THE TECHNIQUE OF FREE SKIN GRAFTING IN MAMMALS

BY R. E. BILLINGHAM* AND P. B. MEDAWAR

From the Department of Zoology, University of Birmingham

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(With Plates 5–7 and One Text-figure)

1. INTRODUCTION

In recent years, skin grafting has been used for the study of a wide variety of biological problems. Some of these problems relate to the properties of skin itself—e.g. pigmentation (Billingham, 1948), hair growth, sensitivity (Haxthausen, 1948), the innate 'racial' differences between the several varieties of epidermal epithelia (Billingham & Medawar, 1948a, b, 1950) and the contributions of dermis and epidermis to the formation of chemically induced tumours (Billingham, Orr & Woodhouse, 1950). With other problems, skin has been used in preference to other tissues because it it is more accessible or easier to handle, or because it gives less ambiguous answers; among these may be mentioned tissue transplantation immunity (Gibson & Medawar, 1943; Medawar, 1944, 1945, 1946a, b, 1948a, b), the measurement of the degree of homozygosity of inbred lines (McDonald & Medawar, unpublished), and the resistance of living cells to procedures such as anaerobic cultivation (Medawar, 1947) or freezing and drying (Billingham & Medawar, 1951).

The peculiar advantages of skin for grafting purposes are, briefly, these: skin is the only tissue which may easily be grafted 'orthotopically', i.e. to an anatomically natural environment; the fate of skin grafts may be watched from day to day and their behaviour easily checked by repeated biopsy and histological examination without prejudice to the well-being of the recipient; and skin may be so grafted as to self-indicate its own survival or death by the presence or absence of epithelial outgrowth from it.

The purpose of this paper is to set forth certain procedures and principles which have emerged from the authors' experience with skin grafting in rabbits, guinea-pigs and mice. The rather special problems of grafting in cows (Anderson, Billingham, Lampkin & Medawar, 1951) and monkeys (Krohn, unpublished) will be dealt with elsewhere.

The union of a skin graft with its bed is a process of healing by 'first intention', i.e. by the direct union of immediately apposed raw surfaces, and as a general rule grafting will be successful if the graft is not too thick and if it is held by light but firm pressure upon a bacteriologically clean and adequately vascular bed until primary union is complete. The greater part of this paper is concerned with

* British Empire Cancer Campaign Research Fellow.
describing in sufficient detail how these conditions can be fulfilled. Only 'free' (as opposed to pedicle or flap) grafts will be dealt with, i.e. grafts which at some stage have been wholly severed from the body; but among these it is convenient to distinguish between two sorts: (a) fitted grafts (Pl. 7, figs. 17, 19, 21–23), which exactly, or almost exactly, fill the defects into which they are transplanted, and (b) open-style grafts (Pl. 5, figs. 4–8; Pl. 6, figs. 12–14), in which more or less of the skin defect is left uncovered by grafted skin. The healing of such incompletely covered defects is achieved partly by outgrowth of skin epithelium from the grafts and ingrowth of epithelium from the edge of the raw area, and partly by the generalized contraction of the wound as a whole. Open-style grafting therefore entails a mixture of healing by first intention and by 'second' intention—i.e. (in this situation) by the progressive resurfacing of the lesion by epithelial migration. It is of particular value when the mere survival of the graft, as indicated by outgrowth from it, is the chief experimental issue.

2. THE ANATOMY OF MAMMALIAN SKIN

The integument of the mammal consists of the following layers: (a) the epidermis, and its appendages (hairs and glands) lying in (b) the dermis or corium, consisting mainly of stout collagen fibres in three-dimensional packing, and containing (under normal conditions) a sparse resident mesenchyme cell population of fibroblasts and histiocytes.

The epidermal appendages, which do not normally abut below the base of the dermis, lie within special basket-works of fine collagen and elastin fibres. Apart from these, the dermo-epidermal interface is by no means a plane surface; looked at from the inner side, the epidermis may be seen to be thrown into one or another of a variety of hill-and-valley patterns that vary from one part of the body to another. The dermal prominences that fit into the epidermal valleys—obviously the one bears the negative imprint of the other—are the so-called dermal papillae. Each dermal papilla contains a tuft of blood and lymphatic capillaries, and the collagen fibres are smaller and in more open packing than elsewhere. Fine elastic fibres are particularly concentrated at the superficial levels of the dermis and are thought to be responsible for holding the epidermis in place.

(c) The superficial fasciae. In the rabbit, the superficial fasciae are revealed by dissection as a number of superimposed planes of connective tissue in which the collagen fibres lie with their long axes in a plane parallel to that of the integument. Thanks to the absence of fibres running perpendicularly to the skin surface, the skin of the rabbit may easily be 'split' off at the level of these fascial planes. In the mouse and the guinea-pig, however, the superficial fasciae are represented by a layer of fatty tissue, the panniculus adiposus, which is rather firmly united to the dermis above and the layer of striped muscle below. The skin of these animals does not therefore split away 'naturally' below the dermis, but must be carefully dissected away.

The principal arteries, veins, lymphatics and nerves of the skin run in a direction parallel to the plane of the skin surface and lie between the superficial fasciae (or
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panniculus adiposus) and (d) the panniculus carnosus, the layer of striped muscle responsible for skin-twitching movements: this layer is absent from man except in the muscles of the jaw and of facial expression. In other mammals it thins out and eventually stops about half way down the limbs.

The fibres of the panniculus carnosus are bound together by an epimysium above and below; the internal epimysium is united to the body wall by very loose areolar connective tissue which allows the integument as a whole to be freely mobile.

A skin graft consists of the epidermis and more or less of the dermis; the graft bed should as a rule be cut down to the vascular fascial planes immediately overlying the panniculus carnosus, leaving the principal vessels of the skin intact (Pl. 5, fig. 4; Pl. 6, fig. 12).

Skin shows considerable variation of minute anatomy from one part to another of the same animal; e.g. the epidermis is thicker and more deeply stratified where the coat is sparse, as it is on most mammals' ears. The dermis of ear skin is thin and the superficial fasciae are fairly loose, so that thin grafts are easier to cut from the ears than elsewhere. These regional variations, and the differences between species mentioned earlier, govern the manner and the ease with which skin grafts of a chosen type may be cut from one part or another of a particular donor. Over and above these mainly anatomical differences, there are also innate differences between the various races of epidermal epithelia—'innate' in the sense that the distinctive properties of the epithelia are conserved indefinitely after grafting to anatomically unnatural positions. Such differences may be used as self-markers to distinguish a graft from the skin that surrounds it (see § 5).

3. ANAESTHESIA

General anaesthesia is essential for grafting operations on small agile laboratory mammals, and it is desirable for the earlier changes of dressings. Nembutal supplemented by ether has been generally satisfactory; ether alone is practicable with the rabbit, but mice of certain strains and guinea-pigs are apt to have spasms or rigors unless the plane of anaesthesia is very attentively controlled.

Nembutal is commercially dispensed as a solution in 10% alcohol containing 1 gr. (=0.065 g.) per ml. The dosages we have found satisfactory are: for rabbits, 1 ml./5 lb. or 2 kg. body weight—administered through the marginal ear vein; for guinea-pigs, 1 ml./lb. body weight of Nembutal solution diluted one in five with Ringer’s solution or normal saline, administered intraperitoneally; for mice, 0.1 ml./10 g. body weight of a solution diluted to one part in ten with normal saline, administered intraperitoneally.

The removal of biopsy specimens under ether alone is made easier by infiltrating the graft bed with 0.25–1.0 ml. of a local anaesthetic, such as 2% ethocaine hydrochloride. Local anaesthetics are as a rule commercially dispensed in solutions containing adrenalin, which helps to check bleeding.

The use of Nembutal makes it desirable to operate on a heated table, to help maintain normal body temperature.
For graft donor and recipient areas alike, hair should be cut or mechanically clipped from an area considerably larger than that which will be included in the actual operation field. The clipped area should then be thoroughly lathered with soap and shaved clean, with an open or 'Durham Duplex' razor. Ear skin may be shaved with an ordinary or miniature safety razor. The excess of soap may be removed with surgical spirit. Hair-bearing skin cannot be completely sterilized, even after shaving, but it can be adequately cleaned by swabbing with o.1 % CTAB (cetyltrimethylammonium bromide, 'Cetavlon') in 70 % alcohol. All the operations described below may now be done by 'No touch' technique, and sterile gowns or rubber gloves are therefore needless refinements.

The only donor area for which this treatment is too drastic is rabbit's general body skin, because of the thinness of its epidermis, but the damage caused to the epidermis by shaving is so soon repaired as to be of no consequence unless a special study is being made of grafts of only 4–6 days' standing.

For ordinary grafting operations, the following instruments should be available: scalpels with blades 11 (14 or 16), 12, 15 and 21; 5 and 3 in. (very fine) dog-toothed forceps; watchmaker's forceps; 'pinch forceps' (Text-fig. 1), with very sharp points, which can be made by bending in the tips of watchmaker's forceps; fine curved scissors; stout straight scissors; ordinary blunt forceps. Grafts should be kept awaiting use by laying them raw side down in small Petri dishes fitted with filter papers damped with sterile Ringer's solution. Plenty of small sterile gauze swabs, most usefully squares of 5 cm. length of side, are needed during operations.

Instruments should be sterile and should be laid when not in immediate use on sterile cloth or on a glass plate sterilized with 5 % Dettol. Stainless steel instruments may be sterilized without deterioration by boiling for 5 min.; plated instruments and scalpels are best sterilized in firmly corked test-tubes by dry heat (140–150° C. for 45 min.). The corks will not blow out if each is pierced by a narrow cylindrical hole firmly plugged with cotton wool. Glassware is also sterilized by dry heat.

Skin grafting requires the use of a number of special dressings. Of these, plaster-impregnated bandage is best bought commercially ('Gypsona'), but vaselined gauze (tulle gras) is best home-made. Tulle gras for larger animals (Pl. 5, fig. 6) is made by cutting fine open-wove bandage into rectangles of the appropriate size, packing them down firmly in a glass or metal box, drying in the oven, and then infiltrating with hot vaseline, the level of which should reach the uppermost layer of bandage. Complete impregnation and sterilization may then be achieved by baking the entire
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vessel at 140–150°C for an hour or more. The same technique, with the substitution of muslin for open-wove bandage, makes a very fine-meshed tulle which, being easy to cut into small squares and more adaptable in shape, is particularly suitable for mice.

The adhesives that are required are surgical spirit gum (e.g. Benzo-Mastiche, Mastisol), available from surgical supply houses, and a rubber latex cement such as Copydex.

5. THE PREPARATION OF GRAFTS

Two sorts of graft are useful in experimental work: (a) the pinch graft, so called from the manner in which it is cut, and (b) the split-thickness or Thiersch graft, named after a French pioneer of plastic surgery.

(a) The pinch graft is the easier to cut. The skin of the chosen donor area is raised into a cone or tent with pinch forceps or fine dog-toothed forceps and sliced off by firm horizontal strokes with a no. 12 scalpel (Pl. 5, fig. 2). Its shape is round or oval, and its diameter (anything from 3 to 12 mm.) clearly depends on the mobility of the skin and the height to which the tent or cone has been raised. Small pinch grafts are button-shaped, and taper from the full thickness of the dermis centrally to the thickness of the epidermis round their edges. Larger pinch grafts, particularly those cut from the ear, have bevelled edges but are of full dermal thickness over the greater part of their area. To expedite healing, the areas from which pinch grafts are cut should be so spaced that each is separated from its neighbour by at least 1 mm. width of normal skin.

Pinch grafts cut from rabbit’s or guinea-pig’s skin automatically split from their substratum at the required thickness. With mice, however, the binding of the layers of skin is such as to make it very difficult to avoid including the fatty layer and the panniculus carnosus beneath it. It is essential for sound healing that both these unwanted layers should be snipped off with curved scissors, or scraped away with firm strokes of a no. 15 scalpel.

(b) The Thiersch graft (Pl. 5, fig. 1; Pl. 6, fig. 10) is thinner than the pinch graft and of a uniform thickness, comprising the epidermis and the superficial layers of the dermis. Thiersch grafts may be cut from the general body skin by holding it taut over the fingers and slicing off a thin shaving of even thickness with a no. 11 (or 14 or 16) straight-edged scalpel. Smearing the donor area with a thin film of sterile vaseline helps the cutting and later handling of such grafts. Trimming to a chosen shape is done after removal. The ear (Pl. 5, fig. 1) is a particularly favourable source of Thiersch grafts, because of the thickness of the epidermis and the sparseness of its coat of hairs. Rectangular grafts of a predetermined size and shape can be cut from the ear by defining their outlines by very light incisions with a no. 15 scalpel before the sheet is sliced off.

Plastic surgeons now use the term split-thickness graft to describe one comprising the epidermis and some but not all of the dermis, and confine the term Thiersch graft to the thinnest split-thickness graft which it is practicable to cut. The present authors propose to use the term Thiersch graft in its wider generic sense, since they have used ‘split skin grafts’ to describe grafts of pure epidermis, i.e. epidermal sheets from which the dermis has been wholly removed.
Pinch grafts, being thicker than Thiersch grafts, take a little longer to heal soundly, but give cosmetically and functionally better results when they do: the hairs that grow from them are of normal (and invariably graft-specific) colour, density and orientation (Pl. 7, figs. 21, 22). Thiersch grafts, which do not contain the hair follicle bases, sometimes fail to regenerate a full normal crop of hairs.

It is obviously impossible to cut Thiersch grafts of adequate size from the very thin ears of mice, but it is quite easy to strip away sheets of skin having the same composition. Two straight but divergent scalpel incisions starting at the base of the ear are made down to the level of the cartilage so that, with the margin of the ear as its third side, the incisions make an equilateral triangle with its apex at the base of the ear. By grasping the skin with fine forceps at the apex the entire triangle may then be peeled away and later trimmed to whatever shape is wanted.

The donor areas of pinch grafts or Thiersch grafts may be dusted thickly with sterile sulphadiazine powder from an insufflator. The powder has a usefully haemostatic function. Other dressings are unnecessary and probably undesirable. The donor areas of Thiersch grafts heal with almost spectacular speed by the upward and outward migration of epithelium from the bases of truncated hair follicles; those of pinch grafts form dry protective scabs, and wound closure is brought about partly by epithelial ingrowth but mainly by general contraction. Pinch graft donor areas remain recognizable by small scars.

It is not obligatory to use skin grafts within a few minutes of their being cut: they are known to remain viable for weeks in the refrigerator and probably for ever at the temperature of liquid air: but if the early post-operative history of a skin graft is of consequence it is probably as well, for uniformity's sake, to use all grafts within a few hours of their removal.

The choice of donor area: 'labelled' grafts. It is sometimes of vital importance that grafted skin should remain distinguishable without possibility of error from the skin of the recipient area around it.

With body skin grafts, a difference of hair colour is the most conspicuous label; differences of hair length or density (as, for example, between belly and back) may also be used. In the guinea-pig, where the superficial epidermis may be pigmented, differences of skin pigmentation as such may also be made use of (Pl. 7, fig. 19). There is only one drawback to the use of colour markers: the loss of pigmentation by destruction of melanophores does not always entail the death of epidermal cells, and, conversely, epidermal cells may sometimes be caused to die under conditions in which the pigmentation associated with them persists (Billingham & Medawar, 1950).

Other innate differences may be used as alternatives to marking grafts by their colour. In mice, for example, Thiersch grafts cut from the skin of the tail remain easily recognizable as such when grafted to the skin of the chest (Pl. 6, figs. 13–16). Ear skin also preserves its specificity of type (Pl. 7, fig. 19), and in guinea-pigs the hairless and rapidly proliferating epidermis of the sole of the foot (Pl. 7, fig. 17) provides a useful and conspicuous marker.

Pure epidermal grafts. Crude pancreatic extracts and commercial trypsin powders
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contain an enzyme (probably an elastase: cf. Balo & Banga, 1949) that causes the epidermis to free itself without damage from the dermis (Medawar, 1941). Pure epidermal or split skin grafts (Pl. 7, fig. 20), as we call them, are therefore easy to prepare from ear skin Thiersch grafts, in which the epidermis is thick and there are insufficient hair follicles to interfere with the clean fission of dermis from epidermis. Some crude trypsin samples contain further enzymes that cause maceration of the epidermal layer, and these make possible the use of epidermal cell suspensions as grafts. Alternatively, the intact epidermal sheet may be floated upon a mixture of 1 vol. of 4% sodium citrate with 4 vol. of normal saline.

Suitable ‘skin splitting’ solutions may be prepared by dissolving 0.25–0.5 g. commercial trypsin powder in Ringer-bicarbonate or Ringer-phosphate (pH 7.4–7.8) containing 1:100,000 phenol red, and filtering the opalescent suspension so formed first through two thicknesses of ordinary filter-paper and then through a sterile Seitz filter. (If Ringer-bicarbonate is used, filtration under negative pressure must of necessity be slow.) The filtrate may be stored in sealed 10 ml. glass ampoules in the refrigerator; it retains its activity for at least 3 months.

Skin that is to be split should for preference be thin, and it is essential that it should be of even thickness. A convenient procedure is to cut Thiersch shavings from the thinly vaselined skin of the dorsum of the ear and to gum them, outermost side downwards, on to a vaselined cover-slip. The whole cover-slip is then incubated in a 37°C water-bath under sufficient trypsin solution to cover it. When splitting is complete, the dermis can be simply peeled off by grasping it at one corner with watchmaker’s forceps; tests should be made after 15 min. incubation and at 10 min. intervals thereafter to make certain that incubation is not unnecessarily prolonged.

For ordinary grafting purposes, pure epidermal grafts are of very limited usefulness, because, if grafted to freshly prepared or even to already granulating raw areas (see below), they do not prevent the closure of the graft bed by generalized contraction.

6. PREPARATION OF RECIPIENT AREAS AND TRANSPLANTATION

The best recipient area for skin grafts is the skin of the chest dorsally or laterally, where the ribs or backbone provide a firm substratum. The lateral thoracic and mammary arteries and their branches, running in the superficial fasciae, provide a perfectly adequate overlapping blood supply throughout the entire region. Venous and lymphatic drainage is in a forward direction, towards the axilla.

Fitted grafts. Fitted pinch grafts (Pl. 7, figs. 17, 19, 21–23) are the easiest to do; the operation amounts to no more than placing one pinch graft in the skin defect or crater left by cutting another. If a pinch graft is replaced in the hole from which it was cut, it no longer fits closely, for the contraction of the graft and the slight gaping of the wound cause it to be surrounded by an annulus of unsurfaced tissue. A good fit, therefore, entails the cutting from the recipient area of a pinch graft rather smaller than the one intended to take its place.

A Thiersch graft is more usually square or rectangular than oval or round; if it is to be an exact fit, therefore, the edges of its intended bed must be defined by
Grafts may be transplanted to the surface of such a raw area immediately after it has been made (Pl. 5, fig. 5); alternatively, the area may be prepared (and suitably dressed: see below) 3–5 days before grafts are transplanted to it. The formation of granulation tissue expedites primary healing and vascularization. This is advantageous when the grafts are obliged to be thick or when they have been treated \textit{in vitro} in such a way as may prejudice their chances of survival. Healthy granulation tissue is dryish, has a fresh, pink colour, and takes on the imprint of its immediate dressing, tulle gras (see below).

In the mouse, raw areas up to 1 cm.\textsuperscript{2} in size may be prepared by extending a bed
of the type cut to receive fitted grafts (Pl. 6, figs. 11, 12). No attempt should be made to prepare large granulating areas in guinea-pigs unless their intake of vitamin C is of known adequacy.

The act of grafting itself amounts to no more than putting the grafts wherever they were intended to go (Pl. 5, fig. 5; Pl. 6, fig. 13). Some pains must be taken to see that Thiersch grafts lie flat. Since wet grafts are apt to be slippery, it is helpful to blot them dry on gauze before grafting. It is not harmful, and may be helpful, to lay the grafts on a bed containing a small quantity of freshly exuded blood, but they should not be laid on clotted blood. The dusting of an open-style bed with sterile sulpha-diazine powder from an insufflator (a pepper pot is not so good: the powder is slightly hygroscopic) checks capillary bleeding, and the powder probably has an antiseptic action.

Pure epidermal grafts are delicate and are best handled by flotation. The vaseline that will have been used to grease their surfaces causes them to float cuticle side up and perfectly flat. A drop of Ringer's solution is placed on their intended bed, and to this they are transferred from the Petri dish in which they await grafting by lifting them up with a thin glass rod with an L-shaped tip. The excess of fluid on the graft bed should be drained away with sterile gauzes or filter paper.

Pure epidermal grafts (Pl. 7, fig. 18) will unite, or at least appose themselves firmly and in a functionally adequate way, either to a freshly cut raw skin bed or (better) to a granulating raw area; but for reasons that will be made clear later it may be best to graft them to a 'half-thickness' bed, such as is left behind when a Thiersch graft is removed. Such beds retain the greater part of the dermal collagen, and do not contract.

Dressings. Tulle gras is the best immediate dressing for skin grafts of all types on all animals. The sheet should be cut to such a size that it overlaps the graft bed all round (Pl. 5, fig. 6; Pl. 6, fig. 14).

The purpose of the outer dressings is to protect the graft physically, to hold it down with a slight perpendicular pressure, and to prevent side-slip. The means by which these conditions can be fulfilled vary from one animal to another.

Rabbits. With open style grafts, particularly in thick-skinned animals, a pressure pad consisting of several thicknesses of surgical gauze cut to the size of the skin defect should be laid over the tulle. The entire thorax should then be firmly wound with 2 yards of 3 in. bandage, great care being taken to keep the pressure on the grafts perpendicular to their surfaces, so as to prevent side-slip. To make the bandage stiff and self-adherent the chest is finally wound with an 18–24 in. length of plaster-impregnated bandage (e.g. 'Gypsona') (Pl. 6, fig. 9). The plaster bandage should be given 5 min. to set before the animal is handled; thereafter it needs no special treatment. With fitted grafts the pressure pad may be omitted.

Guinea-pig. The guinea-pig is more nearly cylindrical in shape than the rabbit, and dressings of the type just described may well slip and cause the grafts to slip with them. This shortcoming may be abolished by painting the skin round the operation field with a surgical spirit gum (Mastisol) after the tulle gras has been applied. Bandage and plaster may then be wound round the thorax with the pre-
cautions just described; the gum sticks the bandage firmly to the skin. An alternative to the use of Mastisol is to cover the tulle with an overlapping square or rectangle of adhesive plaster; this achieves the same effect, but is undesirable because it may cause a severe inflammation of the skin.

For a young adult guinea-pig (approx. 500 g.) 18 in. lengths of both 1\(\frac{1}{2}\) in. plain and 1\(\frac{1}{4}\) in. plaster bandage should be sufficient.

Mice. In mice the use of plain bandage may be dispensed with; plaster bandage is wound directly round the chest, where it sticks firmly to the hairs (Pl. 6, fig. 15). A 7 in. length of \(\frac{3}{8}\) in. wide plaster is sufficient for a 20 g. mouse. This method is trouble-free and very reliable. After drying, the plaster sheath can be painted with picric acid to discourage attempts to gnaw it away. In mice, the use of a small pressure pad between plaster and tulle is desirable only with open-style grafting to relatively large raw areas.

7. INSPECTIONS AND POST-OPERATIVE DRESSINGS

The first inspection and change of dressings should not, if possible, be done before the fourth day after operation, and may well be deferred until the tenth; it is a safe rule that change of dressings should be done no more often than the plan of an experiment requires. Post-operative dressings can be relatively light; a pressure pad may be dispensed with, except perhaps as an absorbent of serous exudate with open style grafts; tulle gras is required only so long as any raw (i.e. not yet epithelialized) tissue remains in the operation field; and as an alternative to the use of plaster the free end of the plain bandage may easily be secured by painting with latex solution.

Although new vessels have begun to penetrate free skin grafts by the 24th hour, so that there is a luxuriant growth of new blood vessels and lymphatics by the 5th or 6th day, the strength of the primary union has by no means reached its maximum at the end of the first week. For this reason, the tulle should be removed circumspectly at the first change of dressings: dried serous exudate can cause the hair stumps on the grafts to stick so firmly to the tulle that the removal of the tulle may sometimes cause a graft to come away with it. So long as any area remains uncovered by skin epithelium, asepsis should be maintained by no-touch technique and the use of sterile apparatus. Fitted grafts have become confluent with the surrounding skin well before the 6th day, and, in theory, no dressings should be required thereafter. But as the reagents used to clean the skin, and the dressings themselves, cause some irritation and itching, it is just as well to keep the dressings on for a week or 10 days longer to prevent the operation field's being excoriated by scratching.

8. THE APPEARANCES OF GRAFTS: AUTOGRRAFTS

Competently transplanted skin grafts (other than pure epidermal grafts and the very thinnest Thiersch grafts) eventually resume the appearance and properties of the skin they were cut from (Pl. 6, fig. 16; Pl. 7, figs. 17, 19, 21–22). To outward appearance, this functional and cosmetic completion of the healing process is to be expected by 20–25 days; the grafts, if of hair-bearing skin, will have grown a pelt of
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hairs of graft-specific number, colour, and orientation (Pl. 7, figs. 21–22); sebaceous glands, after a period of excessive secretory activity, will be normally functional; whatever oedematous swelling may have occurred in the course of primary union will have subsided, and the graft becomes normally mobile and supple. Only microscopy will reveal certain abnormalities, such as the undue richness of the dermis and graft bed in blood vessels, lymphatics and mesenchymal cells, and other evidence of very low grade 'traumatic inflammation' which lasts until, perhaps, the 50th or 60th day after grafting.

The outward appearances of skin grafts between operation and the completion of healing now deserve some mention. A fitted graft and the 'graft centre' of the open-style graft behave alike, except, of course, that the fitted graft establishes an incisive suture line with the skin surrounding it within a few days of its transplantation.

A graft inspected at the 6th day post-operatively is soft to the touch and slightly swollen and (if of weakly pigmented or colourless skin) can be seen to have acquired a delicate pink flush—the consequence of a hypervascularity that slowly subsides from about the 8th day onwards. Though there is not likely to be much sign of it from the outside, the epithelium of a 6-day graft is violently hyperplastic; the epidermis thickens threefold or fourfold or even more, the hair follicles become keratinized trumpet-shaped cysts, and a thick layer of keratinized cells forming a sort of dead cast of the graft epithelium can eventually be stripped away, the original hairs of the graft being trapped in a life-like way within it. We call this cast the 'ghost graft'. The ghost will generally come away as a single sheet if the graft has been left undisturbed for 10 days, either by lifting it from the graft (edge first) with watchmaker's forceps, or simply stuck to the tulle.

The surface revealed by removal of the ghost graft is, in rabbits and mice, firm, white, appreciably waxy or aquafuge (hypertrophic sebaceous glands being now in action), and dotted over with the little depressions that mark the mouths of hair follicles. Guinea-pig skin, particularly ear or sole-of-foot skin, if black or dark brown to begin with, is not likely to be at any stage quite white; but pigmentation becomes transiently much paler, and even a black graft may be no more than a dusky leaden blue at its palest.

The proliferative activities of the epidermis, slowly subsiding over the following 2 weeks, leave behind an anatomical record in the thickened stratum corneum, the outer layers of which can be peeled or stripped off from time to time at inspections. The rudiments of new hairs can be seen microscopically by the 10th or 12th day; if pigmented, they cause a shadow-like discoloration to be visible from the outside by the 14th or 16th day. The young hairs may be expected to pierce the graft surface by the 16th to 20th day. Meanwhile the epidermis settles down to a more normal thickness.

Open-style grafts increase their epithelial area by symmetrical outgrowth over what starts as, or soon becomes, granulation tissue. A 2–3 mm. wide ring of shiny white, thickly keratinized outgrowth should be visible to the naked eye by the 8th day (Pl. 5, fig. 7), and outgrowth proceeds until neighbouring grafts have
coalesced with each other and with the incoming tide of epithelium from the edges of the raw area (Pl. 5, fig. 8). Granulation tissue covered over by epithelium soon becomes collagenized, so turning into young fibrous tissue; as the collagen fibres mature and thicken, fibroblasts become fewer and individually more attenuated.

The epithelium that overlies this substratum of young fibrous tissue is chronically hyperplastic; hairs and sebaceous glands develop sparsely and very late, if at all; its attachment, lacking elastic fibres, is weak, and, unlike the epithelium of the graft centre, it may easily be peeled from its substratum. Except for odd bluntly digitate ingrowths, it keeps throughout a plane surface of contact with the fibrous tissue underlying it, and dermal papillae never form.

'Spread epithelium' of this sort is not a stable tissue. In some manner most of the spread epithelium disappears during wound contracture, and what happens to it is at present a mystery. At all events, the graft centres, which themselves expand during the contracture of the wound, eventually become bunched up together. A pure epidermal or 'split skin' graft (Pl. 7, fig. 18) is obviously comparable to the pure epidermis that spreads from an open style graft, and its ultimate fate during the process of contracture after grafting to a full-thickness skin defect is equally obscure. It manifestly survives if sufficient dermal collagen remains in the graft bed to prevent this contracture. Such a bed is that left behind by removing skin only to the thickness of a Thiersch graft. Unfortunately, its preparation entails leaving the bases of the hair follicles of the recipient area behind. The epithelium of the grafted area will thus be of dual origin.

As an animal grows, its grafts grow with it—casual observation suggests that they grow at the same rate as the skin around them, and to a final size that may exceed the size they would have reached in their original positions.

9. THE APPEARANCES OF GRAFTS: HOMOGRAFTS

Skin is not known to survive orthotopic transplantation from one individual to another of the same species unless the two individuals are (a) identical twins or members of a very highly inbred line; or (b) are dizygotic twins that have desensitized each other, presumably by the interchange of foetal cells made possible by a synchorial placenta—a process at present known to occur only in the cow (Anderson, Billingham, Lampkin & Medawar, 1951); or (c) unless the recipient has been treated with drugs capable of holding the homograft reaction at bay (e.g. cortisone: Billingham, Krohn & Medawar, 1951).

The intensity and quality of the reaction against orthotopic skin homografts, as judged by the epithelial survival time and the nature of the events that precede breakdown, varies somewhat from one species to another. Within any one species, it varies with (i) the antigenic relationship between donor and recipient, (ii) the quantity of skin that is grafted, (iii) the recipient's experience of grafting from the same donor, or from a donor antigenically akin to it; and (iv), presumably, with the level of circulating cortisone-like adrenal cortical steroids in the recipient. Variables (iii) and (iv) are clearly of specialized interest and import, and of the first two, the first enormously outweighs the second. In mice, with all other variables made
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effectively constant, differences of antigenic relationship alone can be responsible for differences of breakdown time as great as between 5 and 80 days.

In general, the homograft reaction may be ‘acute’ (even though dilatory in actual onset) or ‘chronic’. The chronic reaction may be such that it falls short of bringing about a complete breakdown of the homograft epithelium. Instead, it causes the epithelium to thin out and weaken in its attachment and suppresses the differentiation of glands, arrector muscles and hairs, to the accompaniment of a prolonged low-grade inflammatory reaction in the graft dermis. Very often this condition is temporary, and the graft, having lost its pelt of hairs at an earlier stage of the reaction, grows a new one and becomes generally more normal in appearance. A chronic, incomplete, reaction of this sort has been found by McDonald & Medawar (unpublished) in grafts between mice of an inbred strain which had gone some way towards achieving antigenic uniformity; by Anderson, Billingham, Lampkin & Medawar (1951) in some of their grafts between dizygotic twins in cattle; and by Billingham, Krohn & Medawar (1951) in rabbits under treatment with cortisone. The evidence from mice shows that skin grafts can override certain minor antigenic differences between donors and recipients, a fact already known to students of tumour transplantation (see below).

The epithelium of skin homografts undergoing a mild and long drawn-out breakdown reaction may be surreptitiously replaced by the surrounding epithelium of the host, while the collagen fibres of the homograft dermis persist. This phenomenon undoubtedly accounts for many mistaken claims for the success of skin homografting in human beings. To be quite sure that it has not happened, it is essential to use homografts ‘labelled’ by some distinctive property of hair colour or epithelial conformation.

The ‘acute’ reaction, which may be detected as early as the 5th or as late as the 40th day after grafting, is one which, having started, goes rapidly and progressively to completion. A homograft is perfectly capable of growing hairs and differentiating as normal skin, provided only that it lives long enough to do so.

Acute breakdown is always preceded and accompanied by an inflammatory reaction of great violence. The first sign of breakdown is the oedematous swelling of the graft (more prominent in rabbits and cows than in other species). The colour of the graft changes from light to dark pink, through brick red to various shades of yellow and brown. Its superficial epidermis now weakens, and may easily be scraped or picked away to reveal the damp, exuding surface of the graft dermis. At this stage, the migratory epithelium from an open style homograft will already have disappeared, or be represented only by some pussy cuticular debris. With fitted grafts, the perimeter becomes disengaged from the surrounding native epithelium, so that the graft acquires a free rim. When epithelial breakdown is complete, there is nothing to prevent the graft dermis drying in air and becoming reduced to a withered and blackened scab, and this it accordingly does (Pl. 7, fig. 23).

Grafts that submit to acute breakdown are almost invariably undermined by the ingrowth of native epithelium: they are very rarely overgrown. The last remnant of such a homograft is a mushroom-like object attached by a central fibrous stalk. When
this stalk is finally cut through by the advancing native epithelium the scab falls away.

While the breakdown reaction is in progress the proportion of epithelium still surviving cannot be reliably estimated by the grafts' outward appearance. Histological examination of a graft biopsy is essential. Since epithelial breakdown is progressive and takes place simultaneously and to the same degree in all the grafts transplanted on one occasion from one donor to one recipient, it follows that repeated graft sampling provides a faithful and consistent picture of the course of the breakdown reaction in the graft population as a whole.

10. A NOTE ON THE PATTERN OF GRAFTING OPERATIONS

Mention has already been made of the fact that the homografting of skin may be used to estimate the degree of genetic diversity among the animals of a chosen group. For such a purpose skin grafts are clearly preferable to, for example, subcutaneous grafts of spleen (as in the pioneer work of Little & Johnson (1922) and Bittner (1936))—partly because even autografts of spleen grow indifferently, but chiefly because the ‘scoring’ of the survival of spleen homografts can only be done on an all-or-none basis. Skin is also in some important ways preferable to tumour tissue, although tumours are very much the easier to work with. Skin homografts are much more sensitive to the minor antigenic differences between donor and host which tumour grafts can sometimes override (Gorer, 1942; Snell, Cloudman, Failor & Douglass, 1946), and the use of tumours for the assessment of genetic uniformity is mainly confined to testing the homogeneity of an inbred strain in some member of which a spontaneous tumour has arisen, or the degree of divergence of sublines originally derived from it. (It is an added complication that such tumours may lose some degree of antigenic specificity during the course of their maintenance by transplantation: cf. Little & Gorer (1943); Little (1947).) Moreover, all the fragments of a single tumour grafted to, say, a population of mice, are of the same genetic composition: tests for homogeneity making use of tumour grafts are therefore all of the type classified as Type D below—the antigenic composition of the graft becomes a parameter of the experiment instead of one of its most useful variables. Skin, by contrast, can be removed from any individual at will, and grafted at will to any other.

We append brief notes on the five principal patterns of homografting operation which we have employed.

Type A: complete cross-grafting. Each individual among a group of \( n \) members receives one or more grafts from each of the others, making \( n(n - 1) \) distinct pairings of donors with recipients. If of heterogeneous origin, the grafts borne upon any one recipient will elicit reactions of different intensities and survive for different lengths of time. If this property is to be made use of, all the grafts must be transplanted to a chosen recipient on a single occasion (i.e. in one operating session). Otherwise the grafts earlier transplanted will curtail the life of their successors to a degree which depends upon the extent of the overlap of their antigenic consti-
tutions. (Grafts of different genetic origin do in any case act to some degree synergistically when transplanted to a single recipient, for they are certain to share some antigens in common; but they can still be of sufficient disparity to survive for very different lengths of time.)

A complete cross-grafting is specially valuable when the group is presumed to be heterogeneous, and the purpose of the test is to pick out isolated instances of compatibility. The theory of the test has been discussed by Medawar (1945). Tests of types B, C, D and E are merely so many restricted variants of cross-grafting, chosen only because they yield up their information in a more economical way.

Type B: the ‘ring’ test. A group of animals is so operated upon that the first gives a graft to the second, the second to the third, and so on, until the ring is completed by transplanting a graft from the last to the first. This test is of special value when the group is presumed to be homogeneous, and the purpose of the test is to pick out isolated instances of incompatibility. Although any chosen ring arrangement of \( n \) animals is only one among \( (n-1)! \) possibilities, any one such test must (with one exception of no great practical importance) single out an animal whose antigenic constitution is appreciably different from the others'. In its simplest form, the exceptional case is this. Let antigens A and B be supposed too weak to provoke an appreciable immunity when acting singly but strong enough to do so when acting simultaneously. Of three successive animals in a ring, the first may have the constitution AB, the second A alone (or B alone), and the third may lack both antigens. It follows \textit{ex hypothesi} that no incompatibility will be revealed by the ring test in its stated form; but it would have been revealed if the ring had been so arranged that the first animal gave a graft to the third.

Type C: reciprocal interchange of grafts between animals grouped in pairs. Such a test may be used (except with cows: see below) to distinguish monozygotic from dizygotic twins or to decide whether a breeding pair are sufficiently alike to be chosen as the parents of the succeeding generation of an inbred strain. For this second purpose the test is far from exhaustive, for if a group of potential parents contains \( p \) individuals of one sex and \( q (>p) \) of the other, only \( p \) pairings of the \( pq \) that are possible can be set up and tested by graft interchange.

Type D: parallel recipients. Grafts are transplanted from a chosen donor to two (or more) recipients. A test making use of transplanted tumours is essentially of this sort. The homograft that survives longest is borne by the individual having most antigens in common with the antigens of the donor.

Type E: parallel donors. Grafts are transplanted from two (or more) donors to a single recipient. In general, the homografts will survive for different lengths of time, and the homograft that survives longest comes from the donor that has the fewest (or the weakest) of the antigens that are not also possessed by the recipient. Anderson et al. (1951) point out that this is the only transplantation method that could make it possible to distinguish between monozygotic and dizygotic twins in animals such as the cow, to which tests of Type C are inapplicable. The sensitivity of the test can obviously be increased by immunizing the recipient to skin from one of the donors beforehand.
SUMMARY

Methods are described for the execution of free skin grafts in rabbits, guinea-pigs and mice.

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REFERENCES


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EXPLANATION OF PLATES

PLATE 5

Fig. 1. Illustrating the technique of cutting a Thiersch graft from the dorsum of a rabbit's ear. The donor area has been shaved and treated with sterile vaseline to facilitate the cutting.

Fig. 2. Illustrating the technique of cutting a 'pinch' graft from the shaved and sterilized skin on a rabbit's flank. A small tent of skin is raised by pinch forceps and its base is cut free by means of almost horizontal incisions with a no. 12 scalpel.

Fig. 3. Showing the initial stages in the preparation of an extensive rectangular raw area on a rabbit's thorax. The outlines of the rectangle have been defined by means of vertical incisions with a no. 15 scalpel and a flap of skin is being reflected with dog-toothed forceps towards the diagonally opposite corner. By almost horizontal incisions with a no. 21 scalpel the skin is caused to split easily at the level where the dermis is united to the vascular fascial planes immediately overlying the panniculus carnosus muscle.

Fig. 4. Showing the large rectangular recipient area ready for transplantation of the grafts. All traces of dermis have been completely removed. Note the main thoracic vessels and their branches.

Fig. 5. Showing six medium-sized pinch grafts cut from the skin of the rabbit's flank and transplanted to an extensive raw area on its chest.

Fig. 6. To show the sheet of tulle gras in position immediately overlying the grafts. The dressing is self-adherent and semi-transparent.

Figs. 7, 8. The appearance of the operation field illustrated by fig. 6 after 8 days (fig. 7) and after 10 days (fig. 8). The vessel tracks have been obscured by the formation of granulation tissue over the graft bed. Note that there are indications of epithelial outgrowth from the grafts and from the margins of the operation field at 8 days, and that by 10 days the grafts have become surrounded by annuli of well keratinized epithelium and that some have coalesced. Note the faint imprint of the tulle gras.

PLATE 6

Fig. 9. Showing the dressings in position around the rabbit's thorax.

Fig. 10. Illustrating the method of cutting a Thiersch graft from the tail skin of a mouse. The skin has been shaved and lightly smeared with vaseline to facilitate the cutting and subsequent handling of the graft.

Fig. 11. Showing the initial incision in the preparation of an extensive rectangular raw area on the thorax of a mouse.

Fig. 12. Showing the rectangular recipient area ready for the transplantation of the grafts. The lateral thoracic vessel group and its branches can be seen clearly.

Fig. 13. Showing four pigmented tail-skin autografts immediately after transplantation.

Figs. 14, 15. Showing the sheet of fine home-made tulle gras in position over the grafts (fig. 14); and the final outer dressing of plaster of Paris-impregnated bandage in position round the entire thorax (fig. 15).

Fig. 16. Showing a group of pigmented tail skin Thiersch grafts, 64 days after transplantation to the white skin of a mouse's chest. Note that the grafts have completely conserved their characteristic tail-skin appearance (cf. Fig. 10).

PLATE 7

Fig. 17. Showing a pigmented sole-of-foot graft 200 days after transplantation to a guinea-pig's chest. Throughout this period the graft has maintained all the characteristic features of sole-of-foot skin.

Fig. 18. Showing an extensive rectangular recipient area cut in the thoracic skin of the rabbit to which a series of 'pure' epidermal grafts had been transplanted 11 days previously. These grafts have clearly survived, though their individual outlines can no longer be distinguished because of the confluence of their epithelial outgrowth. Note also the ingrowing epithelium from the margins of the operation field; in some places, it has already become confluent with the epithelium from the grafts.
Fig. 19. Showing a black ear skin graft 1000 days after transplantation to the red skin on a guinea-pig's chest. The graft has clearly maintained its specificity (i.e. it still remains characteristically ear skin) and its margin is absolutely incisive.

Fig. 20. Showing a transverse section through a sheet of pure tail skin epidermis prepared from a Thiersch shaving from a mouse's tail by means of a trypsin digestion technique. Ehrlich's haematoxylin and eosin. × 50.

Fig. 21. A 'fitted' pinch graft: mouse's thigh skin transplanted to the chest and so orientated that the hairs point downwards.

Fig. 22. A graft similar to that illustrated by fig. 21 showing a hair crop of graft specific colour, density and orientation.

Fig. 23. Showing a group of eight skin homografts (fitted 'pinch' grafts) and one autograft 12 days after transplantation to a rabbit's chest. Breakdown of the homografts is now complete and they appear as hard, dry, reddish brown scabs, whereas the autograft is perfectly normal.
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