FACTSHEET HUMAN EXPERIMENTATION-05 (SFS11.001)

**Project Name**: Study of Transplantation of Bone Marrow Tissue and Whole Organs, etc.

**Date Started**: June 1960
**Date Terminated**: 3 yr.

**Institution**: Harvard Medical School
**Funding Source(s)**: AEC

**Identification**: TID 6107
**Project Duration**: Dr. Francis D. Moore
**Principal Investigator(s)**:

**Responsible Government Official(s)**: Charles L. Dunham, M.D.

**Objective(s) of Project**: To study the transplantation of tissues and whole organs in the rabbit, dog, and man.

**Short Description**: One patient (in this report) received 250 r total body X-ray irradiation to suppress the immune reaction to a kidney transplant.

Seven other patients received doses of 350 to 450 r. Details in previous reports. Apparently not successful but survival time isn't given in this document.

**Follow-up Data**: 17 months after graft, patient reported to be doing well. Low dose at additional X-ray against progression of a beginning rejection response.

**References**:

**Attachment(s)**:

# A PROGRAM FOR THE STUDY OF TRANSPLANTATION OF BONE MARROW, TISSUES, AND WHOLE ORGANS AND OF RELATED TOPICS IN SURGICAL RESEARCH

ATOMIC ENERGY COMMISSION

TID-6107

Project AT (30-1) - 2265)

PROGRESS REPORT WITH OUTLINE OF CONTINUING WORK

June, 1960

Department of Surgery of the Harvard Medical School at the Peter Bent Brigham Hospital, Boston, Massachusetts

Administration: Peter Bent Brigham Hospital
721 Huntington Avenue
Boston 15, Massachusetts

PBBH Accounting Code #9307

Responsible Investigator: Dr. Francis D. Moore
Moseley Professor of Surgery
Harvard Medical School
Surgeon-in-Chief
Peter Bent Brigham Hospital

## OUTLINE

ATOMIC ENERGY COMMISSION

# A PROGRAM FOR THE STUDY OF TRANSPLANTATION OF BONE MARROW, TISSUES, AND WHOLE ORGANS AND OF RELATED TOPICS IN SURGICAL RESEARCH. PROGRESS REPORT AND OUTLINE OF RENEWAL FOR CONTINUING WORK

June, 1960

Responsible Investigator: Dr. Francis D. Moore

Department of Surgery, Harvard Medical School, Peter Bent Brigham Hospital, Boston, Massachusetts

AEC Number AT (30-1) - 2265 PBBH Accounting Code #9307

## 1. PLAN OF THIS DOCUMENT

The following document constitutes a progress report and renewal proposal of our work, based on research completed since the presentation of our previous proposal, dated June, 1959. It is important to emphasize that our previous documentation (dated June, 1959) covered a two year period, whereas this covers but one year. Each item and area of investigation is referred to very briefly. There is a notation as to pertinent publications or manuscripts; the complete bibliography is listed. No attempt is made to give a complete account of each series of experiments. Additional details are provided in the publications referred to and will be made available by amplification of any section of this report, if so desired by the Commission.

These studies have been carried out cooperatively in the departments of Surgery, Pathology and Radiology. The Department of Medicine has played an important role. Funds from this contract have supported the surgical laboratories both in Building C of the Harvard Medical School and the Peter Bent Brigham Hospital. This criterion, of surgical laboratory support, has been used in selecting the listing of these studies. Many other grants and philanthropic sources are involved and several other biological directions are under investigation but without the support of this particular contract. Related researches carried out in other divisions of

the School or Hospital have not been listed here although these collaborative enterprises have often been of great importance in the progress of this work. Because this contract covers an integrated effort in the study of transplantation, involving several different individuals, subdivisions and departments, it must necessarily list the various phases of the work briefly.

The budget is submitted in the "single column" form, meaning by that that the major share of this work is considered as a unit. A portion of this unit is then considered as being supported by this contract with the Atomic Energy Commission. Because of the productive phase in which this work currently finds itself, and the pressing need for modest salary increases, the budget is slightly larger than that of last year.

2. SPECIFIC AIMS

1. To study the transplantation of tissues and whole organs in the rabbit, dog and man.

2. To study and elucidate the immunologic and genetic factors involved in homograft rejection, and the utility of cytotoxins, immunologic procedures and X-irradiation in enhancing acceptance.

3. To study and perfect surgical techniques and metabolic care ancillary to transplantation, with particular reference to metabolism after injury, whole organ function, and the problems of hospital asepsis.

3. METHODS OF APPROACH

As will be noted, the studies supported under this contract, and the personnel whose support derives herefrom, are engaged in a considerable variety of studies of the transplant problem. This variety of approaches may be analyzed as falling into three broad categories:

1. Studies of the anatomy, surgery, clinical aspects and pathologic course of homotransplant of whole organs, with suitable control studies.

2. Studies of antigen-antibody reactions, immunogenetic phenomena, delayed sensitivity, and the effect of irradiation or cytotoxins in the homotransplant problem.

3. Studies of the transplantation of nonvascularized portions of endocrine glandular tissues, in the millipore filter diffusion chamber, with related studies.

4. ORGANIZATION AND COORDINATION

A program such as this, involving men working in a number of different departments and subdivisions, must be coordinated and correlated if it is to effect the most rapid advance of this field.

Each Tuesday afternoon at five o'clock a transplantation conference is held. These are arranged and planned by Dr. Joseph E. Murray of this surgical department. At these talks, all workers in this field (much of whose work is supported under this and related grants and contracts) report their progress and discuss future plans. In addition, guest speakers and participants from other laboratories are invited to speak. During this year it has been our pleasure to have Professor Medewar of London and Professor Woodruff of Edinburgh participate in these conferences with us at the time of their visit to Boston. The Russian delegation visiting certain universities and medical schools in the United States following the Transplant Conference in New York also participated with us in these Tuesday conferences.

Meetings are held several times each year at which the most senior members of the groups participating meet together to discuss financial plans, mutual support of personnel and future biological directions. At these meetings the Department of Surgery is represented by Dr. Francis Moore and Dr. Joseph Murray, the Department of Medicine by Dr. John P. Merrill, the Department of Pathology by Dr. Gustave J. Dammin and the Department of Radiology by Dr. James B. Dealy, Jr.

5. STUDIES OF WHOLE ORGAN TRANSPLANTATION

a. Kidney Transplantation and Related Topics in Animals

The work on "in vivo banking" has been essentially completed. This consists in the homotransplantation of kidney from one dog to another, and then its retransplantation to the original donor, with studies of its survival and histology after varying periods of habitation in the new recipient. It is of interest that the histologic rejection changes may actually increase for a few days after retransplantation back into the original autologous host, only to be followed by improvement and recovery. This is an important observation in relation to clinical experiences, namely, that the early changes of the rejection response are not necessarily irreversible. Instances of return of renal function have occurred 24, 48 and 72 hours and eight days following temporary residence in an homologous host.

The possibility of using heterologous in vivo banking comes to mind as a method for temporary preservation of human kidneys for transplantation from cadavers

Studies of the enhancement of renal transplants in dogs have been carried out, using spleen cells preserved in glycerol. Homologous live spleen cells are injected intravenously at a dose of about 50 million cells per day for 14 days, and antibody formation against these cells is studied by hemagglutination, hemolysin production, agar gel diffusion and chromated red cell survival. Cytotoxin production is also studied. All of these reactions so far have been negative, indicating no sign of immunity or circulating antibody production from this type of enhancement procedure. Thus far homotransplantation acceptance in paired donors has not been prolonged by these cellular adjuvant procedures.

Kidney slices have been transplanted in laboratory rodents, using the recipient kidney as a bed for the transplant of homologous kidney slices. This has turned out to be a delicate method for the discernment of a graft and host interaction. Although the kidney slice is nonfunctional in the sense of whole organ physiology, cellular viability is readily attained. Thus far, no evidence for a "graft vs. host" reaction has been deduced.

b. Kidney Transplantation in Man

1. Identical Twins

The identical twin series has now been extended to a total of fifteen individuals. A number of pairs of identical twins have been considered for transplantation but rejected because of clinical contraindications either in donor or recipient. Notes on some of the more important recent events in these patients follow:

Pt. Herrick--Now over five years postoperative. Histologic study of the kidney shows no signs of immunologic rejection but does show some subacute glomerular nephritis; the patient has received a very low dose X-ray with the hope of ameliorating this response. In the midst of this X-ray treatment he developed an intercurrent bout of intestinal obstruction which was treated successfully by conventional surgical measures.

Pt. Coxie--Age 48. This patient, somewhat older than other members of the series, required an endarterectomy at the time of transplant and at one year shows normal histology in the transplant biopsy and is in perfect health.

Pt. Rosencranz--This was the first twin to have preliminary bilateral nephrectomy as prophylaxis against contracting the original autologous disease in the isologous transplant. It is noteworthy that we have had two fatal cases of this latter situation. In the case of Rosencranz, at one year after transplantation, there is normal histology in the transplant. The patient is in perfect health and is on low doses of monthly chemotherapy and penicillin as prophylaxis against glomerular nephritis.

Pt. Ray--This patient presents a one-year follow-up. He had acute disease originally, had preliminary unilateral nephrectomy and simultaneously with his graft had a second nephrectomy. The patient is doing well, with normal histology in the kidney at one year.

Ft. Holm--This patient had a normal pregnancy and delivery after kidney transplant and is now normal in every way after four years, and is again pregnant.

Ft. MacDuffie--This is the first twin situation in which the donor has later died. This donor was killed in a road-construction accident when his hand got caught in a piece of machinery, dragging him in to a gear press, with fatal results. His recipient twin is still well and doing nicely.

2. Experience in Non-identical Twins or Non-twin Situations

As mentioned in our proposal last year, we have been faced with the problem of anephric patients on several occasions and on several additional occasions have undertaken whole body irradiation and kidney transplantation in patients who were not identical twins. These experiences will not be recounted here in detail. There has been but a single success. This was in patient Riteris. who had a fraternal twin donor. The patient received 250 r total body X-ray and now, approximately 17 months after graft, is doing well. He rejected his skin graft approximately eight months after the kidney graft was placed. The patient has had low dose additional X-ray treatment for prophylaxis against progression of a seemingly beginning rejection response in the kidney grafts and the result has been gratifying to date.

On eight other occasions we have made attempts to produce homologous kidney graft in patients who were dying of fatal renal disease, or who were anephric. All but one of these have received total body irradiations in doses between 350 and 450 r. In the other patient 6 mercaptopurine was used because a cadaver donor became available suddenly and unexpectedly. This experience with chemotherapy was quite encouraging. Renal function was well maintained for 30 days. The patient died of an intercurrent electrolyte imbalance with cardiac arrhythmia. It is of interest that the renal graft, although showing some unmistakable evidence of rejection, was much better preserved than one would expect in a 30-day homograft. In several of these experiences, family donors were used (two mother-to-daught grafts and one father-to-daughter graft.) At the present time a father-to-son graft is under consideration.

Our approach to these problems has been a cautious one, always differentiating the risk and salvage factors as between kidneys taken from living donors and those kidneys obtained either from cadavers or from infants being treated for hydrocephalus by subarachnoid-ureteral shunt. In the latter case, the kidney is inevitably sacrificed.

The success with patient Riteris and the increasing awareness that the homograft rejection response is subject to modification by immunogenetic procedures short of lethal injury has stimulated continued cautious exploration of this unknown territory. Surgical problems continue to be rather pressing. In at least two instances (Harper and Quental) in the last year, failure has been in part due to changes inthe anastomotic blood supply.

c. Transplantation of the Liver in Dogs

This work has progressed well in an extremely fruitful year and has now reached completion of its second phase.

The first phase consisted in perfection of the operation itself. This was reported briefly in last year's document. It consists of a whole organ hypothermic-to-normothermic homologous canine liver transplant after total hepatectomy in the recipient. The operation involves four major vessel anastomoses and a biliary shunt. Our current success in producing short-term survivors is almost universal. The operation is now done wholly through the abdomen. We have now had many survivors between five and eight days and our longest survivor is 12 days.

The second phase of the work has consisted in a delineation of the pathologic and clinical course of these animals. This has been brought to completion and is currently being published. The liver continues to function very well until approximately 24 hours prior to death. The liver of our longest survivor shows surprisingly little evidence of homograft rejection in the classical sense though some of the other livers show a massive infiltration of plasma cells in the portal areas. The parenchymatous liver cells themselves seem to be spared in the early phases. In one instance we have seen a form of liver cell necrosis that was very atypical and, in the opinion of one veterinary pathologist, was probably acute

hepatitis rather than liver rejection.

d. Transplantation of the Spleen in Dogs

Starting in October, 1959, our interest in whole organ transplantation was extended to the spleen. This has turned out to be an important subject because of the fact that the spleen contains a large mass of immunologically competent cells and is, under certain circumstances, also a multipotential hematopoietic organ. The first two phases of this work have likewise been completed.

First has been the delineation of the requirements for a surgically successful spleen transplant operation and its accomplishment in approximately 60 animals. This consists of a normothermic whole spleen transplant after total splenectomy in the recipient dog. It involves only two major vessel anastomoses. These vessels are rather small, yet viable spleen is readily obtained if the operation is carefully done.

The second phase of this work has been the delineation of the clinical, hematological and pathologic course of these animals. In most of the animals an initial "graft vs. host reaction" is seen in the form of swelling of the splenic follicle with loss of the mantle of mature cells. This phase quickly gives way to one of atretic follicle, going on then to massive infiltration with plasma cells and finally a rejected spleen. The whole sequence takes about 7 to 9 days and is not accompanied by changes in the peripheral blood. It is of interest that in one pair of animals we saw viable spleen at 51 days. This again calls to mind a general phenomena of homotransplantation work that one occasionally encounters remarkable exceptions to the usual laws of immunogenetic rejection. Although these two dogs looked rather alike, they came in different shipments into the laboratory and we had no reason to believe that they were littermates or in any other way related.

This work will be intensified during the coming year. During the last four months we have added whole body irradiation to this protocol. We are starting at 250 r with the concept of studying the effect of this low dose irradiation on the acceptance of spleen. Thus far the results fail to show any clear-cut effect

on the splenic rejection mechanism of whole body irradiation in this dose. We plan during the coming year to increase this dose to 500 r and also to study the effect of chemotherapy on this system. As a first step in the latter category it is essential to study the effect of chemotherapy on an animal when the spleen is shielded from the agent by temporary occlusion of the blood supply to the spleen. This is the chemotherapeutic analogue of the Jacobson experiment on spleen-shielding in irradiated mice. It is important to discern whether or not the protection of the spleen will result in its gradually acquiring a multipotential hematopoietic histology.

As part of these experiments, we are also planning to study during the coming summer and fall the effect of whole body irradiation in the splenectomized dog. There is reason to believe that shielding of the spleen from whole body irradiation has a beneficial effect not only through marrow repopulation but also by abatement of the release of a deleterious humoral substance from the irradiated spleen. Here again this study will be carried out at various dose levels of irradiation, and in close collaboration with the Department of Radiology.

6. IMMUNOGENETIC STUDIES IN MAN AND ANIMALS

The effects of sublethal irradiation on the survival of skin transplants, and the comparison of these data with measurable antibody formation has been further enlarged. This work has been carried out on rabbits exposed to 400 r or to 600 r (LD-10 or LD-50). A number of immunologic parameters have been studied. There has been found very little correlation between immunogenetic rejection of the homotransplant and the formation of circulating antibodies. Whole body irradiation has comparatively little effect on measurable pre-formed antibodies. In similar studies, animals have been given RNA and DNA or various nucleocides immediately after X-irradiation.

Much of the work on experimental and human bone marrow aspiration and injection described in our previous document has now been published as indicated in the bibliography. Active clinical use of bone marrow transplantation is being continued as a part of our study of the antitumor chemotherapeutic perfusion of isolated limbs and organs. For the most part, this latter is no longer a research endeavor nor is it financed from these funds, but has been incorporated into the clinical life of the hospital.

Interest has developed in the effect of irradiation on the course of transplantation when the irradiation is given following rather than prior to the transplant. The theoretical basis for this lies in the concept that immature cellular populations of immunologically competent cells much more readily develop tolerance than do mature cells. Continuous biophysical destruction of mature cells in the continuous presence of antigens might therefore produce tolerance in an adult animal. This work is being carried out in mice.

During the forthcoming year, our studies of chemotherapy and chemotherapy-radiation combinations will be expanded. These studies will be devoted both to the dog and to the rabbit. In the dog, the studies will be devoted to chemotherapy and subsequent attempts to transplant skin or other organs, as well as chemotherapy in relation to splenic histology. In rabbits we will combine chemotherapy with X-ray therapy and injection of specific cellular antigens, in an attempt to modify the

course of skin graft. This study represents a continuation of our irradiation work in rabbits, the initial steps of which have now been published (see bibliography) and the techniques well-standardized.

Studies of <u>skin grafts in human volunteers</u> continue, as a fruitful method of studying the homotransplant phenomenon under highly controlled conditions in man. For this study, normal volunteers have been obtained on whom skin grafts are carried out under local anesthesia. In these studies our first and most basic objective is a complete re-evaluation and redelineation of the normal histochemical sequences of skin graft rejection in man. This seemingly unexciting research needs badly to be done if we are to evaluate types of history compatibility responses, as indices of varying degrees of immune tolerance in man. A second phase of these experiments in man has consisted in local injection of suspensions or lysates of leucocytes. In certain of the protocols the leucocytes are taken from the same donor who has been the source of the skin graft. By this method, cross sensitization can be studied quantitatively. It has been demonstrated in these studies that a skin graft produces sensitization to a subsequent leucocyte injection and that preliminary leucocyte injection will produce a "second set" skin rejection. In certain circumstances, the skin rejection is so rapid as to fall into the "white graft" category. By this term is meant that no vascularization of the graft occurs at all. This may be considered as an extreme acceleration of what has previously been recognized as the "second set" phenomena

Work on <u>indifferent skin grafts in irradiated mice</u> continues to show much promise. In essence, this experimental protocol consists in the irradiation of a mouse and the transplantation into that mouse of bone marrow from a second animal. Then, during the early period of "take" (when this bone marrow transplantation is repopulating the irradiated subject) a skin graft is placed on the animal from a third or "indifferent" mouse. It is of outstanding interest in the field of homotransplantation immunity that this third skin graft often takes and is held very satisfactorily. The reader will recall our work mentioned in the former document,

and reported by Wilson et al., showing that a rabbit given whole body irradiation and bone marrow transfusion pooled from five different animals often showed takes of more than one skin graft. This appears to be another manifestation of the same phenomenon. There are at least two possible explanations--and probably many more--for such a phenomenon. First is the fact that the immature colonies of regenerating immunologically competent cells grow up in the presence of antigen and are tolerant of any one of a variety of homotransplant antigens presented to them during this regenerating phase. The second explanation might be called the "distraction" hypothesis. This would hold that the new bone marrow presents an overwhelming antigenic stimulus to the waning immunity of the irradiated animal, and that the irradiated animal as a whole presents a large antigenic stimulus to the repopulating infused marrow; antigen-antibody activities on both sides are thus wholly taken up with a "graft-vs.-host" and "host-vs.-graft" reaction; in the presence of such intensely antagonistic activity, the small skin graft, dwelling out in the periphery, receives scant attention. Such an hypothesis, while semantically attractive, has as yet no evidence to support it.

Related to the above concepts are the observations made during the past year suggesting that secondary disease or "runt disease" is most marked in mice in whom there is a concomitant graft vs. host and host vs. graft reaction. These reactions have been measured by differential hemagluttinin and red cell survival techniques. It is of importance to emphasize that after whole-body irradiation and bone marrow transfusion, there may be <u>selective</u> cellular survival populations. By this is meant that we have now documented animals after whole body irradiation and bone marrow transfusion, in whom the erythrocyte population belongs to the donor and the leucocyte population to the recipient. A variety of different combinations have been observed. In animals in whom the marrow is clearly rejected, or in animals in whom the marrow is clearly predominant, secondary runt disease is less prominent.

A search for, and attempt to isolate the transplant antigen continues. This work is done particularly in the Department of Pathology, is based on the lysis and chemical fractionation of spleen cells, employs the second set skin rejection response as its titration end-point, and is largely supported from other sources.

Our initial studies of organ preservation have been brought to completion and readied for publication. These have taken the form of normothermic and hypothermic extracorporeal pump oxygenator perfusion of the kidney, and studies of hypothermic preservation of liver, using carbon-14-tagged glucose oxidation to radioactive $CO_2$, as the viability end-point. This important aspect of our work needs to be strengthened and expanded. During the past eight months we have been inactive in this field because of budgetary limitation. It is important to emphasize that in our studies last year we demonstrated that when liver is allowed to cool pari passu with the temperature of the cadaver, its cellular integrity is completely lost in less than an hour and viability is irreversibly compromised. In sharp contrast, if the liver is kept cool by any one of a wide variety of techniques, it maintains excellent cellular integrity as long as 12 hours. Furthermore, we have shown by a comparison of the function of the whole-organ transplanted liver with its in vitro carbon-labelled glucose oxidation rate, that livers that show less than perfect integrated biologic oxidative activity will actually survive and function well after transplantation. Stated otherwise, this particular viability-parameter (glucose oxidation rate) is capable of demonstrating a very wide range of activity from "perfect" (meaning by that essentially normal) on down over a slowly descending scale to a residual activity of about 10% . This latter figure is demonstrated by a completely dead liver and represents the type of residual enzymatic activity that one sees in liver cell lysates.

[redacted]

The studies of endocrine transplantation in millipore filters represent a separate group carried out in the surgical laboratories and, for the most part, by other individuals than those working with whole-organ preparations.

The millipore filter, as it is now employed, has been disappointing as a means of transplanting endocrine tissue in animals and man. It is our conviction, however, that these problems are in part physical, that the method still has much promise and that it should be pursued actively.

Currently our work falls into several categories, as follows:

A concerted attempt is underway to quantify the release of thyroid hormone from millipore filter transplanted thyroid in the dog. For this we are using the radioactive tri-iodothyronine uptake of the red blood cells, which is performed collaboratively by Dr. Hamolsky of the Beth Israel Hospital. It has been found that this tissue survives best if the millipore filgers are placed horizontally between the lobes of dog lung. This technique is currently being pursued very actively and a sizeable statistical series is nearing completion. It should be emphasized that we have good evidence that millipore filter transplanted endocrine tissues maintain their cellular integrity. This has been prover histologically in man for parathyroid, ovary and thyroid, and in animals, additionally, for adrenal and pancreatic islets. Despite histologic cellular integrity, the tissue does not produce hormone normally in man. This may have to do with the thickness of the membrane or oxygen diffusion across the membrane. These parameters are currently under study.

There is substantial evidence that hormones can leave the membrane enclosure quite satisfactorily. We have shown this by the rapid change in tubular phosphate reabsorption upon millipore filter transplantation of either normal parathyroid tissue or parathyroid adenoma in man. This "passive transfer" has also been demonstrated by the transplantation of insulin-producing adenomas of the pancreas in man. In the case of the ovary, production and release of hormone has been shown in monkey and mouse. It is of interest that estrous-producing tissue from the ovary can be

demonstrated when the tissue within the millipore filter has no follicles and is an homogeneous ovarian stroma.

Immunity transfer experiments are being continued with the millipore filter. In many ways this work has proven to be of equal or greater interest than has been the direct work on tissue transplantation. The immunity transfer studies have taken two forms. First has been the demonstration of the transfer of immunity against tumor transplantation by heterologous transfer of tumor-sensitized lymph nodes. This lymph node is enclosed in a millipore diffusion chamber and has been a lymph node that formerly drained an area of successful tumor transplantation. The millipore diffusion chamber evidently protects this lymph node tissue from heterologous transplant rejection, but releases active antibody substances that prevent the "take" of the same tumor in the new recipient carrying the diffusion chamber.

Similarly, the continued production of antibody against bacteria may be demonstrated in lymph node transplanted in a diffusion chamber.

A typical protocol for this experiment consists in immunizing a mouse against Salmonella typhosa. Lymph node tissue from this mouse is then transplanted in a millipore diffusion chamber into a second mouse. This transplanted lymph node tissue continues to protect the second mouse against infection with this organism for a period as long as 30 days. This prolonged protection suggests that the lymph node is living and reproducing within the millipore diffusion chamber and that the reproducing cells of the lymph node are able to manufacture and release antibody even though antigen is absent.

8. RELATED STUDIES IN SURGICAL RESEARCH

1. Studies of Hospital Infection and Immunology

The isolation of patients undergoing renal transplantation requires special aseptic techniques. These have been worked out largely with the support of other agencies. Nonetheless, the establishment of these rooms, of dietary assistance to care for the patients in these rooms, and certain of the studies related to this problem have been supported from this contract.

In addition, a series of researches has been undertaken to measure (by the erythrocyte-adherence technique of antibody titration) resistance against the Staphylococcus aureus. By this technique the natural immunity arising in patients subject to so-called hospital staphylococcal infections has been studied. Our findings indicate that certain patients subject to recurrent cutaneous infections as an aftermath of hospital sepsis are singularly devoid of antibodies against the familiar strains of staphylococci. Other individuals, who are not afflicted with these infections, have a normal antibody titer. This suggests that any "epidemic" of hospital staphylococcal infections must be considered in the light of host resistance as well as in the light of environmental factors.

2. Surgical Metabolism

Studies of surgical metabolism are supported through this grant insofar as it supports ancillary services shared both by the metabolic and transplant studies. An intensive study of fractures, of the effect of hormones on skeletal salt metabolism after fracture, and a continuing study of body fluid volume regulation are under way and will be continued in the coming year, as mentioned in our previous document. Major support for this area arises from other sources.

9. FACILITIES AVAILABLE

Facilities available for these studies are the same as those described in the previous document (dated June, 1959).

As described in that document, these facilities consist of the surgical research establishment of the Department of Surgery of Harvard Medical School at the Peter Bent Brigham Hospital. The laboratories include the fourth and fifth

floors of Building C-II of the Harvard Medical School and the entire first floor area adjacent to the operating rooms in the hospital. In addition, the beds of the hospital and most particularly the five special beds in the Bartlett Metabolic Unit are available for patients being studied under this program. The support of these beds and of this unit is of front-rank importance in the continuation of this work. All of the patients having total body irradiation and bone marrow transfusion have been housed on this unit and its continued use is essential.

The Departments of Radiology and Pathology of the Peter Bent Brigham Hospital and the Department of Bacteriology and Immunology of Harvard Medical School have been very active in collaboration. Animals, materials and biological preparation arising from this support have been extensively studied in pathology and radiology; these departments, by the same token, have made available to surgery the specialized facilities, equipment and skill of their laboratories and personnel.

During the forthcoming year, our Surgical Research Laboratories at the Harvard Medical School will be removed from Building C-II to the ground floor of Building E-I. This represents the first physical move of these laboratories in over fifty years. Our facilities will be somewhat expanded but our most important advantage will lie in the very considerable improvement in light, in space for animal care, in adequacy of animal care, and in service facilities for the research program.

10. <u>OTHER SUPPORT NOW AVAILABLE OR CONTEMPLATED</u>

As mentioned in our previous document (June, 1959) the general laboratory arrangements for this department and this work are supported by an interlocking system of research grants to supplement the university and hospital departmental budgets. The "single column budget" enclosed herein can give but an approximate idea of the order of magnitude of this ancillary support.

The central feature of the financial structure of this department is this contract with the Atomic Energy Commission which historically traces its origin back to our former contract (AT (30-1) - 733) designated as "Injury and Wounding; A Study of Convalescence by Metabolic and Isotopic Methods." Other grants or

contracts involved with this work are listed briefly as follows:

United States Public Health Service: Support in surgical metabolism, millipore filter studies, kidney transplantation and radiologic and pathologic studies.

United States Army, Office of the Surgeon General: Support in liver studies, surgical metabolism, transplantation, support for the department of pathology in transplantation and related studies.

In addition, grants from the American Cancer Society, the Commonwealth Fund, private industry and philanthropy and the Milton Fund of Harvard University are active in helping the support of this work. In the budget provided herewith no attempt is made to differentiate these multiple sources of support in the so-called "single column budget form."

During the past year we were fortunate to obtain a grant of $50,000. per year for two years from the Avalon Foundation in New York. This grant specifically makes it possible for us to cover the hospitalization costs of patients being admitted to the hospital for transplantation. This includes the study of renal disease, the evaluation of donors, the hospitalization of patients, volunteer subjects, donors and recipients in other transplantation fields, and certain aspects of study related to bone marrow transplantation.

11. PERSONNEL

This continues to be a departmental and interdepartmental program that involves, as it did last year, a number of senior personnel as follows:

Dr. Francis D. Moore, Moseley Professor of Surgery and Surgeon-in-Chief
Dr. Gustave J. Dammin, Professor of Pathology and Pathologist-in-Chief
Dr. James B. Dealy, Jr., Professor of Radiology and Radiologist-in-Chief
Dr. Somers H. Sturgis, Clinical Professor of Gynecology
Dr. John P. Merrill, Senior Associate in Medicine
Dr. Joseph E. Murray, Associate in Surgery
Dr. John R. Brooks, Associate in Surgery
Dr. John M. Kinney, Associate in Surgery

A number of postgraduate research assistants have worked with us during the past two years, are working with us now, and contemplate working with us in the coming year. A listing of these indicates the magnitude of our needs for support in this division. Following the name of each man is indicated in parenthesis either the country or city of his origin.

1. Postgraduate Research Assistants working in this program in the period July, 1959, to June, 1960, are as follows:

Dr. Martin Litwin (Birmingham)
Dr. Louis Smith (Los Angeles)
Dr. Ulrich Gruber (Switzerland)
Dr. Arthur Siculer (New York)
Dr. Fred Lee (Boston)
Dr. Norman Sadowski (Boston)
Dr. John O'Connor (Boston)
Dr. Sergio Piomelli (Italy)
Dr. David Hickok (Boston)
Dr. Richard Wilson (Boston)
Dr. Okas Balankura (Thailand)
Dr. Harry Demissianos (Boston)
Dr. H. Brownell Wheeler (Boston)
Dr. Leslie Rudolf (Boston)
Dr. John Cahill (Baltimore)
Dr. Alvaro Rabelo (Brazil)
Dr. Keith Abel (London)
Dr. John E. H. Pendower (London)
Dr. Ronald H. Lewis (Bristol)
Dr. Rameschandra C. Shah (Calcutta)
Dr. John W.D.Henderson (London)
Dr. Daniel E. Pugh (Boston)

2. Postgraduate Research Assistants who will be joining the group in the coming two years, in addition to those above (some of whom will be leaving or have left) are as follows:

Dr. Michael Brady (Dublin)
Dr. Albert Montague (Warsaw) (More recently of New Haven)
Dr. Roy Y. Calne (London)
Dr. John Anderson (London)
Dr. Malcolm Gough (London)

Several Harvard Medical students will be working on these problems with us in the coming summer, including Mr. John P. Dickson, Mr. Peter S. Liebert, Mr. Charles G. Halgrimson and Mr. Douglas M. Behrendt.

12. BUDGET

PETER BENT BRIGHAM HOSPITAL
Contract (AT (30-1) - 2265)
Single Column Budget

Submitted June 15, 1960 for Fiscal Year September 16, 1960 - September 15, 1961

(Note: This single column budget covers a major share of the work described in the enclosed document).

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|---|---|---|---|
| **1. Personnel** | | | |
| 1.1 | Senior Professional . . . . . | $30,000. | |
| | Dr. Moore (1/5 time) | | |
| | Dr. Murray (1/4 time) | | |
| | Dr. Brooks (1/4 time) | | |
| | Dr. Sturgis (1/4 time) | | |
| | Established Investigator . . . . . | 7,500. | |
| 1.2 | Secretarial . . . . . | 10,000. | |
| 1.3 | Technical, Dietetic, Diener . . . . . | 35,000. | |
| 1.4 | Postdoctoral Assistents . . . . . | 17,500. | |
| 1.6 | Total Salaries . . . . . | | $100,000. |
| 1.7 | Social Security - 3% of $100,000. . . . | | 3,000. |
| 1.8 | Salaries plus Social Security | | $103,000. |
| **2. Supplies** | | | |
| 2.1 | Chemicals and Glass | 5,000. | |
| 2.2 | Isotopes | 3,000. | |
| 2.3 | Animals and Maintenance | 10,000. | |
| | | | 18,000. |
| **3. Equipment** | | | |
| 3.1 | Repairs and Purchases | 5,000. | |
| 3.2 | Permanent Equipment | 4,500. | |
| | | | 9,500. |
| **4. Service** | | | |
| 4.1 | Photography and Charting | 1,500. | |
| 4.2 | Postage and Express | 400. | |
| 4.3 | Volunteer Subjects | 800. | |
| 4.4 | Miscellaneous Services | 800. | |
| 4.5 | Travel | 350. | |
| | | | 3,850. |
| **5. Total Expense** | | | $134,350. |
| 5.1 | Available other Sources | | 89,905 |
| 5.2 | Direct Expense Requested AEC | | $ 44,445. |
| **6. Overhead** (35% on $44,445) | | | 15,555. |
| 7. | Total Requested from Atomic Energy Commission | | $60,000. |

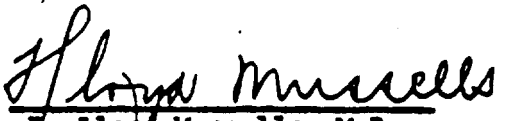
Comments on Budget

This budget involves an increase of $10,000 or approximately 20 per cent in the total requested from the Atomic Energy Commission for the forthcoming year. This increase is necessitated by the scope and pace of the work, by the increasing expense of laboratory equipment and animals and to take care of regular, recurring salary increases of personnel.

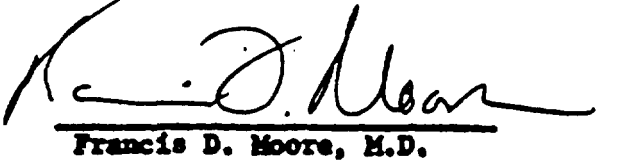
Last year a similar increase was requested and was not granted. Please note that this increase is very severely needed by a research that is in a productive phase, with many personnel working devotedly, in whom salary increases are needed if they are to be maintained as an intact group.

13. APPROVAL

This proposal has been reviewed by and has the approval of the following:



F. Lloyd Hussells, M.D.
Director
Peter Bent Brigham Hospital



Francis D. Moore, M.D.
Surgeon-in-Chief
Peter Bent Brigham Hospital
Moseley Professor of Surgery
Harvard Medical School

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Note: Bibliography of years prior to 1958 is incomplete, as many of the items have previously been reported.


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