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ORS:LM

Oak Ridge, Tennessee 37830

JUL 8 1966

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

REPOSITORY Oak Ridge Operations
COLLECTION Records of Bldg. area
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BOX No. H-75-4 Bldg. 2714-H
No. 2 Cont 2459
FOLDER Med. College of VA

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

Dear Dr. Hume:

The renewal proposal and related material submitted under Contract No. AT-(40-1)-2459 have been forwarded to AEC Headquarters for review. We will let you know of Headquarters' decision relative to renewal of the contract as soon as it is received.

The expenditure statement for the current period contained an arithmetical error of \$100 under the personnel portion of the schedule in both the actual and anticipated amounts; however, one counterbalanced the other and the net result is the same.

Please advise us as to the basis for the new overhead rate of 30.34% of salaries and wages, which is used in the budget for the renewal period.

Also, please furnish us with a report number for the progress report together with a completed Form AEC-427 as requested in our letter of April 25, 1966.

Very truly yours,

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

OAK RIDGE

T-4541

CC: L. D. Crooks, Virginia

DISTRICT

CONTRACTS-2459(UA)

1039035

OFFICE	Res. Ser. Br.	Bio. Br.			
SURNAME	<u>Medley</u> Medley:nhb	<u>Shoup</u>			
DATE	7/8/66	7/8/66			

II. Expenditure Statement

A. Cost of Project to Date

1. Expenditures on AEC Grant thus far 10/1/65 to 6/30/66.

Personnell

S. Aikens	\$	446.06
G. Robertshaw		2,034.75
F. Winkler		531.86
M. Green		2,760.00
E. Borum		4,780.00
J. Dwyer		4,500.00
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		15,052.67
Social Security @ 4.2%		504.22
Overhead 22%		3,421.00
		<hr/>
		18,977.89
Consumable Supplies, Animal Care		10,299.00
Travel		174.76
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Total Expenses AEC		29,451.65
Renewal of Grant 10/1/65		45,391.00
		<hr/>
Balance 6/30/66		15,839.35

2. Institutional Contribution, expenses

Salaries

D.M. Hume, M.D. 35%		8,250.00
H.M. Kauffman, M.D. 50%		4,875.00
H.M. Lee, M.D. 50%		6,563.00
G.M. Williams, M.D. 50%		4,875.00
J.S. Wolf, M.D. 75%		4,781.00
J. Ferre, M.D. 50%		3,000.00
H.J.O. White, M.D. 50%		3,250.00
R. Davis 50%		1,476.00
R. Tinsley 75%		2,025.00
P. Sadler 10%		353.00
		<hr/>
		39,448.00
Social Security @ 4.2%		1,572.00
Overhead 22%		8,678.00
		<hr/>
		49,698.00
Maintenance of Research Facilities		9,000.00
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Total Expenditures, Institutional		58,698.00
Total Expenditures, AEC		29,451.65
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Total Cost of Project (6-30-66)		\$88,149.65

B. Anticipated Expenses for remainder of contract period
(7-1-66 to 9-30-66)

1. AEC Grant

Personnell

S. Aikens	165.48
E. Borum	1,680.00
J. Dwyer	1,500.00
G. Robertshaw	1,200.00
F. Winkler	189.84
M. Green	968.00
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	5,703.32

Social Security @ 4.2%	239.54
Overhead 22%	1,254.60
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	7,097.46

Consumable Supplies	2,375.00
Animals and Care	5,750.00
Travel	615.00
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Total Anticipated Expenses - AEC	15,837.46
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2. Anticipated expenses of Institutional Contribution 7/1/66 to 9/30/66.

Salaries

D.M. Hume, M.D.	2,750.00
H.M. Kauffman, M.D.	1,625.00
H.M. Lee, M.D.	2,182.00
G.M. Williams, M.D.	1,625.00
J.S. Wolf, M.D.	1,594.00
R. Davis	546.00
R. Tinsley	495.00
P. Sadler	138.00
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Social Security @ 4.2%	10,955.00
Overhead 22%	460.00
	2,410.00
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	13,825.00

Maintenance of Research Facilities	3,000.00
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Total Anticipated Expenses, Institutional	16,825.00
Total Anticipated Expenses, AEC	15,839.46

Total Anticipated Cost of Project to 9/30/66	\$32,664.46	15,937.46	32,762.46
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C. Estimate of funds available during remaining period of project.

1. A.E.C.	15,839.35
2. Institution	16,825.00

III. Renewal proposal AT-(40-1)-2459 July 1, 1966

1. Title of project: Effect of radiation on transplantation immunity
2. Institution: Department of Surgery, Medical College of Virginia, Richmond, Va.
3. Project abstract
 - A. Radiation of local graft or circulating blood.
 1. Strontium⁹⁰ extra-corporeal shunt and canine renal homografts.
 - a. Prior to transplantation
 - B. Concurrent with transplantation
 2. Strontium⁹⁰ plus Imuran
 3. Local graft irradiation plus renal-portal drainage
 4. Chemical dosimetry of extra-corporeal Sr⁹⁰ shunts
 - B. Delayed irradiation in treatment of rejecting renal homografts.
 1. Strontium⁹⁰ shunt on day 3 or 4
 2. Imuran plus Strontium⁹⁰ shunt for rejection crisis.
 - C. Irradiation enhancement for investigation of lymphoid stimulating factors.
 - D. Strontium⁹⁰ radiation of blood circulating in teflon-silastic arterio-venous shunts in the human.
 1. Reaal transplant rejection
 2. Pre-treatment of renal transplant recipient.
 3. Acute and chronic lymphatic leukemia
 - E. In-vitro lymphocyte studies following transplantation and irradiation of circulating blood.

1039038.

4. & 5. Scientific background and scope of project.

A. Radiation of local graft or circulating blood.

1. Sr⁹⁰ extra-corporeal shunt and canine renal homografts:

As outlined in the progress report, we have begun studies in four groups of animals receiving Strontium⁹⁰ shunt radiation to circulating blood: 1) animals with thymectomy and irradiation prior to transplantation; 2) animals with only irradiation prior to transplantation; 3) animals with prior thymectomy and irradiation concurrent with transplantation; 4) animals with irradiation concurrent with transplantation. A few animals have been done during the present grant period, after having solved the problems of maintaining the shunts and mounting the Sr⁹⁰ applicator on dogs. During the next year we plan to ascertain 1) whether the thymus is necessary for full immunologic recovery following prolonged blood irradiation and 2) the importance of timing of the blood irradiation with respect to homograft placement. The degree of lymphopenia produced will be observed with respect to the survival of the homograft in attempting to ascertain the importance of the lymphopenic state on the delayed or attenuated state of transplantation immunity.

2. Strontium⁹⁰ plus Imuran:

In attempting to bring the experimental clinical situation closer to the clinical situation, we plan to study a series of dogs who will receive Strontium⁹⁰ shunt irradiation plus therapeutic and subtherapeutic doses of Imuran. In the previous progress report we showed prolongation of canine renal homografts with Yttrium⁹⁰ plus Imuran in subtherapeutic levels.

Another series of dogs will be pretreated with Strontium⁹⁰ shunt before homograft placement to observe whether or not the usual early rejection can be attenuated by making the animal lymphopenic during this period. If this were possible, one might be able to abolish the routine use of corticosteroids in the early post-transplant period; a routine which leads to many life-threatening complications in the transplant patient.

3. Local graft irradiation plus renal-portal drainage.

Previous work in this laboratory has demonstrated that local radiation to the kidney prolongs survival of the transplant. Dogs tested for their ability to reject a second kidney from the same donor display a modification of the second-set rejection phenomenon. It thus appears that local irradiation to the homograft interferes with the normal immunization process.

Work in small animals has demonstrated that injection of antigen via the portal vein results in considerably less sensitization than injections into the systemic circulation. Therefore, routing of the antigenic material through the portal system might offer an additional means of avoiding sensitization. The combination of two techniques 1) local graft irradiation and 2) diversion of renal vein blood from the transplant through the portal system may work synergistically to delay sensitization and prolong the functional survival of a renal homograft.

Preliminary experiments in our laboratory have developed the model for this study, in which the renal artery of the homograft is anastomosed to the donor renal artery, and the renal vein is anastomosed end-to-side to the superior mesenteric vein. The ureter is either placed in the bladder or as a cutaneous ureterostomy. Three of these animals have survived free of technical errors. Two of these animals did not receive local irradiation and survived 11 days, somewhat longer than the mean survival time of untreated canine renal homografts. The third received both local irradiation and portal venous diversion and remains alive and well one month after transplantation with a normal BUN and creatinine, and no other means of immunosuppression. A biopsy at four weeks shows very little cellular infiltrate.

4. Chemical Dosimetry of extra-corporeal Sr⁹⁰ shunts.

In cooperation with the Division of Radiation Physics we will continue to work on development of the ideal geometry of the Strontium⁹⁰ applicators for radiation of circulating blood. Ideally the applicator would combine 1) largest possible radiation dosage to the circulating lymphocyte, 2) shortest possible weight for ease of mobility of the animal or patient, and 3) adequate radiation shielding to protect both the subject and surrounding personnel. With the aid of the very complete Radiation Physics workshop we will continue experimenting with prototypes of applicators. In addition we will construct a replica circulation system with pump, so that we can more accurately predict the cell dosage given by these applicators using the techniques of chemical dosimetry. At present the radiation doses have been empirical and more precise methods are needed to correlate the dose rate per unit time with the lymphopenic response of the animal. Financial aid for a shopworker is requested to continue this portion of the study.

B. Delayed irradiation in treatment of rejecting renal homografts.

1. Strontium⁹⁰ shunt on day 3 or 4:

It has been shown by Zukowski that prolongation of renal homografts can be achieved by starting the administration of 6-mercaptopurine or azathioprine on days 1, 2, or 3 post-transplantation. Histologic and function data indicate that by the 3rd or 4th post-transplant day rejection is well established, and therefore any treatment begun at this time must have to effect either the central immune mechanism or the effector arm of the immune response. Data thus far reported by us would indicate that radiation of circulating blood affects the sensitization process, but it is not known whether lymphopenia would also have an effect in a previously sensitized animal in the early transplant period. This experiment is designed to shed light on that matter.

2. Imuran plus Strontium⁹⁰ shunt for rejection crises.

At present vigorous treatment is required to reverse severe rejection crises both in dogs and in man. Many times the treatment used proves fatal to the host. In this study a series of dogs will receive renal homotransplants and will be placed on low doses of Azathioprine. At time of rejection, these animals will receive blood irradiation by the strontium applicator. This method will be compared with previous studies in which local graft irradiation, increased corticosteroids, and Actinomycin C have been given to reverse the rejection crises.

C. Irradiation enhancement for investigation of lymphoid stimulating factors.

Substantial doses of irradiation given 2-3 days after administration of potent antigens produce in certain experimental animals enhanced production of antibody. The mechanism by which x-ray induces this increased response is unknown. In previous work, Dixon performed thymectomies in a group of adult rabbits on the day of irradiation and found that there was no appreciable effect on the enhanced production of antibody. He reasoned that the most logical explanation was that x-ray produced a depletion of small lymphocytes, thereby allowing the proliferation of cells that had undergone the initial steps toward antibody synthesis and were radioresistant.

However, observations in rats have demonstrated quite clearly that the thymus is the first lymphoid structure to return to normal histological appearance following irradiation. Furthermore, in the rat, the adult thymus appears to be a more active organ than the thymus of the rabbit. Accordingly, preliminary experiments were carried out to ascertain the effect of adult thymectomy upon the phenomenon of x-ray enhancement using soluble Brucellin antigen. The antibody response of x-irradiated controls was not altogether typical of the enhanced pattern, as antibody titers did not greatly exceed those of control non-irradiated animals. However, the thymectomized irradiated animals demonstrated a 1-2 tube depression of mean titers below those of the irradiated group. This difference was not statistically significant because of considerable variation in the response of both the irradiated controls and the irradiated thymectomized animals. On the basis of this preliminary observation three studies are proposed:

1) An experiment identical to the one mentioned above will be carried out, but instead of using Brucellin antigen, Salmonella flagella antigen will be used. This antigen has been shown to be capable of inducing the phenomenon of x-ray enhancement. Four groups of animals will serve as the framework for the experiments. Group 1, normal, non-irradiated; Group 2, thymectomized and irradiated; Group 3, irradiated 2 days after antigen; Group 4, thymectomized and irradiated 2 days after antigen. The antibody response curves to a standard dose of antigen will be determined for each of these 4 groups of animals, and it should be possible to determine clearly whether the thymus is important in the lymphoid stimulating activity that occurs following irradiation.

2) The work of Warner and Schoenberg and that of Good have demonstrated in the chicken that the bursa of Fabricius is necessary for the production of antibody, but not necessarily for the elaboration of reactive lymphocytes. Work done by Archer and Good suggest that in the mammal the appendix and perhaps the lymphoid structures of the Peyer patches might be analogous to the bursa of Fabricius. Accordingly, another group of experiments will be carried out in animals deprived of intestinal lymphoid structures. The same 4 groups of animals will be set up as with those rats deprived of thymic tissue. Preliminary work has shown that it is feasible to excise Peyer patches from the intestinal wall, reconstitute the intestine in adult rats, and have a high percent of survivors. Adult rats, however, do not survive massive small bowel resection in our experience.

3) If either of these experimental protocols obliterates the enhancement phenomenon following irradiation, then animals deprived of thymus or intestinal lymphoid structures might serve as animals for the assay of the particular hormone in question. In addition, the physiology of the lymphoid stimulating factors might be more clearly understood.

References

Taliferro, W.H., Taliferro, L.G., and Jaroslow, B.N.: Radiation and Immune Mechanisms, Academic Press, Inc., New York, 1964.

Dixon, F.J., McConahey, P.J., J. Exp. Med. 117:833, 1963.

Archer, O.K., Sutherland, D.E.R., and Good, R.A.: Nature 200:337, 1963.

D. Strontium⁹⁰ radiation of blood circulating in teflon-silastic arterio-venous shunts in the human.

The initial uses of shunt radiation in renal homograft rejection and in chronic lymphocytic leukemia in man have been summarized in the preceding progress report. In cooperation with the Divisions of Hematology and Radiotherapy we will continue cautious studies in man in three clinical situations: 1) renal transplant rejection, 2) pre-treatment of renal transplant recipients, and 3) acute and chronic lymphocytic leukemia. From the data accumulated from the four patients thus far treated with radiation to the circulating blood, it appears that a lymphopenia can be readily obtained by this method and that it has some effect upon the reversal of the rejection crisis. The short-term effects of the treatment of chronic lymphocytic leukemia have been observed in two patients, and a definite lowering of the peripheral cell count has been seen. The long term effects have yet to be observed, and the dose-time relationship has not yet been explored in the treatment of these patients. We plan to continue blood irradiation with a small number of patients, bringing modifications developed in the laboratory into the clinical situation as they arise.

E. In-vitro lymphocyte studies following transplantation and irradiation of circulating blood.

During the past year, we have gained experience in our laboratories with in-vitro culture of peripheral lymphocytes from both the dog and man. It has previously been shown by Schrehand and Stephani that lymphocytes cultured from both normal and leukemic individuals show a marked decrease in cell survival in-vitro following very modest radiation doses to the culture. It has also been demonstrated by many workers that lymphocytes from normal individuals show blastogenic activity in 80-95% of a cell population following stimulation with phytohemagglutinin, while cells from persons with chronic lymphocytic leukemia show only 10-15% blastogenic activity. We have reported, in the last year, our results of the stimulation with phytohemagglutinin of lymphocytes from human renal transplant recipients. These individuals, even though on immunosuppressive chemotherapy, stimulate to the same degree as normal individuals. We plan during the next grant period to follow the in vitro lymphocyte cultures in both leukemic individuals and transplant

recipients before, during, and after periods of radiation to the circulating blood, both with dye exclusion cell viability studies and with phytohemagglutinin stimulated cultures to ascertain what changes occur in the lymphocyte reactivity during the radiation period. It is hoped that by this method we can assess the proper time dose relationship for ultimate lymphocyte inhibition as well as objectively assess the patient or animal response to the irradiation.

6. Scientific Personnel

Principal Investigator:

David M. Hume, M.D., Professor and Chairman, Department of Surgery, Medical College of Virginia. Time: 25%

Co-Principal Investigators:

H.M. Lee, M.D., Associate Professor of Surgery, 30%; G. Melville Williams, M.D., Assistant Professor of Surgery, 50%; James S. Wolf, M.D., Research Fellow in Surgery, 50%, Fearghus T. O'Foghludha, PhD., Chairman, Division of Radiation Physics, 5%

Newly-Appointed Personnel:

James C. Pierce, M.D., PhD, Instructor in Surgery, 40%; Alan Newhoff, M.D., Research Fellow in Surgery, 50%; Bjarne Semb, M.D., Research Fellow in Surgery, 90%

7. Other Personnel:

Research Associate--James J. Dwyer---	60%
Lab. Spec. B. --E. Borum-----	100%
Lab. Aide --S. Aikens-----	20%
Lab. Tech. B. --M. Green-----	100%
Lab. Tech. B --G. Robertshaw----	50% (undergraduate student)
Radiation Asst. --F. Winkler-----	20%
Shopworker --(Requested)-----	50%

8. Other financial assistance:

No other financial assistance is received for any of the non-professional personnel proposed to be paid from AEC funds except J. Dwyer, who will receive \$4,000 per year from an N.I.H. Institutional Grant. These 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, 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 Clinical Transplant Center and Clinical Research Center 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: \$49,526.00

12. Authentication: Signed for the Medical College of Virginia

Principal Investigator:

David M. Hume
David M. Hume, M.D.

Comptroller:

John H. Crooks, Jr.
L. Daniel Crooks

1039044

10. Proposed Budget for 10-1-66 to 9-30-67

AEC Contribution

<u>* Personnel</u>	<u>% Time</u>	
J.J. Dwyer, Research Associate	60%	6,000.00
E. Borum, Lab Spec B	100%	6,682.00
G. Robertshaw, Student Tech C	65%	5,200.00
M. Green, Lab Tech B	100%	3,968.00
S. Aikens, Lab Aide	20%	717.00
F. Winkler, Lab Tech A	20%	819.00
Lab Mechanic C	50%	3,072.00
		<hr/>
		26,458.00
Social Security @ 4.2%		1,111.04
** Overhead 30.34%		8,027.00
		<hr/>
		\$ 35,596.00

* All State of Virginia employees received 10% general increase on 1-1-66.

** Institutional overhead has been increased from 22% to 30.34%.

Supplies and Equipment

Laboratory glassware, etc.	800.00
Reagents, Drugs	850.00
Pathology (200 X \$1/slide)	200.00
Maintenance and Repair	200.00
Shunt Materials for A-V Shunts	450.00
Shop Materials, construction of extra-corporeal shunts	700.00
Isotopes	800.00
Office supplies and photography	200.00
Telephone and telegraph	50.00
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	4,250.00

Reprints and Publications 300.00

Animals and Care

Operating Expense	3,900.00
Dog Purchase (150 X \$10/dog)	1,500.00
Dog Care (150 X .60/day X 30 days)	2,700.00
Rats (250 @ 1.20 each)	300.00
Rat Care @ .03/day X 30)	180.00
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	8,580.00

Travel 800.00

TOTAL AEC REQUEST \$ 49,526.00

1039045

11. Amount Requested

A.* Institutional Contribution

Salaries

D.M. Hume, M.D.	25%	8,000.00
H.M. Lee, M.D.	30%	4,785.00
Feargus O'Foghludha, M.D.	5%	1,150.00
R. Davis, Animal Caretaker	50%	1,968.00
R. Tinsley, Lab Aide	75%	2,475.00
Lab. Mechanic C	50%	3,072.00

21,450.00

Social Security @ 4.2%	901.00
Overhead 30.34%	6,507.00

28,858.00

Maintenance of Research Facilities	10,000.00
Animal procurement, care, and processing	12,000.00

\$ 50,858.00

B.* Other Non-University Sponsors

Salaries

G. M. Williams, M.D.	50%	7,150.00	NIH
J. S. Wolf, M.D.	50%	4,250.00	NIH
James Pierce, M.D.	40%	6,750.00	NIH
Alan Newhoff, M.D.	50%	3,550.00	NIH
Bjame Semb, M.D.	90%	5,950.00	NIH

\$ 27,650.00

C. Total Institutional and Non-AEC Contribution	78,508.00
Total A.E.C. Request	49,526.00
Total Cost of Project	<u>\$ 128,034.00</u>

* Cost of Clinical Transplant Center not included. This amounts to about \$300,000 per year.