1967

Effect of human chorionic gonadotrophin on skin homografts

Harold Kaiman
University of Nebraska Medical Center

EFFECT OF HUMAN CHORIONIC GONADOTROPHIN ON SKIN HOMOGRAFTS

Harold Kaiman

Submitted in Partial Fulfillment for the Degree of

Doctor of Medicine

College of Medicine, University of Nebraska

February 1, 1967

Omaha, Nebraska

ACKNOWLEDGMENT

The author wishes to thank those who assisted in the preparation of this thesis.

He is grateful to the Department of Obstetrics for the use of its facilities and to Warren Pearse, M.D. and Wayne Ryan, Ph.D. for their suggestions and technical advice.

He is most appreciative of his wife Barbara for her patience and understanding throughout the preparation of the manuscript.

Effect of Human Chorionic Gonadotrophin on Skin Homografts

TABLE OF CONTENTS

INTRODUCTION

In 1951 Blair O. Rogers[1] emphasized the importance of understanding and controlling the phenomenon of skin homografting: "One of the greatest problems in surgery today is the homografting of tissues. One of the ultimate aims of restorative and reconstructive surgery should be the wider use of homografted tissues instead of autografted tissues."

Doctor Rogers went on to say that tissue homografting must be perfected before organ transplantation can be properly mastered. This well-organized summary written in 1951 pointed out errors in previous approaches to the homograft problem. He also emphasized the possibility of there being several factors involved in homograft rejection and that they may have a sequential relationship as yet undiscovered. One of the purposes of this thesis is to discuss some of the hypotheses concerning homografts that have enjoyed credulence.

Dempster's[2] thorough discussion of homotransplantation pointed out that "necessity as the mother of invention" was hard at work during World War II. A great need for skin replacements for burned casualties was recognized during the war. It had been discovered that, with the exception of cornea, cartilage, and blood vessels, all homografts undergo complete disintegration. The rejection problem was intriguing and important enough, however, to draw many investigators to work on its solution.

In 1963 R. E. Billingham[3] stated that the "explosive expansion of interest and effort in transplantation immunology has been due to the increasing confidence that a clinically applicable solution to the homograft problem lies near at hand."

In March of 1966, Pirofsky[4] explained that the ability to transplant various tissues from one human to another without the dangers of rejection, runt disease, or paralysis of the entire immune apparatus will offer a new solution to innumerable medical problems. He went on to say that an understanding of the immunologic relationship between the fetus and mother will furnish a major clue towards the eventual solution of the homotransplantation problem.

It is the consideration of immunity, skin grafting, and pregnancy that makes up the core of this thesis.

Valone[5] in 1952 demonstrated that gestation had a beneficial effect on experimental skin homografts in mice. Heslop, Krohn, and Sparrow[6] in 1954 showed a nearly doubled survival time for skin homografts in pregnant rabbits compared to normal males or unpregnant females. Medawar and Sparrow[7] in 1956 investigated adrenocortical steroids, ACTH, and pregnancy concerning their effects on skin transplantation immunity in mice. They concluded that pregnancy had no significant effect on the grafts.

Serr[8] in 1965 returned to the pseudo-pregnancy type of skin graft investigations suggested earlier by Valone. Serr claimed to show significant graft prolongation with various hormones

including those present in largest concentrations during pregnancy, human chorionic gonadotrophin and progesterone.

Nelson[9] in 1966 described an experiment to investigate the effect of human chorionic gonadotrophin on the thymo-lymphatic system in rats. Using doses of human chorionic gonadotrophin extrapolated from the known human pregnant levels, a significant weight reduction in the thymus was demonstrated. No significant weight changes occurred in the spleen, lymph nodes, or in the lymphoid masses in the intestine when HCG was given. After the animals received HCG with estrogens, the thymus underwent acute atrophy with marked reduction in weight.

In the experiments described in this thesis, I attempted to test the effect of human chorionic gonadotrophin (HCG) on mice skin homografts' survival. Human chorionic gonadotrophin was chosen for a test substance because of its importance in pregnancy in maintenance of the corpus luteum. Formation of chorionic gonadotrophin is thought to be one of the most primitive functions of trophoblastic tissue and one of the most important. The extension of the activity of the corpus luteum results in continued production of progesterone and estrogen and prevents the collapse of the endometrial bed to insure a proper nidation place for the zygote. Certainly progesterone and estrogen could have been studied just as well as various combinations of hormones. The choice of HCG revolved around the fact that chorionic gonadotrophin is exclusively found during pregnancy while estrogens and

progesterone are present in the nonpregnant state. Since we are interested in the question of what makes pregnancy different, a hormone unique to pregnancy seemed most important to test.

METHODS

ANIMALS - Ninety female mice of the congenic resistant strain $B_{10}D_2$ and five female mice of the inbred partner strain, C57BL/10ScSn, were obtained from the Jackson Laboratory of Bar Harbor, Maine. According to George D. Snell, Ph.D.,[10] these strains differ only at the H-2^d and Hc^o gene loci. Snell says that the strain pair ($B_{10}D_2$ and C57BL/10ScSn) has the highest available "strength of histo-compatibility barriers" so that skin grafts between the strains have a median survival time of nine days.

Billingham's "parallel recipients" pattern was used in this experiment. That is, several $B_{10}D_2$ mice received grafts from each C57BL/10ScSn donor.

TECHNIQUE OF SKIN GRAFTING

1) Depilate mice with Nair one to two days before grafting. Depilate area from scapula to just above tail.
2) Sacrifice mouse from which grafts are to be taken (donor).
3) Remove tail skin of donor by cutting with #21 Bard Parker blade, as illustrated, and by pulling skin from cephalic toward caudal end.
4) Place tail skin in Petri dish with filter paper bottom and float in sterile saline.

5) Wash blood from tail skin.

6) Cut tail skin to appropriate size (ca. 3 x 4 mm.).

7) Anesthetize depilated mice individually in "ether machine." (See Figure 1).

8) Secure anesthetized mouse on animal board.

9) Swab depilated area with 70% alcohol.

10) With forceps, pinch skin in middle of back and raise.

11) With #15 Bard Parker blade, slice raised skin, removing a small area (ca. 3 x 4 mm.). Be very careful to leave the thin membrane containing blood vessels (panniculus carnosus). If this is removed, there will be excessive bleeding.

12) Rapidly place donor tail skin from the Petri dish to dry filter paper and then to graft recipient area.

13) Dust with Neosporin Powder and tape securely with 3M brand Elastiderm tape, number with Magic Marker.

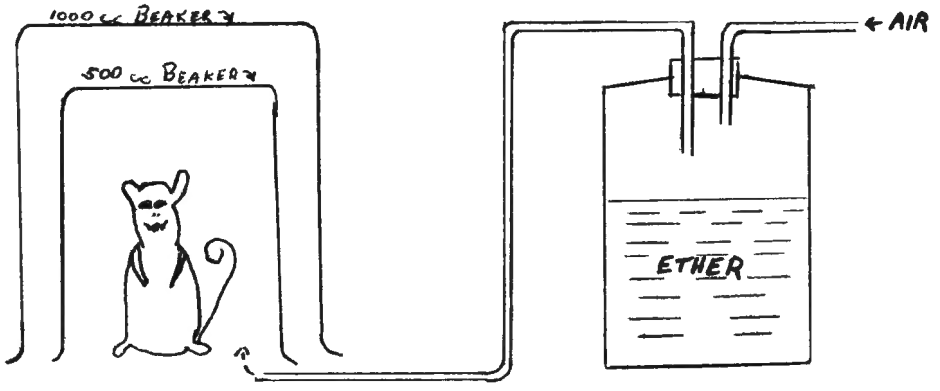

FIG. 1 "ETHER MACHINE"

TEST AND CONTROL ANIMALS

The mice were grafted in random order as they were sedated from their cages and assigned numbers. Each even-numbered recipient was placed in the test group, and each odd-numbered recipient was placed in the control group.

The chorionic gonadotrophin preparation used for the test group was Ayerst's A. P. L., which is biologically standardized in terms of the International Standard. Each unit of A. P. L. represents the specific gonadotrophic activity of 0.1 mg. of a standard preparation held by the National Institute for Medical Research (England) on behalf of the World Health Organization. It was felt that a pregnant human adult female may excrete approximately 800,000 I.U. of HCG per day at her peak in her second month of pregnancy. This excretion of 800,000 I.U. per day in a 50-kilogram female corresponds to approximately 250 I.U. in a 15-gram mouse. When injection of 250 I.U. of HCG was attempted, the mice became quite agitated, their fur stood on end, and they ran wildly around their cages. A dose of 200 I.U. was tolerated much better by the recipients, and this was the daily dose utilized in the test group of grafted mice. The intraperitoneal route was used because it seemed to be less traumatic to the mice than intramuscular or intravenous routes.

Each day new tuberculin syringes and disposable needles were used for the injections. The daily injections began on the day of grafting and ended on the day the graft was completely rejected.

EVALUATING THE APPEARANCE OF THE GRAFTS

Dressings were left intact for seven days, then removed; and the grafts were evaluated by visual examination. The first sign of graft rejection was taken to be swelling of the graft. The color of the graft changed from light to dark pink, through red to shades of yellow and brown. The superficial epidermis seemed to weaken and often fell off, revealing the dampsurface of the graft's dermis. Sometimes the graft's edges disengaged from the surrounding skin. After the epithelium was lost, the graft dermis dried and became a wrinkled black scab. The falling off of this black scab was taken as the end point of graft survival because the earlier changes were too gradual and qualitative in nature for objective observation.

RESULTS

There was definitely a somewhat increased survival of skin grafts given to mice receiving human chorionic gonadotrophin. Table 1 shows that the mean, mode, and median survival times were longer for the group receiving HCG. The standard error of the difference of the two means was calculated and found to equal 0.475 days. The difference between the two means involved in this experiment (13.27 days for the test group and 11.3 days for the control group) is 1.97 days. Thus, a relative deviate can be calculated as 1.97/0.475, which equals 4.21. This relative deviate means that there is a significant difference between the two mean values.

There is actually less than a one per cent chance that the test group and control group would differ this much on the basis of chance alone. Using the principles of statistical logic, we may assume that the difference between these groups is due to the test item, namely the daily intraperitoneal injection of 200 units of HCG.

TABLE 1

	With HCG	Without HCG
Mean day of rejection	13.27	11.3
Median day of rejection	13	11
Mode day of rejection	14	12
Standard deviation	2.23	2.09
Standard error of the mean	0.337	0.335
Standard error of the Difference of Two Means	0.475	
Relative Deviate	4.21	

Table 2 shows that there were significant differences between the test group and the control group on days 9 through 15, as calculated by the four-fold table method of Chi Square determination. Table 2 also includes a comparison of the number of new graft rejections for each day. There appears to be a general but not absolute trend for the control group to have more new rejections daily on days 8 through 12. Then there is a reversal with the test group exceeding the control group on days 13 through 16. Figure 4 attempts to show this concept with the shaded area between the curves approximately equalling the unshaded area between the curves.

Figures 1 and 3 show the differing survivals of skin grafts in the two groups with the test group consistently having a lower rejection rate than the control group.

TABLE 2

Day	No. of New Rejects $\bar{c}$ HCG	$\bar{s}$ HCG	x^2	p	Result
8	2	1	0.222	.60	n.s.
9	1	9	5.57	0.2	significant
10	1	7	12.75	less than .01	significant
11	5	3	10.64	less than .01	significant
12	4	10	19.38	less than .01	significant
13	8	1	7.58	less than .01	significant
14	9	5	6.05	between .02 and .01	significant
15	8	2	5.73	between .02 and .01	significant
16	6	1	All rejected; therefore, cannot calculate (2 zeros in 4-slot table)		

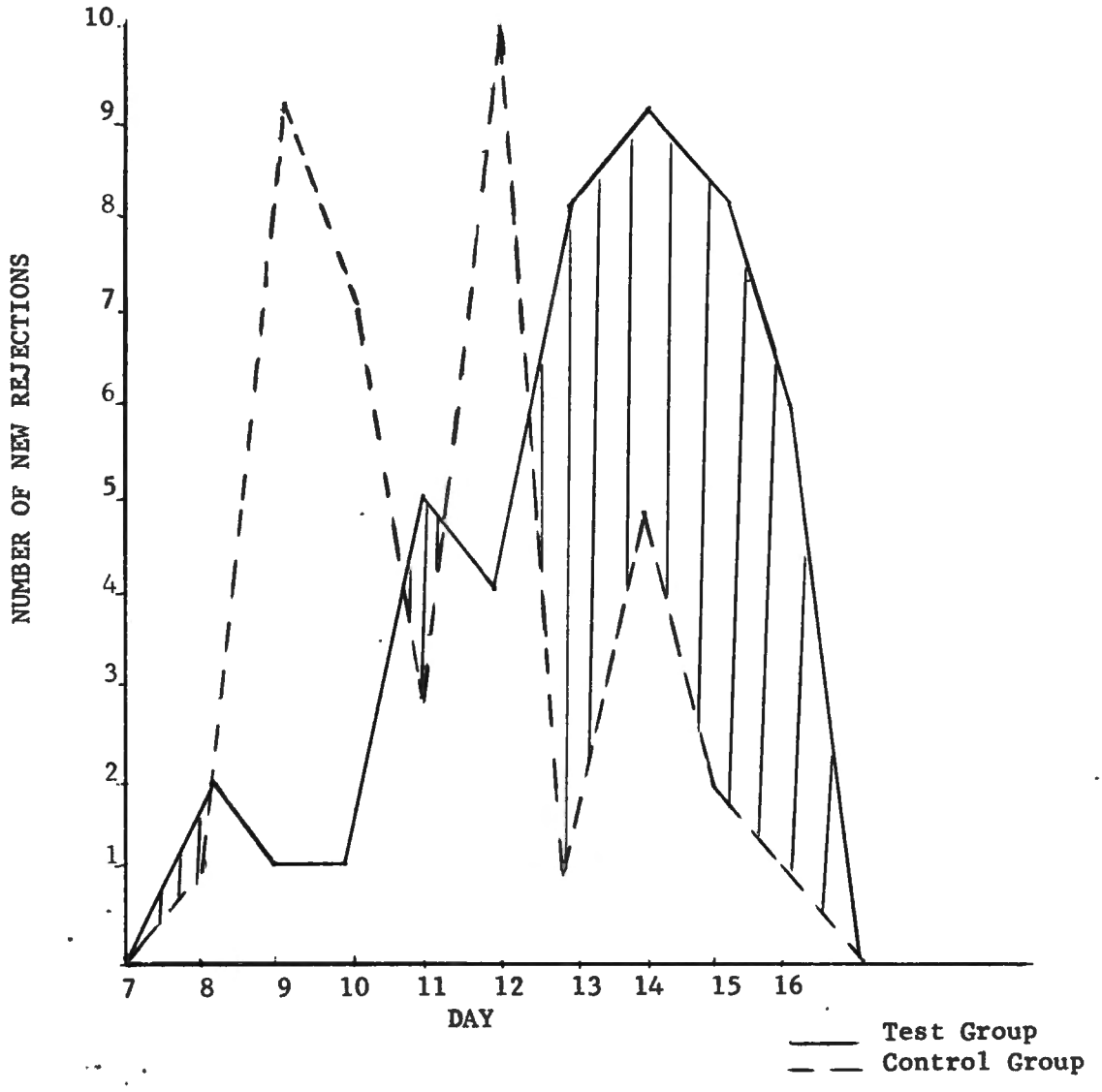

FIG. 2

Number of new rejections per day--comparing test group and control group--showing predominance of control group before day 12 and of test group after day 12.

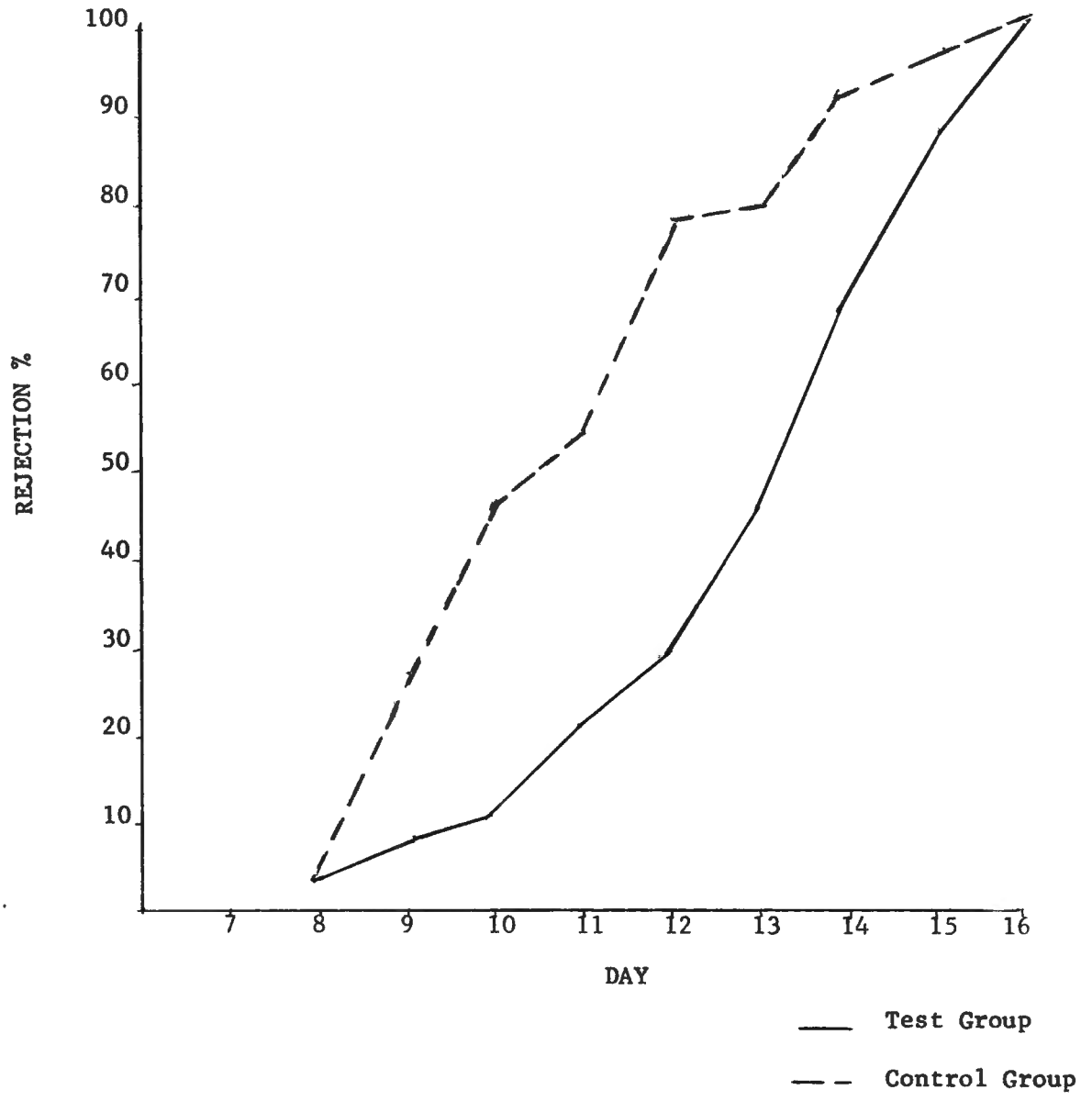

FIG. 3

Per cent of total rejections occurring each day--a comparison of test group and control group--test group tends to reject later.

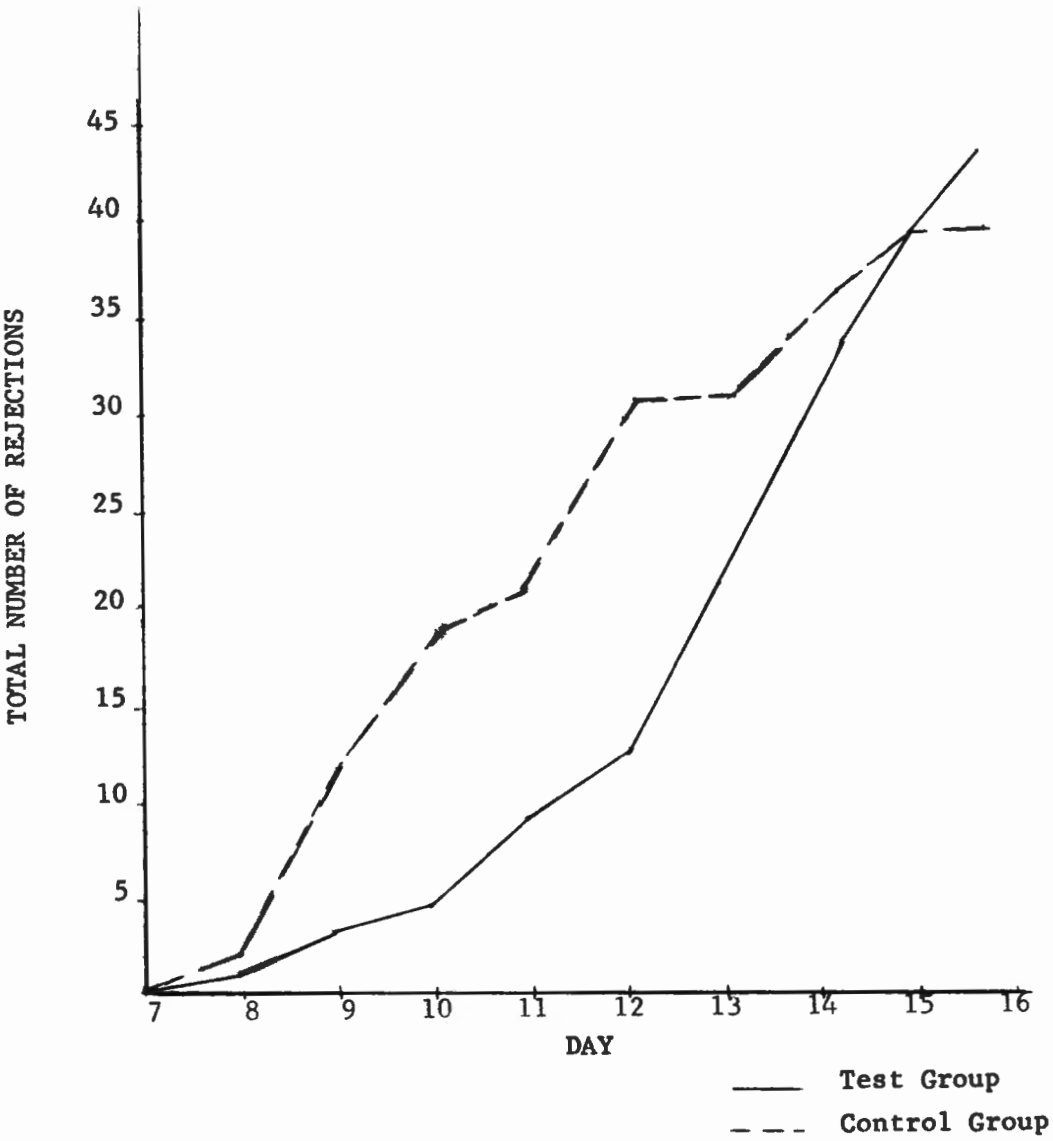

FIG. 4

Total number of rejections accumulated each day--a comparison of the test group and control group showing that from day 8 to day 15 the test group's grafts have longer survival.

DISCUSSION

PAST BELIEFS

The history of tissue transplantation gives some insight into the present state of knowledge on the subject and helps put the main substance of this thesis into perspective.

John Hunter was very interested in transplanted tissues and carried out numerous animal experiments. Although none of his observations are still valid, Hunter is mentioned because his experimental approach to surgical problems stands as a basis for modern investigations of transplantation phenomena.

Kidney transplantation was the subject of a great deal of early experimentation and still is a very active area of investigation. Likewise, skin grafting and basic immunology are subjects that have taken great strides in the past half century. It was in 1902 that the first experimental canine kidney transplant was done in Vienna by a Doctor Ullmann[11]. It was not until 1951 that the first team for human kidney transplants was organized. Lawler, West, et. al.[12] reported their preliminary results from what they claimed to be the first human renal homotransplantation. In 1954 the first identical twin transplantation was performed, and by 1958 Murray[13] could report his work with kidney transplants in seven pairs of identical twins. But further homograft advances required the development of immunosuppressive therapy which, in turn, required a great deal of basic knowledge.

In 1923 Holman[14] at Johns Hopkins worked with human skin grafts and realized that immune mechanisms were involved. Holman questioned very strongly the value and wisdom of ever attempting skin homografts when autografts were possible. He felt that the inevitable antibody production against the foreign proteins of the graft excluded the usefulness ot the graft. But, fortunately, his ominous forecast was not able to prevent further investigation of the subject.

MODERN BELIEFS

Especially since the late 1940's, essential advances have been made in the knowledge of transplantation and immunity on the basic science level. Burnett in Australia and Medawar, Brent, and Billingham in England have done a great deal to help clarify the mechanisms involved in the immune response to tissue transplantation. A good deal of the work accomplished by these men was summarized in August 1964 by Holman[15].

Practical evidence for sensitization in homografting was exemplified by an experiment in which a graft from Animal A placed onto Animal B was rejected in X days. Then if a second graft from Animal A were placed on Animal B, it was rejected in significantly less than X days. The specificity of the response was shown by the fact that a graft from Animal C given to Animal B is not rejected for X days.

Following a sensitizing exposure of one individual to a graft from another individual, several biological events are known to occur. These include the following:

1) There is an accelerated rejection of subsequent grafts from the same donor.
2) Hemagglutinating and cytotoxic antibodies appear in the recipient's serum against the donor's cells.
3) There develops a delayed sensitivity of recipient skin to donor cell suspensions inoculated intradermally.
4) Lymphoid tissue from an animal sensitized to Donor A, if transferred to a nonsensitized third individual, is able to cause accelerated rejection of a graft from Donor A placed at the same time as the lymphoid tissue.

These biological events and their suppression by immunosuppressive agents are considered proof that the homograft response is primarily immunologic in nature. A great deal of work has been done to investigate this immune response, but the whole story is not yet known. The work may be schematically divided into that concerned with the antigens (donor material), antibody (recipient's response), and graft survival.

Knowledge about the donor antigens is meager. Nothing at all is known about the process whereby the specificity of the host response is induced. It has been possible to breed mice which share a gene locus (H-2) which is the major locus responsible for the most potent transplantation antigens. Other mice may be bred so that they differ only at this H-2 locus and others that share various

combinations of loci. It has been shown that the H-2 locus controls the antigen responsible for the most vigorous rejection reaction as well as the antigens responsible for the hemagglutination and cytotoxic antibody responses. The survival of grafts is prolonged when the H-2 locus is identical in the donor and recipient. It is interesting that all grafts made between members of an inbred strain of mice will be accepted except for grafts from a male donor (XY) to a female recipient (XO). In this male-female combination the slow but real rejection is taken to indicate the presence of a gene on the Y chromosome controlling a weak histocompatibility antigen. The antigen problem has been carried even further by men trying to identify the donor cell constituents which act as antigens. Fractions of both nuclei and cytoplasmic material may induce accelerated graft rejection, hemagglutinin formation and cytotoxic antibody formation. Attempts to localize definitely a cellular fraction that specifically and exclusively acts as an antigen for graft rejection have not been successful yet.

However, ways have been found to prolong the survival of homografts by altering the host's immune response, either in a general manner or by inducing a selective unresponsiveness to the tissue antigens of the specific donor only.

General suppression is accomplished by radiation of the recipient, use of radiomimetic drugs, purine antagonists, or

corticosteroids. Also, splenectomy and thymectomy have been used for ablation of lymphoid tissue. Among the disadvantages of these methods may be listed the following:

1) The suppression often is only partial and temporary.
2) These agents impair resistance to infection, and the recipient is subject to the major risks of infection.

Thus, it is felt by many that general immunosuppression by itself will probably never be a satisfactory solution for the homograft problem.

As opposed to general suppression, the term "selective suppression", as used by Holman[15], denotes the induction of unresponsiveness of the host's immune system toward the antigens of the graft without compromising other immunological responses. One might think of a graft between identical twins as an example of selective suppression in that the antigen-antibody phenomenon is largely avoided and that many grafts can survive. But the true unresponsiveness to antigens of a homograft has been possible under special circumstances only. These circumstances are called

1) Tumor enhancement
2) Immunological paralysis
3) Immunologic tolerance

Tolerance is the method most pertinent to this paper, but the other methods deserve a few words of explanation.

Tumor enhancement is concerned with adult animals but has only worked with tumor tissues. It has been found that injection of a tumor extract into a mouse that otherwise would reject a graft from the tumor material permits acceptance of a subsequent tumor graft. The first injection of tumor extract leads to a state of receptivity rather than antagonism toward the graft, but it also leads to the development of hemagglutinating antibodies against the donor.

Immunological paralysis involves soluble tissue antigens rather than whole tissue antigens. Thus, it is not of consequence in homografts of whole tissues.

Immunologic tolerance is induced by injecting cells of one strain of animal into an embryo or very young neonate of another strain. When the embryo reaches adulthood, it accepts grafts from the donor of the original injection and only from donors of the same genetic constitution as the original donor. The immunologic tolerance is specific; other immune responses remain functional. Basic understanding of this phenomenon is incomplete, but Pirofsky[4] credits Medawar with its first description when the latter introduced rat spleen tissue into viable embryonic mice and showed that when these mice matured the challenge of rat tissue failed to introduce an antibody response. It may be inferred from this that the embryo is deficient in its power to produce antibodies or to differentiate between self and non-self, perhaps because of its undeveloped reticulo-endothelial system.

Realizing that the embryo is a privileged individual when it comes to immune reactions and that genetic similarity between individuals is a requirement for homograft survival, many investigators have become curious about the immunologic problem of pregnancy. Except for parthenogenesis, the only situation in which a viviparous fetus does not confront its mother with any foreign antigens is when the parents are members of the same inbred or isogenic strain. But since before recorded time pregnancies in all species of animals have been successful without concern about isogenic parentage. So the immunologic enigma of pregnancy is simply the puzzle of how the fetus survives as a homograft.

The individual with a practical outlook cannot be stopped from wondering if the solution of this problem might be applied somehow to the homograft problem in general. Several hypotheses have been advanced in the past 40 years to explain the survival of fetuses in utero in spite of unavoidable genetic incompatibility between mother and fetus. Some of these hypotheses deserve a brief discussion for their historic value as well as for their contributions to modern knowledge. These include the ideas that

1) The fetus may be antigenically immature.
2) The uterus may be an immunologically privileged site.
3) There maybe an effective physiologic barrier between the mother and the fetus.
4) Immunologic reactivity of the mother may be weakened during pregnancy.

The first of these concepts enjoyed widespread acceptance for nearly 40 years. Little[16] in 1924 advocated the idea that the mother tolerates her embryo because its antigenic potential does not develop until very late in its development. However, in 1963 Billingham and Silvers [3] showed that transplantation antigens appear very early in embryonic life (chicken embryos are antigenic at age four days; mice at 4-15 days). Although the fetal material is antigenic, homografts from fetuses of several species have been shown to have prolonged survival when given to the mother. Billingham claims to have evidence showing that there are peculiar properties of the connective tissue matrix of the grafts that prevent the exposure of the hosts to effective amounts of antigens. There may even be a definite weakening of the hosts' reaction to foreign antigens created by the surviving graft.

The second concept, that the uterus may be an immunologically privileged site, requires a comparison of uterus to the other privileged sites in the body. The brain, the anterior chamber of the eye, and perhaps certain subcutaneous fat pads and the testis have been considered privileged in that they accept homografts with ease. These areas are unique in that none provide grafts with lymphatic drainage although the grafts' blood supply is fully developed. Also, ectopic pregnancy survivals[17] have shown that intra-uterine location is not necessary for solution of the immunologic problems of pregnancy. Schlesinger[18] is credited with providing proof that the uterus has no distinctive properties that

make it favorable for survival of foreign tissue. He studied the fate of strain-specific tumors implanted into the uterine horns of mice and rats. The grafts grew successfully when the tumor and its host were of similar genetic constitution. But when homologous grafts were used, only short growth occurred. Also, intra-uterine tumor homografts underwent accelerated destruction in specifically presensitized animals, no matter if the recipients were nonpregnant, pseudopregnant, or pregnant in one uterine horn. Thus, transplantation immunity can be both evoked and expressed in the uterus in a normal manner.

The third possible explanation is that a physiological barrier exists between the mother and the fetus. Many unsuccessful attempts have been made to influence the incidence or success of pregnancies resulting from mating genetically dissimilar parents. Since no sensitization occurs in a mother made pregnant by a dissimilar mate, as shown by Lanman[19], it is reasonable to suppose an effective barrier between the fetus and mother. However, Lanman's experiments dealt with skin grafting as a means of demonstrating sensitization so that he was not really testing sensitization but graft immunity. It is possible that sensitization occurred during his experiments but that graft rejection was not affected. That there is passage of material between mother and fetus is well documented. Passage of maternal erythrocytes into the fetal circulation is considered a fairly common event, and passage of fetal red blood cells into the maternal circulation occurs in 10 to 20

per cent of normal pregnancies. With the exchange of red cells, it is generally accepted that white cells and platelets are also exchanged. Likewise, Douglas[20] and others have shown that syncytiotrophoblast fragments are transported into the maternal circulation. The presence of these cells with their potential antigenic capacity seems to discredit the placental barrier explanation of "maternal-fetal tolerance".

HORMONAL ROLE IN IMMUNOSUPPRESSION

The remaining explanation is the existence of some manner of weakened maternal immunological reactivity. During pregnancy there is increased production of adrenocortical hormones which may impair the development of sensitivity to homografts as well as to other antigens. On this basis, Medawar[21] suggested that a mother's immunologic capacity may be altered to protect the fetus against immune rejection. With further experimentation, Medawar[7] decided that adrenocortical factors could not explain the phenomena of maternal-fetal tolerance. However, as mentioned in the introduction, Valone[5] in 1952 showed a beneficial effect of pregnancy on skin grafts in mice. He also noted that similar effects could be obtained by creating a state of pseudopregnancy using combinations of the hormones of pregnancy. Serr[8] in 1965 picked up this theme of pseudopregnancy's effect on skin grafts. Serr concluded that cortisone alone did not have any significant influence on homograft survival. Estrogens caused a small but insignificant prolongation of graft survival. Chorionic gonadotrophin caused a prolongation

of 20%, which was given a probability value of less than .001. Progesterone was found to prolong significantly graft survival by 32%. Combined administration of progesterone, estrogens, cortisone, and chorionic gonadotrophins prolonged survival of the homografts by 47.5%, while pregnancy caused a prolongation of 85%. It is noteworthy that only 50 units of chorionic gonadotrophin were used by Serr. In the experiment described in this thesis, a dose four times this great was utilized. Serr's mean length of survival for his control group was eight days with a range of 7-11. For his chorionic gonadotrophin group the mean was 9.6 with a range of 7-14. In my experiment the control group's mean survival was 11.2, range 8-16, while the HCG group had a mean value of 12.6 and a range of 8-16. My results showed a significant difference between the test group and control group on days 9 through 15.

Although the results of this experiment cannot be considered conclusive, I feel that they tend to support the concept that the hormonal changes that occur in pregnancy are intimately associated with the immunologic phenomena that have been previously documented. With further experimental developments and continued interest in this area, I feel that hormone investigations may prove fruitful in shedding light on homograft tolerance.

SUMMARY

In this thesis I have investigated the effect of human chorionic gonadotrophin on the survival of skin homografts in mice closely matched on a genetic basis. The choice of chorionic gonadotrophin

was based on previous experimenters showing that skin graft survival is significantly prolonged during pregnancy and that chorionic gonadotrophin is a hormone of great import and uniqueness during pregnancy.

The discussion reviews the important question of what allows a viviparous pregnancy to survive as a partially foreign, viable graft in intimate contact with the mother's tissues. The answers to this question are far from complete at this time, but it seems likely that the current interest in homografting organs as well as individual tissues will eventually reveal many answers as well as raise new questions.

I feel that the significance of hormonal influences on homograft survival will prove to be great with further investigations such as the one described in this thesis and those done by Nelson[9] and Serr[8].

REFERENCES

1. Rogers, B. O.: Guide and Bibliography for Research into the Skin Homograft Problem, Plastic & Reconstructive Surgery 7:169-201, 1951.

2. Dempster, W. J.: Problems Involved in the Homotransplantation of Tissues, With Particular Reference to Skin, Brit Med J 2:1041-1049 ([redacted]) 1951.

3. Billingham, R. E., and Silvers, W. K.: Sensitivity to Homografts of Normal Tissues and Cells, Ann Rev Microbiol 17:531-564, 1963.

4. Pirofsky, B.: Immunologic Aspects of the Maternal-Fetal State, Annals of Allerg 24:109-111 ([redacted]) 1966.

5. Valone, J. A.: The Effect of Gestation on Experimental Skin Homografts, Plast Reconstr Surg 10 :354-364 ([redacted]) 1952.

6. Heslop, R. W.; Krohn, P. L.; and Sparrow, E. M.: The Effect of Pregnancy on the Survival of Skin Homografts in Rabbits, J Endocr 10:325-332, 1954.

7. Medawar, P. B., and Sparrow, E. M.: The Effects of Adrenocortical Hormones, Adrenocorticotrophic Hormone and Pregnancy on Skin Transplantation Immunity in Mice, J Endocr 14:240-256 ([redacted]) 1956.

8. Serr, D. M.; Biran, S.; and Neuman, Z.: Behavior of Skin Homografts in Mice Under the Influence of Pregnancy Hormones, Harefuah 67:152-154 ([redacted]) 1964.

9. Nelson, J. H., et al: The Effect of Human Chorionic Gonadotrophin on the Thymo-lymphatic System, Obstet Gynec 27:591 ([redacted]) 1966.

10. Snell, G. D.: Congenic Resistant Strains of Mice, Mimeographed pamphlet from Jackson Laboratory, pp. 1-17, Revised May 1965.

11. Williamson, C. S.: Some Observations on the Length of Survival and Function of Homogenous Kidney Transplants, J of Urol 10:275-286, 1923.

12. Lawler, R. H.; West, J. W.; and McNulty, P. H.: Homotransplantation of the Kidney in the Human, JAMA 144:844-845 ([redacted]) 1950.

13. Murray, J. E.: Kidney Transplantation Between Seven Pairs of Identical Twins, Ann Surg 48:343-354 ([redacted]) 1958.

14. Holman, Emile: Protein Sensitization in Isoskingrafting, Surg Gynec Obstet 38:100-106 ([redacted]) 1924.

15. Holman, Halsted R.: Modern Concepts of Tissue Transplantation, Physiol Physicians 2:1-5 ([redacted]) 1964.

16. Little, C. C.: Genetics of Tissue Transplantation in Mammals, J Cancer Research 8:75-95, 1924.

17. Jarcho, J.: Ectopic Pregnancy with Special Reference to Abdominal Pregnancy, Am J Surg 77:273-313, 1949.

18. Schlesinger, M.: Uterus of Rodents as Site for Manifestation of Transplantation Immunity Against Transplantable Tumors, J Nat Cancer Inst 28:927-945, 1962.

19. Lanman, J. T.; Herod, L.; and Fikrig, S.: Homograft Immunity in Pregnancy Survival Rates in Rabbits Born of Ova Transplanted into Sensitized Mothers, J Exp Med 119.2:781-788 ([redacted]) 1966.

20. Douglas, G. W., et al: Trophoblast in Circulating Blood During Pregnancy, Amer J Obstet Gynec 78:960-973, 1959.

21. Medawar, P. B.: Some Immunological and Endocrinological Problems Raised by the Evolution of Viviparity in Vertebrates, Sympos Soc Exp Biol 7:320-338, 1953.