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Immunological aspects of skin homotransplantation

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IMMUNOLOGICAL ASPECTS OF SKIN HOMOTRANSPLANTATION

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INTRODUCTION

Man frequently manifests his dreams and visions in legend and myth. Perhaps in this respect, the ancient legend of the chimaera can be considered man's first exploration into the realm of transplantation. This beast with the head of a lion, the body of a sheep, and the tail of a snake, spewing fire from his nose, represents a composite phenomenon which certainly must have been the work of the gods for man is still grappling with the complex and baffling enigmas involved in tissue transplantation. However, the incorporation of the term chimaera into the terminology of today's scientists concerned with transplantation intimates at this relationship. This dissertation proposes to deal with a single aspect of the transplantation of one organ among members of one species, namely, the immunological aspects involved in the homologous transplantation of skin.

Although the primary subject of this paper superficially appears narrow and restricted, the ramifications and implications associated with this problem are manifold and offer to provide innumerable benefits. The complexities and problems are enormous, with perhaps more perplexing questions to be definitively solved than this review can answer. In order to obtain as complete and

accurate understanding of this problem as possible, it will be necessary to refer to many other areas of knowledge as genetics, endocrinology, chemistry, histology, and others. These will be referred to, however, only to correlate the present information and put into proper perspective the material offered. At present, solution of the immunological problems confronted in homologous tissue transplantation not only offers promise in the field of surgery, but also in the fields of bacteriology, pathology, and cancer research. This year's Nobel Prize in Medicine was awarded to two men who have been intimately associated and involved in this and related subjects for many years--Sir Frank McFarlane Burnet and Peter Brian Medawar. Burnet states clearly and succinctly the present trends and evolving and broadening aspects of immunology.

"Instead of being concerned primarily with the phenomenon of immunity against microbial infection, immunologists are primarily interested today in the way in which the body maintains its genetic and biochemical integrity, and in possible ways by which this mechanism can be circumvented in the interest of therapy or surgical repair on the one hand, or may by its spontaneous malfunctioning give rise to serious disease."(1)

Here again is a demonstration of the impossibility

of segregating science into small compartments. What is the value of this research? What is its practical purpose? This can only be answered by referring to the broader scope of this subject and in the realization that this subject is encompassed in the study of human integrity, perhaps both physically and spiritually.

HISTORY

It is difficult to know where to begin telling the story of our subject for it entails essentially two fields, transplantation and immunology. Lewis Carroll's advice to "Begin at the beginning . . . go on till you come to the end: then stop,"(12) is not always easily followed for our subject too closely resembles his Mad Hatter's tea party. In the past, transplantation has been primarily concerned with autografts and its relationship to plastic surgery, which has been most involved in rhinoplasty. Rhinoplasty itself is an ancient procedure which was known well before the time of Christ. This operation is mentioned in the Ebers' Papyrus (5,6) as well as in the ancient writings of Hindu surgeons of the Tilemakers Caste (4) who utilized skin flaps from the forehead to reconstruct absent noses. Celsus speaks of restoration of the ear.(5,6) Further records of rhinoplasty are scant until the fifteenth century. In 1442, Branca, a

Sicilian surgeon, was capable of reconstructing noses with pedunculated flaps of skin. It is also reported that Branca attempted to reconstruct a patient's nose with tissue obtained from the flesh of a slave's buttock. It is not reported whether he succeeded or not. His son Antonious carried on with his work. In 1597, Gaspar Tagliocozzi published "De Curtorum Chirurgia per Ineltionem" which was the first treatise on plastic surgery and has thus earned him the distinction of being the father of modern plastic surgery. In his treatise he published the description of his Italian or Tagliocozzi method of rhinoplasty. This method was again rehabilitated by Reneaulme de la Garanne in 1712. In 1804 in Milan, Baronio performed an experimental study of autografting with sheep demonstrating the feasibility of free grafts. Except for a report of the Indian method of grafting published in Gentleman's Magazine in October of 1794, no scientific work was published on this subject until 1823 when Bünger presented his experience with free autografts. The first American to enter this field was J. Mason Warren who performed free autografts in 1837. He was followed by Joseph Pancoast. However, with the above exceptions, this field lay dormant for almost half a century, until J. L. Reverdin reported increased rapidity of healing of granulating wounds with the transplantation

of small sections of skin. This report of Reverdin's was confirmed by numerous experimenters including Pollack of London in 1870, Frank Hamilton of New York in 1870, Chisholm of Baltimore and Coolidge of Boston in 1871. L. Ollier of Lyon reported the transplantation of much larger areas of skin in 1872. Thiersch, in 1874, performed whole thickness skin grafts and later, in 1886, reported his refinements in skin grafting to his German medical society. The next significant advancement was made in 1893 when Feodor Krause's report popularized whole thickness skin grafts. During this period great strides were being made in the field of immunology. Schoene, in 1912, (8) suggested an antibody-antigen basis for the homograft reaction. Others, such as Underwood, Holman, Todd, Landsteiner, Stone, Brown, McDowell and Loeb, were all making observations and approaches towards the problem of homografting. Underwood (11), in 1914, reported the case of a severely burned male who was treated with homografts. He observed that all homografts failed except those from the patient's mother. He also noted that a graft from the patient's sister outlasted the other grafts. From these, he states, "one may infer that consanguinity has a favorable influence." Davis (10) stated that he knew of reports of successful zoografts, but he himself had found that after a certain

period of flourishing they seemed to "melt" away. He also concluded, as had Underwood, that "the success or failure of isografts may be dependent on the similarity of blood groups of host and donor. . . ." It is interesting to note that successful homografting was considered quite possible at this time. At any rate, the first steps toward interest in this field of homotransplantation were being made, namely, the recognition that a problem existed. However, I think it is fair to say that this mass of information and knowledge in transplantation, histology, immunology, microscopy and all the others were first recognized as a new discipline by Medawar and Gibson (7,8) in 1943 and 1944 following their reports in the Journal of Anatomy. Medawar particularly was a protagonist in the synthesis and correlation of all this material. His early work laid the foundation for the exploration of this new and intriguing field. A great deal of research had been conducted on tumors in previous years which provided much ground work for this field. Medawar, himself, demonstrated his recognition of a more uniform and direct approach to the homograft problem when he stated, "Although the 'homograft problem,' as that which relates to the grafting incompatibility of tissues may be called, has well-recognized and more or less direct implications for surgery, genetics, serology, and taxonomic zoology,

no systematic attempt has been made to solve it." (7) From Medawar's "systematic attempt" this field rapidly developed and here its history ends and the scientific exploration, an aspect of which this paper will discuss, begins. This field has grown so enormous that in the fourth homotransplantation conference the suggestion was advanced that ". . . the new discipline. . . be termed transplantation biology." (9)

ANATOMY OF THE SKIN

Before proceeding further in describing the behavior of homografts, a brief review of the anatomy of the skin is necessary. The skin is a vital organ as varied and complex in its structure and function as the liver or kidney. It serves vital functions and is necessary to survival. The skin demonstrates variations in composition and thickness in the various areas of the body. It measures from 1/2 mm. in thickness in the eyelids to 4 mm. in thickness in the palms of the hands and the soles of the feet.

Two basic layers compose the skin, the epidermis and the corium of dermis. Below these two layers is the hypodermis which is composed mainly of fat and connective tissue. The epidermis itself is composed of four layers. These are the stratum corneum, or horny layer,

which is the most superficial layer of the epidermis; the stratum lucidum, or clear layer; the stratum granulosa, or granular layer; and the stratum germinativum, or deep layer of Malpighi. The stratum corneum is composed of flat, elongated, cornified cells composed of keratin. The stratum lucidum is composed of cells which have lost their nuclei and cell borders to form a translucent layer. Following this is the stratum granulosa under which lies the stratum germinativum from which the superficial layers are apparently replenished with cells as they emerge on the surface and are desquamated. These four layers are not always present, the stratum corneum and germinativum being the only two layers found in the thinner portions of the skin. The epidermis also contains melanocytes which, along with the blood and inherent color of the skin which is yellow, provide the color to the skin. The epidermis forms undulating structures at its juncture with the dermis; these are called the rete pegs. It has no direct blood supply but instead is nourished by adjacent tissue fluids.

The dermis is also divided into layers, the papillary layer and the reticular layer. The papillae project into the epidermis forming a firm bond with the rete pegs of the epidermis. The dermis is composed mainly of connective tissue in the form of collagen, reticular and

elastic fibers in varying ratios. In addition, the dermis is composed of interstitial fluid or ground substance and fat. Cellular elements include fibroblasts, histiocytes, and fat cells.

The appendages found in the dermis are the sudoriferous glands, the sebaceous glands, the nails and hair with their arrector pili muscles. Of course, the dermis is richly supplied with nerves and nerve endings and receptors. The dermis and hypodermis do not have distinct boundaries but instead gradually and imperceptibly merge into each other.

The skin is richly supplied with lymphatic and blood vessels. The arteries supplying the skin are in the subcutaneous layers. At the approximate junction of the hypodermis and dermis, these vessels form the rete cutaneum. From this, vessels branch both superficially into the dermis and internally into the hypodermis and subcutaneous tissues. The superficial branches form the rete papillare at the juncture of the papillary and reticular layer of the dermis. From here are derived the loops which project into each papilla. Here the blood enters the capillaries and venous system and is drained away by the venous networks which roughly correspond to the arterial channels. This is briefly a superficial description of the complex organ with which this paper deals.(2,3,4,13)

DESCRIPTION OF AUTOGRAFT

In order that we may comprehend where and how the homograft behaves differently from the autograft, we must first understand the actions of the autograft. The following is a description of an autograft take. (4,7,8) (14) Grossly at the time of grafting the specimen appeared pale and blanched. It remained essentially unchanged for approximately 24 hours at which time small areas of pinking appeared. Another change is noted at the site of transplant. This is an area of erythema surrounding the graft area which appears within the first 48 hours. A glistening appearance of the graft is observed with no evidence of edema being present. This period was followed with a generalized pink appearance of the graft by 72 to 96 hours, with disappearance of the area of surrounding erythema and edema. No significant changes are then noted until the sixth post-operative day, at which time the surface epithelium desquamates and the graft and host epidermal layers appear to coalesce with active regeneration occurring. A new, glistening epithelial cover eventually overlies the graft, but this thickens and becomes duller in appearance. The graft appears pale by the ninth to the eleventh day and eventually returns to the appearance of the

normal, surrounding skin by the twentieth day. (31,32,33)

Microscopically, the autograft shows little change during the first three days. A characteristic congestion of the vasculature occurs on the third post-operative day and lasts for five to eight days. (32,33) The dermal vessels become distended with the influx of many erythrocytes. It is assumed that the graft is nourished by a vaguely understood phenomenon termed "plasmatic circulation" (16) prior to its vascularization and subsequent provision with an adequate blood supply. The host bed becomes densely populated with new grown vessels which invade the graft at the attachment of host and graft. These host vessels eventually replace those of the graft to provide the definitive vasculature of the graft.(14) It is not unanimously accepted that host vessels replace those of the graft.(17) At any rate, this revascularization begins on about the fourth day. The lymphatics reach a peak of development about the eighth day. With the exception of the occasional appearance of a few polymorphonuclear cells, lymphocytes, and mononuclear cells, the graft dermis demonstrates little cellular response. An occasional multinucleated giant cell was observed, but these were primarily attributed to the suture material or trauma. In the surrounding host tissues, the cellular response was also meager with few lymphocytes

and other leukocytes observed. All the original hair of the graft is lost.(7) The epithelium reflects the outward appearance with corresponding periods of thickening and thinning. Connective tissue unites the graft and host beginning on about the tenth day. It is important to note that blood connections can be initially established by 48 hours.(15) Medawar divides the phase of autograft establishment into three principal periods (7): a period of primary union, a period of generalized hyperplasia, and a period of partially retrograde differentiation. Within 18-24 days the autograft regains every microscopic and gross resemblance to normal skin.

HOMOGRAFT

With the above description in mind, it is now possible to compare this to the phenomenon termed homograft rejection. Grossly the autograft and homograft appear identical for the first six days, during which time they are indistinguishable. Here, however, the similarity ends. Following the dissolution of the primary erythema described above, a secondary area of edema and erythma encircles the homograft by the sixth to seventh day. It increases in intensity, becoming maximal in intensity by the ninth day. The homograft becomes a cherry-red color with continuing deepening of the pink color originally

established by the sixth day. By the ninth day, the graft develops a definite appearance of cyanosis. There is minimal effort of the homograft to coalesce with the surrounding skin and relatively little desquamation observed which intimates at the lack of vitality of the graft. Accompanying the deepening color changes and cyanosis, the graft becomes swollen and edematous, protruding above the surrounding skin. Medawar, in his original description on rabbit skin homograft, provides a much more colorful description.

"By the eighth day they are so turgidly swollen as to stand out like buttons from the level of the outlying tissue; and this swelling persists thereafter. The very delicate pink flush which autografts acquire with primary union and then lose here deepens to brick-red, dark brick-red, brown, and finally, by the sixteenth or twentieth days, to black. Eventually, the roofing epithelium of the graft can be pulled or scraped off, leaving a pitted and leathery collagenous pad behind. If the epithelium is not pulled off, the grafts acquire a characteristic frilled appearance, the frill being formed from dried articular debris."(7)

B. O. Rogers describes it as a "pneumatic appearance." By the twelfth day graft desiccation and escharification have resulted in a dry and opaque surface.

Finally, the graft evolves into an eschar on the fifteenth day which is sloughed within the twentieth day, all that remains being a dermal pad in the host bed. (14, 7,4) As expected from the gross appearance, the microscopic differences between homograft and autograft are undetectable for the first five days. With the exception of a slightly more intense accumulation of polymorphonuclear cells noted at the junction of the host and graft during these first five days, the above description of the autograft is interchangeable with that of the homograft. Here the similarity ends, and the homograft begins to manifest the ultimately destructive changes which result in the homograft rejection.

Lymphocytes in increasing numbers begin to penetrate the graft. They are also observed in increasing numbers at the site of union of the host and graft and in the neighboring tissues of the host. The graft itself begins to manifest profound changes by the seventh day, concurrent with the insidious increased density of lymphocytes. The vasculature of the graft, both that which is transferred with the transplant and the newly acquired capillaries from the host, is lost. The remaining cellular elements of the homograft dermis vanish and the graft epidermis sloughs. It is noted that the graft vasculature which is engorged and packed with erythrocytes

appear to rupture with the resultant extravasation of hematological elements into the connective tissue spaces. These areas contribute to the gross appearance of the graft and through the subsequent gangrene produce the above mentioned darkening. The homograft epidermis demonstrates degenerative changes by the third to the fifth post-operative day with almost complete death of the epidermis by the tenth day. This devitalized epidermis is sloughed by the ninth to the twentieth day. Remaining is a whitish "dermal pad" which is the only vestige of the original graft. The epidermis is desquamated as an eschar or "art" scab. The remaining graft is slowly deposed by a process of ingrowth of the host epidermis undermining the pad, by revascularization and clearance of graft debris, and by macrophages and multinucleated giant cells. The surrounding host tissues demonstrated reepithelialization from the wound margins, hyperemia and hyperplasia of the host tissues, fibroblastic activity and revascularization. An important aspect to note is the appearance of a multitude of eosinophils at the site of host regeneration and graft displacement. Ultimately, none of the original homograft remains.

TYPES OF GRAFTS

Many variations on grafting have been attempted. The first was the autograft which is simply the condition in which the donor and the recipient are the same individual. This is presently the only type of graft which results in a permanent survival of the graft. The homograft, with which this paper is concerned, is the transplantation of tissues between individuals of the same species. We will discuss a few considerations involved in this relationship below. The remaining type of graft in this classification is the heterograft which has experimental application but is otherwise confined primarily to myth and the imagination of writers who conceive of the animal combination found in literature. (4)

One consideration is the anatomical sites utilized in the transfer of grafts. Grafts which are transplanted into corresponding anatomical sites are termed orthotopic grafts; whereas if they are also transferred into exactly corresponding areas, they are termed isotopic grafts. In other words, skin transferred to other areas of skin are orthotopic grafts. If skin from the right thigh of one animal is transferred to the right thigh in the corresponding area of another animal, it is an isotopic graft. The grafts which are transplanted

heterotopically are those which are transferred to foreign anatomical areas. (4)

Longmire (18) distinguished between homovital and homostatic grafts. The homovital graft is one which must be living at the time of transplantation and remain so, whereas the homostatic graft is one which does not have to be living at time of transplantation in order to serve a useful purpose. Among the former, endocrine glands, skin and kidneys may be considered examples; bone, cartilage and vessels are examples of the latter.

Another distinction made in the science of tissue transplantation is whether or not the donor and recipient exchanging homografts are related. If the two are related as mother and son, brother and brother, etc., the homograft is noted as a syngenesiotransplantation. If this relationship does not exist, it is known as a homoiotransplant.

One more point of differentiation must be observed as it is of particular importance to experimental studies. This is "within" strain and "between" strain homografts. The "between" strain graft is essentially a homograft. The former is essentially an autograft with the exception noted by Eichwald and Silmser. (34) By means of judicious breeding, animals can become so similar in genetic background that they react as if identical

twins. Eichwald and Silmser noted, however, that rejection occurred among males and females of the same strain, whereas takes occurred among these animals if of the same sex. They thus postulated that the rejection must occur on the basis of the y-chromosome which is possessed by only the male members. (34,52)

EARLY CONCEPTS

Three concepts have been in the forefront in an attempt to explain the homograft rejection phenomenon. These were mentioned by Gibson and Medawar (8) in 1943 and more precisely summarized by Medawar (19) a decade later. In his classical work in 1944, Medawar also makes passing mention of these concepts.(7)

The first of these theories related to blood incompatibility. Underwood (11), in 1914, stated that the more closely aligned the blood groups the more likely the survival of the homograft. Davis (5) stated that by judicious blood grouping successful homografts could be obtained. However, in Medawar's earlier work and in his studies conducted in 1946 (21), he discounted the contention that blood compatibility was an important factor in the rejection phenomenon. More exhaustive research in which Medawar collaborated with Brent and Billingham (20) bore out these previously expressed opinions of Medawar.

Great difficulties arise in experimentally excluding the possibility that blood incompatibility does not play a part in this phenomenon, however. The enigma arises due to the factor of the unknown. How can we be sure that we can completely type blood groups? How can we know that due to lack of information, techniques, or detection methods we have uncovered all the factors? The answer is, of course, that we cannot. Gorer (22), as early as 1955, cited evidence which contradicts the earlier work of Medawar et al. In his experiment, Gorer used two combinations of donors and recipients--Balb C. skin on C57 black, and A strain skin on C3H. He also used two types of skin grafts, full thickness and pure epidermal cells prepared according to procedure proposed by Billingham and Medawar. Refer to Table 1.

TABLE 1

The Antibody Response of C57 Blacks to Full Thickness Skin Homografts from Balb. e. (Red Cell Agglutinins)

Red Cells of Strain	A	Balb.C.	C57	C3H
After first Graft	64	16	0	0
After second Graft	1024	128	0	0

Titers are expressed as Reciprocals Adopted from (22)

The author assumes the higher titers obtained with A cells reflect their greater antigenicity. It is also interesting to note the greatly increased response following second grafts. For his tests he used the pooled sera of 3-5 mice stored at -20°C. In this study, a positive antiglobulin reaction to her father's red cells was obtained from the serum of a severely burned girl who had received homografts from her father. These studies merely point out that complete agreement is not yet reached on this subject. However, the weight of experimental study and opinion tends to reduce the importance played by red cell isoantibodies and blood compatibility factors in the homograft problem.

Loeb is the most predominant figure in the theory that a genetic-cellular basis is responsible for the homograft rejection. He is greatly concerned with individuality in relationship to the homograft problem. He was concerned with natural and inherent immunity.(23) "In this system there is one particular substance which characterizes an individual, in contrast to a larger unit, the species."(23) "The character of the individuality differential is determined by and representative of the set of nuclear genes of this individual."(23) His "individuality differential" predicated a natural or inherent type of immune response in contradistinction to

the acquired immunity proposed by Medawar.(7) However, it must be remembered that antigenic differences are gene determined. Some have gone so far as to say that the homograft rejection may revolve around the ribonucleic acid in the cytoplasm of the homografted cell which provides antigenic stimuli to the host for the production of the destructive antibodies.(25) These two theories, that of acquired or inherent immunity, are not entirely compatible; eventually they may even prove to be complementary. The correlation between these two is emphasized by Burnet and Fenner who state: ". . . it has long been realized that the basis of the differences between host and donor tissue are genetic."(24) This concept, however, encompasses an even more basic subject and its continuation will not be followed in this paper.

The next theory and the one in which this paper is most concerned is the one propounding an acquired immunological basis for the phenomenon of the tissue homograft rejection. Gibson and Medawar (8), in 1943, and Medawar (7), in 1944, stimulated the work and research along this line. Gibson and Medawar stated ". . . that resistance to homoplastic grafting is <u>systemic</u> and primarily <u>humoral</u> in nature, and that in one form or another it follows the general pattern of an antigen-antibody reaction."(7) Medawar (8) draws the line

between his theory and that of Loeb's. "Immunity in this technical sense is said to be 'innate' when, as with blood antigens of A, B, O group, the corresponding antibodies are ready made; 'actively acquired' when the antibodies are manufactured de novo in direct response to an antigenic stimulus; and 'passively acquired' when antibodies are transferred in suitable form from one animal to another." Although Medawar was the first to specifically, directly and factually emphasize the actively acquired immunity theory, there were many before who had intimated at this relationship and who had provided much work and research to lay the foundation for Medawar's and subsequent work. Medawar (26) reconfirmed his work in studies one year later. Medawar, himself, noted that his theory was generally disapproved of by most workers.

"The great majority of students of tissue homografting have consistently and systematically denied that immune phenomena play any significant part: they claim that a second dose of homografts, transplanted when the reaction to a first is complete or at its height, provokes a reaction neither more rapid nor more intense than the original."(8)

In order to establish his theory, Medawar first attempted to demonstrate the "second-set" phenomenon. Skin was transplanted to a group of ten rabbits. Sixteen days

later a second series of homografts using the same donors and recipients was done. Medawar found the second-set homograft swollen by the fourth day. He also noted that these grafts were vascularized. These channels were stagnated by the fourth day with disruption, either complete or partial, of their endothelial linings. Due to the rapid disruption of the vasculature, no host leukocytes invade the graft via the vessel walls. Instead, there is only penetration to the degree which is observed in any non-specific traumatic inflammation. The graft demonstrated complete breakdown by the eighth day. Others who have worked on the second-set phenomenon since that time have confirmed this phenomenon.(27,28,29,30,35)

Medawar (8) also demonstrated the effects of dosage on the transplantation of skin. He transferred what he termed "high dosage," "medium dosage," and "low dosage" grafts. He demonstrated that the median survival time of high dose homografts was 10.4 $\pm$ 1.1 days; and in low dosage grafts, it was 15.6 $\pm$ 0.9 days. He also noted that the low dosage grafts had more foreign epithelium at the end of twelve days than all eight of the high dosage grafts had at the beginning.(8) In all, he used ten animals for the low dosage reading, that is, five pairs. Eight were used for the high dosage grafts. Five homografts were used for the medium dosages. This

initial study demonstrated reasonably definitively that a dosage phenomenon exists--at least in rabbits. No such dosage phenomenon has been observed in man, however. Rogers (4) states, "There is apparently neither a direct nor an inverse relationship between skin dosage and survival time of the grafts." We will here also mention the element of time. Rapaport and Converse (37) performed transplants at 21, 22, and 26 days, and found that the second set phenomenon was present. However, when they waited 80 days between the first and second graft, they found that the second graft reacted as though it were an original homograft. They surmised from this experiment that the homograft antigen must remain present in the host in order for the sensitivity and resultant second set phenomenon to occur.(37,4,36,49)

In establishing the immunological basis for the homotransplantation phenomenon, it was important to demonstrate specificity which is a basic characteristic in immunological reactions with few exceptions.(38,39) Such a demonstration would greatly strengthen the acquired immunological theory and the position of its adherents. Medawar (8) again was a pioneer in its demonstration. He found that the reaction was associated only with the graft and not with the host.(8) Recently a graft against host mechanism has been proposed but this will be

discussed later. He showed in a series of three animals that autografts which replaced second set homografts at the site of transplantation in no way differed from primary autografts. Carrying this further, he provided an animal with a mosaic of autografts and homografts alternating them upon the site of transplantation. He observed that without exception the autografts survived and the homografts were rejected. In order to further substantiate the specificity of the homograft rejection phenomenon, he transplanted grafts from two individual donors to one recipient. Although his experiment in this regard was not fully adequate, it did demonstrate with some evidence that both grafts reacted as primary, first set high dosage homografts. This work has been confirmed by Snell, Gorer, Scothorne and Tough along with many others.(25,22,42,40,41,58)

Having established the nature of the homograft rejection, having described it, and having observed many of its various characteristics, the search for a means to alter the homograft response was begun. The importance of establishing a means to modify, ameliorate or completely suppress this rejection is the very substance and heart of these studies, for it is by these means that the homograft may become a practical clinical tool. Among the important concepts in attempts to alter rejection is

that of acquired tolerance.(20,43) Billingham, Brent, and Medawar (43,59) defined acquired tolerance: ". . . as an induced state of specific non-reactivity towards a substance that is normally antigenic--a non-reactivity, moreover, that is due to a primary failure of the machinery of the immunological response." The key words in this definition are "specific" and "antigenic." By including the term "specific," such means of modifying the rejection phenomenon as x-rays and cortisone are excluded for they are not specific in the sense that the rejection phenomenon is suppressed to all tissues by use of this method. "Antigenic" is important for it excludes such phenomenon as syngenesiotransplantation and the acceptance of parenteral grafts by F, hybrid mice. It should be emphasized that this alteration in the immunological reaction is a central failure of the immunological response and not a peripheral one as is the neutralization of toxin by antitoxin.(43)

The impetus for this investigation was an observation by Owen (44) on cattle that dizygotic twin cattle contain red blood cells of dual origin due to the interchange of erythrocytes in fetal life through the synchorial anastomoses. Dizygotic cattle have synchorial circulation, that is, a mixing of placental blood through vascular connections in the placenta. Others have shown that synchorial circulation does

exist in dizygotic human twins but only rarely.(49) Here the term chimaera is introduced which I have referred to in the Introduction. This expression infers a dual or multiple origin of an individual's composition.

In 1951 and 1952, Billingham, Anderson, Lampkin, Williams, and Medawar (51,52) demonstrated that chimaerical dizygotic twin cattle accepted skin homografts in contradistinction to sibling cattle of separate birth who did not do so but reacted as expected. Tolerance is induced by exposing animals to living cell antigens in the period of development before they are capable of responding immunologically due to the functional differentiation of their immune mechanism.(59) The time when it is possible to induce tolerance varies with the animal species involved. Woodruff (58) has shown that the dividing line is not sharp but provides the following times: for mice, 1-2 days before birth; for chicks, hatching; for rabbits and sheep, well before the end of intrauterine life; and for rats, on the day of birth and occasionally up to two weeks old.(76,46, 77,78). Woodruff and Simpson made an earlier report in 1954 on the induction of tolerance in rats in the Proceedings of the University of Otago Medical School. It has been shown that specific immunological tolerance to

skin homografts could be established in dogs subjected to exsanguination-transfusion in the first day of life with blood from the donor.(50,55) It is generally agreed that immunological tolerance is still induced only with living cells that can repopulate the host. (51) In other words, only those tissues capable of producing immunity in adult animals are efficacious, in the establishment of the state of acquired tolerance in the embryonic mammal.(54) Egdahl has named the period when an animal neither develops tolerance nor immunity to an encounter with foreign antigen, a null period.(56) He showed that this period in rabbits was between 3-15 days. During this period the rabbit would be exposed to foreign antigen. With later encounters with this same antigen, the rabbit would react as though he had never been exposed to the antigen. Before this period a state of tolerance would be induced, and following this period a specific immune response would occur. This null period was the same critical period observed by Woodruff.(53,63,64) Another synonym for this null period is the neutral period (46) introduced by Billingham and Brent when they found that tolerance could be produced in mice by the intravenous injection of CBA splenic cells into four-day-old mice, but resistance was encountered on the seventh day and a period

of neutrality lay between. Acquired tolerance is immunologically specific. This can be demonstrated in a number of ways. A mouse made tolerant to one individual donor will reject the homotransplants of all other donors while accepting a graft from the first. Another means of supporting this theory is to inject lymph cells from an immunized mouse into the tolerant host. The recipient and donor of the lymph cells must be of the same strain, otherwise the injected cells would be rejected as any other homograft. By this means, the previously tolerant mouse will reject the graft which it had heretofore been supporting. The graft maintains its original specificity. It is interesting to note that rejection will also result from the transfer of lymph cells without previous immunization of the donor by skin from this donor, albeit more slowly. In the former case, a state of immunization can be reinstituted in ten days, while twenty to forty are required in the later case. From this some important inferences can be formulated. The tolerated graft must be a source of continual antigenic stimulation in order to precipitate a rejection reaction through the transplanted cells.(4,66,65,68) It also must be assumed that the injected normal lymph node cells are capable of responding to this antigenic stimulation, thus resulting in the production of antibodies to the homograft

and its ultimate rejection.(67-70) From the above, one can see that acquired tolerance is a specific immunological phenomenon due to central failure of the immune mechanism which does not interfere with the other aspects of immunity--as Medawar calls them, the efferent and afferent "side of the immunologic reflex mechanism."(4)

ENHANCEMENT

Enhancement is a phenomenon which can be achieved in one of two ways. A recipient may be injected with lyophilized normal or tumor tissue or tissue extracts from the donor. The alternate method is to inject into the recipient specific antisera to the transplanted tissue. Circulating serum antibodies are central to the phenomenon of enhancement. Medawar and Snell suggest that these circulating antibodies may combine with or otherwise inactivate the antigens which cause transplantation immunity.

The first experimental studies concerned with the phenomenon of enhancement were done using tumors.(72,73, 74,75,76) Billingham (71) provides a definition of enhancement as ". . . the induced acceptance of a tumor homograft in a normally resistant host, (it) is the result of pretreatment of immunologically mature animals with desiccates or saline homogenates prepared from the living homologous tissue. It depends upon an active

immunological response on the part of the host--by the formation of hemagglutinins--to antigens of the red-cell type in the innocula."(71) He points out, as do others, that this stimulation by the antigen is incomplete.(71, 78,79,81) It is incomplete in the sense that living cells are not comprised and it does not shorten the life-time of the tumor homografts which are transplanted following the establishment of enhancement. The injections must be administered before the tumor is transplanted or before it makes sufficient connection and exchange between the host. This mode of enhancement is best accomplished by multiple injections rather than with single administrations.(77,81,4)

It is also a characteristic of enhancement that it is specific.(79,82) This has been demonstrated with the use of isogenic strains that differ from one another by only one gene.(82,80) What, then, are the essential differences between acquired tolerance and enhancement? As mentioned previously, the acquired tolerance is due to a central failure of the immune mechanism, whereas the enhancement phenomenon depends on an active immunological response. It has been pointed out by Kaliss (80,82) that the greater the antibody titer, the longer the survival of the homograft. Billingham (71) recognized that tolerance may be complete with no selectivity of the

type of graft, whereas enhancement is only complete in the case of tumors. The materials used to procure enhancement have no power to elicit an immune response (4) which is also in contradistinction to the substances used to establish acquired immunity which can also be used to develop acquired tolerance. It has been conjectured that the phenomenon of enhancement is primarily a desensitization process (81) but as yet this is only a hypothesis. This aspect awaits further development in relationship to the similarity of the tuberculin reaction and the homograft reaction which will be discussed in more detail below.

Adoptive immunity is closely related to many other facets of the homotransplantation problem. Careful separation of the terms adoptive and passive transfer must be accomplished. Passive transfer of immunity is performed by taking already present antibodies and injecting them into the host. By this means the host is supplied with antibodies not of its own production which are capable of reacting with and rejecting the homograft. Adoptive immunity (71,86,84,85,83) is transfer of cells actively made immune which can respond in their new host to the assault of the previous antigen. That is to say, cells capable of actively producing antibodies are transferred to a non-immune animal rendering him immune.(86) "It

depends upon the introduction and continued functioning of immunologically activated cells."(71) The cells capable of producing adoptive immunity are those of the lymph nodes, particularly those draining the area of the site of transplantation, those receiving the regional lymph supply.(87,85,83,90,88,89,66) It has been shown that these regional lymph nodes increase in size. Lymph nodes were measured and weighed and a net increase of 247 mgs. was noted in eleven days in a reported case. (91). Stark et al. (91) refer to enhancement of the homografts' survival by removal of the regional lymph nodes, thus delaying the immune response and the subsequent rejection. I think that enhancement is not the word to use here for, as defined above, enhancement is the result of an active immunological response, whereas this procedure is an interference with the afferent mechanism responsible. In this same paper, a woman, who had undergone a radical mastectomy with resultant lymphedema from the destruction of her lymph drainage, was autografted and homografted, using test sites. A two and one-half times prolongation of survival time is recorded on the homograft on the arm with the obstruction. Scothorne (92) had shown earlier that the lymph nodes in the area of the homograft demonstrate anatomical, histological, and cytological modifications which are also present in

the classical immunological states. The spleen also plays a role in adoptive transfer of immunity for it is possible to establish this immunity with spleen cells just as it is possible with lymph cells.(86,88,90,92) However, the spleen undergoes no characteristic changes.

There are many other instances in which the homograft rejection does not occur or in which it is altered. These include monozygotic twins, uremia, extensive burns, agammaglobulinemia, radiation and cortisone administration. These shall be considered individually in the following paragraphs.

It is known that monozygotic twins can exchange homografts which will react exactly and in every manner as an autograft.(96,93) It is equally well known that dizygotic twins will reject homografts.(94,95,97,98) This needs little comment in this paper. The exact genetic background prevailing in monozygotic twins obviously accounts for their mutual acceptance, whereas dizygotic twins are no more similar than other brothers and sisters and react accordingly. In reality, grafts exchanged between identical twins must be considered autografts.

Damin and his co-workers (99) were interested in determining the effects of uremia on skin homografts. This interest was aroused by their knowledge that skin and kidney are antigenically similar and that kidney function is

prolonged in those animals suffering from uremia.(100) They found that skin homografts were prolonged in patients suffering from uremia. In their studies, they worked with seven patients who had uremia of four to seventy-two months duration. Autografts were also performed as a means of control and comparison. In this work, the survival time of homografts was increased in uremic patients. No explanation of this phenomenon was provided, nor is one available today. Its relationship to immunology, if any, must for the time being remain obscure and unanswered.

Since Underwood's (11) report on his experience with an extensively burned patient, it has been recognized that severely burned human beings tolerate skin homografts better than would ordinarily be expected.(104) Again, an explanation for this phenomenon is not readily forthcoming. An interesting aspect of this problem is related to the size of the antigenic dose administered. (67) It may be that the antigenic dose is so large that a state of immunological paralysis results. This immunoparalysis is predicated on the theory that the dose of antigen is so great as to consume all available antibodies and exhaust the body's capacity for further production.(101) Another possibility is that the stress resulting from the shock of severe and extensive burning

with the resultant increased output of adrenal steroids may account for the prolongation of homografts applied to burned patients. In no case is this a permanent survival. Again, a definitive solution to this question is not available and its exact relationship to the immune mechanism is uncertain.

The next condition I wish to discuss, agammaglobulinemia, has a definite relationship to any immune mechanism that may be at work in homografting. O. C. Bruton (103) reported his work and discovery of agammaglobulinemia in 1952. This disorder is characterized by a triad of findings--absence of gamma globulin in the blood, failure of antibody formation regardless of the strength of the stimulus, and an increased susceptibility to infections. Since the original delineation of this disorder, three distinct forms of agammaglobulinemia have been defined. Transient agammaglobulinemia of infancy, acquired agammaglobulinemia, and congenital agammaglobulinemia are the three recognized forms. The first form is probably the most frequent, but also the least useful from an investigative standpoint in homotransplantation. The following two types are about equally prevalent. Good et al. (102) prefers to call agammaglobulinemia, hypogammaglobulinemia because of his convictions that all these patients do have detectable quantities of gamma

globulin but in varying degrees of concentration and of minute quantity. Good et al. (102) list five immunological failings found in these patients. They are: a failure of response to ubiquitous antigens, a failure of immune response to potent bacterial antigens, an absence of so-called natural antibodies from the circulation, a failure of immune response and absence of toxic reactions to the intravenous or intramuscular injection of mismatched blood and a failure of immune response to potent viral antigens. These above findings demonstrate a rather complete lack of any immunological response by these patients, and thus emphasize the interesting applications of experiment and study agammaglobulinemic patients present in the study of any possible immune mechanism in homotransplantation. Good et al.(102) found also that hypersensitivity was absent in these patients. Of special interest were Good's hematological and lymph node findings. A hypersplenic picture is presented in the blood with added emphasis by the relief of these symptoms following splenectomy. The lymph nodes were found to be small with a thin cortex, poorly developed lymphoid follicles and a medullary structure which contained few cells. Although the lymphocytes appeared to be normal, plasma cells were found to be absent from the lymph nodes. From the above it becomes apparent that the agammaglobulinemic individual has in

actuality three deficiencies, only one being the lack of gammaglobulin. He also lacks the ability to produce plasma cells and antibodies. What relationship and interaction gamma globulin, plasma cells, and antibodies have with one another is not entirely certain. With the above information in mind, Good et al. (102 proceeded to perform homografts with agammaglobulinemic patients serving as hosts and donors. Donor agammaglobulinemic grafts were universally rejected, whereas they themselves accepted homografts from normal donors. Following the successful homotransplantation of these patients, immunological responsiveness was retested to determine whether or not these grafts could provide some amount of immunological competence to these otherwise severely handicapped individuals. Only three patients were so tested and in no case was any antibody formation demonstrated. Another test was conceived to determine whether or not there might be a substance in the blood or tissues of agammaglobulinemics which might have an inhibiting effect on the production of antibodies in normal individuals. In order to determine the validity or non-validity of this hypothesis, normal persons were injected with the serum of agammaglobulinemic subjects being tested prior to and subsequent to these injections for immunological competence. No evidence was derived which supported the

hypothesis of an inhibiting factor in the serum of agammaglobulinemic individuals. It was also shown that the injection of leukocytes subcutaneously or intravenously from previously sensitized normal donors readily transferred bacterial sensitivity. However, the transfer of leukocytes during a state of active antibody stimulation by subcutaneous and intravenous routes produced no detectable amounts of antibodies in the agammaglobulinemic subject. Continuing with the studies of these patients, whole blood was transfused into these subjects to observe the possibility that some as yet unknown blood factor might be absent which was responsible for the immunological unresponsiveness. Again, no such evidence was uncovered as witnessed by the continued state of immunological incompetence. Injections of gamma globulin alone did not establish immunological responsiveness either, although blood levels were raised to nearly normal levels. These injections provoked no formation of plasma cells. This and subsequent experimentation demonstrating the survival of homografts in agammaglobulinemic subjects lend strong support to the immunological basis propounded for the homograft reaction.

The effects of x-irradiation has been another field pursued in an attempt to establish a definitive basis for the homograft rejection phenomenon. In 1947, Rabinovici

(108) irradiated mice with 500 r and then performed homotransplants to determine what effect, if any, radiation had on survival. He found no difference in the time of survival in those irradiated as compared to survival time in normal, non-irradiated mice. However, contrary results have demonstrated prolonged survival time of skin homografts following radiation using 250 r and 300 r (110,111) This work was expanded by radiation experiments followed by bone marrow transplants. Main and Prehn (105) irradiated mice with 800 to 850 r and then injected bone marrow. These man-made chimaeras were then homografted from the same donor strain as the source of the bone marrow, and it was found that rejection did not occur. "It is now firmly established that the lethally x-radiated rat or mouse, resuscitated with myeloid tissue, lives in virtue of borrowed bone marrow." (109) This was also demonstrated by Owen, et al. (106) Radiation alone will result in prolonged homograft survival but ultimate rejection occurs. It is known that radiation drastically reduces, and may abolish, an animal's ability to manufacture antibodies. (107) Thus this is further evidence of the important role of an immune mechanism in the homograft response. A recent study by Makinodan (112) demonstrated that the antigens responsible for bone marrow transplantation immunity were present in the nucleated

cells and absent from the erythrocytes and serum. This study further localized these antigens to the nucleus, mitochondria and microsomes. Here, again, depression of immunological responsiveness with the administration of donor antigens into the unresponsive host produces a state of acquired tolerance.

Cortisone and the steroids have also been used in an attempt to induce permanent survival of skin homograft. It has been demonstrated that cortisone does prolong the acceptance time of a homograft by a treated recipient.(113-115) This may even be accomplished by a local application of cortisone. Toolan (114) reported that human cancers could be induced to grow in mice conditioned by the administration of cortisone. Compounds F and E, cortisol and cortisone respectively, are the only two steroids found to be capable of suppressing the response to tissue homografts. Cortisone has many reactions and it can only be surmised that its ability to reduce antibody production is the elemental cause of the resultant prolongation of homograft survival time. In no case is permanent survival obtained.

Recently it has been observed that the homograft reaction is altered in both Hodgkin's disease (117) and by the use of 6 Mercaptopurine. (116) Kelly et al. were interested in the possible effects of Hodgkin's disease

on homografts by reports that delayed hypersensitivity was not manifested by patients' suffering from this malady. This curiosity was apparently justified, for they found a protracted, delayed and incomplete rejection of homografts in these patients, and in certain cases a complete non-reactivity to the graft. It is presently too early to evaluate the importance of these findings, but like agammaglobulinemia, this disorder presents a valuable opportunity to further investigate the homograft reaction. Meeker et al. (116) investigated the effect of 6-mercaptopurine on homografts. These workers administered 6 mg/Kg/day of 6-mercaptopurine to rabbits. They found that homograft survival was substantially prolonged by this drug. They could not correlate the amount of leukopenia produced with the condition of the homograft. Due to the toxicity of the drug, the maximal effect of this drug could not be absolutely established. Many of their experimental animals succumbed before the homografts had been rejected. They also noted that 6-mercaptopurine had no effect on the prolongation of homografts in mice. This is very similar to the findings with cortisone in that the species used is important with a wide variation in reactivity and effect of the drug. Another interesting aspect of this work was the greatly reduced toxicity of the drug apparent in grafted animals

as compared to the high degree of toxicity in normal, ungrafted animals.

Two theories were proposed as a possible explanation of the mechanism of action of 6-mercaptopurine. First they theorized that a direct metabolic interference with the homograft rejection mechanism was responsible and second that an indirect effect operating through the cytoxic action of the drug might be the mechanism. They favored the first explanation. Other related compounds were also tested and none were found to be effective.

NATURE OF TRANSPLANTATION IMMUNITY

Having examined the homograft reaction thus far, it becomes necessary to ask just what type of reaction is this homograft rejection. What are the antibodies responsible? Is it a classical type of immunological reaction or is it sui generis? In beginning this examination of the nature of the homograft reaction, I would first like to examine its relationship to tuberculin type reactivity. Tuberculin type of allergic response is also called a delayed hypersensitivity of the Koch phenomenon.(39) As Lawrence (121,119) points out, other responses may have aspects in common with delayed reactivity, but this does not make them of the tuberculin

type. Favour (122) summarizes the present knowledge of the tuberculin reaction in his paper at the second tissue homotransplantation conference. First, tuberculin is taken up by selected host cells. The segmented neutrophil plays a prime role in this action. Lymphocytes probably also take up tuberculin. Although certain cells within the skin may also be able to take up tuberculin, it is uncertain that any other body cells are capable of localizing this substance. Lymphocytes are injured per se because of their ability to shed a plasma factor, but the neutrophils are injured only if the plasma factor is already present in the blood or when they become closely associated with sensitized lymphocytes. Usually lymphocytes are intimately associated with injured cells. The circulating blood contains the plasma factors only transiently and in small quantities. Favour describes the tuberculin-type reactivity with this statement. "To a large extent, the tuberculin type reactivity created at other sites in the host, such as by tuberculin testing, may represent a passive transfer of reactivity within the host. Apparently, cellular and humoral components in the blood at any one time do not appear to be a direct measure of the progress of the local tuberculous infection that has sensitized the host."(122) An interesting observation

in regard to the relationship between tuberculin-type reactions and the homograft rejection is that animals which show poor tuberculin responses reject grafts quite as readily as do other more tuberculin active species. Now, in composing the two types of immunological responses, we see many comparisons and similarities.(119) First, both require intact materials to induce sensitivity--the intact bacterial cell and the intact tissue cell. There is a latent period of 10-14 days and 10-12 days in the tuberculin and homograft reactions respectively. The Koch phenomenon and the accelerated rejection of the homografts are quite similar. The time of reactivity in initially inoculated guinea pigs is 10-14 days, but upon reinoculation after a period of six weeks, a highly exaggerated, severe and rapid response occurs in 24-48 hours. This is with tuberculin. This is in parallel with the second set phenomenon of skin homografts. Both demonstrate a variable amount of presence of antibody in the serum. Also there is no correlation between the degree of sensitivity and the quantity of antibody in the blood. In both cases, sensitivity cannot be transferred with serum but can be transferred with cells. A point of difference does exist. Antigens demonstrate no cytotoxicity for explanted cells in the homograft rejection sensitivity, whereas this cytotoxicity does exist

with the tuberculin type. Another point of similarity is the specificity displayed by each. It should also be remembered that the delayed tuberculin reaction is essentially a cellular type of phenomenon.(120) In a recent paper, Rauch and Favour (124) report the transfer of tuberculin sensitivity by plasma fraction. This reactivity lasted two to three weeks.

Many sites in the body will accept homologous grafts without rejection due to an inaccessibility of these sites to the lymphatic and vascular networks essential to establish an immune state. The brain and the eye are two such examples: the former due to the poor lymphatic supply to the eye and thus interference with the efferent mechanism, and the latter due to the inaccessibility of the area to the necessary constituents of the immune mechanism, afferent interference. This led Algire et al. (123) to attempt to duplicate certain of these conditions in order to prolong homograft survival. Using this method homografts in contact with host tissue and homografts isolated by millipore chambers were transplanted to animals. Those not protected by the chamber were rejected, while those provided with the millipore safeguard survived indefinitely. In these experiments the host cells were excluded from contact with the graft. In order to determine if cellular

contact was the prime factor and not exclusion of some other unknown serum component, cells were introduced into the diffusion chambers. If cells from non-immune hosts entered the chamber, no reaction occurred; but if cells from a previously immunized animal entered the chamber, homograft rejection ensued. It was also demonstrated that any cytotoxins could pass freely through the pores of the chamber. Algire, himself, best summarizes his work.

"So far as one can decide from histologic observation, it appears that lymphocytes are involved in the destruction of the homografts, that intimate contact is required, and that both lymphocytes and target cells are destroyed when this occurs. Accordingly, everything necessary for destruction is present when a washed suspension of spleen cells is combined with target cells in diffusion chambers in vivo, and nothing is required from an immunized homologous host. In conclusion, the results of these experiments are in agreement with the hypothesis that cytotoxins to homograft are transported to cells."

However, circulating antibodies to homologous tissue has been frequently demonstrated.(125) There are four types reported: erythrocyte agglutination, leukocytic agglutination, a protective or tumor-neutralizing effect,

and a cytoxic effect. Stetson and Jensen (125) present evidence contrary to Algire's early findings that antibodies are incapable of producing homograft rejection. They attribute Algire's failure to obviate this possibility to inadequate immunization of the animals utilized. He also postulates a "blood-graft" barrier which is capable of isolating the graft from destructive antibodies. This barrier must be destroyed for homograft rejection to occur.

GRAFT VERSUS HOST

In 1953, two workers proposed the hypothesis that a graft competent to produce immune responses might react against the host.(126,127) The implication this hypothesis has in relationship to an immune mechanism theory of transplantation rejection is obvious and thus necessary to explore. This reaction has gone under various names, as the "runting syndrome," "secondary disease," and "homologous disease." Runt disease is characterized by an almost complete absence of lymph nodes, by a discolored, fibrotic spleen normal in size but with precipitously decreased malpighian corpuscles, and retarded growth often resulting in death.

Two hypotheses have been proposed to account for this condition in animals grafted with immunologically

competent tissues. The first, and an inadequate hypothesis, was that pathogens to which these strains had become highly susceptible were transmitted to the recipients along with the graft. The other proposed explanation is much more plausible and is supported by considerably more experimental data than the former. This theory states that the cells transferred to the recipient are capable of reacting against antigens in the homologous tissues of the hosts themselves, thus forming an immunological mechanism active against the host. Here considerable difficulty in terminology arises. Who is to be designated the "host" and who the "donor"? As is so common in this field, a great deal of controversy exists as to the substantiation of this hypothesis and complete accord is not yet reached.(128) However, this work again seems to follow a trend in the field of homologous tissue transplantation which supports Gibson's and Medawar's original hypothesis in 1944 that the homologous tissue reaction is an immune response mediated through an immune mechanism.

SUMMARY

Summation of the above facts related to tissue homografting is not a small task. The knowledge involved includes many aspects not entirely clear at this writing.

However, it will be enough to point out the bare facts of the phenomena and principles involved in homografting.

First, let us examine the three properties of the homograft reaction which identify it as an immunological process. The so-called second set phenomenon was one of the first recognized. This is the accelerated rejection of a second homograft from the same donor to the same recipient. Adoptive immunity is the transfer of immunity to an otherwise non-immune animal through the transference of lymph cell. This is not passive in that the lymph cells are actively producing antibodies and it is not active in that the recipient must be provided with external cells to develop the immunity, thus the term adoptive. In order to provide this adoptive immunity, the lymphoid cells must have been previously activated and the transferred cells must be compatible with the host or they too will be rejected. The third principal factor in the substantiation of the hypothesis of an immunological basis for homograft rejection is that of immunological tolerance. Tolerance is produced by exposing animals at early ages as determined experimentally, and as discussed in the paper to antigens, thus producing a state of immunological non-reactivity. The mechanism for this has been explained in the paper.

Enhancement is another important phenomenon observed in transplantation. It can be induced by injecting the intended host with lyophilized tissue from the prospective donor. Adult, mature animals may be used. In this way a host which would otherwise reject a graft can be induced to accept the transplant for an increased length of time. Enhancement depends upon circulating antibodies and therefore depends upon an active response by the host.

Identical twins will accept homografts from one another with the same ease with which autografts may be transplanted. The chimaera has been discussed in the paper. Agammaglobulinemia in a host permits acceptance of homografts for increased lengths of time and occasionally permanently, as does uremia, extensive burns, and cortisone treatment.

The anatomical and gross and microscopic descriptions of homograft rejection were described, as was the acceptance of the autograft. This was all related to a discussion of the normal anatomy of the skin.

CONCLUSION

Presently the great weight of scientific evidence and opinion supports the theory that homograft rejection is predicated upon an immunological response. This paper

supports such a conclusion. As the investigation proceeds more deeply into the phenomenon, the boundaries between genetics and immunology begin to fade and an intimate relationship can be observed. Genetic variation is apparently the basis for this immune response. Medawar has pointed out that this immune response mechanism provides an explanation for strict isolation of fetus from mother during the months of gestation and I entirely agree with this position.

Where will this investigation lead us? Here is an important question which I am sure cannot be definitively answered for no one can predict the turns to come. Will the possibility of homotransplantation become a reality? This is presently a bleak prospect. The complexities are obvious and an effective approach has not been proposed. However, I do not think the prospects are entirely hopeless. To date, I would have to answer the above question in the negative, but I do not wish to blight the future with no alternative. Greater improbabilities have been overcome by new approaches and new ideas in the past, and I see no reason to reject the possibility of a solution to the problem of homografting in the future.

Lincoln Barnett, in The Universe and Dr. Einstein, observed: "In the evolution of scientific thought, one

fact has become impressively clear: there is no mystery of the physical world which does not point to a mystery beyond itself." Perhaps here is the epitome of the difficulty inherent in concluding a scientific review of this nature--few questions have been answered, but many "a mystery beyond" has been projected.

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