TRANSPLANTATION OF TISSUES AND CELLS

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PREFACE

TRANSPLANTATION OF CELLS AND TISSUES is being increasingly employed in the investigation of a wide range of medical and biological problems. Some of these relate to *specific* properties of particular tissues themselves: e.g., the pigmentation, hair growth, regional variation and regenerative capacity of skin; the functional activity of endocrine tissues; the malignant properties of tumors; the immunological competence of some tissues; the migratory capacity of many types of cell and epithelium, and the re-circulatory habits of the small lymphocytes of the blood and lymph streams. In other problems transplantation affords a simple and convenient method of appraising the ability of certain tissues or cells to withstand various treatments *in vitro* — e.g., exposure to thermal or other physical stresses, such as desiccation, and exposure to drugs and antisera. As a means of evaluating tissue viability, transplantation is frequently superior to cultivation *in vitro* since, under the latter conditions, explants normally lose their histological or anatomical specificity. Orthotopic grafting of a piece of skin will establish not merely cell survival, but survival of skin as an organized, functional tissue.

One of the most important experimental approaches to gerontology requires the production of *age chimeras,* so that transplanted tissues and organs can be maintained in a normal and functionally active state for periods greatly exceeding their normal life expectancy. In immunology, the transfer of certain types of immunity or sensitization from one individual to another can be achieved only by means of *living* cellular "grafts" of appropriate histological type.

Finally, probably the most important contemporary applications of transplantation are in the investigation of two allied phenomena: (a) *tissue transplantation immunity* — the state of sensitization evoked when adult individuals are grafted with cells or tissues from genetically unrelated members of their own species, which soon leads to the destruction of the homografts concerned; and (b) *actively acquired tolerance* of foreign tissue grafts — the specific inability to react against homografts, following exposure to foreign tissue cells at an early stage of life.

A variant of (a) must also be mentioned, where the immunological roles of homograft and host are in effect reversed and the host is *itself* damaged or destroyed as a consequence of the reaction of grafted immunologically competent cells against *it*, the grafts themselves being exempted from rejection by genetic or experimental artifice.

The purpose of this monograph is to present in detail certain procedures and principles for use in the experimental transplantation of tissues and cells, and for the analysis of the immunological responses the grafts may provoke. These procedures have either emerged from the experiences of the various contributors, or have yielded satisfactory results in their hands. Every attempt has been made to indicate the possible applications and limitations of the techniques described.

Because of the tremendous importance of inbred or isogenic strains of animals for the study of many problems involving tissue transplantation techniques, the final chapter describes methods of derivation, propagation, and maintenance of uniformity of these strains.

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Chapter 1

Free Skin Grafting in Mammals

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I. INTRODUCTION

The distinctive advantages of skin for grafting purposes are such that, in addition to its employment in studies relating to the properties of skin itself, it is also the tissue of choice when the type of tissue to be transplanted is not a parameter of an experiment. Its advantages include: its accessibility and abundance; the ease with which it can be handled; its remarkable ability to withstand traumatic and other influences, including temporary deprivation of its blood supply (unavoidable in grafting) with negligible resultant ischemic necrosis; it is the only tissue that can easily be grafted *orthotopically*, i.e., to an anatomically natural environment; the fate of skin grafts can be followed crudely from day to day simply by visual inspection (there are numerous criteria available for appraising the normality or otherwise of a skin graft) or, more precisely, by repeated biopsy without difficulty and without prejudicing the recipient's well-being; skin may be grafted in such a manner as to reveal its own survival or death by the presence or absence of epithelial outgrowth from it; finally, in experiments requiring the exchange of tissue between large mammals, such as cattle or sheep, skin is the only convenient solid tissue. Because of its wide application the grafting of skin will be described in considerable detail.

II. A NOTE ON THE ANATOMY AND REGIONAL VARIATION OF MAMMALIAN SKIN

The mammalian integument consists of the following layers: (a) the superficial epidermis and its appendages (hairs and glands), lying embedded in (b) the dermis or corium, consisting mainly of tough collagen fibers in a three-dimensional packing, usually very sparsely populated by fibroblasts and histiocytes. Apart from the epidermal appendages, which

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do not normally abut below the base of the dermis, the dermo-epidermal junction is rarely a plane surface but presents a variety of "hill-and-valley" patterns that vary from one region of the skin to another (Medawar, '53; Billingham and Silvers, '60). Dermal prominences — the so-called dermal papillae — are exactly complementary in shape to the epidermal valleys as seen in sheets of epidermis viewed from their underside. (c) The superficial fascia unites the skin — usually very loosely — to subjacent parts and in its simplest form consists simply of loose areolar connective tissue. The superficial fascia is not a homogeneous tissue since it may be differentiated into one or more layers of fat with intervening layers of fibrous tissue, and it may contain a sheet of striated muscle, the *panniculus carnosus,* which enables many animals to twitch their skins. With the exception of man, this muscle sheet usually extends over the greater part of the trunk and part way down the limbs.

In the rabbit the superficial fascia is constituted by a number of superimposed planes of connective tissue in which the collagen fibers are disposed mainly in a plane parallel to the skin surface. Because of the absence of fibers running perpendicularly to its surface the skin of the rabbit may easily be "split" off at the level of these natural fascial fission planes. In most other species, however, a layer of fat, the *panniculus adiposus,* occurs in the superficial fascia which is firmly united to the overlying dermis and to the layer of striped muscle below. Consequently the skin of these animals fails to split away naturally and easily below the dermis and has to be carefully dissected or snipped away.

The superficial fascia provides a matrix through which the main cutaneous blood vessels, lymphatics and nerves run in a plane parallel to the skin surface and immediately above the *panniculus carnosus* when this is present.

There is considerable regional variation in the fine anatomy of the skin. The epidermis is thicker and usually pigmented where the fur is sparse or absent, as on the ears, scrotum, plantar surfaces and tails of many species. The dermis of ear skin is usually thin with fine collagenous fibers and the superficial fascia is fairly loose so that the pinnae furnish an excellent source of thin grafts. The skin of the ventrum is almost invariably thinner and more delicate than that of the dorsum and its hairs are shorter and usually lighter in color. These regional differences, and differences between species, determine the manner and facility with which grafts of a chosen type may be obtained.

Superimposed upon these major variations that are largely confined to the anatomy of the fibrous component of the skin, there are innate differences between the various epidermal epithelia which are also conserved indefinitely after grafting to other anatomically unnatural sites in the skin surface (Billingham and Medawar, '48, '50*b*; Medawar, '53). For example, the hairless, smooth, highly stratified and mitotically active epithelium of

the sole of the guinea pig's foot permanently retains these distinctive properties when sole-of-foot skin is transplanted to a site prepared in the hair-bearing skin of the trunk. Such differences as these, and also differences with respect to pigmentation, furnish invaluable "markers" permanently distinguishing a graft from the native skin surrounding it.

III. PRINCIPLE OF SKIN TRANSPLANTATION

A graft consists of the epidermis and a variable amount of the dermis. The graft bed should be cut down to the vascular fascial planes immediately overlying the *panniculus carnosus*, so that the principal vessels of the skin are left intact and can furnish the graft with its blood supply (fig. 1B). Being more fibrous and much less well vascularized the deep fascia do not provide a very satisfactory bed.

Provided that skin grafts are not too thick and are maintained under moderately firm pressure upon a bacteriologically clean and adequately vascular bed, the immediately apposed raw surfaces will become directly united through the proliferative and fibrillogenic activity of mesenchymal cells — i.e., by a process of healing by "first intention." Restoration of the blood supply is probably accomplished initially by a kind of end-to-end anastomosis of some of the small, severed blood vessels in the graft with the vessels of the graft bed (Converse and Rapaport, '56), though the *definitive* vascularization appears to be the result of an invasive penetration of the graft by vessels from its bed which is complete by the fourth day. Although still not very firmly united to its bed, a skin graft of 5 or 6 days' standing possesses an abnormally rich blood supply that leads to a transient phase of intense epidermal cell activity and proliferation reflected in violent hyperplasia (Medawar, '44).

Only "free" (as opposed to pedicle or flap) grafting — i.e., the grafting of skin which has been completely excised from the body — will be described, since free grafts are the only ones of any importance experimentally. For convenience, two categories of grafts will be considered: *fitted* grafts which fill the defects into which they are placed as exactly as possible (figs. 2 and 3), and *open-style* grafts in which the skin defect is incompletely covered by the grafted skin (fig. 1). In the latter case, healing takes place by two concurrent processes: (a) by migratory outgrowth of epithelium from graft and wound margins across the granulation tissue that builds up in that part of the recipient area not covered by the graft; and (b) by a generalized contracture of this resurfaced *ad hoc* mesenchymal wound "fill" (Billingham and Russell, '56*b*). Thus ultimately there is almost complete apposition of the original graft and wound margins, as in the case of fitted grafts. The particular advantage of open-style grafting is simply that it enables the survival of a graft to be indicated macroscopically by the appearance of epithelial outgrowth from it across the surrounding raw wound surface.

IV. ANESTHESIA

General anesthesia is obligatory for grafting operations on small agile animals and it is highly desirable for the primary inspection of skin grafts and for subsequent changes of dressings if the latter are required.

Nembutal (Pentobarbitone sodium), supplemented by ether if required, is very satisfactory for mice, rabbits, guinea pigs, squirrels, Syrian and Chinese hamsters, and chickens. As commercially dispensed, Nembutal contains 70 mg/ml in a solvent made up as follows:

Nembutal	7.0 gm
Ethanol	10.0 ml
Propylene glycol	20.0 ml
Water	70.0 ml
	100.0 ml

Satisfactory dosages and routes of administration are:

For rabbits and monkeys: 0.5 ml/kg body weight administered via the marginal ear vein and the femoral vein respectively, or intraperitoneally.

For chickens: 0.5 ml/kg administered via the wing vein (for very young birds ether should be employed).

For guinea pigs: 1.0 ml/500 gm of a 1:5 dilution of the standard solution in physiological saline or Ringer's solution administered intraperitoneally.

For mice, hamsters, and squirrels, a more dilute solution of Nembutal is required containing 7 mg/ml. This may be prepared by diluting the standard Nembutal 10 times with normal saline, or, more satisfactorily, by dissolving the powder in a more dilute solvent solution:

Nembutal	7.0 gm
Ethanol	10.0 ml
Propylene glycol	20.0 ml
Water	970.0 ml
	1,000.0 ml

This is administered intraperitoneally at a dosage of 0.1 ml/10 gm body weight. It is conveniently dispensed in 25 or 50-ml aliquots and stored in the deep freeze.

Avertin (Winthrop Laboratories, New York City) (0.2 ml/10 gm body weight of a freshly prepared 1.25% solution of Bromethol in normal saline) is another satisfactory anesthetic for mice (Hicken and Krohn, '60). It is administered by the intraperitoneal route.

For rats, an aqueous solution of chloral hydrate (containing 36 mg/ml and administered intraperitoneally at a dosage of 0.75–1.0 ml/100 gm

body weight) has given excellent results (Brightman, personal communication), obviating the mucus secretion in the respiratory tract which is an irritating side-effect of ether anesthesia in this species. If ether anesthesia is employed, mucus secretion can be prevented by a subcutaneous injection of atropine sulphate (0.5 ml/100 gm body weight of a 0.01% solution in normal saline).

It is important to recognize that appreciable intra-specific variations exist in the susceptibility of animals to these anesthetics, though there is considerable uniformity of response in members of the same strain. Some adjustment of the suggested dosages will be required in the light of experience.

The use of Nembutal makes it desirable to take steps to help maintain normal body temperature, e.g., by employing a heated operating table and, particularly in mice, by allowing them to recover at an environmental temperature of about 30°C (an incubator makes an excellent recovery chamber). The duration of the anesthesia is highly temperature-dependent.

V. PREPARATION OF SKIN; INSTRUMENTS; ASEPSIS; DRESSINGS

For both the intended donor and recipient areas of a graft, the hair must be clipped (most conveniently with mechanical clippers) as closely as possible from an area considerably larger than that which constitutes the actual operation field. Since hair-bearing skin cannot be sterilized completely even after shaving, the latter is unnecessary unless very thin grafts are to be cut. Because of the thinness of the superficial epidermis of the trunk of most rodents, it is almost impossible to avoid traumatizing their skin when shaving. This fact must be taken into consideration in planning any studies of grafts of 6 days' standing or less. With "older" grafts this factor becomes negligible because of the rapidity with which damage is made good once primary healing has been accomplished.

Where necessary, the close-clipped sites are lathered with soap and shaved clean with an open or Durham Duplex razor; ear skin is best shaved with a safety razor. Otherwise they are thoroughly cleaned with soap and water or with an aqueous solution of a suitable detergent, the excess of which can be removed with surgical spirit. Final cleaning is achieved by swabbing with a 2% solution of Zephiran, Cetavlon (cetyltrimethylammonium bromide) or similar agent in 70% alcohol which is allowed to dry on the skin. In small rodents when fitted grafts are being employed, and provided that the close-clipped skin surface is not obviously dirty, it is permissible to omit this cleaning procedure for the intended graft recipient area and simply apply a dilute collodion solution (see p. 31) allowing it to dry on the skin. This "fixes" residual hair stumps and epidermal debris, provides perfectly adequate "sterilization" and, in addition, facilitates the cutting of the graft bed with fine curved scissors.

All the operations to be described can be performed with a "no touch" technique; sterile drapes, gowns, rubber gloves, and meticulous scrubbing-up are unnecessary.

For ordinary grafting operations the following instruments should be available: scalpels (e.g., Bard-Parker) with detachable blades nos. 11, 12, 15, and 21 or 22; "pinch forceps" (made by bending inwards the tips of watchmaker's forceps — see fig. 1A and fig. 2A); stainless steel watchmaker's forceps; fine curved scissors; stout straight scissors; stout straight forceps; and both fine and coarse dog-toothed forceps. Instruments should be sterile and when not in use should be placed upon a sterile cloth or laid upon an appropriate surface sterilized with 5% aqueous Dettol (The R. T. French Company, Rochester, N. Y.), or 2% aqueous Zephiran.

Stainless steel instruments may most conveniently be sterilized by boiling for 5 minutes. Scalpels (in fact, all sharp-edged instruments) and glassware are best sterilized by dry heat (140–150°C for 45 minutes). For convenience, scalpels and fine forceps may be stored in a sterile condition, available for immediate use, in 6″ × 5/8″ rimless hard glass test-tubes fitted with Morton stainless steel culture tube caps, or otherwise appropriately plugged.

The grafting techniques require a number of special dressings, etc. *Plaster-of-Paris impregnated bandage* (Gypsona, manufactured by Bauer and Black of Chicago, Ill., has proved superior to all other brands tried) is cut down into strips of appropriate size for the various species. These may then be wound upon small plastic formers and stored in air-tight containers pending use.

FIGURE 1

A. Illustrating the technique of cutting "pinch" grafts from the dorsum of a rabbit's ear using "pinch" forceps and a no. 12 scalpel.

B. An extensive rectangular raw area on a rabbit's thorax on which 9 medium-sized body skin pinch grafts have been transplanted. Five of these grafts are homografts and 4 are autografts. Note the main thoracic vessels and their branches which run over the panniculus carnosus.

C. Showing the sheet of semi-transparent tulle gras in position immediately overlying the grafts.

D. Showing the dressings in position around the rabbit's thorax.

E. Showing the operation field on the 9th post-operative day. The autografts are well healed in, healthy and surrounded by delicate annuli of epithelial outgrowth. Breakdown of the 5 homografts is at an advanced stage. Note their discoloration and the absence of epithelial outgrowth from their margins. Granulation tissue has built up over the entire wound surface, obscuring the vessels.

F. The operation field on the 15th post-operative day. Breakdown of the homografts is now of long standing and they appear as hard, dry, reddish-brown scabs, whereas the autografts look perfectly normal. The entire wound is now completely resurfaced as a consequence of the migratory activity of the epithelium from its margins and from the perimeters of the autografts. Note the approximation of the autografts and the reduction in size of the operation field—the consequences of wound contracture.

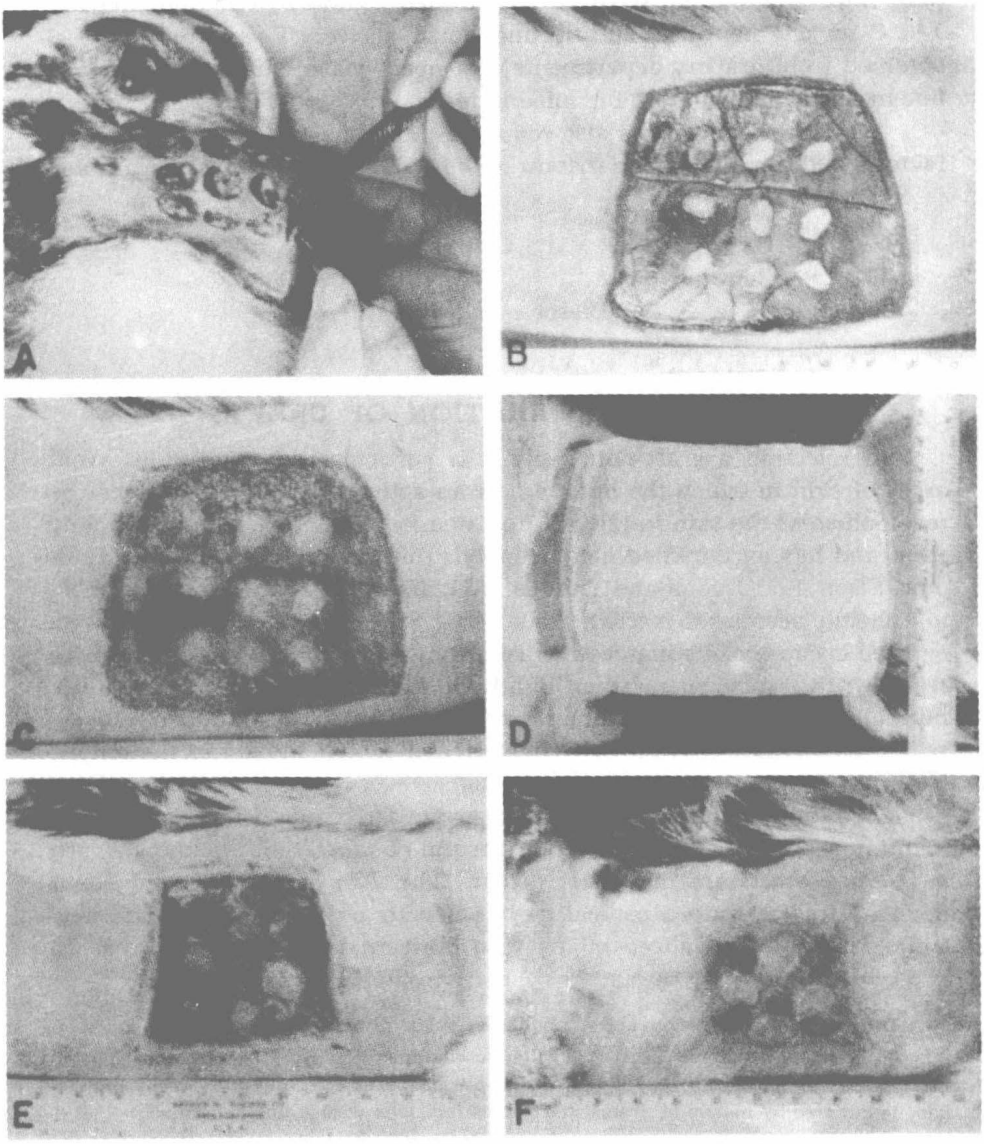

FIGURE 1

Plenty of small sterile absorbent gauze swabs, most useful in 5-cm squares are needed for all the operations to be described.

Tulle gras (vaseline-impregnated gauze) is best home-made because of the coarse texture of the commercial product. Fine open-weave bandage of appropriate size is cut into rectangles, which are then packed firmly in a heat-resistant glass or metal box. After drying in an oven, molten vaseline is poured over the material so that it is completely immersed. Com-

plete impregnation and sterilization are then achieved by maintenance at 140°C for one hour. By substituting "lawn" (a very fine cotton material obtained from drapery departments) for open-weave bandage, a specially fine-meshed tulle suitable for mice is made.

A surgical adhesive is also required. Mastisol, which is highly satisfactory, and completely non-irritant to the skin, has the following formula:

Mastic gum	400.0 gm
Castor oil	12.5 ml
Benzene to	1000.0 ml

Dissolve and filter

VI. THE PREPARATION OF GRAFTS

In selecting a graft donor site it is particularly important to avoid areas of skin in which the hairs are in an active growth phase: since the metabolism of the skin in the affected area is heightened, it is abnormally thick and has an enriched blood supply (Durward and Rudall, '58; Haddow, Elson, Roe, Rudall and Timmis, '45), and it is particularly susceptible to ischemic necrosis if grafted (Rous, '46). Such "active" skin is easily recognized because of its increased thickness, the fact that its hairs are more resistant to displacement when lightly blown in a direction against their normal angle of slope and, when the fur is pigmented, by an apparent deep-seated pigmentation in the skin due to melanogenic activity in the follicle bases.

Essentially only two sorts of skin grafts are required for most experimental purposes: (a) the "pinch" graft, and (b) the "split-thickness" graft.

Pinch grafts are the easier to cut. The skin of the prepared donor area is lifted up into a conical elevation with pinch-forceps or fine dog-toothed forceps and sliced off by firm horizontal strokes with a no. 12

FIGURE 2

The cutting, preparation and transplantation of fitted skin grafts in the rat.

A. Cutting a "pinch" graft from the close-clipped and sterilized skin on the animal's flank. A small tent of skin is raised by means of the "pinch" forceps and its base is cut free by means of almost horizontal incisions with a no. 12 scalpel.

B. Showing adherent fascial tissue being snipped away with the aid of fine curved scissors from the raw surface of a graft. After trimming, the grafts are stored temporarily, dermal side lowermost, on the saline-moistened filter paper which is on the bottom of the Petri dish, until required for transplantation.

C. Showing 4 separate graft beds prepared in the close-clipped and collodion-treated skin of the recipient's thorax. These have been cut with sharp, curved scissors, care being taken to leave intact the panniculus carnosus and the principal vessels overlying it.

D. The grafts in position in the prepared beds. Each is a fairly accurate fit.

E. Showing the rectangle of tulle gras in position.

F. The dressings in position around the animal's thorax.