A STUDY OF HUMAN SKIN GRAFTED UPON THE CHORIO-ALLANTOIS OF CHICK EMBRYOS*

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PLATES 47 TO 49

The possibility of successfully grafting certain malignant tumors of the rat and mouse upon the chorio-allantois of chick and duck embryos was demonstrated by Murphy (1) in 1912. Heterologous grafts of this kind became embedded and vascularized, and grew rapidly and continuously under these conditions until about the 18th or 19th day of incubation. At that time there seemed to be a sudden change whereby the grafts were caused to undergo rapid regression and degeneration.

It seems likely that the chick embryo, up to the last days of incubation at least, cannot respond as adult animals do to the presence of heterologous tissue, and in the absence of such unfavorable reaction the foreign cells are capable of utilizing the nutriment supplied by the developing egg.

The capacity to respond in a way which is unfavorable to heterologous and iso-grafts probably develops gradually in the chick; for the experiments of Danforth and Foster (2) have shown that during the first few days after hatching iso-grafting can still be successfully performed upon them.

Owing to the demonstration of successful hetero-grafts of certain tumors and embryonic tissues upon the chorio-allantois of the chick, and to the demonstration that the chick membrane when subjected

to injury by heat would form granulation tissue, the possibility occurred to us that human skin might be nourished when suitably applied to this highly vascular embryonic tissue. We felt that the study of the fate of such grafts would be of interest in itself, and successful grafts would afford an opportunity for an experimental approach to a variety of problems.

Our experience readily demonstrated the possibility of securing grafts of human and other animal (rabbit, chicken) skin upon the chorio-allantois of chick and duck embryos. Such grafts rapidly became fixed and nourished so that they persisted intact until about the time of hatching. Grafts implanted upon the membrane of 10 day old embryos could be studied for a period of 8 to 10 days, the incubation period of hen eggs being 21 days.

The present paper concerns the technique used, and records our observations upon the mechanisms involved in the survival of grafts of human skin upon the chorio-allantois of chick embryos. It includes also a discussion of certain principles and possible applications of the method.

Technique

Fertile hen eggs were incubated in a commercial hatchery incubator for 10 days. By candling, the large membranal vessels were located and marked on the shell, because it seemed that grafts survived better when placed over them. A square window was then cut in the shell by the use of a rotating carborundum disc according to the method described by Goodpasture and Buddingh (3); and a piece of skin, previously prepared, was applied to the exposed chorio-allantois. The raw corium of the graft was affixed to the presenting vascular ectodermal layer of the membrane; thus the epithelium of the skin presented exteriorly upon the surface as in its natural relationship.

Human skin in three forms was received immediately from the operating rooms of the Surgical Service of the Vanderbilt Hospital. This had been removed in the course of excising tumors or in covering raw surfaces with skin grafts. Pieces of skin, which were trimmed away in fitting various grafts in their beds, were saved and sent to the laboratory. Other pieces surrounding various lesions of the skin were received with the subcutaneous tissue still attached. Additional specimens were of the thickness of thin grafts (Thiersch grafts), while still others were a little thicker but still not of the full thickness variety (split grafts).

The intact skin had been previously shaved and washed with soap, water and alcohol, on the ward, followed by iodine and alcohol in the operating room in preparation for removal. The pieces were stretched upon a sterile board and cut

into squares of ½ to 1 cm., or larger if desired. Each of these was then spread, with the raw corium exposed, upon the smooth surface of a thick metal searing iron having a long handle. A dissecting needle was used to effect these manipulations. By means of the iron the flattened and stretched surface of the corium was directly applied to the chorio-allantois, where it was freed with the needle and laid upon the membrane. Any folded edges or corners were straightened out with no difficulty. The thin graft then lay smooth and flat upon the membrane to which it rapidly became adherent. These manipulations were of course carried out with precautions for asepsis. If skin with subcutaneous tissues attached was received it was stretched and fixed to the board with pins and thin pieces of epithelium with a little corium were shaved off with a triangular fragment of a safety razor blade held in a hemostat. Full thickness skin was never used for grafting.

After the graft was thus affixed to the membrane the window was closed by surrounding the shell opening with a layer of vaseline-paraffin mixture upon which a cover-glass was layed and sealed. The egg was then placed on a wire meshed tray in a bacteriological incubator held at 37°C. and kept moderately moist by means of a vessel of water. The eggs were placed in the tray with the window up.

Through this window the graft was observed at will either with the naked eye or through a binocular microscope. Moisture under the cover-glass was removed by applying to the outer surface a heated, blunt instrument, like the tip of a scalpel handle.

The graft usually remained smooth and flat for 25 to 72 hours, then it tended to become somewhat wrinkled with a diminution in circumference. The wrinkling was probably due to contraction of the proliferating mesodermal tissues of the membrane.

If the graft was successful it remained firm and glistening. If necrosis occurred it became boggy, dull and yellow. Surprisingly rarely did the surrounding membrane become infected or inflamed. At times staphylococci grew upon the keratinized surface of the graft without interfering in any way with its nourishment and survival. If such grafts are clean, thin, fresh and only slightly traumatized, the chances of survival are excellent.

At variable periods of time the membrane with its graft was fixed in Zenker's fluid; and paraffin sections were cut and stained, in order to observe the processes of nourishment of the grafts. The membranal blood vessels in several instances were injected with a 10 per cent suspension of Higgins' India ink, and after fixation, paraffin sections were studied in order to observe the vascular bed.

Attempts were made to study the degree of reestablishment of circulation in the peripheral vessels by direct observation with a Leitz binocular ultropak microscope. Grafts at various stages were observed through the uncovered window. Oils and other substances were applied to the epithelium to make it more transparent. So far, however, we have not been sufficiently certain of our observations to include them.

Structure of the Chorio-Allantoic Membrane of Chick Embryos

At 10 or 12 days of incubation, as Danchakoff (4) has shown, the exterior or ectodermal layer of the chorio-allantois is a thin, highly vascular membrane in which a rich capillary network lies beneath and within the delicate epithelial sheet. Some of the capillary loops seem to be exposed upon the surface without epithelial covering. It is through this membrane that the respiration of the embryo takes place.

On removal of the shell and its adherent fibrous membrane, there is sometimes slight injury to the ectodermal-capillary layer and minute extravasations of blood occur onto the surface; so it seems certain that either because of its original structure or because of slight damage some points of the graft were placed directly in contact with the exposed capillary bed. In effect, therefore, the grafts lay upon a vascular surface analogous to granulation tissue.

Survival of the Grafts and the Processes of Their Organization

Very soon after the application of a piece of skin to the membrane the graft becomes lightly adherent. Microscopic sections at the end of 24 hours show no exudate between it and the membrane, neither fibrin, serum nor leucocytes, if the graft is an entirely satisfactory one. Even at this time, however, there may be wide or narrow areas of the membrane which are entirely denuded of epithelium, and at these places the corium of the graft is closely applied to the embryonic meso-dermal tissue. At other places the ectodermal layer of the membrane may be more or less intact, possibly with occasional microscopic petechiae between it and the corium. Frequently chick polymorphonuclear leucocytes have infiltrated parts of the graft.

At 48 hours one may occasionally see a capillary protruding through the ecto-derm of the membrane beneath the corium. As early as the 3rd or 4th day nu-cleated erythrocytes of the embryo have found their way into preexisting blood vessels of the skin graft, and at times on the 5th day every blood vessel of the graft, even the subepithelial capillaries, is filled with the chick erythrocytes, giving the appearance of a complete establishment of blood circulation through the vascu-lar bed of the skin. This appearance has been particularly marked in grafts from keloids and moles in which the corium is more rigid than in normal skin, and the blood vessels larger. If a graft "takes" satisfactorily there may be little or no cellular exudate to be found in it, and after 5 days sections have been obtained which contain no cellular exudate whatsoever.

In those grafts or parts of grafts which do not take satisfactorily polymorpho-nuclear cell and serous exudates appear early and abundantly in the corium,

and may increase until the entire graft or its epithelium has become elevated by a pustule or a vesicle. These usually die in part or altogether in a few days, although some portions of such grafts may become adequately nourished.

In autografting on human subjects considerable stress has been placed upon the importance of obtaining and maintaining contact of the graft with the underlying wound surface, especially during the first few days after its application. An exudate is prevented from collecting under the graft either by cutting the latter in pieces so small that drainage may occur from under its edges ("pinch grafts") or by perforating the larger grafts at frequent intervals for drainage and maintaining pressure over its surface by a marine sponge or another expansile medium.

In the grafts on the chick membrane an attempt was made to gain approximation by smoothing the skin out but there was no effort to maintain contact. It is probable that in some of the grafts, especially the larger ones, spaces containing plasma or air remained and that this was the cause of permanent collections of exudate and death of these grafts.

On about the 3rd day evidence is often seen of an ingrowth of capillaries from the membrane into the adjoining layer of the corium. These invading vessels may penetrate deeply into the corium during the 10 days of observation.

If the graft is rather thick and nucleated red cells do not early appear within the lumens of the blood vessels of the graft, evidences of inflammation appear and the corium becomes edematous followed by desquamation or necrosis of the overlying epithelium.

Mitotic figures sometimes increase in number in the epithelium of a satisfactory graft at about the 6th day, and hyperkeratosis becomes evident later. Rarely does one find a mitotic figure in a cell of the corium during the period of observation. There is no tendency for the epithelium of the graft to invade the corium.

The Ectodermal Epithelium of the Membrane in Relation to the Graft

One of the most interesting phenomena in connection with a skin graft on the membrane is the perfect approximation of the ectodermal epithelium of the membrane with that of the piece of skin at its periphery. The fusion is so exact and the union so nearly perfect that it would be difficult to detect it, did the smaller chick epithelial cells not take a lighter stain. In some preparations it appears that the two types of epithelium are laced together by cellular fibrils which pass between them producing intercellular bridges or prickles. One gains the impression of an affinity or positive chemotaxis between the epithelium of the host and that of the cut edge of the graft.

On the other hand an opposite relationship seems to exist between the mesodermal tissue of the corium of the graft and the ectoderm of the membrane. In the presence of the graft the latter tends to

disappear from beneath or to aggregate in small foci where it assumes the appearance eventually of epithelial pearls.

In the case of skin grafts from an old man over 70 years of age the cells of such epithelial pearls underwent rapid multiplication with extensive keratinization so that fairly large dermoid cysts were formed. Our observations of this are too limited to draw definite conclusions, but it is suggested that hyperkeratosis of the graft may influence keratosis of membranal ectoderm.

The exact mechanism by which membranal ectoderm disappears or draws apart in the presence of skin grafts has not been determined. It is of interest, however, that mesodermal grafts (*i.e.* of muscle, spleen and cartilage) rapidly sink into the mesoderm of the membrane and the ectodermal layer becomes completely restored and reunited over them.

The observation was made that skin grafts from two human cadavers also tended to sink into the mesoderm of the membrane, but apparently were prevented from becoming completely incorporated, by a union of the epithelial edges with the ectoderm of the host.

Two skin grafts implanted on the same membrane with edges only a few millimeters apart have shown no tendency of the epithelium to grow over the gap in a period of a week. As in single grafts the epithelial margins were sealed by the fusion with them of the ectoderm of the membrane.

The Membranal Fibrous Tissue in Relation to the Graft

In the case of a well nourished graft wherever the collagen and fibroblasts of the corium have come into contact with exposed mesodermal fibrous tissue of the host, a fusion or interlacing of collagenous fibrils and fibroblasts of the graft and host has taken place. There is no separation by fibrin, hemorrhage or exudate under favorable circumstances. There seems to be as much congeniality between these tissues of the graft and host as is observed between the two epithelia.

Considerable hyperplasia of membranal fibrous tissues sometimes occurs beneath a skin graft, and fibroblasts rapidly extend into areas of inflammation or degeneration in the corium and along the edges beneath the epithelium of the graft accompanied by membranal blood vessels.

Without infection there is usually little or no myeloid hyperplasia

in the membranal mesoderm, and the polymorphonuclear leucocytes which pour out of the blood vessels quickly migrate beneath or into the corium of the graft.

Foreign bodies or exudate become surrounded or walled off by foreign body giant cells which form rapidly and in great abundance.

The Blood Vessels of the Membrane in Relation to the Graft

The earliest evidences of an effect of the skin graft upon the underlying capillaries of the membrane are a denuding of the ectodermal epithelium, and minute focal extravasations of nucleated erythrocytes beneath the graft. In some preparations within 48 hours capillaries may be found penetrating the approximating surface of the corium.

Within 3 or 4 days nucleated chick red cells can be seen inside the lumens of the larger blood vessels of the graft. In many places small extravasations of blood seem to intervene between these vessels and the membranal capillaries. In other preparations, especially in keloid grafts, the two vascular beds seem to join directly, the blood passing immediately from the enlarged membranal channels into the preexisting channels of the graft. The latter become filled with chick nucleated erythrocytes, even to the small subepithelial capillaries, and the channels in serial sections appear to be connected and looped in such a way that at least a partial circulation seems inevitable.

There is no doubt that these channels are the original vessels of the graft, for as late as 5 days serial sections have shown persisting non-nucleated human red cells in capillaries continuous with larger channels filled entirely with chick red cells. Furthermore the endothelial lining of the vessels of the graft are preserved, and the human endothelial cells are larger and possess larger and more chromatic nuclei than do those of the chick embryo.

It would appear therefore that the vascular connections between the graft and the host exhibit also the effect of a certain affinity or chemotaxis which causes them to unite, as do the respective epithelial cells and fibroblasts.

So far as representing a restoration of blood circulation is concerned the filling of the blood vessels of the graft with nucleated red blood cells of the embryo may be deceptive. It has been stated that, in serial sections at a 5 day period of a piece of skin graft, capillaries

were found containing human erythrocytes in continuity with larger channels filled with nucleated cells. The presence of human erythrocytes definitely indicates a lack of circulation through these particular channels, and several attempts to demonstrate blood circulation through the persisting vessels of a graft (not keloid nor mole) by India ink injections have failed to demonstrate it. Arterioles and arteriolar capillaries of the membrane and granulation tissue growing into the corium become filled with carbon particles, but adjacent large channels of the skin, although distended by nucleated chick erythrocytes, contain none.

The presence of nucleated red cells of the embryo within the vessels of the graft demonstrates access of embryonic blood to them; and this no doubt provides an adequate plasma circulation even though blood circulation is wanting.

It would seem that a true circulation through the original blood vessels of the graft might in time become established if the incubation period were prolonged and the embryo developed no unfavorable reaction. Under such circumstances no doubt chick endothelium would eventually replace the original lining, for there is evidence in older grafts that the human endothelium gradually disintegrates.

No evidence was found within the period of our observations that capillaries from the membrane grow into the preexisting blood vessels of the graft. However there is a constant increase in capillary, arterial and venous ingrowth from the membrane into the corium of the skin, and in all probability this new vascular organization would eventually take over the circulation which initially is maintained in part through the original channels.

There is no doubt that, in the first days of the graft and probably in part throughout the period of our observations, a plasma circulation of some sort is largely responsible for the survival and maintenance of the grafts. If a piece of skin is very thin, consisting almost entirely of epithelium, no vascularization may appear, yet the graft remains to all appearances in perfect condition. Such grafts indeed are less apt to show evidences of inflammation and degeneration than thicker ones.

Early exudation into the graft, however, seems to indicate injury or degeneration of some sort within it before it is applied. To such injury of the foreign tissue the embryo responds by an outpouring of polymorphonuclear leucocytes and fluid exudate.

Beneath the graft the embryonic blood vessels in a few days increase in size and abundance, evidently in response to influences, perhaps both mechanical and chemical, through fluid exchange, from the applied skin.

Chick red blood cells do not penetrate all the vessels of the graft, and in some instances most of the vessels contain none. Under these conditions there is usually considerable invasion by polymorphonuclear leucocytes and the skin appears to be less well nourished. The access of chick blood to the vascular channels of the graft is a very important element in its nourishment for several days, and grafts from keloids and moles in which vascular channels are larger and more easily penetrated take better apparently than do most specimens of normal skin.

In the keloid grafts especially there are prominent endothelial lined lymphatic channels containing human mononuclear phagocytic cells. Not infrequently mitotic figures are found in these cells. No chick erythrocytes have been observed in lymphatic vessels.

Reimplantation of Skin Grafts

Some attempts were made to remove grafted skin from the first implantations and to regraft them upon a second embryo. This was found to be a rather difficult procedure and the attempts usually resulted in failure, although a few successful second generation grafts were obtained. With the development of a better technique the percentage of successful second grafts no doubt would be greater. The difficulty technically lies in removing the graft, which may be wrinkled, in such a way as to avoid carrying over too much embryonic tissue. The primary graft perhaps also lends itself better to a plasmatic circulation than the secondary, because of its intact blood vessels. Infection also is a common cause of failure of reimplantations.

Grafts have been maintained for periods of 8 days on the first embryo and 6 on the second, before sections were made. These 14 day old grafts show extensive keratinization and active mitosis of cutaneous epithelium. Occasional mitotic figures are also found in fibroblasts of the corium, and in endothelial cells. Human skin grafts can be maintained for at least 2 weeks in this way and probably longer.

The possibility of preserving human skin and other tissues for

purposes of surgical use is obvious, although no demonstration of the practicability of such a method has been made.

Duration of Viability of Skin before Grafting

At present tests are in progress to determine the practicability of preserving skin and other tissues by this method and regrafting them to the same and other human individuals. An attempt is thus being made to determine whether or not those individual hereditary factors or characteristics which are responsible for the failure of iso- or homo-grafts are retained by the tissue after a sojourn of several days on the egg membrane.

We have not directed especial attention to the problem of the duration of viability of skin for grafting, but in the course of our experiments skin removed from the patient 8 hours before grafting, and kept moist at room temperature (22°C.) has yielded successful grafts. In one test human skin kept refrigerated at 5°C. for 24 hours gave excellent takes.

In two instances skin removed from cadavers 2 and 3 hours after death was successfully grafted on chick membranes.

Use of the Method in Experimental Pathology

There are probably many uses to which this method of skin grafting might be put in various experimental studies. Some of our own interests have been directed toward the field of infection and immunity, especially infections caused by viruses.

In order to determine whether or not human skin grafts on the embryo are susceptible to infection by some of these agents, we have successfully inoculated human skin grafts with the viruses of herpes simplex, vaccinia and smallpox. The resulting lesions and cellular changes have been typical of these infections. These studies are being continued and will be reported upon later. The question of cellular immunity is also under investigation.

DISCUSSION

The laws governing the fate of skin when transplanted from one part of the body to another of the same individual and from one individual to another are of great interest alike to the pathologist and to the surgeon.

The experimental work to establish these laws, as far as it has

gone, has met with many difficulties. In human tests the chief difficulty has been that, in order to get complete histological studies at different stages after transplantation, too much skin would need to be sacrificed. In dealing with animals the great amount of hair usually present in the skin makes dressings difficult and infections common when grafting is attempted. If no dressing is applied injury to the surface from mechanical trauma or drying is common and infection will usually ensue.

Davis and Traut (5) avoided these factors to a large extent in their experimental studies on dogs by burying skin grafts, raw surface down, on muscle, and closing the wound edges over them. The grafts were excised later for study. By this method they were able accurately to determine histologically the mode and degree of the reestablishment of circulation at varying intervals after grafting.

Those who have studied the processes involved have recognized three periods in the permanent establishment of mammalian skin grafts which may be enumerated and discussed in relation to our observations as follows:

1. The Stage of So Called Plasmatic Circulation.—This term was first used by Goldmann who was so impressed by the rapid inwandering of mononuclear leucocytes from the host into the vessels and tissues of the corium of the skin, that he felt quite sure the graft, during the first hours of its existence, must be nourished by a sort of circulation of fluids before vascular connections became established (6).

Such a plasmatic circulation seems to be the most important mechanism in the maintenance of human skin grafts on the chick membrane not only in the early stages but throughout the longest period (10 days) of observation.

Unlike the mammalian experiments however, there is in a successful graft only moderate cellular infiltration, and then the invading cells are polymorphonuclear leucocytes. This occurs especially in unfavorable grafts and in and about necrotic blood cells within vascular channels of the graft which are not penetrated by chick erythrocytes, and it seems to be a response to injury and degeneration.

Although there might be an imbibition of fluid directly from the membrane into the corium, the chief nourishment appears to come through a later communication of the two groups of blood vascular channels, whereby first the large and then the small vessels of the

graft become filled with nucleated erythrocytes of the chick, no doubt accompanied by abundant plasma.

2. *The Stage of Vascularization of the Graft.*—In experiments upon dogs Davis and Traut described, as had others before them, the immediate deposit of a layer of fibrin between the tissue of the host and the graft. Within 24 hours a rich network of capillaries grows into the fibrin layer and begins to penetrate the corium. As early as 22 hours fusion of newly formed capillaries with similar or even larger blood vessels of the graft was demonstrated by the method of injection. This evidence indicated the partial establishment of circulation by anastomosis. In the chick embryo, although there is no comparable newly formed granulation tissue found on the membrane, a rich capillary bed is present in the ectodermal layer of the chorio-allantois. There is abundant evidence that anastomoses occur between the vessels, both large and small, of human skin grafts and the blood vessels of the membrane. Sometimes the two vascular channels seem to be united by an extravascular pool of blood. But there is no conclusive evidence that these connections restore intravascular circulation completely. The process seems rather to be one of inturgescence of the vessels of the graft for the most part.

There is, however, an ingrowth of capillaries from the membrane into the proximal layer of the graft after about 48 hours, but this does not lead to an effective blood circulation during the period of our observations. There is no evidence of a complete revascularization of the graft by chick blood vessels, such as took place in about 8 days in the experiments of Davis and Traut upon dogs. Granulation tissue rich in blood vessels forms especially where the corium of the graft is degenerating or disintegrating. The normal corium is apparently relatively impervious to the ingrowth of chick vessels.

3. *The Period of Regeneration and Repair.*—As described by Davis and Traut, the degenerative changes in full thickness grafts implanted in dogs are of an extreme degree, resulting in a slough of practically all the epithelium, except the thin Malpighian layer, and extensive degeneration in the corium including necrosis of the endothelial lining of blood vessels.

This does not occur under favorable circumstances in thin grafts of human skin implanted upon the membrane. All layers remain intact and there is no disintegration of epithelium. On the contrary

it remains normal in appearance. The blood vessels persist and, if the chick blood gains access to them, the endothelium remains intact for several days. If they are not penetrated by chick blood, the contained human white blood cells and endothelium undergo necrosis and the lumen is invaded by chick polymorphonuclear leucocytes.

No evidence of a revascularization by an ingrowth of capillaries and granulation tissue into the lumens of blood vessels of the graft was seen, such as was described by Davis and Traut in their experiments.

There is a greater tendency in the grafts which we have studied for the corium to undergo degeneration than the epithelium, and, as others have observed, epithelium of hair follicles seems to be the most viable tissue present.

Because there is no primary loss of epithelium through necrosis and sloughing there is no particular period of regeneration. Growth of epithelium proceeds apparently continuously, although most mitotic figures are evident about the 6th day. Keratinization ensues, and in grafts 10, and regrafts 14 days old, there is evident hyperkeratosis, for there is no loss from desquamation.

If incompletely nourished the corium tends progressively to disintegrate and there is no period when we have observed regeneration of fibroblasts to any extent. The corium, however, may remain perfectly intact for a week or longer if chick blood readily gains access to the blood vessels of the graft.

The chorio-allantoic membrane of the chick embryo thus offers a favorable medium for the nourishment of human skin grafts during 10 days, which represents the longest period of our observations. Implants made on the 10th day of incubation will survive intact for at least 10 days without any conclusive evidence of a specific reaction of this host against the heterologous tissue. The embryo, until about the time of hatching at least, shows no evidence in these experiments of being able to react unfavorably in a specific way to the grafted foreign tissue.

SUMMARY

Human skin grafted upon the chorio-allantoic membrane of chick embryos adheres and becomes nourished for as long as 10 days.

Occasionally regrafts upon a second egg have succeeded and thus prolonged the vitality of the graft to 14 days.

In successful experiments the epithelium of the chorio-allantois fuses with that of the graft, the collagen fibers of the corium interlace with those of the membrane after the separation or disappearance of the ectodermal layer, and the blood vessels of the chick anastomose, and unite by intervening pools of extravasated blood, with those of the graft. This vascular communication between the two tissues is largely responsible for the nourishment of the graft by affording a plasmatic circulation.

Gradually there is a partial revascularization of the graft by an ingrowth of blood vessels from the chick membrane.

Human skin grafts were susceptible to experimental infection by several viruses.

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EXPLANATION OF PLATES

The stains used for the specimens illustrated were hematoxylin and eosin.
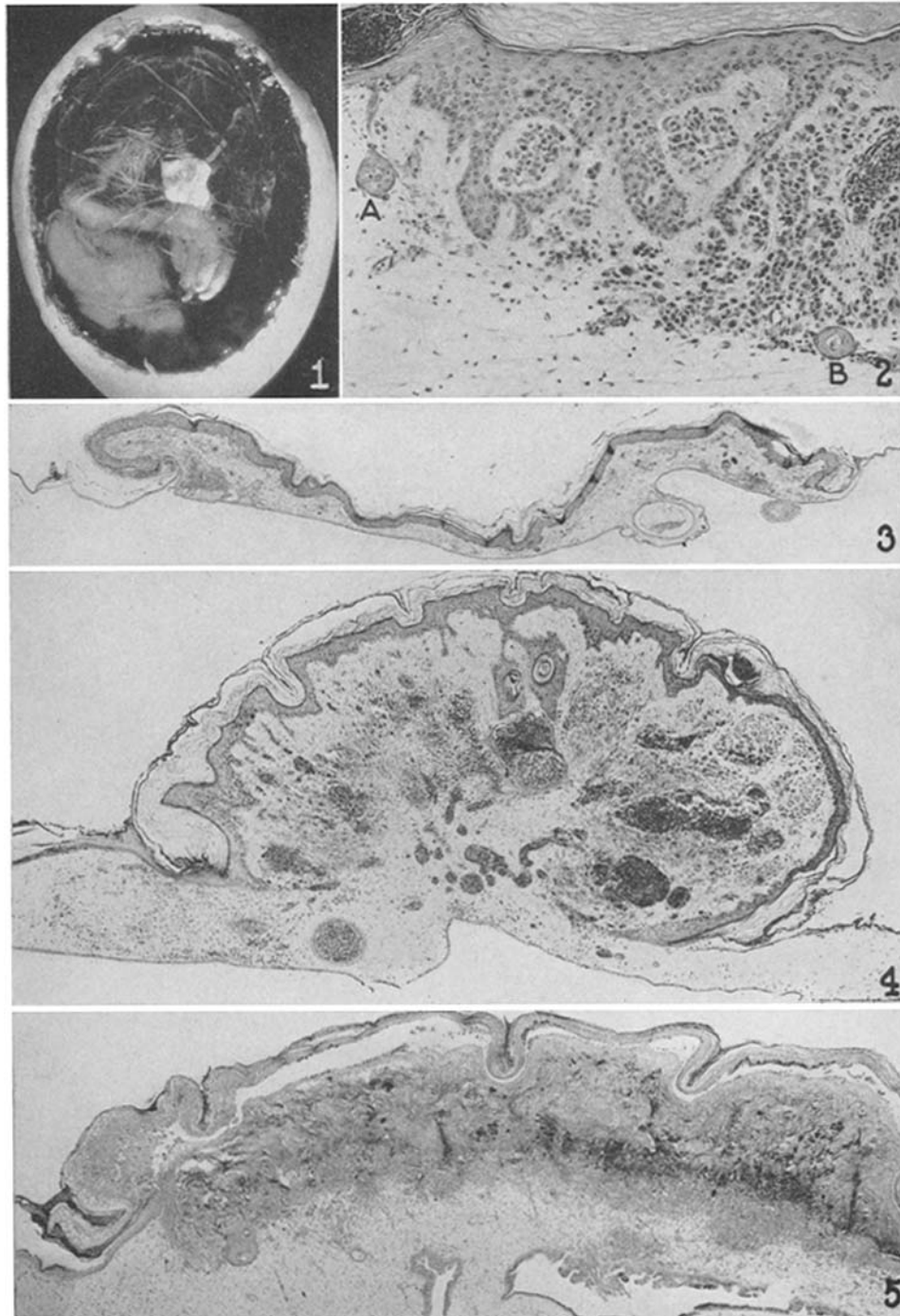
Plate 47

FIG. 1. Graft of human skin on the chorio-allantoic membrane of a hen's egg. Duration of graft 5 days.

FIG. 2. Pigmented mole of human skin grafted 5 days on chick membrane. A and B are epithelial pearls of chorionic epithelium marking position of original ectodermal layer.   × 120.

FIG. 3. Graft of human skin on chick membrane.   Duration 10 days.   × 18.

FIG. 4. Pigmented mole of human skin.   Duration 8 days.   Note blood vessels of membrane enlarged and those of graft engorged with chick erythrocytes.   × 40.

FIG. 5. Graft of human skin, infected and necrotic.   Duration 4 days.   Note zone of inflammatory reaction in membrane.   × 30.

(Goodpasture *et al.*: Skin grafted on chick embryo chorio-allantois)

PLATE 48

FIG. 6. Human skin grafted 5 days. Note (A) vessel of membrane communicating with vessel of graft distended with chick erythrocytes (nucleated). × 225.

FIG. 7. Graft of keloid, duration 5 days. Note communication of blood vessels of membrane and graft. × 225.

FIG. 8. Same as 7 showing (A) human erythrocytes and (B) chick erythrocytes in same blood vessel. × 1100.

FIG. 9. Graft of keloid, 5 days. Note (A, B, C) are of apparently communicating blood vessels of graft filled with chick erythrocytes and communicating with enlarged vessel of the membrane (D). × 150.
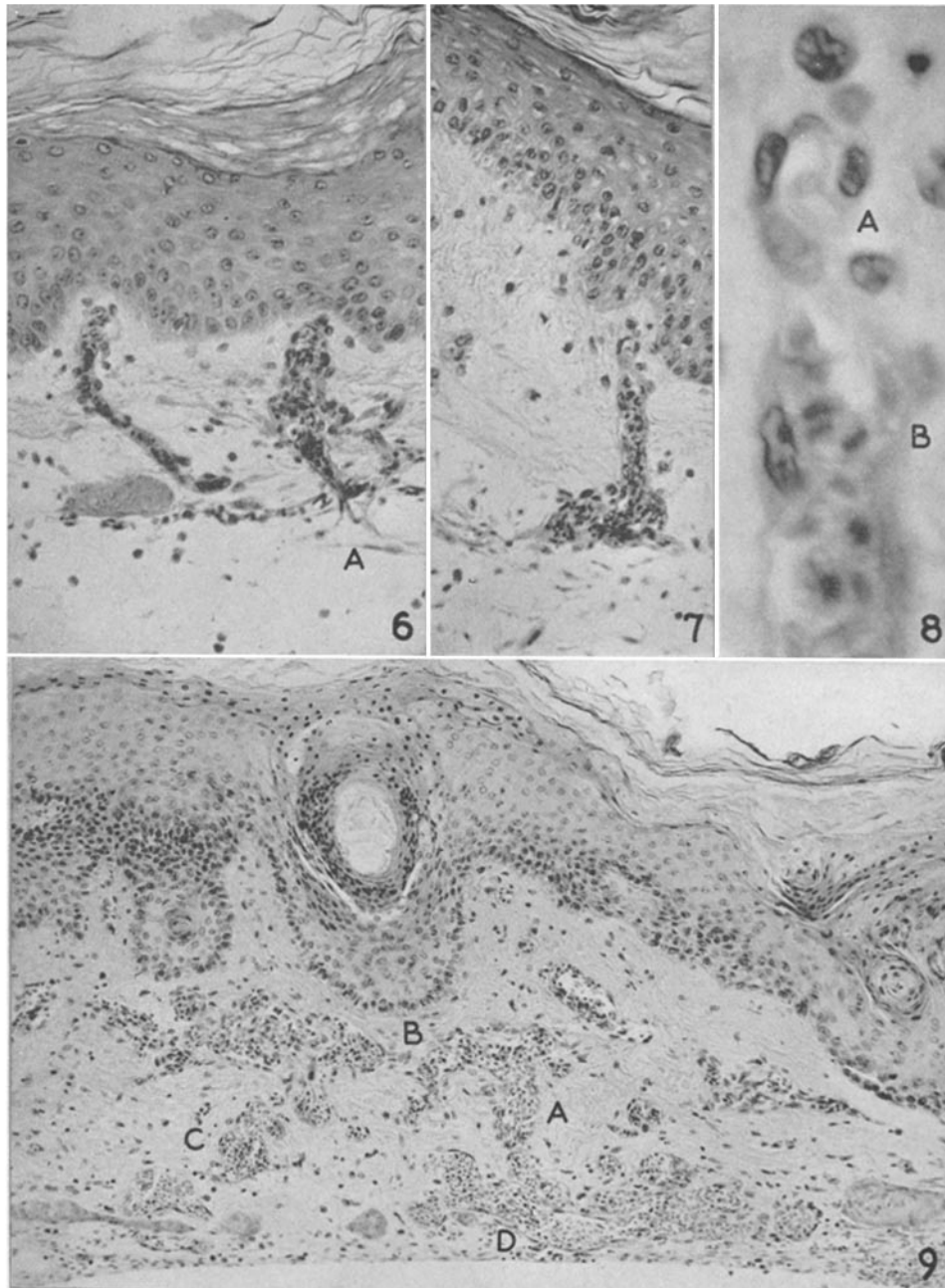
(Goodpasture *et al.*: Skin grafted on chick embryo chorio-allantois)
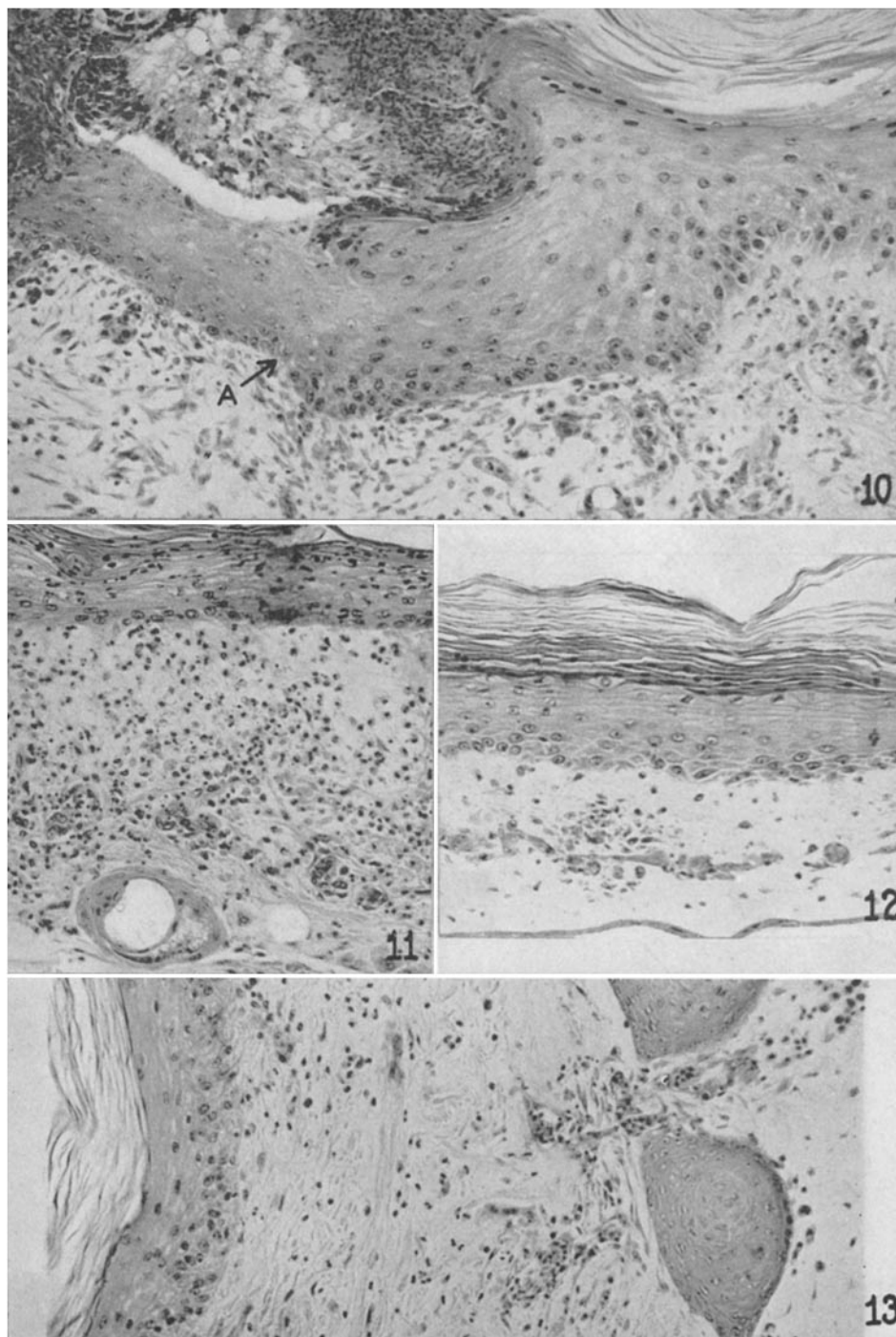
PLATE 49

FIG. 10. Note (A) fusion of membranal ectoderm (left) with epithelium of graft (right). Duration 5 days. × 225.

FIG. 11. Serous and leucocytic exudate in part of graft (8 days) next to a pocket of serum and pus (not shown). × 200.

FIG. 12. Another portion of same graft (Fig. 11) showing good take and no exudate. × 200.

FIG. 13. Granulation tissue from membrane extending into corium of graft between two epithelial pearls of membranal ectoderm. Duration 5 days. × 225.

(Goodpasture *et al.*: Skin grafted on chick embryo chorio-allantois)