anticipated Expenses, Institution 22,294.77

Total Anticipated Expenses 38,616.34

C. Estimate of Funds Available During Remaining Period of Project.

1. AEC 16,321.57

2. Institution \$22,294.77

III. RENEWAL PROPOSAL AT(40-1)2459 JULY 1, 1967

1. Title of Project: Effect of Radiation on Transplantation Immunity.
2. Institution: Department of Surgery and Strauss Surgical Research Laboratories, Medical College of Virginia, Richmond, Virginia.
3. Project Abstract.
 - A. Radiation of Circulating Blood.
 1. Chemical dosimetry and applicator geometry.
 2. Strontium⁹⁰ shunts and anti-lymphocyte serum in canine renal homografts.
 3. Strontium⁹⁰ shunts and imuran in canine renal homografts.
 4. Strontium⁹⁰ blood irradiation in the human.
 - a.) renal transplant rejection crises.
 - b.) pre-treatment of renal transplant recipient.
 - c.) in the leukemias.
 5. In vitro immunologic competence of the lymphocyte following extracorporeal blood irradiation and/or transplantation.
 6. Strontium⁹⁰ irradiation of circulation to ex vivo perfused organ.
 - B. Local Graft Irradiation.
 1. Irradiation of ex vivo perfused organ.
 - C. Total Body Radiation.
 1. Effect of total body radiation on recognition phase of immune response following thymectomy.
 - D. Histocompatibility and In Vitro Immunity Studies.
 1. Lymphocyte cytotoxicity test.
 2. Mixed lymphocyte reaction following irradiation of one population.
 3. Transplantation serum antibody determination following animal and human renal transplantation.
 4. Method for estimation of total lymphocyte mass.
 5. Effects of antibody generated by various antigenic stimuli upon the function of rat kidneys perfused in vitro.

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4. and 5. Scientific Background and Scope of Project.

A. Radiation of Circulating Blood.

1. Applicator Geometry.

We believe that increase in dose-rate to the kilorad/day level mentioned by some authors is inadvisable for reasons connected with red cell and platelet fragility, and we propose therefore to continue working at lower levels (0.5 krad/day) which have the additional advantage that they can be attained with much less hazard to staff.

Adequate dose-rates may be obtained either by using higher r/V ratios or by using higher activity-densities. In general, the use of large r/V ratios involves rather complex threading arrangements with consequent risk of kinking and clotting and greater exposure to staff time if threading is carried out with sources in place. We therefore propose to continue construction of limb-mounted applicators with relatively small volume, compensating for the low r/V ratio by using sources of somewhat higher activity whose juxtaposition to the irradiated fluid will be as close as can conveniently be arranged. A number of designs, based ultimately on shunt No. 4 described above, are under active consideration and we are in touch with the source manufacturers as to the best fabrication techniques. We shall pursue our investigations on the use of low-Z encapsulation (as already tried in No. 4 shunt) as vigorously as possible because of the very significant reduction in radiation hazard. We believe that it will be possible to make a lightweight easily-interchanged applicator which can be worn continuously by the patient, delivering some 1 krad/day to a patient of normal blood volume and that the external hazard from this source will be so small as to call for no undue nursing precautions.

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In continuing our dosimetry studies we propose to investigate the dependence of the mean blood-dose on the wall thickness of the blood tubing, a matter of consequence since tubing of somewhat greater wall thickness than that now used would minimize kinking. We shall also carry further our investigations on the effect of source geometry on the dose delivered, and will investigate the variation of dose between parallel plates when the inter-plate medium is not homogeneous. To study the effect of wall thickness we shall use thermoluminescent techniques in which (as an example) we shall use tubing whose walls are themselves composed of thermoluminescent material of variable lumen size. In this way, the contribution from different annular zones in the tubing can easily be studied. We also propose to use thermoluminescent techniques also in an attempt to mock-up a liquid system in which cells of varying sizes are stimulated by micro-spheres or micro-discs of sensitive material. A study on the use of radioactively tagged cells, in an attempt to discover the temporal pattern of cell flow through exterior shunts, is also in prospect but details have not yet been worked out.

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2. Strontium⁹⁰ Shunt and Anti-lymphocyte Serum in Canine Renal Homografts.

Studies in the production of anti-lymphocyte antiserum were published from our laboratory in 1964 (Sacks, Filippone, and Hume, Studies of Immune Destruction of Lymph Tissue, I. Lymphocytotoxic Effects of Rabbit-Anti-Rat Lymphocyte Antiserum. Transplantation, 2:60, 1964.) In the past year a method of producing a potent dog antilymphocyte antiserum in the horse has been developed (Braf, Z.F., Smellie, W.A.B., Williams, G.M. and Hume, D.M., The Preparation of Specific Potent ALS in the Horse Using Dog Thymocytes. Surgical Forum 1967, (in press)). Studies in the use of this antiserum have resulted in the prolongation of canine renal homograft survival. Its mechanism of action, active constituents, and optimum dose schedule are under current investigation in our laboratory as well as in the laboratories of many other transplantation investigators. However, no one has yet investigated its properties when used in conjunction with radiation of the organ recipient.

It has been proposed by Starzl and his group that lymphocytopenia is not a necessary requirement for the effect of the anti-serum in the immune process. Levey and Medewar have postulated that some form of "Immunologic blindfolding" occurs so that recognition of antigen is interfered with by concurrent use of Anti-lymphocyte serum. Russell and Monaco, conversely, demonstrate a profound lymphopenia in both the mouse and dog during administration of ALS and feel that the lymphocytopenia is an important aspect of the animals' response to the anti-serum.

As the methods of Sr⁹⁰ shunt radiation in the dog are developed to a high degree, and now that a potent dog ALS is available, it is proposed

that these two methods of immunosuppression be combined in an effort to better understand the mechanism of action of each of the methods. Several questions could be answered by this study: 1) Is the ALS committed cell more or less radiosensitive?. 2) Does prior lymphopenia enhance or suppress the effect of ALS?, 3) Could an organism receive protection from the leukopenic effects of radiation by committing the cells prior to radiation injury? The survival of a canine renal homograft following radiation at the time of transplantation and frequent injections of ALS according to our laboratory protocol will be the first study planned using this combination of therapy. Serial tissue biopsies, peripheral lymphocyte levels, health of the animal, and function of the homograft will dictate what alterations will be made to this protocol.

3. Strontium⁹⁰ shunt and Imuran in canine renal homografts.

The pilot studies for this study are reported in this progress report. At the low doses of azathioprine used we were unable to determine any increased survival over animals with blood radiation alone. To attempt to determine the efficacy of adding Imuran it would be given in somewhat larger but still sub-therapeutic dose levels to determine that dose which is most optimal.

Shunt radiation will also be used for acute rejection in animals carried on maximum therapeutic levels of Imuran, for even at these levels of drug a significant percentage of the animals will ultimately reject their transplant. This protocol will then experimentally mimic the method of blood radiation now used clinically.

4. Strontium⁹⁰ blood irradiation in the human.

In the past two progress reports we have discussed our findings in the use of shunt radiation in rejection crises following renal transplantation and in chronic lymphatic leukemia. It has been fairly conclusively shown that this modality is of some usefulness in these conditions. However the amount of information available to date is too scanty to achieve any valid conclusions and must be expanded. Problems of applicator geometry and dose rates, as discussed above, are of prime importance in arriving at optimal response to shunt irradiation and must be solved so that the modality is more predictive and less empirical. However even in the empirical studies performed to date much information has been gained.

We will continue to treat accelerated rejection crises following human renal homotransplantation with Sr⁹⁰ blood irradiation. Patient responses will be monitored by peripheral cell counts and cellular morphology, production of uric acid and other catabolic byproducts, as well as in vitro lymphocyte responses following the radiation. We will continue to assess its role in reversal of rejection by its use with no other modification of the immunosuppressive regimen.

In addition we are beginning a protocol in which one of two patients receiving paired cadaver transplants will receive shunt radiation while on hemodialysis being prepared for transplantation and following transplantation for several weeks. With knowledge of the degree of histocompatibility obtained from lymphocyte cytotoxicity tests, observation of the patient's lymphocyte response, and clinical course of the renal function we will be able to obtain data as to the immunosuppressive abilities of blood radiation in the early phases of transplantation. No other alterations will be made in our basic clinical immunosuppressive regimen, which will continue to include local graft irradiation in the immediate post-transplant period.

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We will also continue to investigate the response to shunt radiation in patients with chronic lymphatic leukemia. The radiation has been shown to be a powerful mechanism for the destruction of circulating leukemic lymphocytes and these patients provide a unique opportunity to study the kinetics of the lymphocyte following shunt radiation. The radiation dose rates have thus far been empirical, and have been far less than the mean cell doses utilized by the Brookhaven group and by Thomas and associates. However with the techniques now being developed in dosimetry and with more efficient Sr⁹⁰ applicators, it should be possible to develop guidelines for the dose-response relationship. As new applicators are developed, they will continue to be evaluated in this group of patients and the response will continue to be evaluated by peripheral cell counts and morphology, and by histologic examination of bone marrow and lymphoid tissue.

5. In vitro Immunologic competence of the lymphocyte after extra-corporeal blood radiation and/or transplantation.

Preliminary findings in this study are described in the above progress report. During the next grant period we plan to continue this study using the already developed techniques of lymphocyte antigenic stimulation in culture. In addition methods of more quantitative reliability are being developed by use of substrate radioactive tagging and cell tagging to assess the degree of lymphocyte stimulation. Indirect methods of agglutination reactions are also being developed after those described by Milgrom and others for assessing the degree of lymphocyte-antigen interaction. These methods are being applied to lymphocytes obtained from animals and man both prior to and during Sr⁹⁰ radiation and with or without corresponding organ transplantation. These studies should lead to some answers to questions of whether there is any alteration of the lymphocyte, short of

death of the cell, that occurs following radiation, and of what time relationships are relative to transplantation and extracorporeal radiation induced lymphopenia.

6. Strontium⁹⁰ irradiation of circulation to ex vivo perfused organ.

Utilizing the previously described ex vivo organ perfusion system and incorporating either a perfusate of known numbers of lymphocytes or diluted whole blood from a single donor animal, canine renal kidneys will be perfused while a Strontium⁹⁰ applicator of known activity is inserted into the circuit. Attempts at dosimetry have resulted in the use of chemical dosimetric methods described in the foregoing progress report. However these methods of necessity measure the radiation dosage of a homogenous liquid. The blood irradiation, however, involves the radiation of particulate matter which probably passes the applicator in a lamellar flow. Thus it is impossible to calculate mean cell doses, but only mean blood doses. However, by perfusion with perfusate with known numbers of lymphocytes and using applicators of known mean blood dose it will be possible to empirically determine the cell death rate for a given radiation dose. By use of microspheres and the thermoluminescent techniques described in a previous section, and by omitting the organ from the circuit it will be possible to determine mean dose of particulate matter to compare with the previously determined mean blood doses.

Studies in transplantation immunology will also be performed using Sr⁹⁰ radiation and ex vivo kidney perfusion. Lymphocyte uptake by the kidney during perfusion has been described in the progress report. These data will be compared with data obtained for lymphocyte uptake after a Sr⁹⁰ applicator has been inserted into the circuit. By using both sensitized and unsensitized lymphocytes in the radiation perfusion system the alteration of the immune capacity of the cells by radiation can be investigated.

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B Local Graft Irradiation--Irradiation of ex vivo perfused organ.

In this year's progress report we have described a system for ex vivo perfusion of the canine kidney which has been shown to have the ability to maintain an organ for many hours with perfusion of either acellular or sanguinous perfusate. It is proposed to utilize this experimental model as an extension of our previous studies of the mechanisms of local graft irradiation. It has already been postulated that one mode of action is to destroy immunocytes attaching themselves to the graft. It has been shown also in studies reported in this progress report that there is an uptake by the homologous rat kidney of perfused lymphocytes. Therefore it would seem a logical extension of this study to evaluate the uptake of lymphocytes by the kidney during ex vivo perfusion while the kidney is undergoing local graft irradiation using various radiation protocols. Since the life span of the kidney in this experimental model is short, there is no need to limit the amount of radiation received and more intensive radiation can be employed than is possible in the experimental animal. Either intermittent or constant irradiation to the organ will be employed. Sampling for lymphocyte uptake by the kidney and organ biopsies will be used to evaluate any acute alterations produced by the irradiation. Both first and second set immunologic reactions will be evaluated.

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C. Total Body Radiation.

1. The Effect of the Thymus on the Cognitive Phase of the Immune Response.

It has been shown that neonatal thymectomy in certain mice strains induces marked lymphopenia and immunological crippling. The ability to respond to antigens can be restored by thymus grafts contained in millipore chambers. However, most of these animals remain lymphopenic. Lymphopenia can be corrected by transplantation of heavily irradiated thymus, which however does not restore immunological competence (Miller, J.F.A.P. The Thymus in Relation to the Development of Immunological Capacity. In Ciba Foundation Symposium, The Thymus: Experiments and Clinical Studies. ed. by G.E.W. Wolstenholme and Ruth Porter). On the basis of these observations, Miller has postulated the existence of 2 thymic factors, one which simply stimulates lymphopoiesis and a second which somehow is responsible for immunological competence. The following experiments were conducted to ascertain if these reactions could be tested for in another system. Spleen cells were obtained from three groups of AKR mice, normal, irradiated, and thymectomized irradiated. Equal numbers of these cells were mixed with Balb/c spleen cells and allowed to react in mixed lymphocyte cultures. AKR spleen cells from thymectomized and irradiated animals were also incubated in the presence of phytohemagglutinin. Activity of the cells in mixed lymphocyte culture is demonstrated by the per cent incorporation of H_3 thymidine into DNA measured by DNA precipitation and counting by liquid scintillation. Our initial experiments indicate that thymectomized, irradiated AKR spleen cells still respond to phytohemagglutinin in nearly normal fashion. However, spleen cells from irradiated thymectomized AKR mice mixed with

1039067

balb/c cells display much less incorporation of tridiated thymidine. It appears as if the cells are capable of proliferation but have somehow lost their powers of recognizing antigenic differences.

D. Histocompatibility and In Vitro Immunity Studies.

1. Lymphocyte Cytotoxicity Test.

In this progress report we discussed preliminary studies in the use of the lymphocyte cytotoxicity test in estimations of histocompatibility. In cooperative studies with Terasaki and with Rappaport we have examined all post transplant patients in our series, and have found some degree of correlation. Using our 12 man donor panel, we will continue to type all pre-operative transplant recipients in an attempt to correlate the clinical course with the degree of histocompatibility.

We also will continue studies of the cytotoxic antibodies produced in patients following transplantation and detectable following transplant nephrectomy. Determination of the specificity and kinetics of this antibody may serve as a useful method of determining histocompatibility and most certainly is useful in retrospectively determining degree of donor-recipient histocompatibility. Our interest continues in cadaver-donor renal transplantation and the value of matching for major antigenic determinants to the success of these grafts must be determined.

2. Mixed Lymphocyte Reaction.

Results of this study to date are discussed in this progress report. We will continue to evaluate this test as an estimation of histocompatibility. However, it is becoming increasingly apparent to us as it is to others that

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the determination of the results of the reaction must be made more precise. One method now under current investigation is the irradiation of the donor cell population so that the reaction only can occur in the recipient cell population. However data must first be compiled about the dosage of radiation effective to this lymphocyte population. Studies of Schrek and others have accumulated data pertinent to this phase of the study. Methods of quantitating the results of the stimulation are also under current study and the most promising appears to be the incorporation of radioactively tagged substrate.

3. Transplantation Serum Antibody Determination Following Animal and Human Renal Transplantation.

Techniques have been developed in our laboratory as well as others for the development of an immune response to target renal cells in tissue culture. We have thus far studied the kidneys of six patients following the removal for chronic transplanted rejection. In all, there can be an increased interaction of recipient lymphocytes with donor rejected kidney cells as evidenced by visualization of lymphocyte adherence to washed kidney cells seen under phase microscopy, as well as by target renal cell death in cultures containing specific lymphocytes.

However in two of these patients target renal cell death was also seen in addition of patient serum only. The nature of this kidney antibody response is under current investigation, and attempts are being made to quantitate this cell destruction. Specific activity and cross reactivity of these sera are being evaluated.

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Indirect mixed agglutination methods of Milgrom are also being applied to this system, and animal transplantation studies are being performed to attempt to classify the time of onset of this antibody activity, means of enhancing or suppressing the activity, and the changes in activity seen after immunosuppressive manipulation of the recipient animal by chemical and irradiation methods.

4. Studies in Determining Lymphocyte Mass.

It is well recognized that peripheral lymphocyte counts do not reflect accurately the total numbers of lymphocytes present in the organism. An estimation of lymphocyte bulk would be an extremely important aid in determining the extent of lymphoid depletion with immunosuppressive agents both in clinical practice and laboratory research.

Adult rats will be subjected to various periods of thoracic duct drainage. Estimates of lymphocyte depletion can be obtained by counting the numbers of lymphocytes lost via drainage. An aliquot of the cells lost by drainage will be tagged with Chromium⁵¹ and injected intravenously. The rate of disappearance of radioactive cells from the systemic circulation will be used as an index of lymphoid depletion, anticipating that a more rapid turnover between lymph nodes and blood stream occurs in animals rendered lymphopenic.

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6. The Effects of Antibody Generated by Various Antigenic Stimuli Upon The Function of Rat Kidneys Perfused in Vitro.

Utilizing the model described above, sera from allogeneic rats stimulated against the antigens of the perfused kidney will be tested for any possible effects upon the renal vasculature. Anticipated immunization schedules call for immunization by allogeneic red cells, spleen cells, and renal homogenate. The purpose of these experiments will be to determine: 1) if any form of iso-antibody has vaso-active effects on the isolated perfused kidney, 2) what immunizing substance is thus able to produce toxic antibody, and 3) at what stage and with what immunoglobulin fraction does optimal activity occur.

Preliminary results have indicated that the antibody produced following injections of homologous spleen cells in adjuvant is inactive, whereas antibody prepared with renal homogenate produces a slight but definite increase in vascular resistance 10 minutes after its addition to the perfusion fluid.

6. Scientific Personnel.

Co-Principal Investigators:

David M. Hume, M.D. [REDACTED], Professor and Chairman, Department of Surgery, Medical College of Virginia. Time:25%; H.M. Lee, M.D. [REDACTED] Associate Professor of Surgery, 30%; James S. Wolf, M.D. [REDACTED], Instructor in Surgery, 50%; G. Melville Williams, M.D. [REDACTED], Assistant Professor of Surgery, 50%; Thomas C. Moore, M.D. [REDACTED], Associate Professor of Surgery, 25%; James C. Pierce, M.D. [REDACTED], Instructor in Surgery, 40%; Fearghus T. O'Foghludha, Ph.D. [REDACTED], Professor and Chairman, Division of Radiation Physics, 5%.

Newly Appointed Personnel:

Ronald Rolley, M.D., Research Fellow in Surgery, 75%; William Gayle, M.D., Research Fellow in Surgery, 75%.

7. Other Personnel:

Research Associate	James J. Dwyer	60%
Lab. Spec. B	E. Borum	100%
Lab. Tech. B	S. Aikens	20%
Lab. Tech. C	P. Fens	100%
Lab. Tech. B	M. Greene	100%
Lab Aide	C. Sung	15%
Student Tech. B	C. Stanley	25%
Shop Mechanic		50%

8. Other Financial Assistance.

No other financial assistance is received for any of the full time non-professional personnel to be paid AEC funds. Part-time personnel

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receive funds for other part-time employment from other institutional and departmental funding. The professional personnel are all paid completely from institutional sources or as instructors or trainees on a departmental N.I.H. Training Grant in Transplantation.

9. Premises, Facilities, Equipment, and Materials to be Furnished by the Contractor:

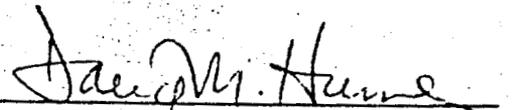
Fully equipped animal operating rooms, hematologic, biochemical, histologic, immunologic, and tissue culture laboratories, isotope-counting equipment, machine shop, animal X-ray (diagnostic and therapeutic), and animal care facilities covering approximately 40,000 square feet are available for this study. The Biometry Department is available for data analysis. The Clinical Transplant Center and Clinical Research Center and Clinical Transplant Self-Care Unit are in operation in the hospital. No other major items of new equipment are needed to carry out the study.

10. Budget: See separate sheets.

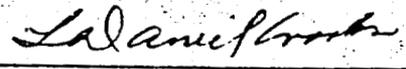
11. Amount Requested: \$55,820.00

12. Authentication: Signed for the Medical College of Virginia.

Principal Investigator:


David M. Hume, M.D.

Comptroller:


L. Daniel Crooks

10. PROPOSED BUDGET FOR 10-1-67 TO 9-30-68

A. AEC Contribution.

<u>PERSONNEL</u>	<u>% TIME</u>	
✓ J.J. Dwyer, Research Associate (\$1,000/month)	60%	\$ 6,000.00
✓ *E. Borum, Lab. Spec. B. (\$667/month)	100%	8,010.00
✓ *P. Fens, Lab. Tech. C (\$483/month)	100%	5,800.00
✓ *M. Greene, Lab. Tech. B (\$375/month)	100%	4,512.00
✓ *S. Aikens, Lab. Tech. B (\$390/month)	25%	1,170.00
✓ C. Stanley, Student Tech. B (\$1.75/hour)	25%	1,000.00
✓ C. Sung, Lab. Aide (\$1.50/hour)	15%	500.00
✓ Lab. Mechanic C (\$500/month)	50%	3,000.00
		<hr/> 29,992.00
Social Security @ 4.4%		1,320.00 ✓
Overhead @ 30.34%		<hr/> 9,100.00
		<hr/> <hr/> \$40,412.00

* State-authorized salary increase on 2-1-67.

SUPPLIES AND EQUIPMENT

Ziess Photo Microscope	3,428.00
Isotopes	1,200.00
Shop Materials, construction of extra-corporeal shunts	600.00
Laboratory glassware, tissue culture materials	800.00
Pathology (300 X \$1.00/slide)	300.00
Disposable oxygenator parts for perfusion circuit	250.00
Shunt materials for A-V shunts	450.00
Maintenance and repair of equipment	200.00
Reagents, Drugs	600.00
Office supplies and photography	200.00
	<hr/> 8,028.00

REPRINTS AND PUBLICATION COSTS

300.00

ANIMALS AND CARE

Operating expense	3,000.00
Dog Purchase (100 X \$10/dog)	1,000.00
Dog Care (100 X \$.60/day X 30)	1,800.00
Rats (250 @ \$1.20 each)	300.00
Rat Care (250 X \$.03/day X 30 days)	180.00
	<hr/> 6,280.00

TRAVEL

800.00

TOTAL AEC REQUEST

\$55,820.00

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B. Institutional Contribution*

SALARIES

✓ D.M. Hume, M.D.	25%	\$ 8,750.00
✓ H.M. Lee, M.D.	30%	6,150.00
✓ F.T. O'Foghludha	10%	2,200.00
✓ R. Davis, Animal Caretaker	50%	2,375.00
✓ Lab Mechanic	50%	3,000.00
		<hr/>
		22,475.00
Social Security @ 4.4%		987.00 ✓
Overhead @ 30.34%		6,819.00
		<hr/>
		30,281.00
Maintenance of Research Facilities		12,000.00
Animal procurement center (care and processing)		10,000.00
		<hr/>
		\$52,281.00

C. Other Non-University Sponsors*

SALARIES

J.S. Wolf, M.D.	50%	7,850.00 (NIH)
G. M. Williams, M.D.	50%	9,050.00 (NIH)
T.C. Moore, M.D.	25%	6,250.00 (NIH)
J.C. Pierce, M.D.	40%	6,750.00 (NIH)
R. Rolley, M.D.	90%	6,300.00 (NIH)
W. Gayle, M.D.	90%	6,300.00 (NIH)
		<hr/>
		42,500.00
Social Security @ 4.4%		1,870.00
Overhead @ 30.34%		12,945.00
		<hr/>
		57,315.00
		<hr/>
Total, AEC Contribution		55,820.00
Total, Institutional and Other Non-University Sponsors		109,596.00
		<hr/>
		\$165,416.00

55,820
710,101 total

* Cost of Clinical Transplant Center not included. This amounts to about \$300,000.00 per year.

William E. Gayle, Jr., M.D.

Date of Birth: [REDACTED] Huntington, West Virginia.

Education:

1. [REDACTED]
2. [REDACTED]

Internship: [REDACTED]

Residency: [REDACTED]

Fellowships:

- A.D. Williams Student Fellowship, 1964.
- Mayo Clinic Student Fellowship, 1965.
- USPHS Research Fellow in Transplantation, July, 1967.

Address:

Medical College of Virginia, 1200 E. Broad Street,
Richmond, Virginia, 23219.

Home Address: [REDACTED]

Honors:

AOA, 1964.

Societies:

Alpha Epsilon Delta.

Research Interests: Transplant Immunology, Hepatic Transplantation.

Ronald Turner Rolley, M.D.

Date of Birth: [REDACTED] Detroit, Michigan.

Education: [REDACTED]

Internship: [REDACTED]

Residency: [REDACTED]

Fellowships: Clinical Research Associate, National Cancer Institute, Surgery Branch, July, 1965 - June 30, 1967.

Address: Medical College of Virginia, 1200 E. Broad Street, Richmond, Virginia, 23219.

Home Address: [REDACTED]

Research Interests: Transplantation and Tumor Immunology.

BIBLIOGRAPHY

1. Rolley, R.T. and Hammond, W.G., Effect of Thymectomy and Total Body Irradiation on Carcinogen Induced Tumor Immunity. Proc. of Am. Ass. for Ca. Res., April, 1967.
2. Stechel, R.J., Doppman, J.L., Rolley, R.T., and Martos, E.J., Rupture of the Aorta After Mechlorethamine HCL Infusion of a Bronchial Artery. JAMA 199:186-89, 1967.
3. Hammond, W.G., Fisher, J., Rolley, R.T., Tumor-Specific Transplantation Immunity to Spontaneous Mouse Tumors. Surgery, 1967 (in press).

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NOTICE OF RESEARCH PROJECT
SCIENCE INFORMATION EXCHANGE
SMITHSONIAN INSTITUTION

PROJECT NO. (Do not use this space)

PUBLICATION BY AEC
HEREBY AUTHORIZED

SUPPORTING AGENCY: **Division of Biology and Medicine
UNITED STATES ATOMIC ENERGY COMMISSION**

NAME AND ADDRESS OF INSTITUTION: Please state also the Division, Department, or Professional School (medical, graduate, or other) with which this project should be identified:

Department of Surgery, Medical College of Virginia, Richmond, Virginia.

TITLE OF PROJECT

The Effect of Radiation on Transplantation Immunity.

Give names, department, and official titles of PRINCIPAL INVESTIGATORS and OTHER PROFESSIONAL SCIENTIFIC PERSONNEL (not including graduate students) engaged on the project, and fraction of man-year devoted to the project by each person. David M. Hume, M.D., Professor and Chairman, Dept. of Surgery (25%); H.M. Lee, M.D., Associate Professor of Surgery (30%); James S. Wolf, M.D., Instructor in Surgery (5%); G. Melville Williams, M.D., Assistant Professor of Surgery (50%); Thomas C. Moore, M.D., Associate Professor of Surgery (25%); James C. Pierce, M.D., Instructor in Surgery (40%); Fearghus T. O'Foghludha, Ph.D., Professor and Chairman, Div. of Radiation Physics (5%).

How many graduate students on project? 3 How many graduate student man-years? 2 1/2

SUMMARY OF PROPOSED WORK - (200 - 300 words. Omit confidential data.) Summaries are exchanged with government and private agencies supporting research, are supplied to investigators upon request, and may be published in AFC documents. Please make your summary SUBSTANTIVE, giving initially and for each annual revision the following: OBJECTIVE; scientific BACKGROUND or REASON for study; proposed PROCEDURE; TEST OBJECTS and AGENTS; and RESULTS TO DATE if any.

This study has been investigating the effects of radiation in the alteration of the immune response to organ transplantation, utilizing several methods of irradiation. Irradiation of the transplanted organ following homografting has been shown by us to be a method of immunosuppression. Laboratory studies have culminated in its use in 120 human renal transplants and its mechanism of action is under current laboratory investigation.

We have developed methods and applicators for extra-corporeal beta irradiation of the circulating blood and have shown prolongation of dog renal homografts by this sole method immunosuppression. It has also been utilized in patients following renal transplantation as well as in patients with chronic lymphatic leukemia.

Recently, increased effort has been placed upon the use of in vitro cell cultures and ex vivo organ perfusion as tools to study transplantation immunology. Precise tools using in vitro transplantation antigen-antibody reactions, kidney and lymphocyte tissue culture, lymphocyte cytotoxicity, mixed lymphocyte reaction, and organ perfusion with specific cells and sera all are being tested in systems inducing cellular alterations by radiation. These precise techniques are being developed to critically evaluate the nature of the immune response in its more basic formulation in order to understand what alterations of the organism will result in immunosuppression.

Fur Period	
Project	Program Category Number
Priority	
Secretary	

Signature of Principal Investigator _____ Date _____

INVESTIGATOR - DO NOT USE THIS SPACE

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