Study of Factors Influencing Induced Tolerance to Skin * Homografts in the Chicken

JACK A. CANNON, M.D., PAUL TERASAKI, PH.D., WILLIAM P. LONGMIRE, JR., M.D.

From the Department of Surgery, University of California Medical Center, Los Angeles, California

OUR STUDY of and experience with successful homografts in the chicken extend over the period of the past six years. The original work was based on that of Danforth [3] who, in 1929, showed that successful homografts could be obtained in a small number of individuals (5 to 10 per cent) when skin grafts were interchanged in one-day-old chicks. A method of collodion "dressing and suture" of the grafts was developed that permitted large numbers of chicks to be grafted in short periods of time. Administration of cortisone, but not of ACTH, somewhat increased the incidence of permanent takes. Variations in the age of graft and host were studied, and it was noted first that successful homografts were almost never obtained when host and graft were over seven days of age. A decreasing incidence of successful takes of grafts onto one-day-old chicks was obtained when the age of the graft was increased to 13 days; when 14 days of age or older, the grafts were never successful.

When a successful homograft was obtained, both host and donor were maintained into adult life. Serious technical difficulties were encountered in grafting adult chicken skin, so that conclusive interpretation of results is difficult. However, when using split thickness grafts, it was noted that if a healthy adult homograft was transferred back to its original donor, a certain take did not occur in more than 50 per cent of a total of 22 host-donor pairs studied (ten previously reported). Furthermore, in 21 instances (five previously reported) a repeat homograft between original, but now adult, host and donor, where the donor was bearing a healthy homograft obtained by grafting when host and donor were one day of age, resulted in only one take of the repeat homograft. In all other such cases, no long-term survival of the repeat homograft occurred; the repeat homograft was frequently seen to slough in the presence of continued viability and health, including feather growth in the original successful homograft. These results would seem to indicate that the homograft may be successful primarily because the one day homograft, not yet having assumed an unalterable tissue specificity, adapts to and at least partially assumes the tissue specificity that is developing in the host before the host, who is being subjected to homagrafting during a developmentally "tolerant" period, recognizes and reacts to the homograft in a destructive manner.

As the result of the work and observations by Owen [5] on twin cattle, it was shown by Billingham *et al.*,[1] Woodruff,[6] and Hašek [4] that the immune mechanism of the host could be made tolerant of skin homografts by the intravenous injection of living cells from the future donor into the embryonic future host. This situation was brought about by various injection technics in the embryonic or new-born infant mouse, rat and chick.

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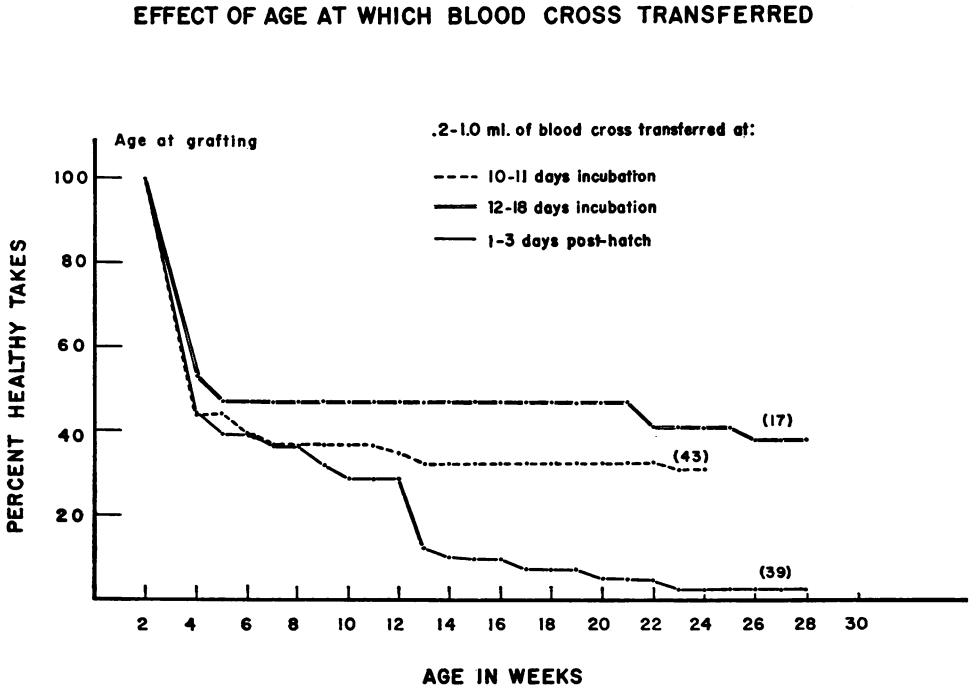

FIG. 1.

METHOD

Our recent work has been concerned with a study of this phenomenon of induced tolerance in the chicken and the successful homografts which can subsequently be obtained in comparison to the successful homografts obtained by the method of Danforth by simply homografting day-old chicks.

Billingham abandoned the use of embryonic chicks for the induction of tolerance because of high mortality and difficulties in technic.[2] However, a technic has been perfected (to be reported in detail in another communication) of cross transfusion of blood between embryonic chicks which results in a hatch rate of 80 per cent as compared to a hatch rate of 85 per cent obtained in control non-injected eggs. The technic, therefore, imposes a mortality of only 5 per cent. The technic involves the use of the usual candling methods to locate the allantoic veins, venipuncture by manipulating the egg against an immobilized #40 hypodermic needle with fine polyethylene tubing used as the blood reservoir, accomplishing the blood interchange by switching the polyethylene reservoirs between the fixed needles, and prevention of fatal hemorrhage by sealing the needle in place in the egg with later removal after clotting in the vein around the needle has occurred. This method involves no complicated or expensive apparatus and a pair of eggs can be subjected to interchange of tolerance inducing amounts of blood in about five minutes.

RESULTS

1. *Effect of varying the age of the embryo at the time of interchange of blood (Fig. 1).* Blood was cross-transferred between chicks incubated ten to 18 days, and chicks one to three days post-hatch. (In the latter instances the interchange was accomplished by simple cardiac puncture.) The amount of blood transferred was 0.2 to 0.4

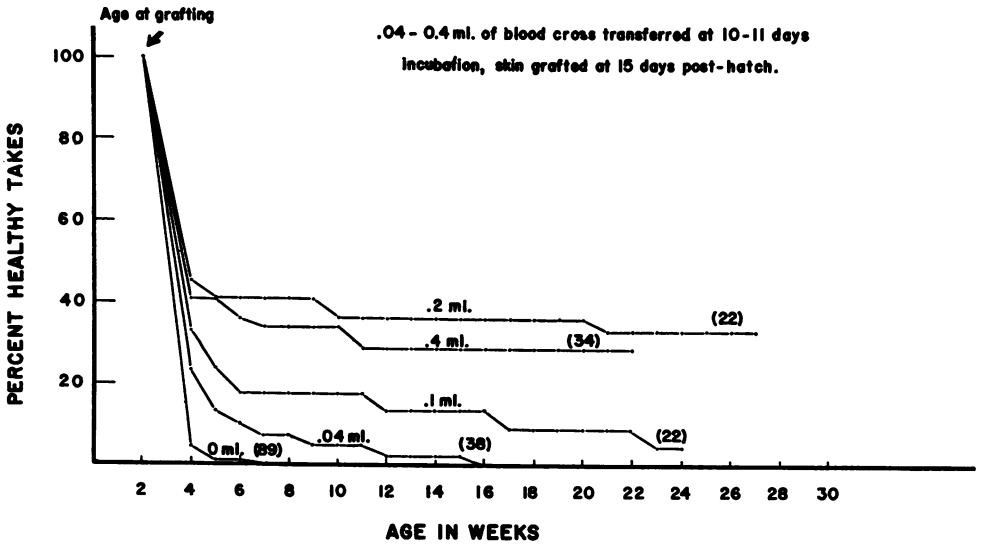

FIG. 2.

ml. in embryonic stages and 0.5 to 1.0 ml. in post-hatch stages. Because we have previously shown that when control chicks 14 days old are cross skin-grafted, a take of the homografts is never obtained, we did not test the experimental chicks for tolerance by skin homografting until the fifteenth day of age. Crossing of blood between embryos at 10 to 11 days of incubation resulted in 14 chicks out of 43 who maintained healthy homografts at least 18 weeks. Upon crossing blood at a later stage in development, 12 to 18 days of incubation, the percentage of healthy homograft takes increased slightly to 47 per cent. Cross-transfusion of blood in the first three days after hatching resulted, initially, in takes of homografts in approximately 40 per cent of the 39 chicks tested. This effect, however, was not as long lasting as the effect produced by injection of blood in embryonic stages. Starting at the sixth week, the percentage of healthy takes dropped down to 2.5 per cent by the twenty-third week after grafting. We note here that the conclusions made by Billingham when tolerance was induced by injecting newly-hatched chicks are based on observations on the homograft usually for a period of 30 to 45 days. By contrast, in our group of 39 chicks tested the percentage of surviving healthy homografts dropped from 40 per cent to 2.5 per cent *after* the forty-second day or sixth week.

2. *Effect of amount of blood transfused* (*Fig. 2*). Varying quantities of blood from 0.04 to 0.4 ml. were cross-injected between 10 to 12 days incubated chicks. Fifteen days after hatching the chicks were cross-grafted. Increasing the dosage from 0.04 ml. to 0.2 ml. increased the percentage of takes at six weeks from 8 to 38 per cent. However, there was little difference between the results obtained with 0.4 ml. as compared to 0.2 ml. With the larger dosages, the effect was more permanent. At 19 weeks after grafting of the 56 chicks previously injected with 0.2 and 0.4 ml. of blood, 18, or 32 per cent,

EFFECT OF AGE AT WHICH CHICKS ARE GRAFTED

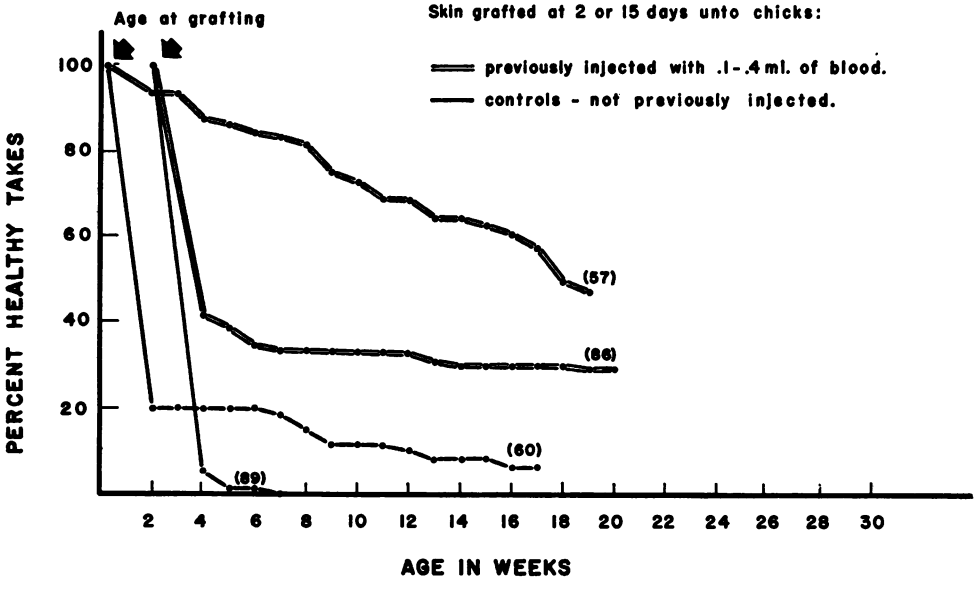

FIG. 3.

had surviving homografts. Of 60 chicks injected with 0.04 ml. to 0.1 ml., only 4 per cent had surviving homografts.

3. *Effect of age at which skin is grafted* (*Fig. 3*). Upon previous cross-transfusion of 0.1 to 0.4 ml. of blood in embryonic stages, chicks grafted at two days had 37 out of 57, or 65 per cent, healthy homograft takes at 13 weeks. Untreated control chicks grafted at the same time had 6.7 per cent takes at 13 weeks. Of the 86 chicks grafted at 15 days, 25 (29 per cent) showed homograft survival to the eighteenth week after grafting. In the 89 control chicks also grafted at 15 days, no instance of homograft take was found four weeks after grafting. There is, therefore, a marked significant difference between the effect of grafting at two and 15 days post-hatching when tolerance is induced by cross-transfusion of ten to 12 day embryos.

4. *Effect of repeat grafting on the same chick from the same donor* (*2 and 15 days post-hatching*) (*Fig. 4*). Upon cross-transfusion of 0.2 to 0.4 ml. of blood at 11 to 16 days of incubation, the same chick pairs were cross-grafted at two and again at 15 days post-hatching. At eight weeks of age, 19 out of 21 (81 per cent) of the first homografts, grafted at two days post-hatchings, were surviving, while only 11 out of 21 (52 per cent) of the second homografts, grafted at 15 days, were healthy. In control untreated chicks grafted in a similar manner at two and 15 days, when eight weeks of age, 10 out of 108 (9.2 per cent) had healthy first homografts, while three out of 108 (2.8 per cent) had surviving second homografts.

DISCUSSION

Although much work has been done on the phenomenon of acquired tolerance, to our knowledge this is the first report in which 1) the embryonic stages have been injected with negligible mortality and in

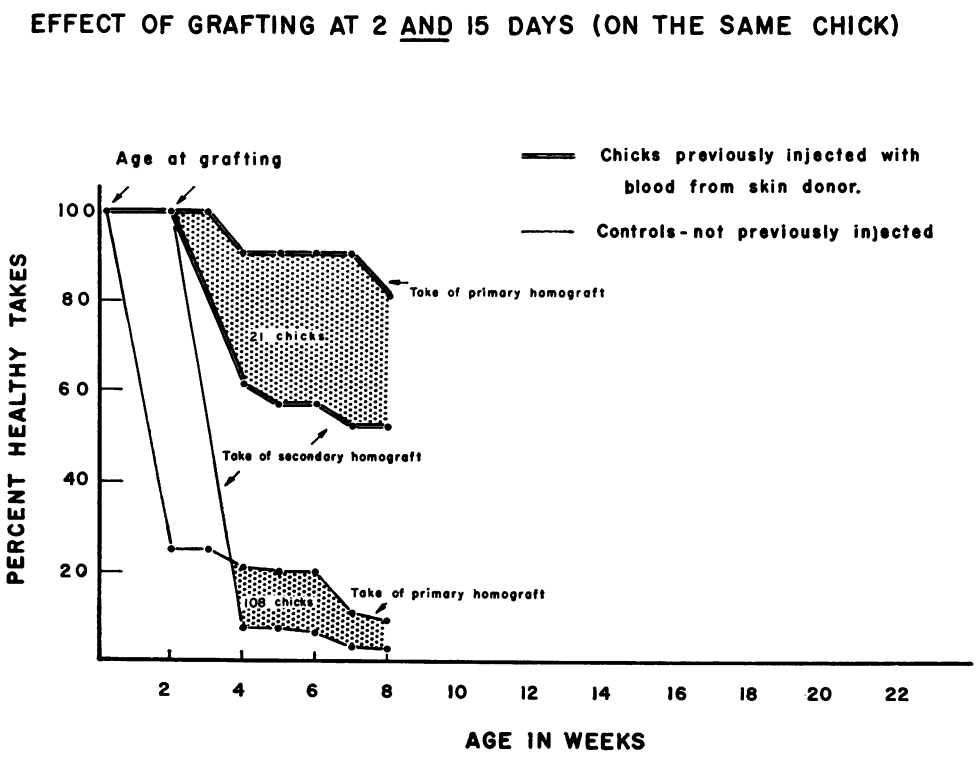

FIG. 4.

significant numbers, 2) different stages of embryonic development have been tested, and 3) most importantly, the long term effect (at least six months) of induced "tolerance" has been observed. Approximately 40 per cent of chicks injected in embryonic stages from mid-development on, have surviving homografts six months after grafting as compared to 2.5 per cent in chicks injected shortly after hatching.

It is interesting to note that this "tolerance" effect is markedly different when the chicks are grafted at two or 15 days post-hatching, subsequent to cross-transfusion of blood in the embryonic stages. Grafting of skin at two days resulted in a 42 per cent initial take. Homograft take then declined rather rapidly in chicks grafted at two days, while remaining relatively constant in chicks grafted at 15 days. Nevertheless, chicks grafted at two days had a significantly higher percentage of take at six months. How much of this difference is due to the age of the host and how much to the age of the graft is yet to be determined.

Interpretation of the data in experiment number four is difficult and the results are to us somewhat confusing. We are unable to interpret these results on the basis of assuming the presence of host tolerance alone as being responsible for the incidence of homograft survival which resulted. At eight weeks of age, 11 out of 19 individuals appeared to be tolerant of both grafts, i.e., that applied from the same donor at two days of age and that applied at 15 days of age. Eight or 40 per cent of the 19 were still tolerant of and supported healthy primary grafts but at the same time had already sloughed the secondary grafts from the same donor. It would appear that in this experiment only one immunological system was involved in the host, but skin homografts in two radically different (infantile

vs. essentially adult) stages of development were used. The results of this experiment, as well as those we have perviously reported, would appear to raise additionally the question of whether tissue specificity factors as well as immunological tolerance factors in the host are involved.

Finally, it would seem pertinent to raise the question of whether the preparations which so far have resulted in skin homograft survival in the chicken did so by identical mechanisms. At the present time, we would presume to distinguish three such preparations. The oldest, and the one with which we have had the most experience, might be termed the Danforth preparation, the second, beautifully conceived and extensively studied by Billingham in the chicken, might be referred to as the Billingham preparation and the third, which ingeniously duplicated in the chicken the natural embryonic parabiosis noted by Owen to occur in cattle, might be termed the Hašek preparation.

In the Danforth preparation, our interpretation of our evidence would indicate that alterations in the tissue may be more important, or at least as important as tolerance- producing alterations in the host immune mechanism. Some of our evidence would seem to question whether the latter occurs or is routinely permanent in the Danforth preparation.

In the Billingham preparation, tolerance-inducing alterations in the host immune mechanisms must certainly occur, though alterations in the grafted tissue may also occur. This preparation, like the Danforth preparation though to a lesser degree, is apparently never more than 50 to 60 per cent permanently effective and is sensitive to many variables

The Hašek preparation, like the situation occurring in fraternal twin calves, produces permanent and mutual tolerance between host-donor pairs. Blood cell "chimerism," however, exists in both instances.

We consequently would question, at least tentatively, whether conclusions which can be made from data collected in experiments on one preparation can be applied without reservation to the other preparations. We submit that further comparative studies, similar to the ones contained in the above preliminary report, are indicated and may yield highly significant information.

SUMMARY

Some factors involved in the phenomenon of "acquired tolerance," such as the age of the host when blood is cross-transferred, dosage of blood injected, and age of the host when homografted, were studied in chicks. Upon cross-transfer of blood in embryonic stages, approximately 40 per cent of the 60 chicks tested showed skin homograft survival six months after grafting. In 89 control untreated chicks, also two weeks old when grafted, no instance of homograft survival was found after five weeks. Upon cross-transfusion of blood shortly after hatching, skin homografts took initially in 40 per cent, but dropped to 2.5 per cent by six months. The percentage of take increased with increasing dosage of blood from 0.04 to 0.2 and 0.4 milliliter.

Subsequent to embryonic cross-transfusion of blood, skin that was cross-grafted 2 days post-hatching resulted initially in 94 per cent takes out of 57 chicks tested, while in 86 other chicks cross-grafted at 15 days, 42 per cent took initially. The take of skin grafted at two days declined steadily to 47 per cent by the nineteenth week, at which time the take of skin grafted at 15 days was 29 per cent. In another group of embryonically cross-transfused chick pairs grafted twice between the same host-donor pair, at two and 15 days, the skin grafted at two days again took in a large percentage initially (100 per cent), while the 15 day graft took in 62 per cent. The implication of this experiment and our previous work upon the question of graft adaptation is discussed. Differences in the behavior of host and homograft, in what

are tentatively referred to as the Danforth, Billingham, and Hašek preparations respectively, are noted and certain of the possible implications are briefly discussed.

The evidence of rather marked "late" homograft destruction in a group of chicks made highly tolerant in their early post-hatching period by embryonic cross-transfusion, together with evidence from our previous experiments, strongly suggests that the presence of a viable skin homograft is frequently not sufficient to perpetuate the state of tolerance. If the antigen-antibody reaction does govern the survival or destruction of homografted tissue, the failure of the viable skin homograft to perpetuate tolerance may be a result of an inadequate "dose of antigen" arising from the graft or the "tolerance-producing antigen" may not be produced by the skin homograft at all. Continued survival of the skin homografts, if the latter condition prevails, may result from graft adaptation during the transient tolerant period produced by cross-transfusion during embryonic life.

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