MCV/Q
MEDICAL COLLEGE OF VIRGINIA QUARTERLY
VOLUME FIVE • NUMBER FOUR • 1969

TRANSPLANTATION
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Manuscripts, submitted in duplicate, should be prepared according to recommendations in the *Style Manual for Biological Journals*, 2nd Ed., published in 1964 by the American Institute of Biological Sciences, 2000 P Street, N.W., Washington, D.C. 20036.

Subscription rates in the USA and Canada: 1 year, $4; 2 years, $7; 3 years, $9. All other countries: 1 year, $5; 2 years, $8; 3 years, $10. Interns, residents, and students: 1 year, $2.

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CONTENTS

Fortieth Annual McGuire Lecture Series: TRANSPLANTATION

Laboratory and Clinical Studies of Cardiac Transplantation
RICHARD R. LOWER, V. ERIC KEMP, WALTER H. GRAHAM, DAVID H. SEWELL, HERMES A. KONTOS AND GEORGE M. WILLIAMS, Richmond, Virginia 167

The Role of Preservation in Transplantation
FOLKERT O. BELZER, San Francisco, California 171

Isolated Organ Perfusion: Physiology and Application
WILLIAM E. GAYLE, JR., Richmond, Virginia 173

Induction of Immunological Tolerance to Tissue Allografts with Antilymphocyte Serum
ANTHONY P. MONACO AND MARK A. HARDY, Boston, Massachusetts 182

Tissue Typing
RONALD T. ROLLEY, Richmond, Virginia 190

Relation of Tissue Typing to Results of Clinical Transplants
HYUNG M. LEE, Richmond, Virginia 195

Cellular Rejection
JAMES S. WOLF, Richmond, Virginia 200

Contributors to this Issue
205

Table of Contents for Volume Five
209

Index to Volume Five
211

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Fortieth Annual McGuire Lecture Series
OCTOBER 31—NOVEMBER 1, 1968

TRANSPLANTATION

PROGRAM

Thursday, October 31

NEW TECHNIQUES—Dr. Hume, Moderator

David M. Hume, M.D.
Introduction to Transplantation Immunology

Folkert O. Belzer, M.D.
The Role of Preservation in Transplantation

William Gayle, M.D.
Isolated Organ Perfusion: Physiology and Application

Fritz H. Bach, M.D.
Histocompatibility Genetics

Ronald Rolley, M.D.
Tissue Typing

H. M. Lee, M.D.
Relation of Tissue Typing to Clinical Transplant Results

CLINICAL ASPECTS—Dr. Lower, Moderator

Robert S. Schwartz, M.D.
The McGuire Lecture
Grafts of Immunocompetent Cells: Graft Versus Host Disease, a Model for Lymphoma

Gordon B. Avery, M.D.
Pregnancy: A Common Homograft

David M. Hume, M.D.
Experiences in Clinical Renal Homotransplantation

G. Melville Williams, M.D.
Hyperacute Rejection and Antibody Formation

James C. Pierce, M.D.
Splenectomy and Transplantation

Richard R. Lower, M.D.
Experimental and Clinical Transplantation of the Heart

James S. Wolf, M.D.
Cellular Rejection

George R. Prout, M.D.
Urological Complications in Renal Homografts

Thomas E. Starzl, M.D.
Experimental and Clinical Transplantation of the Liver

NEW DIRECTIONS—Dr. Pierce, Moderator

Robert S. Schwartz, M.D.
The McGuire Lecture
Modification of the Immune Response to Transplantation

Anthony P. Monaco, M.D.
The Induction of Homograft Tolerance Using Antilymphocyte Serum

Thomas E. Starzl, M.D.
Clinical Experiences with Antilymphocyte Serum

Fritz H. Bach, M.D.
The Future of Tissue Matching

Friday, November 1

PROBLEMS—Dr. Williams, Moderator

John P. Merrill, M.D.
Causes of Transplant Failure

John S. McPhaul, M.D.
Recurrent Disease in Transplanted Kidneys
Laboratory and Clinical Studies of Cardiac Transplantation*

RICHARD R. LOWER, V. ERIC KEMP, WALTER H. GRAHAM, DAVID H. SEWELL, HERMES A. KONTOS AND GEORGE M. WILLIAMS

Division of Thoracic and Cardiac Surgery, Medical College of Virginia, Richmond 23219

Cardiac transplantation was carried out on four patients at the Medical College of Virginia between May and October of 1968, in an effort to salvage them from the terminal stages of otherwise uncorrectable heart disease. Despite a strikingly good early recovery from operation in each case, three of the patients died of acute homograft rejection in one to three weeks; our second case is living and well, ten months after operation, and is at this writing the world's third longest survivor. The world experience to June of 1969 includes about 130 cardiac transplants. Of the first 100 patients operated on over six months ago, 20 are surviving, and the majority of these have returned to a productive existence, demonstrating the feasibility of complete rehabilitation of at least some terminal patients after cardiac transplantation. The high mortality rate—significantly higher than was anticipated—has resulted from acute and chronic homograft rejection and from the equally difficult problem of infection. Certain lessons have been learned from our own experience and from the world experience with this procedure, and these will be reviewed in an attempt to establish the current status and future potential of cardiac transplantation.

Background: Animal Experimentation

The information gained from ten years of our laboratory experience in part set the stage for the clinical trials of cardiac transplantation initiated by Barnard in December of 1967. These investigations were begun first in the laboratories of Dr. Norman E. Shumway of Stanford University and later continued at the Medical College of Virginia. The feasibility of the procedure was first established in December of 1959 when dogs were shown to recover completely after orthotopic homotransplantation of the heart (Lower and Shumway, 1960; Lower, Stofer and Shumway, 1961). The animals lived from 4 to 21 days without immunosuppressive therapy and exhibited remarkably normal activity until death from rejection supervened, providing convincing evidence that the transplanted heart could recover excellent function despite the interruption of nerves and lymphatics. In a subsequent study two dogs recovered after transplantation of the heart and both lungs (Lower et al., 1961). The animals died after five days, but showed normal ventilation and gas exchange prior to the onset of rejection.

* Presented in part by Dr. Lower at the Fortieth Annual McGuire Lecture Series, October 31–November 1, 1968, Medical College of Virginia, Richmond.
† Supported by research grants from the American Heart Association, the Richmond Heart Association and the National Heart Institute.
Further laboratory studies (Lower, Dong and Shumway, 1965a,b; Lower, Dong and Glazener, 1966) revealed that the most sensitive and reliable test for impending cardiac rejection was a fall in the QRS voltage on the electrocardiogram, often with the additional finding of abnormal intraventricular conduction. With the advent of immunosuppressive drugs, specifically azathioprine and methylprednisolone, survival of animals was prolonged by treating each recognized rejection crisis with transiently high doses of the drugs which were then tapered to lower maintenance doses in an attempt to diminish the high incidence of toxicity and infection. A few animals survived more than a year and provided a significant stimulus to the subsequent clinical application of cardiac transplantation. Extensive physiologic tests of the transplanted heart revealed that, although function was often depressed for the first 24 to 48 hours, thereafter cardiac output, even in response to stress, was remarkably adequate (Dong et al., 1965). In some animals made to breathe 7% oxygen, cardiac outputs could be markedly increased (Kontos and Lower, 1969).

Evidence of autonomic reinnervation was seen in the majority of transplants within a few months after operation and was confirmed by appropriate immediate responses to direct electrical stimulation of vagal and sympathetic nerves (H. A. Kontos, M. D. Thames and R. R. Lower, unpublished data). The return of sinus arrhythmia was also seen as a useful indicator of vagal reinnervation (Thames, Kontos and Lower, 1969), and the ability of the heart to respond reflexly to peripheral hypertension also reappeared (H. A. Kontos et al., unpublished data). Recently, fluorescent staining of sympathetic fibers has confirmed their regeneration within the graft after several months.†

Although these observations fostered considerable enthusiasm concerning the application of cardiac transplantation to human disease, this enthusiasm was tempered by certain additional observations. In about 25% to 30% of acute rejection crises, it was difficult or impossible to control the rejection process with drugs. Where continuous or high dose immunosuppression was needed, infection was often inevitable. Moreover, on histologic examination of animals surviving over three months, all showed some evidence of compromise of coronary arterial lumens by thickening of the intimal layer. In some of the longest surviving cases a few of the coronary arteries were nearly occluded. These findings were presented in 1967 (Lower and Cleveland, 1968), but it was our hope that improved methods of histocompatibility matching along with better drugs for immunosuppression, such as antilymphocyte globulin, might make cardiac transplantation in man less susceptible to both acute and chronic rejection. Unfortunately these aims have not been entirely realized.

Selection of Recipients and Donors

The selection of patients for cardiac transplantation has generally been reserved for those in the terminal stage of their illness with no alternative hope for recovery. This policy is attested to by the large numbers of patients who have died in our hospital and elsewhere while awaiting a suitable heart donor. In the Stanford series, for example, the mean survival of patients accepted for transplantation, but for whom no suitable donor could be obtained, has been four weeks, with a maximal survival of 12 weeks (N. E. Shumway, personal communication).

Two of our patients had sustained multiple myocardial infarctions with the subsequent development of refractory heart failure. The other two patients had cardiomyopathy of undetermined etiology with severe biventricular failure. Cardiac catheterization in each case revealed pulmonary hypertension, elevation of the left ventricular end diastolic pressure, low cardiac output and extremely poor contractility of the left ventricle. The patients were all considered to have no alternative hope for recovery from their cardiac disease and were therefore, accepted for cardiac transplantation.

The selection of donors required the establishment of irretrievable brain death by an independent team of neurosurgeons and neurologists. Brain death in these cases was caused by extensive intracerebral hemorrhage or by massive brain trauma. The criteria included no pupillary reaction, absence of spontaneous respiration or movements, absence of reflexes and an isoelectric electroencephalogram. The heart in the donor invariably required vasopressors for support and had usually sustained one or more episodes of arrest. It is of interest that, despite its failing status in the donor, the heart in each case adequately supported the circulation in the recipient as soon as coronary blood flow was reestablished.

Prospective histocompatibility typing by the microlymphocyte-toxicity techniques of Terasaki was carried out in each case. However, despite an excellent match with no demonstrable major incompatibility in the first case, the patient died of acute fulminating rejection at one week and was the first patient in the world experience to die from this cause. The course in this patient illustrated that typing, as currently practised, does not in fact detect, with sufficient sensitivity, all forms of incompatibility. The second patient, however, was also an excellent match; and, although

† Studies performed by Dr. John E. Norvell, Department of Anatomy, Medical College of Virginia.
he has sustained three rejection episodes, each one responded well to a temporary increase in immunosuppressive therapy, and he remains well.

The third patient in this series demonstrated a principle which may continue to receive increased attention in the preoperative matchings of potential recipients. Despite the absence of preformed antibodies detected by the routine preoperative serum-lymphocyte cross match, retrospective studies of the patient’s serum by the technique of immune adherence established that some degree of prior sensitization did exist, as antibodies were present against cultured kidney cells from the same donor. The patient’s clinical course was characterized by a fulminating rejection episode at one week which was unresponsive to massive immunosuppressive therapy. The fourth patient, mismatched for histocompatibility antigen HLA-3, initially responded to therapy for a rejection episode at one week, but subsequently became refractory to treatment and died of rejection at 18 days. Antibody was eluted from the heart postmortem in each of the three fatal cases. The possible role of antibody in acute cardiac rejection has been discussed further in another report (G. M. Williams et al., unpublished data).

Surgical Technique

The basic surgical technique which proved successful in animal transplantation (Lower et al., 1961) was employed in our own cases and has been generally utilized by other transplant surgeons with few modifications. The posterior atrial wall, containing the openings of the vena cavae and pulmonary veins, is retained in the recipient to facilitate anastomosis of the donor heart. Anastomosis of the aorta and pulmonary artery in the supravalvular region completes the procedure. Surgeons have varied most in their management of the donor heart. In our own cases the donor heart was cooled by immersion in saline at 8 to 10°C to afford the myocardium protection during the 60 to 90 minutes that coronary circulation was interrupted and no coronary perfusion was used.

Postoperative Management

Postoperative management of the recipient has varied little from the care of the routine cardiac surgery patient with the exceptions that immunosuppressive therapy is administered in an effort to prevent rejection, and monitoring efforts are directed toward the early detection of a rejection crisis (Lower et al., 1968; Sewell, Kemp and Lower, 1969). It now seems apparent that the cardiac transplant recipient has an immunologic capability that is less impaired than the chronically uremic renal transplant patient, and the cardiac patient, therefore, requires more intensive immunosuppressive treatment, at least in the initial weeks, to control rejection. This was not appreciated early in our experience but has evolved as the world results have been discussed. The current recommendations which have received widest acceptance are that the recipient be given azathioprine in the largest dose tolerated, usually 3 to 4 mg/kg, and prednisone in a dose of 200 mg/day during the initial two to three weeks. Drugs are later tapered to a lower maintenance dose to decrease the risk of infection and the other side effects of steroid administration.

The role of antilymphocyte globulin is less clearly established. Some patients have been managed successfully without it; others have developed fatal, acute or chronic rejection despite its use. The optimal preparation, route of administration and dose have not been agreed upon. Some patients reportedly have rapidly developed antibodies against the horse globulin with evidence that its immunologic effectiveness is rapidly dissipated (Butler et al., 1969). A few investigators have therefore considered it most useful in the treatment of a difficult rejection crisis or where there are wide histocompatibility differences.

Early Detection of Rejection

The early detection of a rejection crisis requires close monitoring of the electrocardiogram on at least a daily basis for the first few months. The primary signs of impending rejection are a decrease in the QRS voltage, a rightward shift in the frontal plane axis, delayed intraventricular conduction and atrial or nodal arrhythmias. Rejection is characterized clinically by the insidious onset of right heart failure as evidenced by weight gain, edema, venous distention with increased pulsations, right ventricular gallop, and, eventually, the development of murmurs of relative tricuspid or mitral insufficiency. Early in the course of rejection the lung fields appear radiographically clear and even oligemic, in reflecting the predominant right heart failure. Thus, it is suggested that digitalis preparations and diuretics be discontinued as soon as is practical after operation to avoid masking this important collateral evidence of impending rejection.

While serum enzyme abnormalities do occur with some rejection episodes (particularly elevation of the lactic dehydrogenase isozymes I and II and the creatine phosphokinase), these abnormalities occur as a late manifestation of rejection and indicate a more severe degree of myocardial injury. It is apparent from our own observations and the experience of others that a typical rejection crisis can occur and be diagnosed by other clinical and ECG criteria without detectable abnormalities in the serum enzymes. However, enzyme levels were markedly elevated in the terminal stages of each of the fatal rejection episodes in our patients.

The other nonspecific signs and symptoms occurring in conjunction with rejection, such as pericardial
friction rub, fever, leukocytosis and malaise, can all occur with other postoperative complications, e.g., infection, embolism or the various post-perfusion syndromes, and are thus of limited value in the diagnosis of impending rejection.

Treatment of Acute Rejection

The successful treatment of a rejection crisis requires the immediate administration of prednisone in transiently high doses of 1,000 mg daily until the clinical and radiographic signs of rejection improve with subsequent tapering to maintenance levels. In addition, local graft irradiation, actinomycin and antilymphocyte globulin may be used. Recent laboratory studies in dogs with cardiac transplants have established by serial myocardial biopsies the effectiveness of these measures in the treatment of the rejection crisis (Graham et al., 1969). It would appear from the recently discussed world experience that about 70% of rejection crises can be successfully managed in this way.

Yet even with successful control of the acute rejection problem, a process which has been termed chronic rejection may develop in the graft, consisting of irregular thickening of the intimal layer of the coronary arteries identical to that which was seen in the transplanted dog hearts (Lower and Cleveland, 1968) and in other organ homografts. It is presumed that antigen-antibody complexes injure the endothelium of the vessels and cause agglutination and adherence of platelets at areas of maximum injury with subsequent organization of thrombi. Unfortunately, the development of such lesions cannot be easily detected clinically, except perhaps by arteriography; and if the process goes unchecked, serious compromise of cardiac function will inevitably result. Whether prophylactic antithrombotic measures can forestall the process until eventually the donor endothelium is populated by host cells has yet to be established. More precise histocompatibility matching and more effective immunosuppression would at the moment seem to offer the best insurance against loss of the graft due to chronic rejection.

References


The Role of Preservation in Transplantation*

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Introduction

Let us look first into the advantages and disadvantages of preservation in clinical transplantation. I should mention that we are talking of short-term preservation up to 72 hours, rather than preservation for indefinite periods of time. The latter will have to wait until problems of freezing and thawing have been solved, but this still appears to be something for the distant future. What, then, are the advantages?

First, it minimizes the total anoxia time. Although it is well known that human cadaver kidneys can maintain viability up to ten hours by simple hypothermia, it is our feeling that this is not good enough. In cadaver kidneys, the organ is already damaged during the agonal period of the donor, and the additional cold storage time undoubtedly further damages the organ, perhaps permanently. Furthermore, if preservation for more than ten hours is necessary, some method of perfusion is needed.

Second, it allows potential recipients to stay at home either on central dialysis or chronic dialysis until a properly matched kidney becomes available. The patients actually do not have to be called until a matched kidney is known to be present, thus eliminating unnecessary admissions and great disappointments. Furthermore, if the potential recipient cannot be reached immediately, he is still not excluded from receiving that particular kidney. It, of course, allows the total recipient pool to be tremendously increased, because patients who are dialyzed in different centers or in different cities can be selected by computer and can be considered for a transplantation.

Third, adequate preservation allows for histocompatibility testing between the donor and a pool of recipients and the direct cross match between the donor white cells and the best matched recipients.

Fourth, function and damage can be evaluated before the actual transplant, thus eliminating those organs which have been irreversibly damaged during the agonal period of the donor. This aspect is still in the investigational stage, but it appears that the perfusion characteristics are of the greatest importance.

Fifth, adequate preservation is essential for organs such as the liver and heart, because preparation of the recipient is more time-consuming. This problem at the present time is usually eliminated by preservation in the donor, the so-called heart-beating cadaver.

Sixth, it makes the transplant an elective instead of an emergency operation, which can be of the greatest importance if the kidney becomes available during a period of time when all operating rooms are filled.

Seventh, if preservation and selection of organs could be perfected, a much larger source of donor organs would become available. If we are going to be using the so-called neurological deaths only, not enough kidneys will become available to treat all patients in chronic renal failure. However, if we could use kidneys from other patients, such as automobile accident victims or other trauma cases who either enter the emergency room dead on arrival or die within the first 20 minutes, the number of available organs would be tremendously increased.

Finally, it does allow a smaller staff on the transplant team, since the donor nephrectomies, as well as the transplantations, can be done by one team.

Methods

Perhaps at this point we should briefly mention the different methods that have been used for preservation of whole organs. They are hypothermia, hyperbaric oxygen, isolated perfusion, metabolic inhibitors, and, finally, a combination of the above methods. When our studies were initiated, we felt that perfusion would theoretically be the best method, as oxygen could be supplied, and CO₂ and metabolic waste products could be removed. To eliminate the problems of microemboli, we started with an acellular perfusate such as serum or plasma. However, we were plagued initially by the same problems that all other investigators have had with isolated perfusion—increasing perfusion pressure, decreased flow, and tissue edema. On implantation, even after short periods of preservation, the organs did not function; or, if they did function, they showed evidence of some permanent damage.

We then noticed that, if the plasma were frozen and quickly

* Presented at the Fortieth Annual McGuire Lecture Series, October 31–November 1, 1968, Medical College of Virginia, Richmond.
thawed, a precipitate developed in the plasma, producing a completely opalescent solution. We found that the most efficient way of removing this precipitate was by serial filtration through Millipore filters, and that, by so doing, a clear solution was obtained. Perfusion with this cryoprecipitated plasma eliminated the problems of rising perfusion pressure and tissue edema. When fat stains were used on the kidneys perfused with normal plasma, multiple fat droplets were seen in the capillaries, the glomeruli and the tubules. Fat stains of kidneys perfused with cryoprecipitated and filtered plasma did not show these fat emboli; thus, it became obvious that the etiology of the rising perfusion pressure was due to fat emboli obstructing the capillary system. When blood is used, these emboli can come from blood cell aggregates; but even if the cellular components are removed, the end stable lipoproteins denature, and their aggregates are just as destructive. Furthermore, this process is self-perpetuating. Once decreased capillary flow is produced, it results in tissue hypoxia, which is shown to produce cellular swelling, especially when combined with hypothermia. The cellular swelling by itself produces increased capillary resistance and further decreased capillary flow, and the cycle continues until death of the cells and the organ results.

Results

We next performed 24-hour and 72-hour preservation studies in dogs, and autotransplantation was combined with immediate contralateral nephrectomy. All animals in both groups survived. The average BUN rise in the 24-hour group was 80 mg%, occurring usually on the third or fourth day, while in the 72-hour group the BUN rose to an average of 140, again on the third or fourth day. However, there was a subsequent rapid decline in the BUN, so that the 24-hour groups had normal BUNs by the seventh or tenth day, while in the 72-hour group the BUN returned to normal by the third week. The animals were then retested after six months, and conventional as well as electronmicroscopy studies revealed normal architecture. Paraaminohippuric acid and inulin clearance studies were within normal limits, and none of the animals developed hypertension. In order to be sure that there was no acceleration in the rejection process, we did several homotransplants using 24-hour perfused kidneys, and the animals rejected their kidneys in the normal period of time ranging from 7 to 21 days.

In August, 1967, we did our first human preservation study; in this, the kidney was preserved for 17 hours. The patient subsequently developed tubular necrosis lasting two weeks, after which the kidney started to open up. His creatinine clearance went as high as 40, at which time he had his first rejection episode. This was partially reversed, but he subsequently had two more rejection episodes requiring maintenance on fairly high doses of steroids. Six months after transplantation he entered the hospital with a ruptured gallbladder secondary to amyloidosis, from which he later succumbed. Of particular interest is the fact that no amyloid was found in the transplanted kidney six months after transplantation.

At that time, our equipment was not transportable, and we found that if the kidney was obtained in one hospital and had to be transferred to the Medical Center before preservation could be started, the anoxia time was still considerable. The preservation unit was thus made transportable, so that it could be moved to the operating room where the donor kidneys were obtained. The perfusion circuit, details of which have been published in several other articles, basically consists of a pulsatile pump, a heat exchanger, an organ chamber, a membrane oxygenator, a cooling system, a pressure recorder and a battery system. For perfusing human kidneys, we use fresh frozen AB+ plasma which is cryoprecipitated immediately before use. The final filter in the Millipore filter system is 0.22µ and acts also as a bacterial filter. Immediately before use, several additives, such as phenolsulphthalein, magnesium, steroids and antibiotics, are added to the perfusate. We have used this method in 42 patients, and the longest period of preservation has been 36 hours. The kidney functioned immediately.

Summary

Although it is too early to draw any definite conclusions from these results, as a period of two years is probably necessary to evaluate cadaver kidney transplantation in humans, it appears that our well-matched patients have much fewer problems than our poorly matched patients. It is hoped that by preservation methods such as those discussed, improved methods of tissue typing, and better immunosuppressive therapy, we will be able to raise the survival level of cadaver kidney transplantations to the level between 80 and 85% presently being obtained in transplantations using living, related donors.
Isolated Organ Perfusion: Physiology and Application*

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History

The perfusion of isolated organs is certainly not a new idea (Table 1). Le Gallois, although probably not the first to perfuse isolated organs, predicted in 1812 that life could be maintained in organs by ex vivo perfusion (Norman, Covelli and Sise, 1968).

Elaborate mechanical perfusion systems were developed by Ludwig and Schmidt in 1868 and Lindbergh and Carrel in the early 1900's (Carrel, 1912; Perry et al., 1968). Our current techniques have added significantly to knowledge in many fields, including physiology, transplantation, immunology, toxicology, pathology and pharmacology. I will attempt to cover some of the more interesting and important recent developments in perfusion and show its application to understanding fundamental physiology.

Systems

Basically, there are three types of isolated perfusion systems. An isolated in vivo system has an intact animal in which a specific organ is isolated by cannulation. The importance here is that the neural system remains intact while the "isolated" organ and its responses are being studied. An isolated ex vivo system has an organ removed from an animal, and that animal (isologous) or another animal (homologous or heterologous) has its blood circulated through the organ.

TABLE 1

<table>
<thead>
<tr>
<th>Year</th>
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<tr>
<td>1812</td>
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<td>Ludwig and Schmidt</td>
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<td>1875</td>
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<td>1913</td>
<td>Carrel</td>
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<td>1931</td>
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<td>1935</td>
<td>Carrel and Lindbergh</td>
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* (Carrel and Lindbergh, 1938.)

* Present at the Fortieth Annual McGuire Lecture Series, October 31–November 1, 1968, Medical College of Virginia, Richmond.
which is usually contained in a box or bag. In this system, the neural factors are severed whereas, the hormonal factors remain intact. Finally, a complete isolated in vitro perfusion system utilizes an artificial pump and various perfusate mixtures or anticoagulated blood. Neither hormonal nor neural response mechanisms are intact in this system, and the circulation is regulated by the pump and the intrinsic neural mechanisms.

**Problems in Perfusion**

With these systems in mind, let us look at some of the problems in organ perfusion. In the intact in vivo system, problems of cell death are seldom encountered unless the drugs administered to the organ or the perfusion apparatus directly damage the cells. In vitro systems inevitably result in cell death no matter how elaborate the system or how nutritive the perfusate. It is imperative to resolve the etiology of cell death in ex vivo and in vitro systems in order to establish better maintenance of organs and thereby prolong survival for the purposes of study and, perhaps more importantly, transplantation.

Whether or not cell or organ death is due to vaso-occlusive phenomena, lack of endothelial integrity, or plugging by embolic fatty products is not yet clear. One or several of these phenomena may be causative, depending upon such factors as the mechanisms of perfusion, the choice of perfusate, the oxygenation and the organ. It has been suggested (Belzer et al., 1968) that one cause of organ death in some isolated systems is emboli of lipid aggregates. According to Belzer, these emboli could cause tissue hypoxia with resultant cellular swelling, anaerobic metabolism, acidosis and death. The common denominator in cell and organ death is hypoxia due to inadequate perfusion. It has been recently demonstrated (Cohen and Folkman, 1968) that the pathogenesis involved in hypoxic cell death is inadequate perfusion or inadequate oxygenation. Hypoxemia leads to anaerobic glycolysis within the cell and produces an intracellular lactic acidosis. As a result, there is a lack of energy because of the anaerobic metabolism. Without energy the sodium pump mechanism fails, and water is imbibed into the cell, producing edema. The lysosomes swell and the resultant release of enzymes caused by acidity, temperature rise, or decreasing osmotic pressure produces self-destruction of the cell.

**Assessing Viability**

For transplantation purposes, it is imperative that organ viability be assessed with some degree of assurance prior to reimplantation.

How can one, then, detect cellular death early in the perfusion of isolated organs so that something can be done? The answer to this question is far from being simple depending, as it does upon many factors. It is dependent upon the organ under study. If one is studying the kidney, for example, it is difficult to correlate specific enzyme changes with cell death. Other factors, including urine output, blood flow and appearance, do not necessarily correlate with ultimate viability of the organ. A group of investigators (Belzer et al., 1968) have recently demonstrated that there may be some correlation between LDH isozymes and the prediction of viability of perfused kidneys. Rising perfusion pressure has also been shown to correlate with total renal function in isolated organs.

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**Fig. 1**—The pump regulates arterial flow (A pump) and venous flow (V pump) separately through the liver. A bubble oxygenator is used with a heat exchanger. Pressures and flows (AF and VF) are continuously monitored. (Reprinted with permission from W. E. Gayle, Jr., G. M. Williams and D. M. Hume in *Organ Perfusion and Preservation*, Appleton-Century-Crofts, 1968, p. 807.)
and probably indicates renal death (Belzer et al., 1968). The pathologic mechanism is obstruction to the renal vasculature, and the perfusion pressure rises because of inadequate perfusion of these small vessels. In an organ which has an active enzyme system, such as the liver, there are more readily detectable enzymatic changes which correlate with cell death. The major question here is: To what degree enzyme change indicates whether the liver will or will not survive and immediately support life after reimplantation. This is dependent upon the amount of anoxia it suffers during extirpation, perfusion, preservation, and reimplantation. There are many parameters for studying acute changes of liver damage, the more common being the measurement of SGOT (serum glutamic oxaloacetic transaminase), SGPT (serum glutamic pyruvic transaminase), potassium and glucose changes and lactate-pyruvate ratios.

Regarding the applicability of isolated organ perfusion to transplantation, one should closely examine the various ways of assaying viability of different organs prior to reimplantation. Appearance of the organ is often helpful in detecting the presence of edema, poor venous return, or the presence of inadequate oxygenation, as, with these conditions, there may be blotchiness or poor color in general. Excretory output can also be measured, although this does not necessarily correlate with function.

Chemical assays, including those of enzyme changes in the kidney, liver, and heart, are important, as changes in perfusate pH, temperature, oxygen concentration, and carbon dioxide content. Pressure changes in the chambers of the heart as a measure of contractility may be beneficial in assaying the viability of the heart. Tetrazolium bromide dye is a rapid method for attempting to assay cardiac viability (Maginn and Hadjimichalis, 1968). In studies by Idezuki et al. (1968), pancreatic viability was determined by giving an isolated pancreatic graft a glucose stimulation test which, if the graft was viable, brought about the immediate production of insulin. Even with minimal output of insulin after 48 hours of perfusion, this test indicated a viable graft that functioned on reimplantation.

As mentioned previously, in an ex vivo perfusion system, the excretory functions of organs, such as the kidney and liver, were not totally reliable as adequate assays of viability. The kidney can put out large amounts of urine which is isosmolar with the perfusate and represents simply an ultrafiltrate of a dying kidney. Similarly, the liver can put out bilious-appearing material which, if assayed, proves to be a poor quality of true bile and probably represents an ultrafiltrate type of material. Flow rates through organs depend upon several factors: whether the perfusion is normothermic or hypothermic; the method of perfusion; the type of pump used; whether or not the pressure is constant; and the amount of vascular resistance within the organ system. One elaborate system (Norman et al., 1967) provides the ability to monitor the intake and output via a computer-type setup and, thereby balance and control the precise amount of flow. In our laboratories we utilize a pressure-sensitive Edwards-Bosher† pump, which detects resistance and, as the resistance increases, automatically and proportionately diminishes the flow (Fig. 1). Diminishing flow rate provides us with immediate evidence of intravascular resistance secondary to vasoconstriction, occlusive disease, or other problems which raise resistance and diminish inflow. We feel that this is a highly physiologic system in that, essentially, it allows the organ to control its own flow by intrinsic autoregulation.


Clearance studies, such as excretion of dyes in the isolated kidney preparation or excretion of bromsulphalein or ammonia in the isolated liver preparation, are also utilized for detection of viability. These are probably somewhat more reliable than the simple measurement of excretory rate alone, because excretion of the dye demands cellular function.

Another method of assaying viability is pathology, including light and electron microscopy. Because it takes time for the preparation of sections, these methods prevent obtaining conclusive evidence of viability immediately prior to implantation.

The sine qua non of assaying viability, of course, is normal function after reimplantation. This is the only reliable indicator of adequate viability of the organ. The obvious problem here is that, where human transplantation is concerned, this implies surgical manipulation of the patient (recipient) in order to detect viability of the organ involved. The immediate need, of course, is for some quick, reliable, and accessible method of assaying viability of isolated organs being perfused for transplantation. Various aspects of such a method, including chemical changes, excretory clearance functions and vital dye studies, are presently being pursued with the greatest interest at the present time.

Mechanics of Perfusion

Returning to more basic physiology, let us consider some of the actual mechanics of perfusion of isolated organs. There are many variables in an isolated system. The first of these is temperature. Perfusion at normothermia (37 C), at room temperature (25 C), or at considerable hypothermia (4-10 C), for the purposes of prolonged preservation, depends upon the desires of the investigator. Almost all physiologic, pharmacologic, toxicologic, and immunologic studies are per-
formed at body temperature, but, for the purposes of studying preservation techniques, hypothermia is usually utilized, since the metabolic rate is markedly diminished. However, it produces problems, such as associated vasospasm and sludging of blood when cellular products are present, inactivation of heparin at low temperatures, and inactivation of certain drugs.

Another basic consideration in the mechanics of perfusion is the presence or absence of hyperbaria. Belzer (Belzer, Ashby and Dunphy, 1967) has adequately demonstrated that hyperbaria is not necessary for the preservation of kidneys for a period of up to 24 and, perhaps, even 72 hours with the use of hypothermia and a plasma solution. Other investigators (Brett-schneider et al., 1968) have demonstrated that, for their purposes, hyperbaria is necessary to maintain viability during preservation of human and canine livers. Recently it has been demonstrated that hyperbaria sufficiently suppresses cellular swelling in the liver for a period of 24 hours (Huntley et al., 1968). However, after 24 hours the enzymes are markedly altered, and the liver is not sufficiently functional to support life. The perfused liver gains weight at a rapid rate after the first 24 hours, whether or not it is in a hyperbaric atmosphere. Hyperbaria may be necessary for prolonged preservation of the pancreas and, perhaps, the heart and the lung. However, there are certain inherent disadvantages to hyperbaria: 1) Compression and decompression take several extra hours; 2) There is potential danger of an accident with the high pressure system; and 3) Excess oxygen will damage in vitro tissue culture cells, although it is difficult to assess its effect on whole organs. It has been shown that hyperbaria (using nitrogen or helium) will accomplish effects similar to those accomplished by hyperbaric oxygen, and it is speculated that pressure alone may be the important factor (Lyons, Dietzman and Lillehei, 1966).

A third consideration in perfusion mechanics is the type of pump and oxygenator utilized. As previously mentioned, we use the Edwards-Bosher pump, because we think it is physiologically sound. Belzer and Folkman both utilize different types of pumps and obtain very satisfactory results. These pumps are basically pulsatile. It has been demonstrated by work in our own laboratory (Robertshaw and Gayle, unpublished data) and by others (Tait and Eisenman, 1966) that pulsatile perfusion allows lymphatics to empty during the diastolic phase and, therefore, diminishes edema formed in the parenchyma and around the capsule of the organs. The type of oxygenator is also important. It has recently been demonstrated that disc oxygenators, which have a large surface contact with the actual perfusate, have increased screen filtration pressure (Ashmore, Svitik and Ambrose, 1968) and as previously mentioned (Belzer et al., 1968), may cause problems with embolization of fatty materials. Membrane oxygenators have been utilized for bypass over periods of 12 to 24 hours and have a very minimal increase in screen filtration pressure. We have recently switched to a membrane oxygenator for our preservation system but have, however, been able to successfully preserve some kidneys for 24 hours with a disc oxygenator. A fourth area of importance in perfusion is the choice of perfusate. Again, there is much controversy as to the exact type of perfusate to use. This is dependent upon the type of study desired by the individual investigator. If the system is to be as physiologic as possible, a whole blood or dilute whole blood solution is utilized. Problems involved, however, are sludging and outflow block—especially in the liver—if a whole blood solution is utilized. Regardless of the perfusate used, perfusion of the lungs, without ventilation, is problematic for maintenance of viability (Stevens et al., 1968). In preservation (Table 2), dilute whole blood solutions have worked satisfactorily with lung and liver. With kidney preservation, the use of a plasma solution has been shown by Belzer to be the most satisfactory (Belzer et al., 1967). The use of crystalloid solutions has been shown by some investigators to be nearly as effective as that of plasma. However, one does run into the problem of edema after occurring long preservation. Depending upon the study, many additives have been utilized in perfusates, including magnesium, steroids, antibiotics, adenosine (Stewart and Wilbrandt, 1968), chlorpromazine, buffering agents, glucose and insulin.

The question of inclusion of cells in the perfusate has been a very controversial one. Up until recently it was thought that platelets might be harmful; however, it appears that

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<td>Per fusates for Preservation</td>
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<tr>
<td>Kidney— homologous undiluted ACD plasma, room air, pulsatile perfusion, moderate hypothermia</td>
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<tr>
<td>Liver— dilute heparinized whole blood, hyperbaric oxygen, hypothermia</td>
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<td>Lung— ventilation, ? perfusion (dextran-ACD-blood), ? hyperbaria (2A)</td>
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<tr>
<td>Pancreas— non-perfusion relatively successful to 22 hours</td>
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<td>Thymus— Eagle’s MEM, fetal calf serum</td>
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W. E. GAYLE, JR.
Fig. 2—Isoproterenol added to an isolated perfused liver with a falling venous flow produced immediate increase in flow.

Folkman has renewed the desire to have platelets present to insure physiologic preservation of the endothelium (Gimbrone et al., 1969). He and his colleagues have demonstrated that the platelet, which is thought by some to contribute to problems in isolated organ perfusion, may be an important factor in maintaining the integrity of the vascular endothelium. They have recently demonstrated this by perfusing dog thyroid glands with platelet-rich plasma and comparing them to thyroids perfused simultaneously with platelet-poor plasma under identical conditions. The endothelium seems to be maintained with adequate function after re-anastomosis in organs perfused with platelet-rich plasma for 24 hours. Platelet-poor perfused thyroids, however, after a period of three to four hours, swell and have petechial and purpuric hemorrhages in their subcapsular area on reimplantation. This is perhaps the newest and most exciting concept in organ perfusion and preservation and goes against the theory of many workers in the past (Humphries, 1967).

One final perfusate which has been used for maintenance of perfusion in isolated organs (Paton et al., 1968) and in the total rat model (Geyer, Monroe and Taylor, 1968) is the fluorocarbon perfusate (Pluronic F-68). Geyer et al. have described a highly intriguing application in which rats were completely exchanged with a fluorocarbon-serum emulsion without cells, maintained at 100% oxygen atmosphere, and kept alive for six to eight hours. If washed erythrocytes were added to fluorocarbon emulsion, the animal survived indefinitely after being perfused for up to 140 minutes.

MCV Data

At this point I would like to relate some of the work recently done in our laboratory to the aspects of organ perfusion and transplantation. Doctor William Harlan (O'Brien, Harlan and White, unpublished data) has recently shown that palmitic acid, which is probably the major source of energy for the renal cortex, is markedly diminished in canine renal allografts as compared to isografts and normals. It is hoped that we can apply this work and its implications to the study of rejection.

Other studies relevant to transplantation are being performed by one of our medical students, Mr. Harold Levinson, who is using an isolated rat perfusion system modeled after that of Doctor Judah Folkman to study in vitro aspects of rejection. Utilizing PA and Lewis
rat strains, lymphocytes can be obtained from the thoracic duct of one rat strain. Either a culture medium which is isologous to one rat strain or a medium, such as Medium 199, which is indifferent to both strains, is used to suspend the cells. Thoracic duct lymphocytes, tagged with chromium 153, are utilized to perfuse syngeneic and allogeneic rat strains. Counts of the venous effluent as well as counts of the total kidney are monitored after a standard perfusion time. These data appear to demonstrate that the allogeneic kidney took up more sensitized lymphocytes than did the syngeneic kidney. Preliminary data on the venous effluents, however, have not proved consistent in their results. Utilizing tagged red cells to reveal whether the kidneys were being

![Diagram](image)

**Fig. 3**—Intermittent intravenous isoproterenol drip produced an immediate rise in venous flow. (Reprinted with permission from W. E. Gayle, Jr., G. M. Williams and D. M. Hume in *Organ Perfusion and Preservation*, Appleton-Century-Crofts, 1968, p. 812.)

![Diagram](image)

**Fig. 4**—Vasospasm with arterial and venous flow diminution is produced with epinephrine. (Reprinted with permission from W. E. Gayle, Jr., G. M. Williams and D. M. Hume in *Organ Perfusion and Preservation*, Appleton-Century-Crofts, 1968, p. 810.)
adequately perfused, Levinson demonstrated that the kidneys are not always being perfused equally in the cortical and medullary areas.

Doctor Gilbert Robertshaw has published an excellent report on the presence of serum antibody which is not cell-bound (Robertshaw et al., 1967). One of a set of unrelated mongrel dogs was subjected to a first-set transplant that used a kidney which was allowed to reject and was then removed. After a short rest period, a second-set graft was performed utilizing either skin or spleen. Skin grafts were subsequently performed until a "white-graft" reaction was obtained, implying hyperacute rejection. The kidneys of the original donor were placed in a perfusion circuit, and the serum of the hypersensitized recipient dog was added to the perfusion system after a baseline was obtained. It was immediately demonstrated that the donor kidney swelled, and the urine output diminished rapidly. A recipient kidney was used as a control. Upon analyzing these data, Robertshaw found that there was a decrease in renal arterial flow, oxygen uptake across the kidney, urine output and urine osmolality in the donor kidney. Pathologic examination revealed proteinuria and increased edema.

In a more recent study (Robertshaw et al., unpublished data) the donor kidney, after being perfused, was replaced in the donor animal and allowed to perfuse for 30 minutes to 2 hours. Urine output remained low. On pathologic examination, polymorphonuclear leukocytes were shown to adhere to the endothelial lining of the kidneys, demonstrating continuance of the hyperacute rejection phenomenon.

Work done with isolated liver perfusions in our laboratory has demonstrated that there are probably beta-adrenergic receptors within the vasculature of the liver which are affected by isoproterenol (Figs. 2, 3) and tend to cause vasodilatation of the postsinusoidal sphincter mechanism (Gayle, Williams and Hume, 1968). This causes relaxation and allows increased flow, especially if the system has been previously subjected to vasospasticity with either norepinephrine or epinephrine, both of which produce marked vasoconstriction and diminution of flow through the liver.

Fig. 5—Norepinephrine produced similar results.
(Figs. 4, 5). With the administration of isoproterenol, increased flow is more marked in the venous phase than in the arterial phase (Figs. 6, 7). Lymphatic output, as measured by direct cannulation of perihilar lymphatics, is increased with the administration of vasoconstrictive agents. With the relaxation of the sphincter accomplished by isoproterenol, the lymphatic output diminishes. It is our feeling that if post-hepatic transplantation support to the cardiovascular system were needed, isoproterenol would probably be the drug to choose, since vasopressor agents, such as norepinephrine or epinephrine, would harm the liver by causing vasoconstriction. However, not enough work has been done with human livers to definitely corroborate this in the human system. We are also, at present, working on effects of certain anesthetic agents on hepatic flows and pathologic changes.

Conclusions

Isolated organ perfusion provides us with an excellent tool for investigating the physiology and the function of a variety of individual organs. It enables us to explore these organs, their pharmacologic reactions, their ability to withstand various stresses, their pathology after stress and their suitability for preservation and transplantation. Isolated perfusion has allowed us to better understand hemodynamics, effects of various perfusates, and toxicity of drugs on individual organs. We have recently been enlightened about the vital role of platelets and endothelial integrity. We now have a model for assessing individual hormonal systems and qualitative and quantitative endocrine and exocrine functions. Organ perfusion has provided us with a method for preservation and a means for studying organ viability prior to transplantation. We now have an invaluable isolated system in which we can study certain
aspects of immunology, including the rejection mechanism, sensitization, and the evaluation of serum and cell-bound phenomena. Our immediate goals include detection of cell or organ death prior to re-implantation by some rapid and accurate method and prolongation of preservation time for in vitro organ systems.

References


Induction of Immunological Tolerance to Tissue Allografts with Antilymphocyte Serum*

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Introduction

At the present time successful clinical organ transplantation depends on chronic administration of immunosuppressive agents to prevent the persistent tendency of the recipient to reject the graft. Chronic immunosuppression—although often successful in maintaining graft function and viability—is frequently associated with numerous and often fatal complications. These associated problems include: 1) inherent toxicity of immunosuppressive drugs per se, e.g., Imuran (hepatitis), steroids (osteorosis, gastrointestinal bleeding, etc.), Cytoxan and actinomycin C (thrombocytopenia, leukopenia); 2) occurrence and persistence of low-grade rejection in the graft, e.g., endarteritis in renal and cardiac allografts, while function is maintained; 3) occurrence of severe and bizarre infections, usually those associated with cellular immunity (particularly of the viral, protozoan and fungal varieties); and, finally, 4) spontaneous appearance of malignancy, presumably due to the abolition of the immunological surveillance mechanism which is probably operative in preventing the de novo appearance of malignancy in most normal human beings. For these reasons specific immunological tolerance remains the ultimate and eventual solution to the widespread application of clinical organ transplantation.

Immunological Tolerance

The phenomenon of actively acquired immunological tolerance was discovered during studies of tissue transplantation. This term was used to describe a specific state of unresponsiveness to an antigen or antigens in adult life as a consequence of exposure to antigen in utero or in the neonatal period. In a study of the immunogenetic consequences of vascular anastomoses between cattle twins in utero, Owen (1945) showed that most cattle twins at birth are erythrocyte chimeras as they possess erythrocytes of their own genotype as well as those of the opposite twin. This chimeric state was shown to persist beyond the life span of the erythrocyte, suggesting that erythropoietic cells (stem cells) were exchanged in fetal life and continued to produce erythrocytes of characteristic serological type throughout adult life.

The wide significance of Owen's observation was emphasized by Burnet and Fenner (1948) in a general theory of the immune response which predicted the phenomenon of tolerance. To explain the failure of adult animals to react immunologically to their own tissues, these authors suggested that the body cells possess some type of self-marker component, and that the capacity to recognize this self pattern develops during embryonic
or early postnatal life. They predicted that exposure to an antigen in embryonic life would cause that antigen to be recognized as self in later life, and, consequently, no immune response would be made to it. In future studies, it became apparent that erythrocyte chimerism was not the only manifestation of tolerance in dizygotic cattle twins which appeared after mixture of placental circulations. Dizygotic cattle twins were shown to accept skin grafts from each other, and this mutual tolerance was specific since skin grafts transplanted from third parties were quickly rejected (Anderson et al., 1951). These observations in nature set the stage for the production of actively acquired tolerance by Medawar and colleagues in the laboratory. These investigators injected mouse embryos of the CBA strain in utero through the anterior abdominal wall with a suspension of living spleen cell lymphocytes of the A strain (Billingham, Brent and Medawar, 1953). The CBA recipients, regularly accepted A-strain skin grafts when grafted in adult life. This tolerance of A-strain grafts was specific, since CBA mice tolerant of A-strain tissues easily rejected allografts from the unrelated AU strain. At the same time, Hašek reproduced Owen’s observations in chickens by the ingenious method of making a deliberate synchorial parabiosis of embryos via the chorioallantoic membrane (Hašek, 1953). At hatching, the parabiots were separated and were found to be tolerant not only of each other’s red cells, but skin allografts. Subsequently, it was found that in utero injection was unnecessary, and that intravenous injection during the early neonatal period also produced tolerance. In these experiments tolerance could be abolished by the injection of tolerant animals with lymphoid cells from normal adult members of the same syngeneic strain. Furthermore, careful testing showed that the tolerant mice were, in fact, chimeras insofar as their lymphoid cell populations were concerned (Billingham, 1958). Tolerance induced in the neonatal period was frequently permanent. This was in sharp contrast to the transient nature of the tolerance to chemical antigens observed by a number of other investigators when non-living, non-replicating antigens, such as proteins, were injected into neonatal animals for varying periods from birth to adulthood. The demonstration by Smith and Bridges (1958) and Mitchison (1959) of the necessity of repeated injections of non-replicating antigens to maintain tolerance toward these antigens, coupled with the observation of chimerism in tolerant mice in Medawar’s experiment, emphasized the concept that persistence of antigen was necessary for the maintenance of tolerance. Another important aspect of tolerance induction involves the genetic relationship between the donor and the immature host. It was found that the more distant the histocompatibility relationships between donor and host, the more difficult it was to induce tolerance. When strong histocompatibility barriers existed, large inoculums of cells were required, the intravenous route was obligatory, and the tolerance-responsive period extended only to a short time after birth. On the other hand, when the genetic and, therefore, antigenic disparity between donor and host was relatively weak, such stringent requirements disappeared. In fact, when mice of very weak histocompatibility differences were used, the tolerance-responsive period extended even into adulthood (Shapiro et al., 1961).

Graft-Versus-Host Reaction

Just as the phenomenon of actively acquired tolerance was discovered in tissue transplantation research, so also was the graft-versus-host reaction (hereafter, GVH reaction) basically an outcome of research in immunological tolerance. Simonsen (1957) found that adult splenic cells, when injected into newborn or embryo chickens and mice, produced a disease that could only be interpreted as an outcome of an immunological response by these grafted cells against the host. Simultaneously, Billingham and Brent (1957), in their attempts to induce tolerance in newborn mice, found that, in certain strain combinations, all injected mice died from a peculiar wasting disease. They concluded that a GVH reaction was the likely explanation of this pathological condition which they named runt disease. GVH reactions result when a recipient of a graft comprised of immunologically competent cells is incapable of rejecting the cellular graft. This situation typically arises in very young individuals (embryos and newborn recipients). It also occurs in adults when a graft from a homozygous donor, i.e., genetically AA, is given to a host that is an F1 hybrid between the donor strain and a dissimilar strain, i.e., genetically AB. A graft A transplanted to an AB host represents a situation in which the hybrid cannot reject the graft by immunological means even though the host may be entirely competent immunologically. This is true because the graft does not possess any antigens that are foreign to the host. The graft, however, can react against the host, since the latter contains foreign antigens derived from the dissimilar parent of the hybrid. The mouse is the classic animal for the production of GVH reactions and runt disease. In severe reactions, mice grow normally for about a week, but thereafter growth ceases, and there is subsequent weight loss, associated with diarrhea, alopecia, dermatitis and eventual death. This pathological condition has been demonstrated in numerous other species. It is of great clinical significance that human recipients of bone marrow
transplants, especially after immunosuppressive treatment with whole body irradiation or cytotoxic drugs, have shown all the symptoms of classical GVH reactions similar to those observed in lower species. The most important parameters which determine severity of GVH reactions are: 1) the immune competence of the cells utilized; 2) the dose of the cellular inoculum; and 3) the degree of histocompatibility difference between the donor and the recipient. The pathological symptoms and the mortality rate increase as these parameters increase.

Immuno logical Tolerance and Antilymphocyte Serum

Induction of specific immunological tolerance to either tissue allografts or non-replicating, chemically defined antigens, such as proteins, is by no means limited to neonatal or immunologically incompetent young animals. Tolerance can be induced in adult, immunologically competent animals under a number of special circumstances. For example, it may be induced by the injection of massive amounts of antigen (so-called immunological paralysis or antigen overloading) or by the injection of antigen during certain times of immunosuppression or lymphocyte depletion. Immunological depression in adult animals to abet the induction of tolerance has been achieved in a number of different ways, i.e., irradiation, cytotoxic drugs, steroids or a combination of these (Russell and Monaco, 1965). In our laboratory, heterologous antilymphocyte serum (ALS) has been used as an immunosuppressive agent to facilitate the induction of tolerance to tissue allografts in adult animals. It is the purpose of this report to review our experiments in this particular area.

Heterologous ALS is an extraordinary immunosuppressive agent. Mice treated with this reagent show a dramatic inability to reject tissue allografts and xenografts (Gray et al., 1966; Monaco et al., 1966). Recent evidence indicates that ALS acts mainly on peripheral circulating lymphocytes (Taub and Lance, 1968). The mechanism of action of ALS continues to be uncertain, but evidence suggests (Monaco, Wood and Russell, 1965a) that its immunosuppressive property depends on its effects on thymus-dependent, immunologically competent lymphocytes. ALS frequently induces peripheral lymphopenia and tissue lymphocyte depletion, and the treated animals are nonspecifically immunosuppressed. Some sera, equally immunosuppressive, do not necessarily produce a dramatic and sustained lymphopenia. It appears, therefore, that for ALS to be immunosuppressive, it must act on a select group of peripheral lymphocytes which is probably quite small in number, and its effectiveness is not dependent on the lymphopenia per se, but rather on the depletion or destruction of a certain population of immunocompetent, thymus-dependent cells. Recent work suggests that ALS is highly effective in depressing cellular-type immunities, as typified by reaction of tissue allografts, whereas the humoral antibody response to certain antigens, especially bacterial ones, is much less affected.

The depression of immune capability following treatment with ALS may be due to the decreased number of mature lymphocytes and/or to the immune incompetence of remaining lymphocytes. From our experiments it appears that the lymphoid cells exposed to ALS in vivo are not affected, while those sensitive to GVH reactions in susceptible recipients or tolerance and chimerism in neonatally injected histoincompatible mice. This supports the concept that the cells remaining in lymph nodes after rabbit anti-mouse lymphocyte serum (RAMLS) treatment are immunologically incompetent. These remaining cells (or their descendents) eventually become immunologically competent, perhaps under the influence of the thymus or a thymic product.

Experiments have been done in mice and rats to determine whether or not tolerance is a property associated with the thymus or thymus-derived cells. Galton, Reed and Holt (1964) showed that allograft tolerance occurs with the achievement of thymic chimerism. This suggested that the presence of allogeneic cells within the thymus is essential for the induction and maintenance of a tolerant state and that the target cell for tolerance induction must be the lymphoid precursor cell within the thymus. The presence of the antigen within the thymus and the existence of tolerance may, however, be coincidental, as has been suggested by Miller and Osoba (1967). Other investigators (Follett, Batisto and Bloom, 1966) have shown that the lymphoid system in adult thymectomized animals can be rendered specifically tolerant to defined antigens. Thus, the presence of the thymus is not necessary for tolerance induction. This does not exclude the lymphoid precursor cells as the target cells for tolerance induction. These may be present in the thymus-dependent areas of lymphoid organs (Holborrow, 1968) and be influenced by a thymic humoral factor (Hardy et al., 1968). The breakdown of tolerance occurs when immunologically competent cells are recruited from precursors which have not been exposed to the tolerance-inducing antigen. This recruitment appears to be dependent on the thymus or on a thymic humoral factor. Claman and Talmage (1963) showed that, in mice previously rendered tolerant to horse serum albumin, adult thymectomy delayed the spontaneous escape from tolerance. Argyris (1965) showed that the transplantation of C3H/He thymus tissue accelerated the breakdown of tolerance in C3H/He mice tolerant.
IMMUNOLOGICAL TOLERANCE AND ANTILYMPHOCYTE SERUM

to CBA skin grafts. Goldstein et al. have recently shown that tolerance to A/Jax skin in neonatally thymectomized CBA mice can be abolished by a thymic humoral factor (unpublished data). It appears, therefore, that the thymus or a thymic humoral factor plays an important role in the induction and the abolishment of a tolerant state, presumably by its influence on the development and the recruitment of immunologically competent cells.

Although ALS is highly effective in depressing cellular type immunities—as typified by rejection of tissue allografts—while preserving relatively intact the humoral antibody responses to certain bacterial antigens, it must be emphasized that ALS-induced suppression of tissue graft rejection is still nonspecific. Thus, third-party allografts placed on mice treated with ALS while an earlier test allograft is alive are rejected at the same time as the test allografts (Monaco et al., 1966).

Furthermore, adult thymectomy and ALS treatment produce a prolonged period of nonspecific immune incompetence in the capacity to express cellular type immunities. Adult-thymectomized, ALS-treated mice frequently bear healthy allografts for over 100 days. Third-party grafts applied at that time also frequently show prolonged survival. This profound immune incompetence induced by adult thymectomy and ALS seemed analogous to the unresponsiveness seen in neonatally thymectomized mice. Because of this similarity, we felt it might be possible to induce tolerance in adult animals treated in this manner.

In the initial experiments (Monaco, Wood and Russell, 1966) adult-thymectomized and normal A/Jax mice were treated with ALS and infused with C3H/He × A/Jax F1 hybrid cells intravenously. F1 hybrid cells were used to avoid a GVH reaction (Fig. 1). C3H/He skin allografts in adult-thymectomized, ALS-treated, cell-infused mice showed a markedly prolonged survival, as compared to adult-thymectomized animals receiving ALS but no cell infusion. Furthermore, thymectomized, ALS-treated, cell-infused mice rejected third-party C57BL/6 J skin in a normal fashion while retaining their first and, even, second C3H/He skin allografts (Fig. 2). This emphasized the fact that they were specifically tolerant to C3H/He tissues. These tolerant mice were found to be lymphoid cell chimeras, containing lymphocytes of both A/Jax and C3H/He genotypes. The tolerance was abolished by injection of normal (syngeneic) A/Jax lymphocytes, a finding similar to that noted.
by Medawar and colleagues in their tolerance experiments in neonatal animals. This acquired state of tolerance associated with a state of lymphoid cell chimerism was seen in all the thymectomized mice which received both ALS and a cell infusion.

Since hybrid donor lymphoid cells would never be available in clinical situations, attempts were made to reproduce the above experiment using non-hybrid (homozygous) allogeneic lymphoid cells. Severe GVH reactions resulted when high doses (300 × 10^6) of cells were used. To avoid the GVH reaction, we used allogeneic histocompatibility antigens in a cell-free form from the supernatant of mechanically disrupted spleen cells (Monaco, Wood and Russell, 1965b) of the skin graft donor. The addition of this cell-free antigen (CFA) to a course of ALS increased the prolongation of skin allografts slightly over that achieved with ALS alone. The addition of CFA to a course of ALS in adult-thymectomized mice almost doubled skin allograft survival as compared to that in ALS-treated thymectomized mice (Abbott, Monaco and Russell, 1969). Of major importance was the finding that third-party grafts were rejected relatively normally in the thymectomized animals receiving ALS and CFA, and, therefore, the prolonged survival of the original allograft was a form of specific acquired tolerance in the adult.

The tolerance induced with CFA in such lymphocyte-depleted animals may be long lasting but is not permanent. Furthermore, the number of animals in any group showing a great degree of tolerance is less than when replicating lymphoid cells are used as the donor antigen. Presumably this results from a regeneration or increase in the number of immunologically competent cells which can, again, react to the foreign antigen. Since these cells are probably thymus-dependent, as we indicated earlier in this article, one may justifiably question the reappearance of immunocompetent cells in an ALS-treated thymectomized mouse. It is probable that a small number of immunologically competent cells can regenerate from

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Fig. 2—A. An adult-thymectomized A/Jax mouse which received ALS for seven days followed by 300 × 10^6 C3H/He lymphoid cells, as in Figure 1. The mouse bears a C3H/He graft in perfect condition for 100 days.

B. The same mouse bearing the same first C3H/He graft as well as a second C3H/He graft on day 150.

C. A similar adult-thymectomized, ALS-treated, cell-infused mouse bearing a perfect C3H/He graft over 100 days but simultaneously rejecting a third-party C57B1/6J skin allograft—i.e., indicating specific tolerance.

D. The mouse visualized in B after infusion with normal syngeneic A/Jax lymphoid cells, showing rejection of previously well-tolerated C3H/He grafts—i.e., the abolition of specific tolerance.
the bone marrow in the absence of the thymus. Another possibility is that residual stem cells in the peripheral lymphoid system (lymph nodes, Peyer’s patches, etc.) may differentiate to immunologically competent cells under the influence of a thymic humoral factor which persists in such tissues after thymectomy. It may be possible to circumvent this regeneration by the addition of small doses of immunosuppressive agents (perhaps in association with specific antigen) to prevent the regeneration of new immunocompetent cells. Apparently, when a chimeric state is established, regeneration of competent cells and loss of the tolerant state is delayed or prevented by persisting donor antigen in the form of chimeric lymphoid cells.

Until recently we were unable to induce any form of specific tolerance in the non-thymectomized, ALS-treated adult mice. On the contrary, we found that such animals were sensitized after infusion with allogeneic F1 hybrid lymphoid cells. These seemingly incompatible results may be explained by Mitchison’s (1965) elegant demonstration that the antigen dose frequently determines whether tolerance or sensitization results from antigen introduction. This investigator showed that tolerance to bovine serum albumin in mice resulted when the protein was administered in two dose zones, i.e., in high and low ranges. Doses in other ranges either produced sensitization or no effect. The concept of high zone and low zone tolerance was thus introduced. We tested the effects of various doses (0.01, 0.1, 1.0, 10 and 100 x 10^6) of homozygous allogeneic C3H/He lymphoid cells in five groups of ALS-pretreated (non-thymectomized) A/Jax mice. Controls received ALS but no cells. Figure 3 shows the survival curves of the test allografts in the various groups. C3H/He skin allografts survived longest in A/Jax mice which received 10 x 10^6 cells, thus indicating a potentiation of ALS immuno-suppression by cell infusion, i.e., partial tolerance. The grafts in the groups receiving 0.1 x 10^6 or 100 x 10^6 had a shorter survival time than the ALS controls, indicating a partial sensitization by this dose of cell infusion. Mice receiving 0.01 x 10^6 or 1 x 10^6 cells rejected their grafts at the same time as the controls treated with ALS alone, showing that there was no effect of doses of cells in these ranges. These observations in ALS-treated mice receiving cell infusions are analogous to those of Mitchison on various protein antigens in which the resulting reactions of sensitization and/or tolerance are probably dependent on the dose of antigen utilized. Of importance in these experiments, from the point of view of potential clinical application, was the observation that in these lower dose ranges where partial tolerance resulted, there was little if any observed GVH reaction.

Lance and Medawar (1969), in a similar experiment, have emphasized the effect of the timing of injections of cells on the probability of their potentiating or sensitizing the recipient of the graft. Furthermore, these investigators demonstrated that GVH reactions can be avoided by using cells with decreased immune competence, i.e., thymocytes, bone marrow cells, and cells from ALS-treated donors.

That this phenomenon of acquired partial tolerance induced with donor antigen is not peculiar to skin allografts in mice is well established. Organ allografts appear to be less antigenic than skin allografts; hence, it will probably be easier to develop a tolerant state
to an organ allograft than to a skin allograft. We must further remember that organs are attached by direct vascular anastomosis and that the antigen is chronically released. This may be important in maintaining a tolerant state. Thus, a permanent state of chimerism might not be necessary to achieve a permanent state of tolerance. Seifert et al. (1966) obtained significant improvement in renal allografts by pretreating dogs with subcellular (cell-free) antigen and 6-mercaptopurine and adding methylprednisolone after transplantation. Dagher et al. (1967) used a two-week pretreatment course with soluble cytoplasmic antigen from viable donor spleen cells and demonstrated a prolonged renal allograft survival in dogs when minimal doses of azathioprine and methylprednisolone were given after renal transplantation. These studies of whole organ allograft survival suggest that the prolongation was augmented by the donor antigen. The use of specific donor antigen appears promising as an adjunct in immunosuppressive therapy of whole organ allografts.

Conclusion

Our interest in the problem of tolerance induction is directly concerned with clinical organ transplantation. ALS is highly effective in depressing cellular immunities. Since at least initial allograft rejection is predominantly a cellular phenomenon, one would expect ALS to be highly effective clinically. Our initial observations in this regard support this concept. However, non-specific depression of cellular immunity may also lead to an increased number of viral, fungal, and protozoan infections. Experiments, such as those presented, strongly suggest that a specific state of tolerance to organ grafts in man should be attainable with the aid of ALS followed by introduction of appropriate antigen. A number of avenues are open to increase the likelihood of success for clinical induction of tolerance. Improved methods of histocompatibility typing will aid in tolerance induction, since tolerance is more easily established when histocompatibility differences are minimized. Much effort is being expended in isolation of human cell-free histocompatibility antigens in a soluble form (Kahan and Reisfeld, 1969). Experimental evidence so far suggests that soluble antigens are less immunogenic and more tolerogenic. Development of optimal (non-sensitizing, tolerogenic) antigenic dose schedules—to be administered after immunosuppression has been achieved—should facilitate clinical tolerance induction. If antigens are utilized in a cellular form—as is our tendency at present—then replicating lymphoid cells from ALS-treated donors, replicating non-immunologically competent cells (thymocytes, bone marrow cells), or nonreplicating parenchymal cells of appropriate organs (liver, kidney, etc.) might, theoretically, be utilized. It is apparent that organ transplantation cannot be fully applied until the problem of specific allograft tolerance is solved. A practical solution to the problem may be closer at hand than we realize.

References


Tissue Typing*

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Introduction

The problem of homograft rejection in man remains unsolved. Any organ transplanted from one individual to another (except in the case of identical twins) will be rejected, unless the rejection process can be abated by some method. Unfortunately, homograft rejection still occurs all too frequently, and the toxicity of the drugs used presents problems. However, in other animal systems the rejection process is directly related to the degree of antigenic difference between the donor and the recipient. Recently, various transplantation centers have tried to achieve antigenic similarity between the donor and the recipient as a means of improving the homograft survival rate. In order to do this, the antigens of the potential donors and the recipient are measured in vitro. This is called tissue typing, or histocompatibility matching.

Humans have been shown to develop antibodies against donor cells following multiple blood transfusions, multiple pregnancies, organ homografting and deliberate immunization using repeated injections of leukocytes or platelets. Such antibodies are cross-reactive in varying percentages of the random population. A panel of such antibodies or antisera can be tested against any individual's cells to determine his antigenicity. This can then be compared with the results of typing from other individuals prior to transplantation.

Antibodies have been identified against an antigen system known as HL-A (Table 1). Tissue typing studies performed in families have shown that this system is the result of antigenic determinants present on the same pair of chromosomes at several subloci. However, the exact interrelationship of these determinants is not fully known. The use of the HL-A terminology for the antigens has simplified the exchange of information in this field. The original terminology of various investigators for these antigens is also shown in Table 1.

Methods

The methods most commonly employed in tissue typing are: 1) lymphocytotoxicity; 2) leukoagglutination; 3) platelet complement fixation; 4) mixed cell agglutination; 5) immune adherence; and 6) mixed lymphocyte culture. The lymphocytotoxicity test is the most commonly employed test and requires viable lymphocytes, a foreign complement source (generally, rabbit complement), and a battery of antisera. Cell death occurs if an antigen-antibody-complement reaction takes place. The most common method of detecting cell death is a dye exclusion method. Whereas dead cells imbibe the surrounding fluid, including the dye, and appear larger and darker on the inverted phase microscope, living cells with intact cell membranes exclude the surrounding fluid and dye. Terasaki and co-workers have developed one form of this technique (Terasaki, Vredevoe and Mickey, 1967; Mittal et al., 1968). An alternate method of determining cell death is to preincubate the lymphocytes with $^{51}$Cr and then determine the release of this isotope as an indication of cell death (Rogentine and Plocinik, 1967).

Reproducibility of these tests is good, with about a 3% error. However, the tests cannot be done with cells other than lymphocytes, e.g., kidney cells. An assumption is made that lymphocytes react similarly to cells from transplantable organs, i.e., kidney, heart. However, this may not be the case. The need for viable lymphocytes to perform the test precludes the later testing of cadaver donors unless the lymphocytes are specially freeze-preserved or maintained in tissue culture. An outline of the lymphocytotoxicity test employed at the Medical College of Virginia is shown in Table 2.

Leukoagglutination is also a commonly employed method of tissue typing. In this method leukocytes (whole "buffy coat") are reacted with the panel of antisera without any complement (Zmijewski et al., 1967). An antigen-antibody reaction leads to agglutination of the white blood cells. There are a number of antibodies which are more reactive in the leukoagglutination test than in the lymphocytotoxicity test, as well as several antibodies which are demonstrable only by leukoagglutination. However, these later antibodies presumably

* Presented at the Fortieth Annual McGuire Lecture Series, October 31-November 1, 1968, Medical College of Virginia, Richmond.
are not recognizing important transplant antigens.

The platelet complement fixation test requires platelets, complement, and a battery of antisera (Shulman et al., 1964). This test is no longer widely employed. Dausset (1969) recently described new antigen-antibody reactions—related to the HL-A system—which occurred with this test but were not demonstrable in the lymphocytotoxic or leukoagglutination tests.

All three of these tests require a large panel of antisera. Whereas erythrocyte typing requires only a single anti-A serum and a single anti-B serum. This is because the transplantation antigens as well as the antisera employed for detecting these antigens are poorly defined.

The three other techniques of tissue typing are not widely employed at present but may have wider application in the future. Mixed cell agglutination requires that the target cells be grown in tissue culture monolayers (Milgrom et al., 1966). However, any cell line capable of being grown in tissue culture may be used. Attempts to employ this test with dispersed, fresh cells, cryostat sections, or fresh tissue slices have been unsuccessful. This test requires a special indicator cell system consisting of human erythrocytes precoated with an antibody directed against human erythrocytes and an anti-human antibody capable of reacting with both the first antibody and the test antisera. At the Medical College of Virginia this indicator system consists of Rh+ erythrocytes precoated with anti-Rh antibody and a goat anti-human antibody. The test antisera is layered over the monolayer of target cells, followed by thorough washing and the addition of the coated indicator cell system. Agglutination of the indicator cell system indicates that the test antisera has reacted with the target cell (Fig. 1).

The immune adherence test requires a cell suspension, potent complement source, and human
erythrocytes (Nelson, 1956). It can be performed with various types of cells—dead or alive. If an antigen-antibody-complement reaction takes place, the erythrocyte indicator cells will adhere to the target cell. The immune adherence and mixed cell agglutination tests are much more sensitive than the previously described tests. The mixed lymphocyte culture technique has been described in full by Bach (1966).

In the immune adherence test a comparison of reactivity of lymphocytes and kidney cells from one individual against the same antisera revealed these results in the five individuals studied: 37 negative reactions against lymphocytes and kidney cells, 15 positive reactions against lymphocytes and kidney cells, 29 reactions in which the antisera reacted positively with kidney cells and negatively with lymphocytes, and four instances in which the reverse relationship existed. This is presumptive evidence for an antigenic difference between kidney cells and lymphocytes. Whether or not it is a qualitative or quantitative difference is not shown by these data. At any rate, the data are against the assumption that, by typing leukocytes, one can determine the full antigenicity of an organ to be transplanted.

A similar discrepancy has been demonstrated by testing lymphocytes and kidney cells from the same individual—through use of the lymphocytotoxicity and mixed cell agglutination tests, respectively. In retesting an individual’s cells against an antiserum in which the lymphocytotoxicity reaction was negative and the mixed cell agglutination reaction positive, the difference in reactivity appeared to be quantitative, as prior absorption with the individual's leukocytes removed the reactivity against kidney cells, despite there originally being no reactivity demonstrable by lymphocytotoxicity testing.

Since the antigenic determinants for the HL-A system occur on the same pair of chromosomes, there is a 25% chance that a sibling will be identical with any other sibling for the HL-A system. By tissue typing a family, it can be determined which individuals share 0, 1, or both chromosomes. Amos et al. (1969) have shown that skin grafts between nonimmunosuppressed siblings known to be identical for the HL-A system have a mean survival of about 25 days. The failure of permanent graft take might indicate that there are other antigenic systems present on other chromosomes which are not yet defined.

Detailed absorption studies have been done in our laboratory to demonstrate the presence of HL-A antigens in various human tissues, i.e., kidney, liver, heart, blood vessels, lung, skin, pancreas, ureter and fat. Kidney and liver tissue were found to contain all eight of the well recognized HL-A antigens, whereas fat had no HL-A antigens. The other tissues studied were not tested for all eight antigen groups but did contain every antigen for which tests were run (Rolley, Williams and Hume, 1968). These studies were sufficiently controlled to exclude the possibilities of nonspecific loss of antibody activity through surface absorption phenomena or by anti-complementary factors. The studies indicated that the HL-A system is widely distributed in human organs, but this does not preclude the possibility that tissues possess important histocompatibility antigens not detectable on leukocytes. The possibility of such tissue transplantation antigens being poorly expressed on leukocytes has been shown in the ABO erythrocyte system.
Histocompatibility Matching

Of the six tissue typing techniques discussed above, only mixed lymphocyte culture involves direct interaction between donor and recipient cells. The other five tests all use a comparison of results against a panel of antisera. A histocompatibility match may be reported on an A, B, C, or D scale. The terms A and B are comparable to the terms matched or compatible; the terms C and D are comparable to the terms mismatched or incompatible. Table 3 shows two examples of an A match. In the upper example of that table, the antigenicity of the donor and the recipient for these eight antigens is the same. However, a great deal of attention is also given to individual serum reactions, since not all sera have been classified for one of these particular antigen groups. In this example 4% of the sera reacted with antigens of the donor but not of the recipient. Theoretically, the recipient is capable of reacting to such foreign antigens.

In the second example of an A match in Table 3, the recipient has more antigen groups than the donor. At our present level of knowledge, this is assumed not to affect graft survival and therefore, the example is not considered a mismatch; but rather an A match.

Table 4 shows an example of a B match in which no recognized antigen groups are present on donor cells but absent on recipient cells. However, more than 5% of the individual sera detected antigens on donor but not recipient cells; hence, this match is rated B rather than A.

Table 4 also shows an example of a C match in which a well recognized antigen group is present on donor cells but absent on recipient cells. A D match (Table 4) indicates a donor-to-recipient mismatch of two antigen groups.

Preformed Antibodies

Tissue typing tests determine neither the presence of preformed antibodies against donor antigens, nor the presence of organ-specific auto-antibodies, such as the anti-glomerular basement membrane antibodies. To look for preformed antibodies against histocompatibility antigens, it is necessary to perform a cross match test using the recipient's serum and donor's cells. This can be done by any one of the five tissue typing methods discussed in this paper. If preformed antibodies are found and a transplant performed, there is a great probability that the transplant will be rejected very quickly. A discussion of the detection of auto-antibodies is beyond the scope of this paper.

At present, histocompatibility matching is an inexact science, and there are undoubtedly many histocompatibility antigens either poorly defined or undefined. With further definition of such antigens, improvement can be expected in the correlation of tissue typing with homograft function and survival. Further work also needs to be done to determine which method of tissue typing is best.

| TABLE 4 |

Examples of Lymphocyte Typing Results

<table>
<thead>
<tr>
<th>Donor</th>
<th>+</th>
<th>+</th>
<th>-</th>
<th>+</th>
<th>-</th>
<th>-</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

12 of 100 sera "killed" donor's but not recipient's sera.

"C" Match

<table>
<thead>
<tr>
<th>Donor</th>
<th>+</th>
<th>-</th>
<th>+</th>
<th>-</th>
<th>+</th>
<th>-</th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

1 group mismatch in "major direction."

"D" Match

<table>
<thead>
<tr>
<th>Donor</th>
<th>-</th>
<th>+</th>
<th>+</th>
<th>+</th>
<th>-</th>
<th>+</th>
<th>-</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

2 group mismatches in "major direction."
References


Relation of Tissue Typing to Results of Clinical Transplants*

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The theoretical background for tissue typing has already been given to us by Dr. Bach with his usual simplicity and clarity. Methods currently in use have been described by Dr. Rolley. In the following brief article I will report on the correlations that we and other groups have seen between tissue matching and the results of clinical transplants.

Tables 1 and 2 show the terms we use relating to the clinical ranking of the course and the tissue matching grade.

Living Related Donors

In spite of the technical difficulties and the many variables involved in clinical transplantation, correlation of the tissue matching with living related donor transplants has been shown to be reasonably good (Vredevoe et al., 1965; Terasaki et al., 1966; Lee et al., 1967). Table 3 shows a retrospective study of the living related donor patients in Richmond who survived at least six months. Since it is a retrospective study and does not include acute failure, it is not a pure study of the statistical evaluations; however, quite a significant clinical trend can be seen. Patients who had good tissue matching almost always appeared to have had a good clinical course, though there were a few exceptions. These exceptions themselves are interesting because of the possibility of recurrence of the original disease which may not be within the framework of histocompatibility tissue matching. An interesting point to note is that in the group of patients whose tissue typing grade is C, i.e., only one major group mismatches, there is a fairly even spread between those whose clinical course is good and those whose clinical course is poor. Similar results have been observed by others, particularly Dr. Terasaki, who compiled fairly large numbers from multiple centers.

Chi-square analysis of the distribution of $2 \times 2$ tables is given in Table 4 with the AB group as a match and CD group as a mismatch. The distribution falls short of statistical significance with a p

* Presented at the Fortieth Annual McGuire Lecture Series, October 31–November 1, 1968, Medical College of Virginia, Richmond.

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**TABLE 1**
Evaluation of Donor-Recipient Histocompatibility Testing Grade

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Less than 5% major mismatches and no major group mismatches</td>
</tr>
<tr>
<td>B</td>
<td>More than 5% major mismatches and no definite major group mismatches</td>
</tr>
<tr>
<td>C</td>
<td>One major group mismatch with less than 25% major mismatches</td>
</tr>
<tr>
<td>D</td>
<td>Two major group mismatches OR more than 25% major mismatches</td>
</tr>
</tbody>
</table>

**TABLE 2**
Results of Kidney Homotransplants

<table>
<thead>
<tr>
<th>Clinical grades</th>
<th>Serum Creatinine</th>
<th>BUN</th>
<th>Creatinine Clearance</th>
<th>Proteinuria</th>
<th>Blood Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>$&lt;1.5$</td>
<td>$&lt;20$</td>
<td>770</td>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>B</td>
<td>1.5–2.0</td>
<td>20–40</td>
<td>70–40</td>
<td>+</td>
<td>Controlled without Medication</td>
</tr>
<tr>
<td>C</td>
<td>2.0–3.0</td>
<td>$&lt;100$</td>
<td>40–20</td>
<td>++</td>
<td>Controlled with Medication</td>
</tr>
<tr>
<td>D</td>
<td>$&gt;3.0$</td>
<td>$&gt;100$</td>
<td>$&lt;20$</td>
<td>+++ or +</td>
<td>Difficult to Control Medication</td>
</tr>
</tbody>
</table>
TABLE 3
Correlation of Clinical Results and Histocompatibility Typing

<table>
<thead>
<tr>
<th>Lymphocyte Antigen Matching</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>D. L.*</td>
<td>K. G.</td>
<td>M. P.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M. H.</td>
<td>B. L.</td>
<td>J. M.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F. C.</td>
<td>F. C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>W. F.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N, McC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E. P.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. M.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>J. We.</td>
<td>D. P.</td>
<td>R. R.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G. M.</td>
<td></td>
<td>N. McE.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>W. C.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. C.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. A.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>R. E.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. J.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>L. D.</td>
<td>E. A.</td>
<td>W. L.</td>
<td>M. D.</td>
</tr>
<tr>
<td></td>
<td>J. M.</td>
<td>J. DeR.</td>
<td>P. M.</td>
<td>E. R.</td>
</tr>
<tr>
<td></td>
<td>L. V.</td>
<td>R. G.</td>
<td>V. M.</td>
<td>R. A.</td>
</tr>
<tr>
<td></td>
<td>E. J.</td>
<td>G. F.</td>
<td></td>
<td>B. C.</td>
</tr>
<tr>
<td></td>
<td>H. B.</td>
<td>O. L.</td>
<td></td>
<td>S. S.</td>
</tr>
<tr>
<td></td>
<td>C. O.</td>
<td>N. R.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K. R.</td>
<td>R. P.</td>
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<td>M. H.</td>
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<td></td>
<td></td>
<td>S. H.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>W. P.</td>
<td>W. T.</td>
<td>E. R.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>J. L.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>W. J.</td>
<td></td>
</tr>
</tbody>
</table>

* Initials refer to patients’ names.

However, if one divides and separates clinical rank A from the remaining B, C, D, a statistically significant distribution with a p value of 0.005 is noted (Table 5). This means that the majority of poor clinical results belong to the mismatch group, and most of the patients who matched well belong to the group with a good clinical outcome. The other point is that, of the group whose matching grade is C, almost half of them have done quite well in spite of mismatch. This spread of results has been explained (in speculation) by mistyping due to technical difficulties, incomplete antigen panels, variability of the effectiveness of immunosuppression on different patients, and the variability of responsiveness of different patients. Patients doing well who received a mismatched transplant would have been missed if typing had been used for selection.

In comparing the group of patients having sibling-to-sibling relationships with the group having parent-to-child relationships, Singal, Mickey and Terasaki (1969) noted that there is a higher correlation with the sibling group (Table 6). This is not surprising when one considers the probable ease with which the genetic similarity can be defined among the siblings. Dr. Bach has already enlightened us on this aspect.

TABLE 4
Correlation of Histocompatibility Typing with Clinical Course
(Living related, Richmond, October, 1968)

<table>
<thead>
<tr>
<th>Lymphocyte Antigen Matching</th>
<th>Good</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matched</td>
<td>18</td>
<td>4</td>
</tr>
<tr>
<td>Mismatched</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>p &lt; 0.14</td>
</tr>
</tbody>
</table>

TABLE 5
Correlation of Tissue Matching with Clinical Course

<table>
<thead>
<tr>
<th>Lymphocyte Antigen Matching</th>
<th>A</th>
<th>BCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matched</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>Mismatched</td>
<td>8</td>
<td>22</td>
</tr>
</tbody>
</table>

Unrelated Cadaver Donors

Since tissue typing has neither been done very long nor very frequently on the cadaver donor series, we do not as yet have a good statistical study. However, some of the results are beginning to come in. Dr. Terasaki’s recent review of the results, based on data from many centers, seems to show some correlation, though not as clearly nor as significantly as among living related donor groups (Patel, Mickey and Terasaki, 1968). Table 7 shows these results. He also found some significance when he compared re-
TABLE 6
Correlation of Clinical Rank and Typing in Patients with More Than 6 Months
Graft Survival

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Clinical rank</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>Sib-Sib*</td>
<td>35</td>
<td>7</td>
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<td>Parent-Child†</td>
<td>25</td>
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<td>43</td>
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<tr>
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<td>13</td>
<td>13</td>
<td>1</td>
<td>11</td>
<td>63</td>
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* Sib-Sib: Matched vs Mismatched t = 3.66; p < .001
† Parent-Child: Matched vs Mismatched t = 2.54; p = .01

Data from Singal, Mickey and Terasaki (1969).

TABLE 7
Clinical Rank and Typing
(Over 4 months survival)

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>F</th>
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</thead>
<tbody>
<tr>
<td>Matched</td>
<td>6</td>
<td>10</td>
<td>1</td>
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<tr>
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<td>4</td>
<td>14</td>
<td>7</td>
<td>3</td>
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p < 0.02

Data from Patel, Mickey and Terasaki (1968).

TABLE 8
Correlation of Rejection and Tissue Matching

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<th>C</th>
<th>D</th>
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<tr>
<td>Matched</td>
<td>9</td>
<td>8</td>
<td></td>
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<tr>
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<td>4</td>
<td>8</td>
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</tbody>
</table>

p < 0.001

Data from Patel, Mickey and Terasaki (1968).

TABLE 9
Creatinine Clearance and Tissue Typing

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<th>Donor</th>
<th>Creatinine Clearance</th>
<th>Mean</th>
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<tr>
<td>Matched</td>
<td>NR*</td>
<td>88.0</td>
<td>78.5</td>
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<tr>
<td></td>
<td>C†</td>
<td>74.2</td>
<td></td>
</tr>
<tr>
<td>Mismatched</td>
<td>NR</td>
<td>69.6</td>
<td>50.1</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>44.7</td>
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Cadaver Matched vs Mismatched p < 0.01

* Nonrelated
† Cadaver

Data from Patel, Mickey and Terasaki (1968).

When he compared renal function, he also found a fairly significant difference in creatinine clearance between the matched group and the mismatched group in cadaver transplant patients (Table 9). The p value in the matched cadaver versus the mismatched cadaver source was 0.01. Two other groups have reported their experiences, though on rather small numbers (Morris, Kincaid-Smith and Marshall, 1969; van Rood et al., 1969). Both of these groups have found significant statistical correlations of typing and clinical results in unrelated donor-recipient pairs (Tables 10 and 11).

Our cadaver typing results have been rather small in number, so although we cannot attribute any statistical significance to the results, they are of some interest. Table 12 shows the following prospective study. Among the seven patients whose clinical results were poor in the matched group, four, in a subsequent study, had a positive cross match against the donors' kidney cells. Even though the numbers are small and the period of observation short, an encouraging trend can be seen.

We have attempted to show that a correlation exists between the results of tissue typing as practiced using leukocyte group antigens, the HL-A system, and the outcome of renal homotransplantation. We, as well as Dr. Bach, agree that the correlation is not as close as one would wish and is particularly low with unrelated cadaver donor source. To make matters more complicated and difficult, there is the problem of ABO system incompatibilities. The detrimental influence of the ABO group incompatibility upon the clinical result of the human renal homotransplant has been well substantiated (Starzl et al., 1963; Hume et al., 1964; Starzl, 1964; Gleason and Murray, 1967).

There is evidence that preexisting host anti-donor antibodies,
TABLE 10
Correlation of Leukocyte Typing of Unrelated Donor-Recipient Pairs and Rejection Grade After Renal Transplantation

<table>
<thead>
<tr>
<th>Rejection grade</th>
<th>A</th>
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<tr>
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<td>1</td>
<td>0</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mismatched</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>$\chi^2 = 4.9$</td>
<td></td>
<td></td>
<td></td>
<td>p &lt; 0.05</td>
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TABLE 11
Correlation of Leukocyte Typing of Unrelated Donor-Recipient Paris and Renal Homograft Survival

<table>
<thead>
<tr>
<th>HL-A Mismatched</th>
<th>% Functioning</th>
<th># of Cases</th>
</tr>
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<tbody>
<tr>
<td>0–1</td>
<td>75%</td>
<td>8</td>
</tr>
<tr>
<td>2–3</td>
<td>60%</td>
<td>12</td>
</tr>
<tr>
<td>4 or more</td>
<td>30%</td>
<td>17</td>
</tr>
<tr>
<td>$p &lt; 0.02$</td>
<td></td>
<td></td>
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</table>

Data from van Rood et al. (1969).

Summary

Current data reveal that, when matching is very good, there is also very good correlation in living related donor results, particularly in the sibling donor source. When there is slight mismatching, correlation is not as good; however, many patients seem to do well in spite of some mismatch. Correlation in the unrelated cadaver group is not as precise or significant.

The current status of tissue typing is still not as precise as it should be, in order for us to rely on it as a selection criterion for renal homotransplantation. However, with rapidly advancing knowledge of antigen groups in the HL-A system and a more refined and simplified method of tissue typing, the significance of the correlation between tissue typing and clinical results of the homotransplant should improve.

TABLE 12
Unrelated Cadaver Donor Source (Richmond, 1969)

<table>
<thead>
<tr>
<th>Typing</th>
<th>Good</th>
<th>Poor</th>
</tr>
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<tbody>
<tr>
<td>Matched</td>
<td>8</td>
<td>7*</td>
</tr>
<tr>
<td>Mismatched</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

* 2—long ischemia, pretransplant; 1—died from hepatic failure and gastrointestinal hemorrhage; 4—positive cross match between donor lymphocyte or kidney cells and recipient serum.
References


Introduction and Background

Delayed or cellular hypersensitivity is the primary immunologic mechanism in the rejection of organ homografts. Mounting evidence implicates the small lymphocyte as the cell responsible for implementing this reaction.

The immunologic role of the lymphocyte in the graft-versus-host reaction was first recognized by Simonsen (1957). He showed that if newborn or heavily irradiated mice are injected with foreign adult lymphoid cells, the animals will waste and die. He further demonstrated that the cells causing the graft-versus-host reaction are present in the peripheral blood of the mouse. Other investigators have since demonstrated that this cell is the small lymphocyte.

Strober and Gowans (1965) have demonstrated that there is interaction between small lymphocytes and a foreign organ graft. In this study, lymphocytes were first perfused through isolated allogenic kidneys and then injected back into the animal from which the lymphocytes were derived. It could then be demonstrated that these animals reacted to a skin graft from the kidney donor with a secondary response, indicating prior sensitization. This study seemed to indicate that the small lymphocyte is involved in the afferent (sensitization) limb of the homograft response.

There is also experimental evidence to suggest that the small lymphocyte is intimately involved in the efferent (destruction) limb of the homograft response. The earliest indication of this was histologic interpretation of the events surrounding the homograft reaction. In serial biopsies taken daily following renal transplantation in dogs, the events can be examined unmodified by immunosuppressive agents. In biopsies from such dogs, very little histologic alteration can be seen for the first 48 hours, but by the third day there are well-established pyronine-positive mononuclear cells in the perivascular interstitial areas. In the ensuing days this infiltration spreads and tubular cell degeneration occurs, followed by infarction. The glomeruli remain remarkably normal during this entire process of primary graft rejection. In the immunosuppressed animal this perivascular infiltration of immunocytes does not occur, and organ function is prolonged.

Experimental Data

Interested in this observed phenomenon, my colleagues and I designed several experiments to study the relationships of the lymphocyte to the efferent arc of transplant immunity and the effect of lymphocyte depletion upon the temporal and histologic events of the homograft response.

Because of the marked radiosensitivity of the lymphocyte, it seemed possible that lymphocyte depletion could be produced in the experimental animal by the use of radiation. Hume and Egdahl (Hume et al., 1960) had previously reported that lethal whole-body radiation in the dog abrogated the immune response, whereas sublethal doses of radiation did not alter the immune response.

Our first experiments (Wolf et al., 1966) utilized small pellets of Yttrium90, a powerful beta emitter having a half-life of 64 hours. The pellets were encased in Silastic tubing and implanted into the abdominal aortas of dogs. Thus, all the blood flowing through the aorta was irradiated. These animals all showed a prompt and profound lymphopenia (Fig. 1), which persisted for two to three weeks following the cessation of radiation and dropped to levels of 10% of control values.

Serial biopsies of mesenteric lymph nodes in these animals showed progressive lymphocyte depletion from germinal follicles. Bizarre cells were seen in the circulating blood of these animals during the radiation period; the cells showed clumping of the cytoplasm and nuclear vacuolization. A group of these animals received renal homografts in addition to blood irradiation (Fig. 2). The untreated control kidneys had a functional survival of 5.2 days, while the irradiated animals had a mean functional survival of 16 days, the longest functional survival being 34 days. From this study, it appeared that a relation-
CELLULAR REJECTION

Fig. 1—Dogs receiving \(^9\text{Y}\) implants show a marked and prolonged lymphocytopenia.

ship existed between total lymphocyte mass in the dog and its ability to produce homograft destruction.

To examine further the effect of the lymphocyte on the efferent arc, we designed an experiment in which we tried to keep the afferent arc constant (Wolf, McGavic and Hume, 1969). To do this, we simultaneously transplanted two kidneys from the same donor into the same recipient. One kidney was placed in the pelvis and the other in the neck. One of the two kidneys received local graft irradiation in a dose of 150 roentgens on days one, three, five and seven post transplantation. Thus, the animal had one kidney to which he was normally sensitized and which had received no form of immunosup-
pression, while the other kidney received only local graft irradiation. Serial biopsies were then taken simultaneously in both kidneys in several dogs. In each biopsy the unirradiated kidney showed more marked cellular infiltration on any given day than the irradiated kidney. The nonirradiated kidneys rejected in a mean time of 5.7 days, while the irradiated kidneys had a mean survival time of 12.1 days, with 24 days the longest period for a surviving kidney.

It would appear from this experiment that the animal was normally sensitized—as indicated by rejection in the normal time of the untreated kidney—but that the local irradiation served as an immunosuppressant, probably by destroying sensitized lymphocytes in the kidney. This experiment, then, gives further evidence that the lymphocyte is involved in the primary immune response.

To further manipulate the cellular environment in an attempt to study the effect of the lymphocyte on the efferent arc of transplant immunity, we designed some tissue culture experiments (Wolf, Fawley and Hume, 1969). In these studies, renal cells were cultured from transplanted kidneys removed from patients who had rejected either chronically or acutely. These cells were grown in tissue culture at 37°C, using Eagle’s Minimum Essential Medium to which was added 20% serum and penicillin, streptomycin, and L-glutamine. After these monolayer cultures had been established for a period of five to seven days, lymphocytes—from the patient, from the kidney donor, or from an indifferent donor—were added to the cultures. Figure 3 shows one such renal cell monolayer culture to which indifferent lymphocytes have been added. The lymphocytes did not appear to have any effect on the cultures, and the culture continued to grow in a normal fashion. However, as seen in Figure 4, when the patient’s own lymphocytes were added to the culture, even in the absence of complement or autologous serum, there was destruction of most of the kidney monolayer within 12 to 14 hours. Nine human kidneys which had been previously transplanted for periods of 1 to 18 months have thus far been studied, as have been six non-transplanted kidneys. In seven of the nine transplanted kidneys which had histologically established rejection patterns, the recipients’ lymphocytes were markedly cytotoxic to the kidney cells, showing destruction of most of the mo-

Fig. 3—Renal cell monolayer culture reacted with indifferent lymphocytes and showing no evidence of renal cell destruction (× 380).
nolayer within 12 to 14 hours, even in the absence of complement source and regardless of the source of the serum. Indifferent lymphocytes, donor lymphocytes, or lymphocytes from other transplant recipients did not have this same effect.

**Conclusion**

From these studies we have concluded that: 1) sensitized lymphocytes in close physical contact to the target cell can produce destruction of the target cell; 2) deficiency of lymphoid mass within the organ homograft recipient can produce attenuation of the homograft reaction; and 3) the lymphocyte target cell reaction in vitro is individual-specific, but not complement-dependent, is rapid, and proceeds in the absence of autologous serum source.

Mounting experimental data suggest that the homograft reaction is intimately related to the small lymphocyte. It is evident that both the afferent and efferent arcs of transplant immunity are dependent upon the intactness of the lymphoid system. The lymphocyte has the ability to recognize the foreign antigen, is involved in the activity of cell-bound antibodies, and may be involved in the inductive processes leading to antibody formation.

**References**


Contributors to This Issue

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**TABLE OF CONTENTS**

1969 • NUMBER ONE

<table>
<thead>
<tr>
<th>Title</th>
<th>Author(S)</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset of the Immune Response</td>
<td>S. G. BRADLEY, Minneapolis, Minnesota</td>
<td>9</td>
</tr>
<tr>
<td>Investigations on Congenital and Induced Osteopetrosis</td>
<td>DONALD G. WALKER, Baltimore, Maryland</td>
<td>17</td>
</tr>
<tr>
<td>Thermodes and Theories</td>
<td>N. HERBERT SPECTOR, Richmond, Virginia</td>
<td>20</td>
</tr>
<tr>
<td>Current Concepts in the Field of Neurochemical Mediation</td>
<td>BERTA SCHARRER, Bronx, New York</td>
<td>27</td>
</tr>
<tr>
<td>Abstracts of Theses for Graduate Degrees</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>Contributors to this Issue</td>
<td></td>
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</tr>
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1969 • NUMBER TWO

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<thead>
<tr>
<th>Title</th>
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<tr>
<td>Histamine and Thyroid Hormone Function</td>
<td>RICHARD W. SCHAYER, Orangeburg, New York</td>
<td>44</td>
</tr>
<tr>
<td>The Interplay of Defense Mechanisms Against Infectious Diseases</td>
<td>QUENTIN N. MYRKV, Winston-Salem, North Carolina</td>
<td>49</td>
</tr>
<tr>
<td>On the Antiquity of Man</td>
<td>ROLAND SCHMIDT, Morgantown, West Virginia</td>
<td>56</td>
</tr>
<tr>
<td>The Role of Operations Research in a University Hospital</td>
<td></td>
<td>61</td>
</tr>
<tr>
<td>A Review and Bibliography</td>
<td>S. J. KILPATRICK, Jr., Richmond, Virginia</td>
<td>69</td>
</tr>
<tr>
<td>Unilateral Aphakia and Contact Lenses</td>
<td>HERBERT WIESINGER, Richmond, Virginia</td>
<td>69</td>
</tr>
<tr>
<td>Days, Old and New</td>
<td>WILLIAM T. SANGER, Richmond, Virginia</td>
<td>72</td>
</tr>
<tr>
<td>Book Review</td>
<td>S. J. KILPATRICK, Jr., Richmond, Virginia</td>
<td>77</td>
</tr>
<tr>
<td>Contributors to this Issue</td>
<td></td>
<td>78</td>
</tr>
</tbody>
</table>
Twenty-Second Annual Stoneburner Lecture Series: PSYCHIATRY IN MEDICAL PRACTICE
GEORGE KRIEGMAN, Guest Editor

Psychiatry in Medical Practice
GEORGE KRIEGMAN, Richmond, Virginia 84

The First Stoneburner Lecture:
Changing Concepts of Deviance
DOUGLAS D. BOND, Cleveland, Ohio 85

The Second Stoneburner Lecture:
Perspectives in the Behavioral Sciences
DOUGLAS D. BOND, Cleveland, Ohio 90

I. ASPECTS OF COMMUNICATION IN MEDICAL PRACTICE
Whom, Why and How to Refer
ZIGMOND M. LEBENSOHN, Washington, D.C. 94

What Referring Physicians Can Expect from the Psychiatrist
DAVID R. HAWKINS, Charlottesville, Virginia 101

Recognition and Management of Psychiatric Emergencies
JOHN A. EWING, Chapel Hill, North Carolina 106

II. THE FAMILY AS A FACTOR IN MEDICAL PRACTICE
Family Tension and Psychophysiological Illness
MILTON ROSENBAUM, New York, New York 112

Physical Illness, the Family and the Physician
MARC H. HOLLENDER, Philadelphia, Pennsylvania 116

Social and Community Psychiatry and Its Effect on the Family
WILLIAM W. JEPSON, Minneapolis, Minnesota 120

II. PSYCHOPHYSIOLOGIC CONDITIONS
Psychiatric Issues in Therapeutic Abortion
HENRY D. LEDERER, Richmond, Virginia 126

Sexual Counseling in Medical Practice
GEORGE KRIEGMAN, Richmond, Virginia 130

Anxiety, Defense and Cognition: A Theoretical Basis for Practical Handling of the Surgical Patient
STANLEY L. BLOCK, Cincinnati, Ohio 135

The Older Population and the Aged Patient
EWALD W. BUSSE, Durham, North Carolina 137

IV. PSYCHOSOCIAL MANAGEMENT OF EMOTIONAL FACTORS IN MEDICAL PRACTICE
Community Resources—The Role of Other Professionals
LUTHER CHRISTMAN, Nashville, Tennessee 143

The Physician’s Role in the Assessment of Normal Behavior
MELVIN SABSHIN, Chicago, Illinois 147

Psychopharmacological Procedures
HEINZ E. LEHMANN, Montreal, Canada 151

Contributors to this Issue 161

Fortieth Annual McGuire Lecture Series: TRANSPLANTATION

Laboratory and Clinical Studies of Cardiac Transplantation
RICHARD R. LOWER, V. ERIC KEMP, WALTER H. GRAHAM, DAVID H. SEWELL, HERMES A. KONTOS AND GEORGE M. WILLIAMS, Richmond, Virginia 167

The Role of Preservation in Transplantation
FOLKERT O. BELZER, San Francisco, California 171

Isolated Organ Perfusion: Physiology and Application
WILLIAM E. GAYLE, JR., Richmond, Virginia 173

Induction of Immunological Tolerance to Tissue Allografts with Antilymphocyte Serum
ANTHONY P. MONACO AND MARK A. HARDY, Boston, Massachusetts 182

Tissue Typing
RONALD T. ROLLEY, Richmond, Virginia 190

Relation of Tissue Typing to Results of Clinical Transplants
HYUNG M. LEE, Richmond, Virginia 195

Cellular Rejection
JAMES S. WOLF, Richmond, Virginia 200

Contributors to this Issue 205

Index to Volume Five 208
Volume Five
SUBJECT INDEX

A
Abortion, therapeutic:
psychiatric considerations in, 126–129
Acarina. See Arachnida
Acetylcholine:
as messenger substance, 27
Acoustics:
effect on tobacco mosaic virus (abstract), 35
Actinomycin D. See Dactinomycin
Agglutination tests:
in tissue typing, 190-191
Allopurinal:
use in gout, 7
Amines:
biogenic, in neurochemical mediation, 30
Amphibia:
effect of thyroid hormone levels, 45–46
Analeptics:
in treatment of depressive states, 158–159
Anoxemia:
in perfusion, 174-176
Anoxia:
minimized by preservation in transplantation, 171
Anthropology, physical:
domestication of plants and animals, 58–60;
growth and development of man, 56–60
Antibodies:
development of, against donor cells, 190
host anti-donor, histocompatibility testing of, 193, 197-198
Antidepressive agents:
therapeutics of, in mental disorders, 158-159
Antigen-antibody reactions:
and the lymphocyte, 200-203
in infectious disease, 49–52;
in tissue typing methods, 190-192
onset of the immune response, 9–15
Antigens:
consequences of exposure to, in embryonic life, 182-183
histocompatibility testing, 171, 195-198
HL-A terminology for, 190;
leukocyte group in tissue typing, 196–198;
tissue typing (histocompatibility testing), 190-193
Anxiety:
and promotion of disorganization, 116–118;
as psychiatric emergency, 107;
in surgical patient, 135
Arabinose:
L-arabinose utilization in Escherichia coli (abstract), 33
Arachnida:
sex-determining mechanisms in (abstract), 35–36
Attitude:
in prejudice, 91–92
B
Bacteriophage:
normalization of, 10
Behavior:
assessment of normal, in humans, 147–150;
deviant, in humans, 85–89;
emotional reactions of surgical patient, 135–136;
in psychiatric emergencies, 107–109;
treatment of disorders in, 120–124
Behavioral sciences:
ideal characteristics of scientists, 87
Benemid. See Probencid
Birds:
inmunoelectrophoretic analysis of proteins of House Sparrow (abstract), 37–38
Bone resorption:
and osteopetrosis, 17–19
Body temperature:
radio-frequency electrodes and hyperthermia, 23
Body temperature regulation:
instrumentation, 20–26;
methods and theories of brain heating and cooling, 20–26
Brain:
temperature-sensitive areas in, 23–24
C
Carbon monoxide poisoning:
in Great Britain, 53–55
Carbamamide. See Uricosuric agents
Catecholamines:
relationship to thyroid hormone, 46
Central nervous system:
thermal stimulation of, 24
Chemotherapy. See Drug therapy
Child welfare:
history of, 91–92
Chromatography:
DEAE-cellulose, 12
Cinchophen:
use in gout, 7
Cyclic 3', 5'-AMP:
in sodium permeability (abstract), 38
Cognition:
effects of stress on, in surgical patient, 135–136
Cognition disorders:
as psychiatric emergencies, 107
Colchicum:
use in gout, 5
Communication:
interprofessional and patient relationships, 93–110
teaching as major contribution of psychiatrist, 103
Community health services: and interprofessional relations, 143–146
Community mental health services: importance of, in psychiatric therapy, 120–124
Scarcity of, 145
Complement fixation tests: in tissue typing, 190–191
Contact lenses: and unilateral aphakia, 69–71; obstacles to fitting of aphakic patients, 71
Counseling: for sexual problems, 130–134
Creatinine: clearance and tissue typing, 197
Culture: influence on the individual, 92
Curriculum: in medical schools, 120
Cyclophosphamide: effects on normal and induced immune responses, 10–11
Cytogenetics: in the Acarina (abstract), 35–36

D

Dactinomycin: effects on normal and induced immune response, 10
Death: by accidental carbon monoxide poisoning, 53–55. See also Suicide
Decision making: operations research in, 62–65
Defense mechanisms: in parental response to child illness, 116–119; in surgical patients, 135–136
Delusions: drug therapy for, 152, 153–154
Depression: in the elderly, 140
Disease: as form of deviance, 85–89; interaction of family and physician in, 116–119
Drug addiction: in treatment of psychiatric disorders, 154–155
DNA: in antibody specificity control, 9
Drosophilia: equinoxials, mating pattern of (abstract), 34
Drug tolerance: in treatment of psychiatric disorders, 153–159

E

Emergencies: psychiatric, recognition of, 106; psychiatric, treatment of, 108–110
Environment: human and early influences of, 90–92; importance of adaptation to, 86–89; relationship to treatment of psychological disturbances, 120–121
Epinephrine: influence on ion exchange (abstract), 38
Epithelium: cells of, in anaerobic utilization of L-lactate (abstract), 36–37
Escherichia coli: L-arabinose utilization in (abstract), 33

F

Family: as factor in psychophysiological disorders, 112–115; influence on individual psychology, 122–124
Family therapy: in medical practice, 111–124; in mental illness, 122–124; in therapeutic abortion, 128; role of physician in, during illness, 116–119
Frogs: acid-base behavior and ion fluxes in (abstract), 34; muscles, contraction of (abstract), 36; sodium permeability of skin (abstract), 38

G

Gamma globulin: and onset of the immune response, 9–15
Geriatrics: effects of disease and treatment on older population, 137–141
Gingiva: anaerobic streptococci indigenous to (abstract), 37
Gout: classification and pathology of, 3–8; etiology of, 2–3; therapy in, 5–7
Guilt: and promotion of disorganization, 116–119

H

Hallucinogens: experimental use in treatment of depression, 153
Health: concepts of, 85–87, 89
HeLa cells: induction of fusion in (abstract), 33
Histamine: in thyroid hormone function, 44–48
Histological techniques: relation to clinical transplants, 195–196

I

Immune serums: activity of, 10–15; induction of tolerance to tissue allografts, 182
Immune tolerance: actively acquired, 182–183; relation to antilymphocyte serum, 184–188
Immunoelectrophoresis: in egg white, yolk and plasma protein analysis (abstract), 37–38
Immunology: defense mechanisms and infection, 49–52; onset of immune response, 9–15
Immunosuppressive agents: complications of, 182; influence on graft-versus-host reactions, 183–188
Indomethacin: use in gout, 6
Ion exchange: in frog skin (abstract), 34, 38
Infection: mechanisms against, 49–52
Institutional practice: role of state hospital in care of mentally ill, 121–122
Interprofessional relations: and patient anxiety, 94; attitudinal differences between physician and psychiatrist, 101–102 in assessing normal behavior, 147–150 in health professions, 143–146 in psychiatric emergencies, 106; planning for and evaluation of, 143–146 referring physician and the psychiatrist, 101–105
Hospital psychiatric departments: functions of, 104–105; significance of, to medical practice, 112–115
Hyperbaric oxygenation: in organ preservation, 171, 175–176
Hypercalcemia: as related to osteopetrosis, 17–19
Hyperthyroidism: physiopathology of, 45
Hypocalcemia: as related to osteopetrosis, 17–18
Hypochondriasis: in the elderly, 140
Hypoparathyroidism: as explanation for osteopetrosis, 17
Hypothalamus: and brain temperature, 20–26
Hypothermia: and circulating liquid thermodilution, 20–21
Hypothermia, induced: in organ preservation, 171–172
Hypothyroidism: vascular responsiveness in, 46
Isolation perfusion: 
physiology and application of, in organs, 173–181
systems, 173–174

Judgment:
moral, in determination of deviant behavior, 86–89

Kidney:
preservation of, 171

L

Lactates:
an aerobic utilization of (abstract), 36–37
Learning:
and human development, 90–91
Legislation, medical:
concerning abortion, 126–129
Lymphocytes:
and transplantation cellular immunity, 49–52;
effect of depletion on homograft response, 200–201;
immunologic role of, 200
Lymphoid tissue:
and immune capability, 184

M

Mental disorders:
drug therapy in, 151–160
Mental health:
concepts of, 86–87;
prevention of psychiatric emergencies, 110
Mercaptethanol:
in early and late antibody differentiation, 12
Metabolic clearance rate:
in organ preservation, 171–172
Monkeys:
Macaca mulatta, intrinsic back musculature of (abstract), 35
Mosaicism:
erthrocyte chimerism in cattle twins, 182–183
Muscle contraction:
in frogs (abstract), 36
Muscles:
spinal, classification of (abstract), 35
Mutation:
enhancement of L-arabinose utilization in Escherichia coli by (abstract), 33

N

Nephrocalcinosis:
as related to osteopetrosis, 17
Neural transmission:
classification of chemical media, 27
Neurohormones. See Neurohormors
Neurohormors:
as chemical mediators, 27–30;
as distinguished from neurohormones, 27
Neurons:
as neurosecretory cells, 27–30
Neurosecretory cells. See Neurons
Neuroses:
psychoneurotic reactions in adults, 140–141
Neutralization tests:
phage-neutralization assay, 10
Noradrenaline. See Norepinephrine
Norepinephrine:
as messenger substance, 27;
in cold adaptation, 45

O

Operations research:
dynamic programming, 62;
linear programming in, 62;
methods of and use in university hospital, 61–68;
network analysis, 65
Osteoblasts:
activity in osteopetrosis, 18
Osteoclasts:
activity in osteopetrosis, 18
Osteogenesis:
and osteopetrosis, 18–19
Osteopetrosis:
pathology of congenital and chemically induced, 17–19
Oxygen consumption:
in frog muscles (abstract), 36
Oxytocin:
in direct action on "target" tissue, 29

P

Paleontology:
evaluation of Australopithecus and other fossil types, 57–58;
use in determining earliest known man, 56–59
Para-influenza viruses:
induction of fusion in cultured cells by para-influenza 3 virus (abstract), 33
Parathyroid hormone:
use in osteoprotic studies, 17–19
Passer domesticus. See Birds
Patients:
handling emotional reactions to surgery, 135–136;
the older population as, 137–141
Perceptual disorders:
drug therapy in, 152
Perfusion:
importance of perfusate choice, 176–177;
in organ preservation, 171–172
Peripheral nerves:
Schmidt-Lanterman incisures in (abstract), 32
Phagocytosis:
as defense in infectious disease, 49–51
Phenybutazone:
use in gout, 6
Physician-patient relations:
and anxiety, 94–95
Physicians:
and sexual counseling, 130–134;
role in assessing normal behavior, 147–150

Plasma proteins:
immonoelectrophoretic analysis of (abstract), 37–38
Population:
characteristics of the older, 137;
vital statistics of older, 137–140
Postoperative care:
in cardiac transplantation, 169
Preservation, biological:
advantages and disadvantages of, in clinical transplantation, 171–172
methods of, for organs, 171–172
Probability:
stochastic programming in operations research, 62
Probenecid:
use in gout, 7
Professional-patient relations:
in management of psychiatric emergencies, 108–110
Proline:
tritiated, use in osteogenic evaluation, 18
Psychiatry:
in medical practice, 84–164;
in society and the community, 120–124;
revolution in, 122–123;
trends in, 147–150
Psychopharmacology:
development of psychiatric treatment, 151–159
Psychophysologic disorders:
and family tension, 112–115;
treatment of, 112–115
Psychosurgery:
in medical practice, 125–141
Psychoses:
as psychiatric emergencies, 107
Psychoses, senile:
concepts of etiology and treatment, 139–140
Psychosexual development:
factors in, 130–131
Psychotherapy:
in medical practice, 142–160;
management of, 104–105
Public opinion:
and treatment of deviants, 87–88

R

Rabbits:
in osteoprotic studies, 18
Radio-frequency heating. See Body temperature regulation
Referral and consultation:
choice of patient for, 94–95;
consultation methods, 102–104;
in psychiatric emergencies, 107–110;
preparation of patient for, 99–100;
problems in, 94–100;
role of the psychiatrist, 101–104
Regeneration:
in immunologically competent cells, 186–187
RNA, messenger:
in mediation of antibody specificity synthesis, 9
Schools, medical:
Medical College of Virginia, history, 72–76

Serology:
studies and homotransplantation, 195–198

Serums. See Immune serums

Sex:
factors determining sexual identity, 130–131;
problems, 131–132

Sex behavior:
factors in, 130

Sex behavior, animal:
in Drosophila equinoxialis (abstract), 34

Social change:
as catalyst in interprofessional relations, 143–145

Social isolation:
of mentally ill, 121–122

Social problems:
and therapeutic abortion, 127–129

Social service, psychiatric:
role in psychophysiological therapy, 114–115

Sociopathic personality:
drug therapy for, 152

Sodium:
transport across frog skin (abstract), 38

Sodium salicylate:
use in gout, 7

Steroids:
use in gout, 6

Streptococcus:
an aerobic, isolated from human sub­

gingival crevice area (abstract), 37

Stress:
anxiety-defense model in surgical pa­

tient, 135

Suicide:
by carbon monoxide poisoning, 53;
risk of, in pregnant women, 126–127

Sulfonamides:
physicochemical properties of, in re­

tation to biological activity (ab­

T

THERMODYNES. See Body temperature reg­
ulation, 20–26

Thymectomy:
and induction of immune incompe­

tence, 184–185

Thyrocaltcitonin:
effects on bone formation, 18

Thyroid gland:
role in osteopetrosis, 17

Tissue donors:
methods of tissue typing, 190

Tobacco mosaic virus:
mechanical breakage of (abstract), 35

TRANQUILIZING AGENTS:
neuroleptic and anxiolytic seda­
tives, 153–158;
therapeutic applications in psychia­


disorders, 153–158

Transplantation:
graft-versus-host reactions, 183–184

Transplantation, homologous:
and tissue typing, 190–198

cardiac, current status and future po­
tential of, 198;
cardiac, selection of patients for, 168–
169
detection and treatment of rejection, 169–170
rejection of homograft, 190

Transplantation immunology:
mechanisms of, 200–203

U

Uracil mustard:
effects on normal and induced immune
response, 10

Uric acid:
in gout, 7

Uriconic agents:
use in gout, 7

V

Vasopressin:
in direct action on “target” tissue, 29

Vision:
binocular testing, 69–71;
unilateral aphakia and contact lenses, 69–71

Wine:
relationship to occurrence of gout, 7–8

W
## Volume Five

### AUTHOR INDEX

References to articles are in boldface; references to photographs and biographical sketches are in lightface; references to abstracts are in italics.

<table>
<thead>
<tr>
<th>Name</th>
<th>Pages Referenced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allen, Richard Charles</td>
<td>32</td>
</tr>
<tr>
<td>Azzam, Nabil A.</td>
<td>32</td>
</tr>
<tr>
<td>Belzer, Folkert O.</td>
<td>171, 205</td>
</tr>
<tr>
<td>Block, Stanley L.</td>
<td>135, 161</td>
</tr>
<tr>
<td>Bond, Douglas D., Stoneburner Lecturer</td>
<td>85, 90, 161</td>
</tr>
<tr>
<td>Bost, Robert G.</td>
<td>33</td>
</tr>
<tr>
<td>Bradley, S. G.</td>
<td>9, 39</td>
</tr>
<tr>
<td>Busse, Ewald W.</td>
<td>137, 161</td>
</tr>
<tr>
<td>Christman, Luther</td>
<td>143, 161</td>
</tr>
<tr>
<td>Copeman, W. S. C.</td>
<td>2, 39</td>
</tr>
<tr>
<td>Ewing, John A.</td>
<td>106, 162</td>
</tr>
<tr>
<td>Fleetwood, Mildred Kaiser</td>
<td>33</td>
</tr>
<tr>
<td>Friedman, Ruth Torvik</td>
<td>34</td>
</tr>
<tr>
<td>Gayle, William E., Jr.</td>
<td>173, 205</td>
</tr>
<tr>
<td>Geeraets, Ragnit</td>
<td>34</td>
</tr>
<tr>
<td>George, Robert M.</td>
<td>35</td>
</tr>
<tr>
<td>Graham, Walter H.</td>
<td>167, 205</td>
</tr>
<tr>
<td>Hamrick, Philip Edward</td>
<td>35</td>
</tr>
<tr>
<td>Hardy, Mark A.</td>
<td>182, 205</td>
</tr>
<tr>
<td>Hawkins, David R.</td>
<td>101, 162</td>
</tr>
<tr>
<td>Heinemann, Richard L.</td>
<td>35</td>
</tr>
<tr>
<td>Hollender, Marc H.</td>
<td>116, 162</td>
</tr>
<tr>
<td>Jepson, William W.</td>
<td>120, 162</td>
</tr>
<tr>
<td>Kemp, V. Eric</td>
<td>167, 206</td>
</tr>
<tr>
<td>Kilpatrick, S. J., Jr.</td>
<td>61, 78; book review 77</td>
</tr>
<tr>
<td>Kontos, Hermes A.</td>
<td>167, 206</td>
</tr>
<tr>
<td>Kriegman, George</td>
<td>84, 130, 163</td>
</tr>
<tr>
<td>Lebensohn, Zigmund M.</td>
<td>94, 163</td>
</tr>
<tr>
<td>Lederer, Henry D.</td>
<td>126, 163</td>
</tr>
<tr>
<td>Lee, Hyung M.</td>
<td>195, 206</td>
</tr>
<tr>
<td>Lehmann, Heinz E.</td>
<td>151, 163</td>
</tr>
<tr>
<td>Lower, Richard R.</td>
<td>167, 206</td>
</tr>
<tr>
<td>McCarter, R. J. M.</td>
<td>36</td>
</tr>
<tr>
<td>Mant, A. Keith</td>
<td>53, 78</td>
</tr>
<tr>
<td>Molinary, Samuel V.</td>
<td>36</td>
</tr>
<tr>
<td>Monaco, Anthony P.</td>
<td>182, 207</td>
</tr>
<tr>
<td>Myrvik, Quentin N.</td>
<td>49, 78</td>
</tr>
<tr>
<td>McGuire Lectures, 1968</td>
<td>167–203, 205–207</td>
</tr>
</tbody>
</table>

Rolley, Ronald T. | 190, 207 |
Rosenbaum, Milton | 112, 164 |
Sabiston, Charles Barker Jr. | 37 |
Sabshin, Melvin | 147, 164 |
Sanger, William T. | 72, 78 |
Scharrer, Berta | 27, 39 |
Schayer, Richard W. | 44, 79 |
Schmidt, Roland | 56, 79 |
Seibert, Charlene A. | 37 |
Sewell, David H. | 167, 207 |
Spector, Herbert N. | 20, 39 |
Stoneburner Lectures, 1968 | 84–160, 161–164 |
Walker, Donald G. | 17, 39 |
Watlington, Charles O. | 38 |
Wiesinger, Herbert | 69, 79 |
Williams, George M. | 167, 207 |
Wolf, James S. | 200, 207 |
the “spasm reactor” in your practice
The Machine Age man still possesses a Stone Age stomach; sometimes the job of merely coping with today's environmental stress may prove too much. For some (the "spasm reactors" in your practice), tension, anxiety and worry may find expression through the voice of gastrointestinal or other smooth muscle spasm. To treat these patients with antispasmodics alone is often to miss the point of origin of their disturbance; to rely solely on tranquilizers often proves discouragingly slow or ineffective in relieving spasm and pain.

To quiet and quell Donnatal can promptly and effectively quell the spasm and quiet the tensions that trigger it. Prescribed by more physicians than any other antispasmodic-sedative, Donnatal continues to provide the classic answer.

The "Donnatal Effect" The characteristic, over-all effect of Donnatal has been observed in many thousands of children and adults, clearly establishing its value as a versatile sedative-antispasmodic. Outstanding in effectiveness, safety, economy, uniformity of composition and dosage convenience, Donnatal continues to be desired and prescribed by a majority of physicians.

In a multiplicity of indications Particularly useful when anxiety and tension accompany, aggravate or account for smooth muscle spasm, Donnatal is indicated for the symptomatic relief of recurring, persistent or chronic visceral spasm.

Brief summary Blurring of vision, dry mouth, difficult urination, and flushing or dryness of the skin may occur on higher dosage levels, rarely on usual dosage. Administer with caution to patients with incipient glaucoma or urinary bladder neck obstruction. Contraindicated in acute glaucoma, advanced renal or hepatic disease or a hypersensitivity to any of the ingredients.

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<thead>
<tr>
<th>Ingredient</th>
<th>Content</th>
<th>Equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyoscine hydrobromide</td>
<td>0.0065 mg.</td>
<td>0.02 mg.</td>
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<tr>
<td>Atropine sulfate</td>
<td>0.0194 mg.</td>
<td>0.0582 mg.</td>
</tr>
<tr>
<td>Hyoscyamine sulfate</td>
<td>0.1037 mg.</td>
<td>0.3111 mg.</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>(¼ gr.) 16.2 mg.</td>
<td>(¼ gr.) 48.6 mg.</td>
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</tbody>
</table>

Each tablet, capsule or 5cc. of elixir (23% alcohol) Extentab®

The "Donnatal Effect" QUIETS THE STRESS/QUELLS THE SPASM