EFFECTS OF HEREDITY ON REACTION TO SKIN-GRAFTS IN GUINEA PIGS

by

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INTRODUCTION

John Hunter of England was the first worker in the field of transplantation when prior to 1825 he unsuccessfully attempted to transplant teeth from one individual to another. Since Hunter's failure, numerous attempts to transplant tissues and skin have resulted in varying degrees of success.

Skin grafts transplanted from one area to another in the same organism are known as autografts. Autografts and those between identical twins are expected to survive permanently. However, homografts, those from the body of one organism to that of an unrelated or distantly related individual do not usually survive longer than about a week. The short duration of survival and ultimate death of homografts and the successful survival of autografts of the same tissue are due to differences in genetic relationship between the donor and the host. Billingham (1959) reported that isografts (the grafting of skin between closely related highly inbred individuals) survived permanently like grafts exchanged between members of a pair of identical twins. His reasoning was that the donor and host had many of the same genes and consequently possessed many of the same gene determined cellular antigens.

The availability of inbred guinea pigs at Kansas State University made it possible to study the effects of widely varying genetic relationships between donor and host on reaction to skin grafts. The objectives of the present study were: (1) to develop a skin grafting technique applicable to the guinea pig; (2) to develop a numerical scoring system to be used to indicate the host's macroscopic reactions to homografts; (3) to determine the association between longevity of skin grafts and the coefficient of relationship between inbred guinea pigs; and (4) to study the effects of genetic relationship on the differential counts of circulating leucocytes in guinea pigs involved in skin grafting.

REVIEW OF LITERATURE

Theories

There are several theories of why grafts fail to survive. The first is one of athrepsia or lack of nutrients by the host, postulated by Ehrlich, (as cited by Dempster, 1951), after observing an increase in the percentage of takes in successive transfers of certain homotransplanted tumors. Additional support of this theory is the evidence of Gorer (1948), as cited by Dempster (1951) that tumors may undergo antigenic simplification during the course of serial transplantation, (a) mice can be rendered susceptible to transplantation tumors and to leukemia by total body irradiation of the host prior to transplantation, Murphy (1926), Krebs et al. (1930), Furth et al. (1933), Richter and MacDowell (1935), all cited by Dempster (1951); (b) Trypan blue injected daily into rabbits can render them susceptible to certain transplanted tumors by blocking the response of the reticulo-endothelial system,Gabrielli (1955); and (c) human skin can grow in serial fashion on the chorio-allantoic membrane of the chick, Blank et al. (1948).

The second theory of graft-failure is based on blood group incompatability. Rogers (1957) supported this theory because survival time of grafts was prolonged when there was agreement between the blood groups of host and donor. The situation was made complex by the results of Longmire et al. (1947) who found that when skin grafts were interchanged among a mother, father and son who possessed the same ABO and MN blood group classification, no successful grafts were obtained. Medawar (1946) was unable, however, to demonstrate antigenic relationship between the red cells and the skin of rabbits.

The third theory implies that there is the development of stroma and the vascular supply into the graft. Medawar (1948) has shown that if a rabbit is specifically immunized against the skin of a given donor and later receives a skin homograft into the anterior chamber of the eye, the homograft will be destroyed only if it is invaded by blood vessels. He says that vascular stagnation is not connected with the breakdown of skin homografts since the graft is not dependent on direct blood supply for survival. He concludes that rupture of blood vessels does occur, but only after the disintegration process has set in.

The fourth theory for graft failure is based on local cellular reaction, i.e. "Individuality Differentials" theory (Loeb, 1944). Loeb regarded the individuality differentials as cytotoxins and contended that the lymphocytic infiltration which occurs around the homotransplant is the main reaction of the host. He denies that there is a significant systemic reaction. Others who oppose Loeb's view interpret the individuality differentials as merely equivalent to tissue antigens. Blumenthal (1941), using several species of animals, confirmed the theory of local cellular aggregation of lymphocytes, but also found lymphocytosis in the peripheral circulation.

The fifth theory is that homograft disintegration may be interpreted as an activity-acquired immunity reaction. Even though there is evidence that tissue antigens are derived from genes, the exact relationship is not clear. Owen et al. (1945) working with dairy cattle, found the differences in frequency of certain cellular antigens between Guernsey and Holstein cattle to be statistically significant. Previously, Landsteiner and Miller (1926) found large individual differences in the ability of a given species to react to blood serum from different species. They repeated their work using rats and obtained similar results. They felt that reactions are not dependent on the race of the animal in such a way that a certain race is associated with a certain serological type, exclusively. However, Oudin (1956) was able to produce precipitating antibodies in the blood serum of some rabbits against the blood serum of other rabbits. Kozelka (1933) found no absolute racial differences in factors concerned with hemaglutination in fowls. He found that highly inbred strains showed a relatively high degree of homogeneity in their agglutinogens as compared with unrelated birds. Medawar (1944), from his work with rabbits, hypothesized that at least seven independently-combined antigens are responsible for skin graft reactions and says there are at least 23 such groups in man. Gorer (1938) felt that antigenic differences were determined by dominant genes. Loeb and Wright (1947) concluded that the genes present in the host of a transplant determined its success or failure. Snell (1948), on the basis of his work with mice, estimated that there were six to 14 gene loci involved in determining the fate of tumor transplants. The presence of antibodies has never been clearly demonstrated in the serum of animals that have received homotransplants of normal tissue.

Reactions

In an extensive series of tissue and organ transplantations carried out by Loeb and associates: [Hesselberg and Loeb (1918); Loeb (1902), (1902), (1918), (1918), (1920), (1921), (1926), (1927), (1930), (1930), (1935), Loeb and Addisson (1909), (1911); Loeb and Herter (1936)] as reported by Blumenthal (1939), it was shown that various types of transplantations are characterized by reactions against the transplant on the part of lymphocytes, polymorphonuclear leucocytes and the connective tissue cells and blood vessels in accordance with the relationship of host and transplant donor.

In 1916 Little and Tyzzer, as quoted by Loeb and Wright (1927), found that tumor transplantation in mice from hybrid to parental strain gave negative results as far as the number of takes was concerned, while reverse transplantation gave 100 per cent takes.

Little and Johnson (1921-22) found in an inbred strain of Japanese waltzing mice that transplantation of pieces of spleen from waltzing mice to hybrids from these animals and white mice, behaved like autotransplants; while spleen transplanted from such hybrids to waltzing mice behaved like very pronounced homotransplants, and were destroyed within a short time.

Loeb and Wright (1927) worked with highly inbred guinea pigs. They concluded that the reaction against the transplant depends on the presence in the host of genetic factors of the donor. The lack in the donor of genetic factors present in the host is apparently of little or no consequence. Apparently, absence of genes in the transplant is without significance. It is the presence of strange genes in the transplanted tissue which causes the inferiority in the latter in respect to the survival and regenerative power.

Loeb and King (1927) worked with highly inbred rats and transplanted thyroid, cartilage, uterus, ovary, parathyroid, liver, kidney, spleen and bone marrow. Their results indicated a great difference between transplantation in inbred rats and inbred guinea pigs. It is certain that through long continued inbreeding in rats there was no appreciable approach to homogeneity of the "individuality differentials", such as was to a certain extent accomplished in the guinea pigs.

Of course it is confirmed that while the absolute severity of the reactions differs according to the relations between the "individuality differentials" of host and transplant, the relative severity is the same in all the organs and depends mainly upon the character of the individuality differentials.

Loeb and McPhee (1931) worked with inbred guinea pigs and instead of transplanting tissues from one member of an inbred family of guinea pigs to another member of the same family, they transplanted from a hybrid between two inbred families to another hybrid of the same kind. Their results became more unfavorable in accordance with the greater difference in average genetic similarity between host and donor, as was to be expected under those conditions.

On the whole their results confirmed the conclusion that reactions of a host against a transplant represent a finely graded indication of the genetic relationship between individuals.

Danforth and Foster (1929) successfully transplanted tissue between two breeds of chickens.

Kozelka (1932) reported that genetic relationship was established with the permanent survivals of integumental grafts in one-to-three-day-old chicks. A significant correlation existed between the coefficient of inbreeding and the percent of grafts persisting. Percentages of graft survival were: 18 percent of the grafts between unrelated birds, persisted, 27 percent between a population consisting of half and full brothers and sisters, 50 percent between full brother and sister, 68 percent between offspring from father-daughter matings, 42 percent from matings of half sisters mated to unrelated males. There was no assurance that when a graft from a given donor would persit, a reciprocal graft would also persist. In a majority of cases where reciprocal grafts were made, the grafts on only one host persisted. The time of absorption of the grafts indicated that other than genetic factors were responsible for the regression of some of the grafts. Sex apparently is also a factor in determining absorption. A significantly larger number of grafts was absorbed when female tissues were grafted upon male hosts.

Stone et al. (1935) transplanted thyroids and parathyroids of dogs after they had been cultured in blood serum of the future host.

Danforth (1935) made grafts between unrelated individuals in pheasants without drug therapy.

Landaner and Aberle (1935) showed that normal skin on frizzle birds lasted longer than frizzle skin on normal birds. They thought that physiology of the bird is involved in the severity of immune reaction.

According to Murphy (1926), Krebs et al. (1930) and Furth et al. (1933) mice can be rendered susceptible to transplantation of tumors and to leukemia by total body irradiation of the host prior to transplantation.

Blumenthal (1939) worked in guinea pigs, rats, pigeons, chickens, and recorded higher leucocytic counts after grafting when genetic relationship of donor and host was more distant. The importance of donor and host relationship to grafting was also demonstrated by Loeb, King and Blumenthal (1943).

Exceptional cases of two successful father to son grafts have been reported by Wolf (1946) as quoted by Longmire and Smith (1951), and cases of mother and father to son by Kearns (1949 as quoted by Longmire 1951). But when skin grafts were interchanged among mother, father, and son who possessed the same O and MN blood group classification, no success was obtained, (Longmire et al. 1947).

Craig and Hirsch (1957) worked with chickens and significant associations between relative increases of lymphocytes in the circulating blood following skin transplantation and relationship coefficients were found in three trials. Greater responses were obtained when grafts were made between breeds of chickens than for grafts within a breed. But unlike Loeb and Wright (1947) and Loeb and McPhee (1931) where asymetrical results between reciprocal donors and hosts may occur, Craig and Hirsch (1957) obtained symmetrical responses between reciprocal donors and hosts of the same breed in their study.

Berry, Craig and Underbjerg (1958) worked with skin grafts and skin implants between chickens of known genetic relationship. The ratio of post-operative to pre-operative percentages of circulating lymphocytes and increases in leucocyte totals were used as measures of the severity of reaction. Reactions to skin grafts were significant in terms of percentage increases of lymphocytes from the differential white cell count and indicated that the post-operative increment in percentage of circulating lymphocytes was inversely associated with relationship of donor and host. Skin implants as measured by changes in percentages of lymphocytes failed to elicit a significant reaction. Changes in total counts of leucocytes failed to show any consistent associations with relationship for either skin grafts or implants.

Berry and Craig (1958) used adjusted three-day post-operative lymphocyte percentages as the measure of intensity of the immune reaction, but the technique failed to indicate the greater genetic diversity between strains and breeds as compared with that between presumably unrelated birds of the same strain.

Law (1954) formulated three generalities regarding growth of transplantable tumors which (in mice): (a) usually grow in all members of the strain of origin, (b) very likely do not grow progressively in unrelated strains of mice, and (c) grow in all F_1 progeny when one of the parents is from the strain of origin of the tumors.

Counce et al. (1956) showed that the determinants of resistance and susceptibility of normal and abnormal tissues may be similar.

Werder, Hardin and Morgan (1958) observed that the fate of homologous full-thickness skin grafts in certain inbred mice appears to be determined by a number of factors that may not be related to the histocompatible genes at the H-2 locus that are known to occur in these strains. Antigenic components of the pigments characterizing the coat colors of the mice might have an influence on the acceptance and rejection of the homografts. The variability of the fate of isologous skin grafts among the F_1 progeny indicated that the parents were heterozygous for factors that affect the acceptance of the transplants. Werder and Hardin (1955) working with these strains of mice previously showed that hair color was a determining factor for the growth of homologous skin. The homologous skin from the white strain grew readily and skin from the strain with a black coat was rejected. The acceptance of skin from the two brown mouse strains occupied an intermediate position.

It was possible to graft certain tumors in spite of normal immune reaction. An explanation of such tumor growth was given by Gorer as quoted by Dempster (1951) that tumors may undergo antigenic simplification during the course of serial transplantation. The high transplantability of blood vessels, cartilage and corneal tissue may be due to low dosages in grafts, or to weak antigens, or no antigens.

Behavior of transplanted adipose tissue is recorded by Hansberger (1959) in hereditarily obese mice. Using a larger number of animals, normal and obese anlages were transplanted to normal as well as potentially obese hosts. Hosts and donors were sacrificed several months later and the transplants were either found to be absorbed or replaced by connective tissue.

Lawler (1950) reported successful transplanting of human kidneys. Generally glandular transplants follow Halstead's law (1909) as quoted by Dempster (1951) that the success depends on the deficiency in the host of the particular secretion of the gland which is transplanted. Turner (1939) also found this situation when transplants were made to the anterior chamber of the eye. Russell and Gittes (1959) recorded that parathyroid homografts between rats of proven genetic diversity may be rejected as promptly as skin, but the majority survive for an indefinite period, there being no intermediate class. Dempster (1953), Lefebvre (1950), Simonsen and Sorensen (1949) and other investigators studying the course of homo-transplanted kidneys have reported essentially similar observations concerning function, survival and histological changes of such transplants, some of the common findings are adhesions to the kidney and thickening and necrosis of the capsule. Lang, Dammin and Miller (1955) reported that as long as the donor kidney was maintained with its own circulation independent of that of the recipient, there was little or no tissue reaction at the junction between donor and recepient tissue during the 21 days they observed their animals.

Craig, Polley and Wearden (1960) reported that the rate at which skin grafts undergo regressive change is associated with the genetic diversity between donor and host. Differentiation was highly significant between all adjacent regression lines for full sib, half sib, unrelated-of-the-same-strain, and different-breed donor tissue in the first experiment and between all adjacent regression lines for full sib, half sib, unrelated-of-the-same-strain, and cross bred donors in the second experiment. Sex of the host appeared to have no significant effect on homograft reactions.

Techniques

The skin grafting technique developed by Polley, Grosse and Craig (1960) was a modification of that of Cannon and Longmire (1952). Polley et al. claimed their technique was a successful one for use with young chicks in genetic studies. The down was removed from the backs of donor and host. Flexible collodion was applied a few minutes prior to grafting to stiffen the skin of both the donor and host. Physiological saline was then injected subcutaneously to separate the skin from the underlying tissues. A regular full-thickness skin graft (about 8 x 10 mm) was then removed, turned 180° and fitted into a previously prepared graft bed on the host. A dressing consisting of a plasticized vinyl chloride film coated with a pressure sensitive adhesive and with a bleached, un-napped flannel pad in the center, was then placed over the graft. No suturing was necessary. Chickens were held by an assistant during these operations and no anaesthetic was used. All 44 autografts in the experiment survived.

Boyd and Smith (1960) used intraperitoneal thiopentone anaesthesia in rats. The skin over the back of the neck and intrascapular area was shaved, cleaned with spirits, dried and a full thickness circular graft of skin 2.0 c.m. in diameter was removed. Care was taken not to include in the graft underlying muscle fibres. Having been transferred to the graft bed which was also 2.0 c.m. in diameter, the graft was held in place by 8-12 sterile black silk sutures placed regularly round the circumference. Many grafts were protected by plastic buttons of larger diameter than the graft and these were held in place by 4 sutures which were placed out with the grafted site. Other grafts and adjacent skin were sprayed with "Nobe cutane" (Evans).

Earlier, many other workers tried to develop a successful skin grafting technique by adopting different methods and procedures. A short account of all those attempts are given below.

According to Dempster (1951) chick embryo tissue lacks the power to produce antibodies. Burke and co-workers (1944), as quoted by Dempster (1951), showed that the immunity response to homotransplanted embryonic tissue varied at different stages of development of that tissue.

Cannon, Weber and Longmire (1954) showed that tissue specificity or antigenicity of chick skin developed at the time of hatching and was completely formed by the 14th day. The ability to resist homografted tissue also developed at the time of hatching but was not complete until the 7th day after hatching. They used less-than-three day old chicks as hosts and donors of skin for homografts with birds up to 16 days old.

Danforth and Foster (1929) also obtained successful results much earlier by using very young chicks of different breeds.

Baetzner and Beck (1928), as quoted by Longmire (1951), gave donor serum to the host and thus was successful in prolonging the life of skin grafts.

Rohde (1925), as quoted by Longmire (1951), tried donor plasma, serum, skin extracts and skin autolysates for desensitizing the host. But it was found that in comparison, the homografts of the untreated hosts lasted longer than those of the treated hosts.

Snell _et al_. (1960) recorded that when mice receiving tumor homografts were preinjected with killed tumor tissue, or with nonliving preparations of certain normal tissues from the strain to which the tumor was indigenous, the growth of the tumor was often markedly enhanced. The treated host died and the untreated controls survived.

Kaliss (1958) recorded such enhancement as due to some physiological alteration in the tumor, induced by its contact with antiserum which insured its survival despite the hostile responses of the host.

Snell (1956, 1957), Billingham, Brent and Medawar (1956) postulated that the effect of antiserum was to block the development of the cellular type of immunity in the nodes and other lymphoid tissues draining the graft. Billingham et al. referred to the postulated effect as an "afferent inhitition of transplantation immunity" and Snell as a "walling off" of the graft by circulating antibody.

Robinovici (1947), as quoted by Longmire (1951), found that irradiation of the recipient prolonged the period before sloughing of the graft and that the number of lymphocytes was reduced in rats.

Craddock and Lawrence (1948) recorded that x-ray treatment if given before the onset of antibody formation inhibited the antibody formation in rabbits. But once antibody production had begun x-ray had no effect on further production. Dempster et al. (1950) recorded similar behavior in skin, when lymphocytes and plasma cells played active parts.

Blumenthal (1941), Baxter and Entin (1947) froze the tissue as a method of modifying the transplant reaction, but without any good result. But, Casey et al. (1948) were able to transport tumor in this way with considerable success.

Blumenthal (1941) attempted to modify transplant reaction with heat treatment of tissue to be transplanted and found no host reaction. Dempster

(1951) suggested that these tissues were dead. Kiskadden and McDowell (1951), as quoted by Longmire et al. (1951), achieved success with this method in guinea pigs.

Host reaction was inhibited by Blumenthal (1941) by immersing the tissue in chemicals. But Dempster said the cells might have been killed.

Some workers tried the technique of desensitization. Gaillard (1948), as quoted by Dempster (1951), transplanted parathyroids from stillborn babies after the parathyroids had been cultured in the blood plasma of future host. Thyroids and parathyroids of dogs were transplanted earlier by Stone et al. (1935) after culturing them in blood serum of the future host.

Martinex, Smith and Good (1958) recorded that acquired tolerance for homologous transplantation of ovaries and pituitaries in mice was achieved by the intravenous injection during the neonatal period (within 24 hours after birth) of homologous living spleen cells taken from adult mice of the same strain as the prospective donors of the endocrine glands. In case of ovaries, the functional adequacy was reflected in the occurrence of normal cyclic changes in vaginal cells. In case of pituitaries normal growth was maintained. In one instance conception, gestation and lactation occurred.

Saphir and Appel (1943) tried mechanical blockage of the host's reticulo-endothelial system in rabbits by the use of trypen blue (daily intravenous injection) and cancelled the immunity reaction in some animals.

Rhode (1925), Allen et al. (1952) and Billingham and Medawar (1953) reported that skin extracts prepared from homologous skin in animals of various species were used in an attempt to prolong the life of transplanted homografts. The results indicated divergent success. Allen and associates (1952) reported that the survival time of homografts in rabbits was extended

16 days by "desensitization" of the recipient with specific donor antigen. They used phenol as a preservative in the preparation of the skin extracts.

Billingham and Medawar (1953) re-investigated the above technique and showed that the life-time of skin homografts transplanted to rabbits may be prolonged by the injection of a weak solution of phenol in saline. These workers were unable to prolong the survival of skin homografts by the injection of phenol free donor skin extracts into the recipient rabbits.

Hardin and Werder (1955) recorded that subcutaneous injections of homologous skin extracts before, after, or on the day of transplantation, resulted in permanent viability in the majority of the homologous skin transplants.

Werder and Hardin (1954), postulated that the application of consecutive homologous skin grafts or the injection of homologous skin extracts influences the immunity mechanism in such a manner as to result in permanent viability of the homograft.

Foster and Hanrahan (1948) reported a skin graft from a white man to a negro woman lasting at least 90 days when the host was given pyribenzamine for sixty days.

Sparrow (1953) found that cortisone and cortisone acetate were ineffective in prolonging the life of grafts when administered intraperitoneally to guinea pigs, but that 25 mg. of cortisone acetate injected subcutaneously, doubled the life of the grafts. The oral administration of extra ascorbic acid to guinea pigs was also ineffective.

Boyd and Smith (1960) recorded that histamine plays no essential part in the homograft reaction but that its depletion in the skin by retarding the normal granulation tissue response for healing may postpone the circumstances which are normally necessary for the homograft reaction.

MATERIALS AND METHODS

Guinea pigs were selected from eight of the inbred lines (X, D, B, S, F, T, U, and Z) maintained at Kansas State University. Each of the lines was formed from different foundation stocks, so relationship coefficients between members of different lines were considered as zero.

There were eight trials involving animals from the X and D lines in which there were a donor and three hosts in each trial. Each host received two grafts on the dorso-lumbar region, one anteriorly and the other posteriorly. The three hosts in each trial were closely related, distantly or less closely related, and unrelated, respectively, to the donor. Another series of 12 trials was conducted in which pigs from the same inbred line served as reciprocal donors and hosts.

Each pig's identification was assured by using a letter, a number to the left, and one to the right of the decimal. The letter indicated the inbred line to which the pig belonged; the number to the left of the decimal was the number of litters recorded within that line since the renumbering began on July 1, 1958. The number to the right of the decimal was the pig's number within its litter. For example, pig X57.1 was in the 57th X line litter born since July 1, 1958 and was the first pig recorded in the litter. Pigs possessing the same letter and number to the left of the decimal in their identifications, such as X57.1 and X57.2, were full sib littermates.

Animals used and the experimental design were as follows:

(1) X57.1 ↙ ↓ ↘ X51.2 X54.2 U55.2

(2) X57.1 ↙ ↓ ↘ X57.2 X61.1 R84.1

(3) X63.2 ↙ ↓ ↘ X63.3 X53.1 G59.2

(4) X62.2 ↙ ↓ ↘ X62.1 X7.3 W1.1

(5) D70.2 ↙ ↓ ↘ D70.1 D43.1 X59.1

(6) D73.3 ↙ ↓ ↘ D73.1 D50.2 C17.2

(7) D73.3 ↙ ↓ ↘ D73.2 D74.2 Z37.1

(8) D71.2 ↙ ↓ ↘ D71.1 D66.3 I10.1

(9) B68.2 ⇄ B68.1

(10) B69.1 ⇄ B69.2

(11) S31.1 ⇄ S31.2

(12) S32.3 ⇄ S32.2

(13) T20.1 ⇄ T20.2

(14) T17.2 ⇄ T17.3

(15) U57.2 ⇄ U57.1

(16) U51.1 ⇄ U51.2

(17) F2.2 ⇄ F2.1

(18) Z31.1 ⇄ Z34.1

(19) F4.2 ⟶ F4.1

(20) Z31.1 ⟶ Z31.3

(21) X64.1 ⟶ X64.2

Technique

The one-man technique used in this experiment was a modification of those of Blumenthal (1939), Cannon and Longmire (1952) and Polley, Grosse, and Craig (1960). The technique can be described as follows:

A. Differential counts of blood leucocytes were taken before grafting.

B. 1. The guinea pig was prepared, and grafting was done 18 to 20 hours after last feeding.

2. Ether was used as the anaesthetic. It took two to three minutes to anaesthetize the guinea pig.

3. Guinea pigs were shaved on the back region, approximately 2 1/2 inches in width and 3 inches in length and cleaned with soap and water.

4. Alcohol was applied to the shaved area and was allowed to dry.

5. Graft beds approximately 1.5 sq. cm. were prepared in the host.

6. An area of approximately 1.5 sq. cm. was cut to the full thickness of the skin from the donor.

7. The skin was spread over a flat, smooth, sterile surface and in most of the cases was turned 180°.

8. The skin graft was then fitted to the graft bed previously prepared on the back of the host.

9. The skin graft was covered by sterile gauze of almost the same size.

10. In case of bleeding, blood was soaked by extra sterile gauze and grafting was delayed until bleeding stopped.

11. No suturing was done, but water repellant adhesive tape was used (1 inch in breadth) for keeping the graft in position.

Precautions were taken to not displace the grafts. Adhesive tapes (1" x 1 1/2") were applied with mild pressure over the grafted area, so that the grafts were held in position and in contact with the underlying bed.

In some cases a dressing consisting of a plasticized vinyl chloride film coated with a pressure sensitive adhesive with a bleached un-napped flannel pad in the center was also used over the graft. But, as this dressing was not satisfactory, it was replaced by the water-repellant adhesive tape. As an extra precaution, clips were used in some cases to keep the tape in position.

The survival of 56 autografts in a series of separate trials conducted before the experiment was actually started, indicated the success of the technique.

C. On the third day after the operations the adhesive tapes and clips were removed and the grafts were examined.

D. Differential blood counts were made on the third, sixth, and fourteenth days.

E. From the third to the 20th day, grafts were macroscopically scored according to their condition (Table 1).

Table 1. Scores used to describe the condition of homografts.

Points	Condition of Grafts
5	Normal, Healthy
4	Some discoloration and/or inflamed
3	Slightly shrunken, deep discoloration
2	Black color, shrunken
1	Crusty, black, shrunken
0	Sloughed off

Controls and Precautions

The differential counts made before transplantation served as control values. These values were taken as the base value for determining the effects exerted by the experimental procedures. Precautions were taken to control age, nutrition, temperature and source of blood.

Age. All guinea pigs used as hosts in this experiment weighed from about 300 to 500 gms. and belonged to what Blumenthal (1939) called the young adult group.

Timing. In order to avoid variation in blood counts due to differences in food intake, graftings were done approximately 16 to 20 hours after the last feeding.

Season. (Effect of temperature) The number of neutrophils and lymphocytes vary in different seasons. They increase in hot weather (Blumenthal 1939), so the experiment was designed to cover three months: February, March and April of 1961, during which period atmospheric temperature underwent very little variation in Manhattan, Kansas, and moreover the temperature in the guinea pig laboratory was controlled.

Source of Blood. Differential counts of blood taken from the ear vein and from the heart of the guinea pig revealed that there was an average of 55 percent lymphocytes in the heart blood as compared with 62.8 percent in the blood taken from the ear vein (Blumenthal, 1939). In this experiment, all differential counts were made from blood taken from ear veins of the guinea pigs.

Staining. The blood smears were fixed in methyl alcohol. Wright's stain (C. L. Blood Stain) was used with buffer solution in the proportion of 2:1. The stain was mixed with buffer solution by blowing on the slide.

After five minutes of staining the slides were flushed with distilled water.

The buffer solution was prepared in the following way:

Potassium phosphate Sorensen's Kh_2PO_4------------------ 6.63 gm.

Sodium phosphate, dibasic anhydrous Na_2HPO_4------------ 2.56 gm.

Distilled water to make 1 liter

Ph 6.4.

The blood smears were examined under oil-immersion and differential counts were taken.

The appearance of different kinds of guinea pig leucocytes when stained with Wright's stain is shown in Plate I and Plate II.

RESULTS AND DISCUSSION

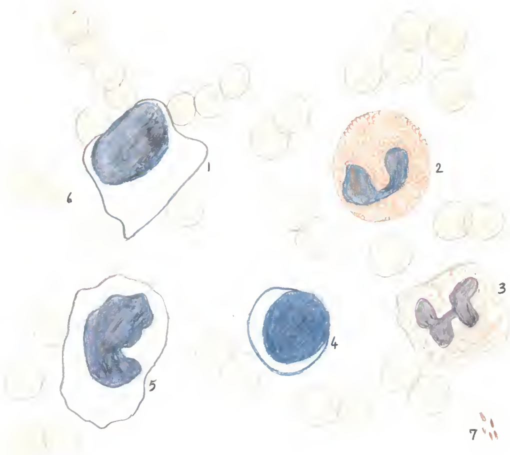
The percentages of healthy grafts (scoring 5 or 4) within each genetic relationship group at different days post-grafting are shown in Table 2. In poultry, Polley, Grosse and Craig (1960) reported that even in the case of the most diverse exchange of skin, i.e., from Rhode Island Red to White Leghorn, 100 percent of the grafts were healthy at 6 days post-grafting when donors and hosts were one and two days old at surgery. The results of the present investigation with guinea pigs were unlike the above findings. In young adult guinea pigs, skin grafted to unrelated individuals of another inbred line showed 100 percent healthy grafts only to the third day, and skin grafts exchanged between distantly related animals in the same inbred line showed 100 percent healthy grafts only up to the fourth day. The skin grafted between closely related guinea pigs of the same inbred line (with relationship coefficient of donors and hosts varying from .56 to .94) showed 100 percent healthy grafts up to five days only. On the sixth day post-operative,

EXPLANATION OF PLATE I

Cells found in guinea pig blood

1. Large lymphocyte
2. Eosinophil
3. Neutrophil
4. Lymphocyte
5. Monocyte
6. Erythrocytes
7. Thrombocytes

PLATE I



EXPLANATION OF PLATE II

Different kinds of lymphocytes commonly found in high lymphocytic reaction after skin grating.

1. Small lymphocyte.
2. Lymphocyte of intermediate size.
3. Lymphocyte with indented nucleus.
4. Large lymphocyte with indented nucleus, unevenly dark-blue cytoplasm, and pointed cytoplasmic projections.
5. Large lymphocyte with azurophilic granules.
6. Large lymphocyte with irregular cytoplasmic contours.

PLATE II

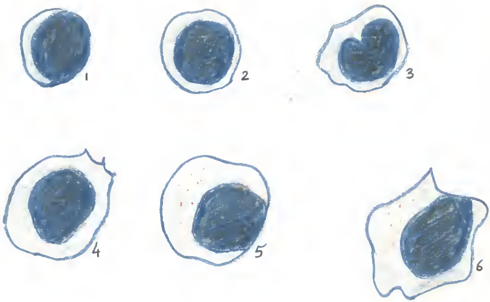


Table 2. Percent healthy grafts[1] corresponding to genetic relationship.

Relationship coefficient of donor and host	Number of hosts	Percent healthy grafts at days post-grafting: 3	4	5	6	7	8	9	10	11	12
.56 to .94[2]	31	100	100	100	94.7	87.7	80.7	65	26.31	7	Nil
.06 to .50[3]	8	100	100	87.5	62.5	56.25	31.25	12.5	Nil	Nil	Nil
Zero[4]	8	100	87.5	68.75	62.5	12.5	Nil	Nil	Nil	Nil	Nil

[1]Healthy grafts were defined as those which scored 4 or 5 (see Table 1)

[2]Closely related donors and hosts were mostly full sibs, with one parent-offspring relationship, in the same inbred line.

[3]Three of the guinea pigs in this group were full sibs but not inbred. Other hosts were distantly related to the donors and were in the same inbred line.

[4]A relationship coefficient of zero indicates that the donor and host were from different inbred lines and without any known relationship.

the percentage of healthy grafts in both unrelated and distantly related guinea pigs was the same, 62.5 percent; whereas, the percentage of the same type of grafts in guinea pigs with relationship coefficients from .56 to .94 was 94.7 (Table 2). On the 9th day post-operative the percentage of healthy grafts in unrelated animals was zero, that in the distantly related guinea pigs was 12.5, but the percentage in the closely related group was still 65.0.

The correlations between visual scores recorded by the author and those recorded independently by Dr. J. D. Wheat and Dr. H. G. Spies were .76 and .77, respectively. Both correlations were significant at the .01 level of probability.

The longevity of the healthy grafts (number of days for which the graft receives a score of at least 4) in unrelated guinea pigs was 7 days, in distantly related guinea pigs (i.e., with relationship coefficient from .06 to .50) it was 9 days and in the closely related ones (i.e., with relationship coefficient from .56 to .94) the longevity of healthy grafts was 11 days.

Pre-operative and post-operative differential counts of the leucocytes were made. The relative percentage increase or decrease in lymphocytes was then calculated. The mean pre-operative percentages and the relative changes in lymphocyte percentages following the skin grafting are shown in Table 3. It appears that the relative increase in the percentage of lymphocytes was highest on the third day in close and distantly related guinea pigs; whereas for unrelated guinea pigs, for some unknown reason, the relative increase in lymphocyte percentage was highest on the sixth day. However, the value on the sixth day was only slightly higher than on the third day.

Table 3. Pre-operative percentages and relative changes in percentage of lymphocytes, corresponding to genetic relationship, following skin grafting.

	Relationship of hosts and donors		
	Close[1]	Distant[2]	Unrelated[3]
Preoperative	53.6	44.0	44.9
Relative change on third day post-operative	+6.7	+18.6	+25.4
Relative change on sixth day post-operative	+3.6	+15.0	+26.5
Relative change on fourteenth day post-operative	-2.2	+7.6	+4.6
Number of observations	31	8	8

[1]Close - inbred donors and hosts with relationship coefficients between .56 and .94.

[2]Distant - the donors and hosts had relationship coefficients ranging from .06 to .50.

[3]Unrelated - the donors and hosts were from different inbred lines and had no known relationship.

When the donor and host relationship was close, the relative increase of percentage lymphocytes was only 6.7 percent on the third day and 3.6 percent on the sixth day. The percentage increase of lymphocytes in the case of distantly related individuals was 18.6 percent on third day and 15.0 percent on the sixth day. Whereas in the unrelated guinea pigs increases were 25.4 percent on the third day and 26.5 percent on the sixth day post-operative.

From Table 3, it is apparent that generally the reaction was least when the relationship between the donor and host was close, intermediate when the donor-host-relationship was distant, and highest when the donor and host were unrelated.

Table 4 shows the regression and correlation coefficients between the pre-operative percentage of lymphocytes and the relative change in the percentage of lymphocytes shown by closely related, distantly related and unrelated guinea pigs after skin grafting. It is apparent that the negative correlation on the sixth day post-operative is highest (-.91) in case of unrelated guinea pigs whereas it is lowest in case of highly related guinea pigs with relationship coefficient ranging from .56 to .94.

Table 4. Correlation (r) and regression (b) coefficients between the pre-operative percentage of lymphocytes and the post-operative change in lymphocytic percentage.

Relationship Coefficients	No. of Hosts	Regression and correlation coefficients					
		3rd day post-operative		6th day post-operative		14th day post-operative	
		r	b	r	b	r	b
.56 to .94	31	-.14	-.24	-.56**	-.75	-.63**	-.66
.06 to .50	8	-.64	-.73**	-.61	-.68	-.29	-.34
Zero	8	-.59	-.79**	-.91**	-1.87	-.05	-.11

**Significant at .01 level.

It is apparent from Table 4 that the guinea pigs which showed a higher initial lymphocytic count showed a comparatively lower rise in lymphocytic number after the skin grafts were made.

Regression and correlation for the third day in closely related guinea pigs indicate that if the relationship between host and donor is high one can expect a relatively lower amount of lymphocytic activity. If the relationship is lower, one can expect a larger increase in lymphocyte activity. The same statement is true for correlations and regressions for the 14th day (Table 5).

Table 5. Regression (b) and correlation coefficients (r) between the relative percentage change in lymphocytes and relationship coefficient between host and donor of a skin graft.

Relationship Coefficient	No. of Hosts	3rd day reading r	3rd day reading b	6th day reading r	6th day reading b	14th day reading r	14th day reading b
.56 to .94	31	-.13	-9.66	.36*	32.13*	-.13	-9.08
.06 to .50	8	-.26	-16.62	-.68	-28.91	-.10	-4.58

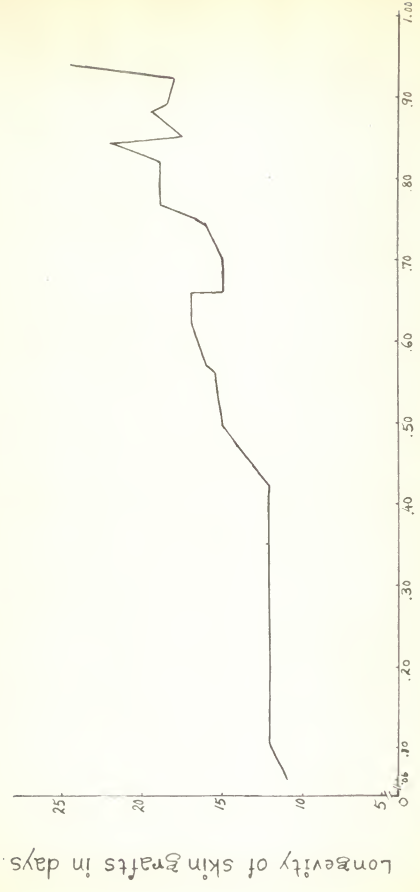
*Significant at 0.05 level.

The regression and correlation coefficients involving closely related hosts and donors for the 6th day indicated an inverse association, i.e., the largest increase in lymphocytic activity was observed when genetic relationship was closest.

Lymphocytic reaction was negatively correlated with an increase in the relationship coefficient between donor and host. Both the regressions and correlations for 3rd day, 6th day and 14th day indicated that the lymphocytic increase in the circulating blood was comparatively less in cases where the relationship coefficients were high between hosts and donors. This indicates that throughout the whole period of two weeks post-operative, the lymphocytic reactions were less where the relationship coefficients were high (Table 6).

The effects of different levels of genetic relationship in inbred guinea pigs on the longevity of skin grafts are shown in Fig. 1. The longevity of skin graft was calculated up to the day prior to sloughing. In the 94.4 percent related guinea pigs, the average longevity was 24.5 days, whereas

FIG. 1.



Relationship coefficients between donors and hosts of skin grafts in inbred guineapigs.

Table 6. Regression (b) and correlation coefficients (r) between relative percentage change in lymphocytes and relationship coefficient of host and donor of skin graft.

No. of Hosts	Relationship Coefficients	3rd day post-operative r	3rd day post-operative b	6th day post-operative r	6th day post-operative b	14th day post-operative r	14th day post-operative b
39	.06 to .94	-.69**	-28.45	-.24	-11.36	-.39*	-15.14

*Significant at 0.05 level.
**Significant at 0.01 level.

for the .779 to .926 range of relationship coefficients, the average longevity of skin grafts was found to be 16 days. Fifty percent related guinea pigs had an average longevity of 15 days for their skin grafts. In the guinea pigs with relationship coefficients varying from .062 to .426, the average longevity was only 12 days. In the cases where the guinea pigs were not related, the average longevity was found to be approximately 10 days. The average day on which deterioration and sloughing off of the skin grafts began in both related and unrelated guinea pigs are shown in Tables 7 and 8. The day deterioration began was considered as the first day the graft received a score of 3 as per Table 1. In the case of unrelated guinea pigs, the deteriorations of the skin grafts started on the 5th day and in distantly related guinea pigs (i.e., from .06 to .5 relationship coefficients), the deterioration started on the 6th day. In the highly related guinea pigs (with relationship coefficients varying from .56 to .94) the deterioration started on the 8th day post-operative. Table 7 also shows the different percentages of deteriorations on different days post-operative.

Table 7. Average day on which deterioration of the skin grafts began.

Relationship coefficients between hosts and donors	No. of hosts	Percent deteriorated on days post-operative							
		5	6	7	8	9	10	11	12
.56 to .94	31				9.6	19.4	32.3	29.1	9.6
.06 to .50	8		25		37.5	12.5	25		
Zero	8	25	12.5	50	12.5				

Table 8. Average day on which sloughing of the skin grafts began.

Relationship coefficients between hosts and donors	No. of hosts	Percent sloughed off on days post-operative																	
		9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
.56 to .94	31					3.2			19.4	22.7	12.9	3.2	16.2	6.4		3.2		9.6	3.2
.06 to .50	8				12.5	50			37.5										
Zero	8	12.5	12.5	50	25														

The correlation between the relationship coefficient and the number of days required for a graft to be sloughed is positive for both highly related (relationship coefficient .56 to .94) and distantly related (relationship coefficient .06 to .50) guinea pigs (Table 9). In case of highly related guinea pigs the correlation coefficient was +.57 and in distantly related guinea pigs the correlation coefficient was +.85. It is apparent from this table that the longevity of graft is positively correlated with the genetic relationship of the host and donor.

Table 9. Correlation between relationship coefficient and the day a graft received a zero score.

Relationship Coefficient	No. of Hosts	Correlation Coefficient
.56 to .94	31	+0.57**
.06 to .50	8	+0.85**

**Significant at 0.01 level.

The negative correlation coefficients between the visual scores and the relative change in the percentage of lymphocytes in the groups of guinea pigs with relationship coefficients varying from .56 to .94 and from .06 to .50, on the 3rd day and 14th day post-operative, suggest that with the rise in visual scores, there was a corresponding decrease in the change in percentage of lymphocytes (Table 10). The positive correlations between visual scores and change in lymphocytic counts on the 6th day, though they were nonsignificant, possibly suggest the occurrence of unexplained biological phenomena between the 3rd day and 14th day post-operative.

Table 10. Correlation (r) between the visual scores and the relative change in the percentage of lymphocytes.

Relationship Coefficient	No. of Hosts	Dorrelation Coefficients 3rd day post-operative	6th day post-operative	14th day post-operative
.56 to .94	31	-.06	+.13	-.11
.06 to .50	8	-.68**	+.26	-.13
Zero	8	+.15	+.09	Zero

**Significant at 0.01 level.

Visual scores of skin grafts were positively correlated with relationship coefficient between the donor and host.

A comparison of Table 6 and Table 11, regarding the superiority of either the visual scoring system or the percentage change in the lymphocytic count, reveals that on the 3rd day post-operative, which is considered as the day of highest reaction, the change in lymphocytic percentage appears to be a far better method of estimating reactions than visual scores. Whereas, when the reaction is subsiding, that is, on the 6th and 14th days post-operative, the visual scoring system appears to be a better method of studying reactions.

Table 11. Correlation between relationship coefficient of host and donor and visual scores.

No. of Hosts	Relationship Coefficients	Correlation Coefficients 3rd day post-operative	6th day post-operative	14th day post-operative
39	.06 to .94	+.25	+.48**	+.69**

SUMMARY

This study was undertaken (1) to develop a suitable technique for skin grafting in guinea pigs; (2) to evolve a system of visual scoring in skin homografts to study the effects of genetic relationship on macroscopic reactions of homografts; (3) to find the proportionate longevity of skin grafts in respect to the coefficient of relationship of inbred guinea pigs and finally (4) to study the effects of genetic relationship on the lymphocytic reactions to skin grafts in guinea pigs.

The technique developed for skin grafting included the use of ether as an anesthetic, the site of grafts was the back of the guinea pigs which was shaved and cleaned. Alcohol was used as an antiseptic. The graft beds were made approximately 1.5 cm. square. Grafts of the same size were taken from donors and placed on the graft beds. Sterile gauze was placed on the grafts, taking precaution not to displace them, adhesive tapes were used with mild pressure to keep the grafts in position and in contact with the underlying bed.

Differential blood counts were taken on the 0 day before the operation and on the third, sixth, and fourteenth days post-operative. The adhesive tapes were removed on the third day and visual scoring was started the same day. The scoring system developed was one in which 5 points were given for normal, healthy grafts, 4 for healthy and/or inflamed ones, 3 for slightly shrunken and slight discoloration, 2 for shrunken and discolored grafts, 1 for black and crusty grafts, and 0 for grafts which had sloughed.

Longevity of the grafts was studied in respect to the relationship coefficients of inbred guinea pigs available from the Kansas State University

guinea pig laboratory. Longevity of skin grafts was more in case of guinea pigs in which high relationship coefficients existed between the hosts and donors. In 94 per cent related guinea pigs, the longevity of skin grafts was 25 days.

It was apparent that guinea pigs with higher initial lymphocytic counts showed a comparatively lower rise in lymphocytic number after skin graft.

It was found that with a rise of relationship coefficients, there was a rise in visual scores and a decrease in the change in percentage of lymphocytes.

The correlation between relationship coefficient and change in lymphocytes on the third day post-operative was -.13 in case of closely related (relationship coefficient from .56 to .94) and -.26 in case of distantly related (relationship coefficient .06 to .50) guinea pigs. The post-operative lymphocytic reactions were least in case of highly related guinea pigs, intermediate in case of distantly related ones, and highest in unrelated guinea pigs.

The correlation between relationship coefficient and change in lymphocytic percentage was -.69,(significant at 0.01 level) Correlation between relationship coefficient and visual scores was .25 on the 3rd day post-operative. Thus the change in lymphocytic percentage appeared to be a far better method of studying reactions than visual scores on the 3rd day post-operative which was considered to be the day of highest reaction. The correlations between relationship coefficient and change in lymphocytic percentage on the 6th and 14th days post-operative were -.24 and -.39 (significant at 0.05 level) respectively; whereas the correlation between relationship coefficient and visual scores on the 6th and 14th days post-operative were +.48 (significant at 0.01 level) and +.69 (significant at 0.01 level), respectively. Thus it appeared that on the 6th and 14th days post-operative the visual scoring system was a better method of studying reactions.

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LITERATURE CITED

Allen, H. L., R. D. Williams, C. G. Longood and E. H. Ellison. 1952. The effect of donor skin desensitization and ACTH on survival of skin homografts in rabbits. Ann. Surg. 135, 239.

Baetzner, W., and S. Beck. 1928. Uber homoio transplantation. Zentrafbl. f. Chir. 55, 272.

Baxter, H., and M. A. Eutin. 1947. Experimental and clinical studies of reduced temperatures in injury and repair in man: Structure and potentialities of human skin in temperature control and in defence against thermal trauma. Plastic and Recon. Surg. 2, 569.

Berry, J. E., J. V. Craig, and G. K. L. Underbjerg. 1958. A comparison of the effects of skin implants and skin grafts on the cellular constituents of chicken blood. Poultry Science, 37, 312.

Berry, J. E., and J. V. Craig. 1959. Estimation of genetic diversity among strains and breeds of chickens by lymphocyte increases following skin grafting. Poultry Science 38, 439.

Billingham, R. E., and P. B. Medawar. 1953. Resensitisation to skin homografts by injections of donar skin extracts. Ann. Surg. 137, 444.

Billingham, R. E., L. Brent, and P. B. Medawar. 1956. Enhancement in normal homografts, with a note on its possible mechanism. Transplantation Bulletin 3, 84.

Billingham, R. E. 1959. Reactions of grafts against their hosts. Science, 130, 947-953.

Blank, H., L. L. Coriell, and T. F. McNair Scott. 1948. Human skin grafted upon the chorioallantois of the chick embryo for virus cultivation. Proc. Soc. for Expt. Biol. and Med. 69:341-345.

Bluementhal, H. T. 1939. Effects of organismal differentials on the distribution of leucocytes in the circulating blood. Arch. Path. 27. 510.

Blumenthal, H. T. 1941. Arch. Path. 31, 295.

Blumenthal, H. T. 1941. Organismal differentials: Further investigations of their effects on the distribution of leukocytes in the circulating blood. Arch. Path. 31:295-303.

Boyd, J. F., and A. N. Smith. 1960. The effect of compound 48/80 on the autograft and homograft reaction. The Brit. J. of Expt. Path., 41, 359-368.

Cannon, J. A., and W. P. Longmire, Jr. 1952. Studies of successful skin grafts in the chicken. Annals of Surg. 135, 60-68.

Cannon, J. A., R. A. Weber, W. P. Longmire, Jr. 1954. Factors influencing the survival of successful skin homografts in the chicken. Effects of varying age of donor and recipient. Annals of Surg. 139, 468-472.

Casey, A. E., L. Meyers, and G. R. Drysdale. 1948. Proc. Soc. Exp. Biol. N. Y. 69, 579.

Counce, S., P. Smith, R. Barth, and G. D. Snell. 1956. Strong and weak histocompatibility gene. Differences in mice and their role in the rejection of homografts of tumors and skin. Ann. Surg. 144, 198-204.

Craddock, C. G., Jr., and J. S. Lawrence. 1948. The effect of roentgen irradiation on antibody formation in rabbits. J. of Immun. 60, 241-254.

Craig, J. V., and L. J. Hirsch. 1957. Genetic relationship and reactions to skin grafts. J. Heredity, 48, 235.

Craig, J. W., C. R. Polley, and S. Wearden. 1960. Estimation of genetic diversity by skin graft. Reactions in young chicks. Poultry Sci. 39, 1533-1540.

Danforth, C. H., and F. Foster. 1929. Skin transplantation as a means of studying genetics and endocrine factors in the fowl. J. of Exp. Zool. 52, 443.

Danforth, C. H. 1935. Different potentialities of male and female skin in Reeves pheasants. Proc. Soc. for Exp. Biol. and Med. 33, 291.

Dempster, W. J., B. Lennox, and J. W. Boag. 1950. Brit. J. Exp. Path. 31, 670.

Dempster, W. J. 1951. Problems involved in the homostrains plantation of tissues with particular reference to skin. Brit. Med. Jour. II:1041-1049.

Dempster, W. J. 1951. Problems involved in the homotransplantation of tissues, with particular reference to skin. Brit. Med. J. II, 1041-1049.

Dempster, W. J. 1953. Arch. Intern. Pharmacodynamic 95, 253.

Foster, D. G., and E. M. Hanrahan. 1948. Observations on a skin homograft after 60 days of pyribenzamine therapy. Bull. John Hopkins Hospital, 82, 501.

Furth, J., H. R. Siebold, and R. R. Rathbone. 1933. Amer. J. Cancer, 19, 521.

Gabrielli, E. R. 1955. Raticulocndothelial activity during antibody response. Ann. N. Y. Acad. Sci., 59:337-342.

Gaillard, P. J. 1948. Growth 2, 1939.

Gorer, P. A. 1938. The genetic and antigenetic basis of tumor transplantation. Jour. Path. and Bact. 44:691-697.

Hansberger, F. X. 1959. Behavior of transplanted adopose tissue of hereditarily obese mice. Anat. Record 135, 109-113.

Herden, C. H., and A. A. Werder. 1955. Effects of skin extracts on the viability of homologous skin grafts in mice. Annals of N. Y. Aca. of Sci. 59, 381-383.

Kaliss, N. 1958. Immunological enhancement of tumor homografts in mice. Cancer Res. 18, 992.

Kozelka, A. N. 1932. Integeemental grafting as a means of analyzing the factors determining the secondary sexual characters of the Leghorn fowl. Jour. Exp. Zool. 61, 431.

Kozelka, A. N. 1933. Individuality of the red blood cell of inbred strains of fowl. Jour. Immun. 24:519-530.

Krebs, C., H. C. Rask Nielsen and A. Wagner. 1930. Acta Radio, Science, Suppl. 10, 1.

Landaner, W., and S. C. Aberle. 1935. Studies on the endocrine glands of frizzle fowl. Am. J. Anat. 57, 99.

Landsteiner, K. and C. P. Miller, Jr. 1924. On individual differences of the blood of chickens and ducks. Proc. Soc. for Expt. Biol. and Med. 22:100-102.

Lang, S., G. J. Dammin, and B. F. Miller. 1955. Transplantation of rat kidneys in parabiotic and pseudoparabiotic animals. Annals of New York Academy of Sciences, Vol. 59, 401.

Law, L. W. 1954. Genetic studies in experimental cancer. Advances in Cancer Res. 2, 281-352.

Lawler, R. H., J. W. West, P. H. McNutty, E. J. Claudy, and R. P. Murphy. 1950. J. Am. Med. Assoc. 144, 845.

Lefebvre, L. 1950. Arch. Intern. Physiol. 57, 429.

Little, C. C., and E. E. Tyzzer. 1916. J. Med. Res. 33, 393.

Little, C. C., and B. W. Johnson. 1921-22. Proc. Soc. Exper. Biol. and Med. 19, 163.

Loeb, Leo. 1921. Biol. Bull. 40, 143.

Loeb, Leo. 1926. Am. J. Path. 11, 99, 301.

Loeb, Leo, and S. Wright. 1927. Transplantation and individuality differentials in inbred families of guinea-pigs. Am. J. Path. 3, 251.

Loeb, Leo, and H. D. King. 1927. Transplantation and individuality differential in strain of inbred rats. Am. J. Path. 3, 1943.

Loeb, Leo, and H. C. McPhee. 1931. Transplantation of tissues in hybrids of inbred families of guinea pigs and individuality differentials. Am. Naturalist, 65, 385.

Loeb, L., H. D. King, and H. T. Blumenthal. 1943. Transplantation and individuality differentials in inbred strains of rats. Biol. Bul. 84, 1-12.

Loeb, L. 1944. The biological basis of individuality. C. C. Thomas Publishing Co., Springfield, Ill.

Longmire, W. P., Jr., H. B. Stone, A. S. Daniel, and C. D. Croon. 1947. Report of clinical experiences with homografts. Plastic and Recon. Surg. 2:419-426.

Longmire, W. P., Jr., and S. W. Smith. 1951. Homologous transplantation of tissues. A.M.A. Archives of Surgery 62, 443.

Martine, Z. C., J. Wm. Smith, and R. A. Good. 1958. Acquired tolerance to homologous transplantation of endocrine glands in inbred strains of mice. The Brit. J. of Expt. Path. 39, 574-581.

McNair, Scott. 1948. Human skin grafted upon the chorioallantois of the chick embryo for virus cultivation. Proc. Soc. for Expt. Biol. and Med. 69:341-345.

Medawar, P. B. 1944. The behavior and path of skin autografts in rabbits. Jour. Anatomy 48:176-199.

Medawar, P. B. 1946. Relationship between the antigens of blood and skin nature. 157:161.

Murphy, J. B. 1926. The lymphocyte in resistance to tissue grafting, malignant disease and tuberculoisis injections: An experimental study. Monogs. Rockfeller Inst. Med. Res. 21.

Oudin, J. 1956. The "allotypy" of certain protein antigens of serum. Abst. of papers presented at the Acad. of Sciences. France. 242: 2606-2608.

Owne, R., D. C., Startmont, and M. R. Irwin. 1945. Genetic differences between two breeds of dairy cattle as shown by incidence of cellular antigens. Abst. of paper prepared for 1944 meeting of the Genetics Soc. of America Genetics. 30:16-17.

Polley, C. R., A. E. Grosse, and J. V. Craig. 1960. A skin grafting technique for use in genetic studies with chickens. Transplantation Bull. M, 425-428.

Robinoke, N. 1947. The fate of skin homotransplants performed on previously x-rayed rats. Plastic and Recon. Surg. 2, 413.

Rogers, B. O. 1957. The genetics of skin homo-transplantation in the human. Ann. N. Y. Acad. Sci. 64:741-766.

Rohde, C. 1925. Uben Versuche Zur Uberwindung der Auheilungschwi erigkeiten transplantate.

Russel, P. S., and R. F. Gilles. 1959. Parathyroid transplants in rats. J. of Exp. Med. 109, 571-588.

Saphiro and M. Appel. 1943. Cancer Res. 3, 767.

Simonsen, M., and F. Sorensen. 1949. Acta. Chir. Scand. 99, 61.

Snell, G. D. 1948. Methods for the study of histocompatability genes. Jour. Genetics, 49:87-103.

Snell, G. D. 1956. The suppression of the enhancing effect in mice by the addition of donor lymph nodes to the tumor inoculum. Transplantation Bulletin. 3, 83.

Snell, G. D. 1957. The homograft reaction. Ann. Rev. Microbiology. 11, 439.

Snell, G. D., Henry J. Winn, Jack H. Stimpfling, and Stephen J. Parker. 1960. Depression by antibody of the immune response to homografts and its role in immunological enhancement. J. of Exp. Med. 112, 293-314.

Sparrow, E. M. 1953. The behavior of skin autografts and skin hemografts in the guinea pig, with special reference to the effect of cortisone acetate and ascorbic and on the homografts reaction. J. of Endocrinology 9, 101-113.

Stone, H., J. Owings, and G. O. Gey. 1935. Living grafts of thyroid and parathyroid. Surg. Gynec. and abst. 60, 390.

Turner, C. D. 1939. Anat. Rec. 73, 145.

Werder, A. A., and G. A. Hardin. 1954. The effect of spleenectomy on the survival rate of homologous skin grafts in C. F. W. mice. Surgery 35, 405.

Werder, A. A., and C. A. Hardin. 1955. Importance of hair color to successful skin transplantation. A.M.A. Arch. Surg. 70, 407-410.

Werder, A. A., C. A. Hardin, and P. Morgan. 1958. Observations on the genetic relationship affecting the transplantability of skin in inbred mice. Annals N. Y. Acad. of Sci. 743, 722-725.

EFFECTS OF HEREDITY ON REACTION TO SKIN-GRAFTS IN GUINEA PIGS

by

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MASTER OF SCIENCE

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1961

This study was undertaken (1) to develop a suitable technique for skin grafting in guinea pigs; (2) to evolve a system of visual scoring in skin homografts to study the effects of genetic relationship on macroscopic reactions of homografts; (3) to find the proportionate longevity of skin-grafts in respect to the coefficient of relationship of inbred guinea pigs and finally (4) to study the effects of genetic relationship on the lymphocytic reactions to skin-grafts in guinea pigs.

The technique developed for skin grafting included the use of ether as an anesthetic, the site of grafts was the back of the guinea pigs which was shaved and cleaned. Alcohol was used as an antiseptic. The graft beds were made approximately 1.5 cm. square. Grafts of the same size were taken from donors and placed on the graft beds. Sterile gauze was placed on the grafts, taking precaution not to displace them, adhesive tapes were used with mild pressure to keep the grafts in position and in contact with the underlying bed.

Differential blood counts were taken on the 0 day before the operation and on the third, sixth, and fourteenth days post-operative. The adhesive tapes were removed on the third day and visual scoring was started the same day. The scoring system developed was one in which 5 points were given for normal, healthy grafts, 4 for healthy and/or inflamed ones, 3 for slightly shrunken and slight discoloration, 2 for shrunken and discolored grafts, 1 for black and crusty grafts, and 0 for grafts which had sloughed.

Longevity of the grafts was studied in respect to the relationship coefficients of inbred guinea pigs available from the Kansas State University guinea pig laboratory. Longevity of skin grafts was more in case of guinea pigs in which high relationship coefficients existed between the hosts and donors. In 94 per cent related guinea pigs, the longevity of skin grafts was 25 days.

It was apparent that guinea pigs with higher initial lymphocytic counts showed a comparatively lower rise in lymphocytic number after skin graft.

It was found that with a rise of relationship coefficients, there was a rise in visual scores and a decrease in the change in percentage of lymphocytes.

The correlation between relationship coefficient and change in lymphocytes on the third day post-operative was -.13 in case of closely related (relationship coefficient from .56 to .94) and -.26 in case of distantly related (relationship coefficient .06 to .50) guinea pigs. The post-operative lymphocytic reactions were least in case of highly related guinea pigs, intermediate in case of distantly related ones, and highest in unrelated guinea pigs.

The correlation between relationship coefficient and change in lymphocytic percentage was -.69 (significant at 0.01 level). Correlation between relationship coefficient and visual scores was .25 on the 3rd day post-operative. Thus the change in lymphocytic percentage appeared to be a far better method of studying reactions than visual scores on the 3rd day post-operative which was considered to be the day of highest reaction. The correlations between relationship coefficient and change in lymphocytic percentage on the 6th and 14th days post-operative were -.24 and -.39 (significant at 0.05 level) respectively; whereas the correlation between relationship coefficient and visual scores on the 6th and 14th days post-operative were +.48 (significant at 0.01 level) and +.69 (significant at 0.01 level), respectively. Thus it appeared that on the 6th and 14th days post-operative the visual scoring system was a better method of styding reactions.