THE BEHAVIOUR AND FATE OF SKIN AUTOGRAFTS AND SKIN HOMOGRAFTS IN RABBITS

(A Report to the War Wounds Committee of the Medical Research Council)

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INTRODUCTION

The 'natural' process of wound healing is always necessary, but not always sufficient, to secure an efficient functional repair of damaged tissue. The repair of injuries involving an extensive loss of skin has long been recognized to be a surgical problem; one for which, in the majority of cases, the operation of *skin grafting* is a fully adequate solution.

Skin grafting becomes a makeshift when the area of skin loss is larger than a patient can make good from his own resources. No more can be done, in such a case, than to graft the area of loss with isolated islands of skin in the shape of discs (pinch grafts), shavings, or strips. Skin from *another* human being will not serve as a permanent graft: neither in the human subject, nor, it will be shown, in the rabbit, is there any evidence that normal cellular tissue can survive transplantation between individuals of ordinary genetic diversity. The possibility of using skin homografts in clinical practice has been almost, if not quite, universally discredited.†

* Working with a part-time personal grant from the Medical Research Council.

† The literature of skin homografting has been reviewed by Gibson & Medawar (1943) and Medawar (1943).

Although the 'homograft problem', as that which relates to the grafting incompatibility of tissues may be called, has well-recognized and more or less direct implications for surgery, genetics, serology, and taxonomic zoology, no systematic attempt has been made to solve it. There is an urgent need for a straightforward description of the behaviour of homografts, and of how their behaviour varies with the quantity of tissue that is grafted and with the recipient's previous record of graftings from the same donor source. Skin is the tissue of choice, because it can be made to provide a quantitative measure of the time of survival of foreign tissue under a variety of different conditions, and because like nerve and bone (but unlike glandular tissue) it can be grafted 'isotopically'—into an anatomically natural environment. The pinch or discrete graft has proved to be superior to the continuous sheet graft for the following reasons: (*a*) it is technically the easiest to work with; (*b*) it can be cut to a fairly uniform size, and so makes possible a regulation of the dosage of grafts that an animal receives; (*c*) it grows by lateral spread of epithelium around its margin, and the extent of this outgrowth provides a useful quantitative measure of the efficiency of grafts of various types; and (*d*) single members of a uniform population of pinch grafts may be re-

moved at regular intervals for histological examination, with the object of constructing a serial record of their behaviour and ultimate fate.

The rabbit was chosen as experimental animal, more for its size and ease of supply than for any intrinsic merit. Its serological peculiarities do, however, throw some light on the homograft problem. The 181 rabbits that are reported on here were taken from the miscellaneous stock of half a dozen different suppliers, in the belief that the use of material which is completely heterogeneous in the genetical sense is the correct alternative to using properly inbred stock. No such stock is, in any case, available.

TECHNIQUE

The standard operation is the transplantation of pinch grafts from the outer aspect of the thigh to the lateral thoracic wall. Differences of detail between experimental and orthodox surgical practice are governed by the difference in structure between human and rabbit skin, and more particularly by the fact that the integument of the rabbit is freely mobile over the body wall.

Structure of rabbit skin. The superficial epidermis averages 0·006 mm. in thickness, and consists of one or of two 'staggered' (offset) layers of plate-like cells bounded externally by a very delicate cuticle. Stratum granulosum and stratum lucidum are absent. The principal part of the dermis which underlies it, 0·40–2·00 mm. in thickness, is composed of stout collagen fibres in the typical three-dimensional packing of compact connective tissue. It grades superficially into the papillary layer, where the fibres are smaller and in more open packing; and below, rather sharply, into a layer where the fibres are orientated two-dimensionally in the plane of the integument. At this deeper level, therefore, the skin 'splits' naturally from its substratum. The principal arteries, veins, lymphatics and nerves of the integument travel in the fascial layer, and they likewise run in the plane of the integument.

The dermis is bounded beneath by the *panniculus carnosus*, a layer of striped muscle formed in the thoracic region by the cutaneous maximus, which divides the plane-orientated fibres of the dermis from the loose areolar tissue which unites the integument to the body wall. The fibres of the panniculus are knit together by a distinct epimysium above it and below.

The density of packing and the degree of slope of the hair follicles varies from one place to another. The sebaceous glands are simple acini ranging from 0·025 to 0·030 mm. in diameter, and containing perhaps a dozen cells. They open into the lumen of the hair follicle about two-thirds of the way up from its base to its external aperture.

Preparation of the donor area and cutting of grafts. The grafts are cut from the very closely *clipped* skin of the outer aspect of the thigh. (Although the trauma caused by shaving is ultimately repaired, it complicates the interpretation of histological specimens; and if the donor area is shaved some days before operation, the traumatic thickening of the epidermis destroys one of the most valuable measures of the extent of cellular activity in the grafts.)

Immediately before operation, the skin of the donor area is rubbed for 1 min. with soap and water, rinsed for a few seconds in a fine jet of surgical spirit, and dried with a sterile swab. A tent of skin is raised by means of towel forceps with points sharpened and so adjusted that they only just overlap, and then sliced off by a horizontal incision through its base. No more than 0·05 mm. of the fascial planes that immediately underlie the compact connective tissue of the dermis is included. If the skin is thick, the graft has the button shape characteristic of human pinch grafts (Text-fig. 1*a*); but more usually (Text-fig. 1*b*) the graft is a flat oval disc, less like a pinch graft than a 'Wolfe' (full-thickness) graft of that size and shape.

As each graft is cut, it is laid raw side down on the bottom of a *dry* sterile Petri dish. The donor area requires no dressing other than a thick dusting of sulphanilamide powder, which need not be sterile.

A *large* pinch graft has been used in all experiments except some of those relating to the significance of graft dosage. Fifty such grafts from ten different donors, weighed in ten batches of five, gave a mean wet weight of 0·058 g. ranging from 0·028 to 0·103 g., with a modal class of 0·045–0·055 g. Median sections of twenty-five further grafts from twenty-five separate donors ranged from 0·37 to 1·90 mm. in thickness with more than a hint of bimodality (cf. the weights):

mm.	
0·35–0·50	****
0·51–0·65	*********
0·66–0·80	****
0·81–0·95	*
0·96–1·10	*
1·11–1·25	**
1·26–1·40	**
1·41–1·55	*
1·90	*

The average diameter of the grafts, measured along the major axis of the oval after fixation in full extension, was found to be $8{\cdot}5 \pm 0{\cdot}3$ mm. standard sampling error. The epithelial area of the 'large' graft is thus reasonably uniform; its thickness is the principal variable.

The *small* graft (Text-fig. 1*c*) used in a number of experiments on the significance of graft dosage gave a mean weight of 0·0058 g., ranging from 0·0048 to 0·0074 g., the estimate being based on fifty grafts weighed in five batches of ten. Their diameter, not accurately computed, ranged between 2 and 3 mm. 'Large' and 'small' grafts are hereafter used as technical terms.

Pigmented skin has not been used for grafts. Animals bearing homografts were in no case used as graft donors.

Preparation of the recipient area and transplanta-

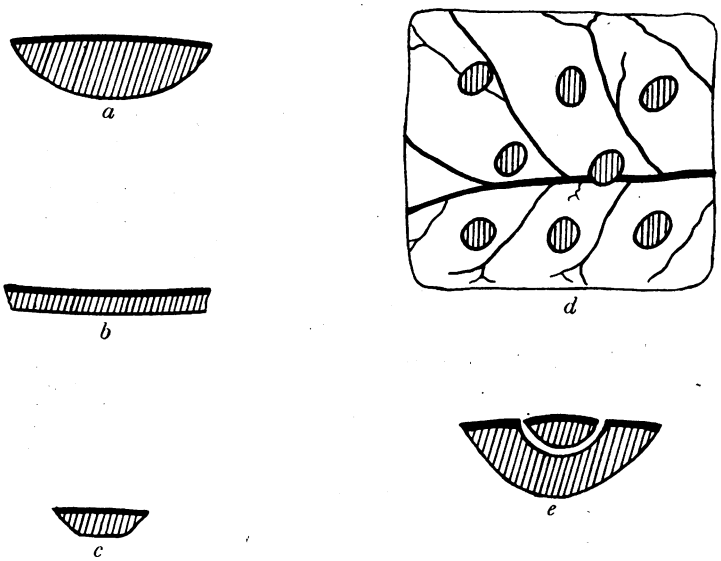
tion of grafts. The grafts are transplanted within half an hour of removal to a rectangular raw area on the lateral thoracic wall stripped down to, but not including, the vascular fascial planes immediately overlying the panniculus carnosus. The ribs offer a firm substratum for the grafts; and if the integument moves, the grafts move with it.

The side of the thorax is shaved over a wide area and rubbed with 2% v/v Dettol, which is allowed to dry on, immediately before the boundaries of the recipient area are defined by light scalpel incisions. The size of the raw area should be such that the grafts may be spaced 1½–2 diameters distant from each other and from the edges of the area. Smooth, almost horizontal strokes with the scalpel will cause the skin to 'split' in the required plane. The cutting of small ascending vessels is inevitable, and these should be lightly cauterized before the grafts are planted. Thin grafts always shrink and may curl up in the interval between cutting and planting; when they are in position they must therefore be smoothed out by firm strokes from the centre outwards. As a prophylactic measure, the operation field is lightly dusted with sterile sulphadiazine powder (140° C. dry heat for 1½ hr.). (I have not examined the consequences of omitting this precautionary step; since the rabbit is fairly resistant to infection, they may not be serious. But experience has shown that if sulphadiazine powder is used, it *must* be sterile.)

It is extremely useful to make a map of the operation field showing the relationships of the grafts to the principal blood vessels (Text-fig. 1 *d*). Grafts that are immediately underlain by the main longitudinal vessel group (which traverses the raw area somewhat below its midline) should be avoided when the first biopsy specimen is removed.

For homograft operations, and for the cutting of grafts, ether anaesthesia is sufficient. Autograft operations are somewhat longer, since grafts are cut from and transplanted to the same animal; for these, anaesthesia may be induced by Nembutal (1 grain/kg.) and maintained by ether.

Dressings. It has been established by surgical practice, and here confirmed for experimental, that pinch grafts will take in 100% of cases if they are transplanted to a clean vascular bed and held there under moderate pressure until primary healing is complete. Since conditions are ideal on the former score, everything depends upon the nature of the dressings and the manner in which they are applied. The following routine has given uniformly successful results.



Text-fig. 1. The shape of (*a*) a large pinch graft cut from thick skin, and (*b*) from thin skin; (*c*) the bevel-edged shape of the small pinch graft; (*d*) a typical operation-field map, showing the distribution of pinch grafts in relation to the main vessel tracks; (*e*) a 'mosaic' graft: small autograft within large homograft.

First, the operated area is covered with a 3 × 4 in. rectangle of 'tulle gras', home-made by impregnating fine open wove bandage with vaseline and liquid paraffin in such proportions (about 2:1) that the tulle is tacky and self-adherent at body temperature. (Commercial tulle is too widely meshed and too expensive.) Over the smoothed-out tulle is placed a similar-sized pad made from two thicknesses of folded surgical gauze. The entire thoracic wall is then firmly and evenly wound with 2 yd. of 3 in. bandage. This is the critical stage; with experience, it soon became possible to keep the pressure on the grafts perpendicular for the first few turns.

In theory, the animal should require no further dressing; but it is most desirable that the bandage should be made self-adherent and (incidentally) unpalatable. For this purpose I have used 1 yd. of 4 in. bandage ('Cellona') impregnated with plaster of Paris. The plaster does not and is not intended to form a rigid capsule: it can be cut off with scissors when the occasion arises. Only in preliminary experiments, in which I used a pressure pad of cotton-wool in conjunction with the other dressings, did I find that the animals suffered any distress; and here it was associated only with the mistaken use of pads cut to the size of the operated area, instead of covering the whole thoracic wall. Pressure pads in general soon proved to be wholly unnecessary.

Change of dressings and removal of biopsy specimens. Animals from which biopsy specimens are removed every 4 days have their dressings changed accordingly; otherwise dressings are changed at 6-day or 8-day intervals. The first change is more difficult than the remainder, because the hair stumps on the grafts often stick firmly to the tulle. The old dressings must therefore be removed with the greatest care after the tulle has been thoroughly wetted with sterile Ringer solution. Failure to observe this precaution may result in the graft being pulled out from its bed, which forms a shallow, flat-bottomed vascular crater in the raw area.

The raw area should be dryish and of a delicate salmon pink colour. The track of the principal vessels of the vascular layer is still recognizable at 4th day post-operatively, but becomes obscured thereafter. I have found it best to remove the graft serving as biopsy specimen, with the tissue around it, by bold rectangular incisions through the panniculus carnosus and down to, but not including, the areolar tissue beneath it. Much less trauma is caused by this radical excision than by attempts to slice off the graft in some intermediate plane. Bleeding can usually be checked by the application of light pressure with a swab for 1 min.; but if one of the larger vessels of the integument traverses the graft bed, clamping with mosquito forceps may be necessary.

After biopsy, the raw area as a whole is lightly dusted with sulphadiazine powder, and the biopsy donor area rather more thickly. Dressings are similar to those applied post-operatively, except that the plain bandage may be reduced to 1 yd., and the plaster-impregnated bandage to ½ yd.

In later dressings there is no danger of the grafts pulling out by sticking to the tulle gras, which may be removed without special precautions. The raw area closes by outgrowth of epithelium from the grafts, ingrowth of epithelium from its sides, and by contraction, principally across the dorso-ventral axis. The contraction of the raw area has the effect of bringing the grafts closer together than they were at first planting.

Histological technique. A uniform histological routine has been adopted for all biopsy specimens from which measurements are taken. The specimen is laid raw side down on squares of filter paper and fixed for 24–30 hr. in Zenker's fluid without acetic acid (and without added formaldehyde).

Thereafter the procedure has been: running tap water overnight; iodized 50% alcohol 2 hr.; methylated spirit 2 hr.; 1½ hr. in each of three changes of absolute alcohol; first cedarwood oil overnight; second cedarwood oil for the following day; ligroin overnight; then immediate immersion in the first of three successive 3 hr. impregnations with 57° C. paraffin wax, in which the specimens are finally imbedded.

The blocks are cut vertically and serially from end to end by alternating a fixed number of microtome strokes at 8μ with a fixed number, usually 30, at 20μ. The thinner strips are assembled in order. The first section from each strip is mounted on one slide, the second from each strip on a second slide, and so on up to five or six. Each slide therefore mounts an average of ten equally spaced vertical sections through the block. Two have been stained with Ehrlich's haematoxylin followed by aqueous orange G and alcoholic eosin; a third with Regaud's (a speeded-up variant of Heidenhain's) haematoxylin with or without one of a variety of background stains; a fourth with celestine blue or haematoxylin and picro-fuchsin (van Gieson); and a fifth with haematoxylin, xylidene red, and light green (Masson).

This technique is similar in essentials to that described by Gibson & Medawar (1943). Its advantage is that a close neighbour of a section stained by one method is stained by a variety of others.

Terminology. In the classification of specimens and in the terms used to describe them, I have again followed Gibson & Medawar (1943). The epidermal layer on top of the graft is the 'graft roof', and that part of the raw area on which the graft is planted is distinguished from the rest by being called the 'graft bed'. The tissue between the grafts and overlying the panniculus carnosus is called the 'outlying tissue'; the term 'raw area', which describes its surface before, in due course, epithelium covers it, would clearly be misleading. Epithelium of new formation is 'spread epithelium', and is differentiated into inner and outer rings and margin (Gibson & Medawar, 1943, Text-fig. 1). The inner ring is that which has a cuticle sufficiently well developed to be visible to the naked eye. When grafts coalesce with each other or with epithelium that has grown in from the margins of the raw area, all the spread epithelium acquires 'inner ring' character.

THE BEHAVIOUR OF AUTOGRAFTS

The autograft operation is one in which eight large pinch grafts are transplanted from the thigh to the thoracic wall in the manner just described. The behaviour of autografts has been reconstructed from the serial record provided by six animals, from each of which a biopsy specimen was removed every 4th day from the 4th to the 24th days inclusive. There were no consistent differences between one set of

grafts and another, and the description which follows applies without reserve to them all.

Outward appearance. By the 4th day, the grafts acquire a delicate pink flush. Outgrowth over the raw area is not usually visible to the naked eye, though histological examination shows that it has, in fact, taken place. By the 8th day outgrowth is obvious: the grafts are more or less symmetrically ringed by 2½–3½ mm. of thickly cuticularized epithelium, and their pink colour is either lost or obscured by the exfoliations over the graft roof. Coalescence between neighbouring grafts, and between the marginal grafts and the ingrowing epithelium from the edges of the raw area, is usually complete at the 12th day but for diamond-shaped interstices framed between them. The grafts, which at the 8th day are lifted above the surface of the raw area, now sink flush with it. Contraction of the raw area, most prominent dorso-ventrally, supplemented by ingrowth from its sides and outgrowth from the grafts completes the epithelial surface by the 16th day. Exfoliation of cuticle remains abnormally vigorous; but if the excess layers are scraped away, fine new hairs may be seen to have pierced the graft roof. Their orientation is governed by the original orientation of the graft.

Dermis, blood vessels, and graft bed. Primary healing, and the through-and-through invasion of the grafts by blood and lymphatic vessels, is complete by the 4th day. The vessels in the lower reaches of the dermis have differentiated into well-defined arterioles and venules (cf. Pl. 1, fig. 13), the former up to 0·03 mm. in diameter of lumen, the latter somewhat larger. The size of these differentiated vessels falls off from the deeper layers of the graft towards the surface: arterioles of the papillary layer are little larger than capillaries.

These vessels may be said to form the 'definitive' circulation of the grafts. The process of vascularization is, however, accompanied by mild traumatic inflammation, one expression of which is the fact that many of the capillaries become dilated and engorged with red cells (cf. Pl. 1, fig. 15). Such 'wound vessels', as they may be called, are by far the most prominent in the graft, for though they retain the simple endothelial lining and extremely tenuous collagen sheath of capillaries, they commonly range from 0·05 to 0·07 mm. in diameter of lumen. Wound vessels are end-products. At first they stagnate (an inference which can be drawn from the presence of pyknotic or otherwise degenerate leucocytes within them), then the endothelial lining disrupts and the lumen of the vessels is obliterated. The great majority have disappeared by the 8th day. Rarely, one or two persist until the 12th.

The 'definitive' vessels of the graft become reduced in size and more particularly in number from the 4th day onwards. By the 24th day they are little larger and hardly more abundant than in normal skin (Pl. 1, figs. 8, 9). Lymphatics, however, reach the peak of their development between the 8th and 12th days, when they may reach 0·075 mm. in diameter. In the earlier stages, particularly at the 8th day, they may be found to contain mononuclear phagocytes, miscellaneous degenerate leucocytes, and even occasional red cells; and although this cellular matter soon disappears from their lumina, lymphatics still remain abnormally large and abundant at the 24th day.

New collagen is laid down in the graft dermis during its union and subsequent differentiation in association with (*a*) the blood vessel walls, (*b*) the bold dermal papillae defined by the indentations which the graft roof acquires when the original hairs are thrown off (a process described fully below), (*c*) the new depots of glandular epithelium and the new hair follicle primordia, which are supported in a delicate collagenous scaffolding of new formation (Pl. 1, figs. 10–12). This collagen *might* have been formed by cells carried over with the graft itself, or by their descendants; but evidence from the study of homografts, discussed later, suggests that the cells forming new collagen in all three places come exclusively from the graft bed.

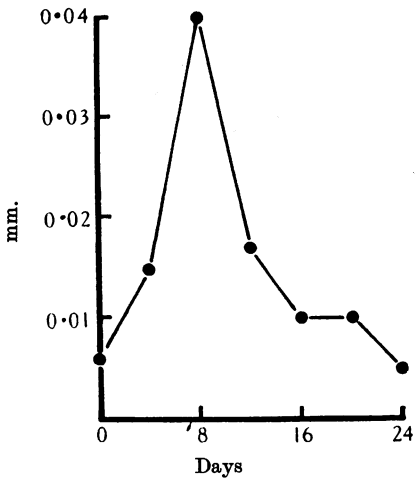
The traumatic inflammation associated with primary healing and the establishment of a circulation is represented partly by the vascular proliferation already noticed; partly by a feeble outward migration of monocytes from the vessels, so that the cellular population of the graft becomes abnormally rich; and partly by oedema. The oedema leads to a slight swelling of the dermis and of the fascial planes of the graft bed, which reaches its peak at the 8th day. Thereafter the swelling subsides, and the collagen-fibre packing returns to normal. The fascial organization of the graft bed, temporarily disturbed by capillary and fibroblastic proliferation, re-establishes itself between the 16th and 24th days (Pl. 1, fig. 9).

Epithelium. The behaviour of the epithelial elements provides a delicate and efficient record of the progress and differentiation of the graft. Outgrowth of a thin but multilayered epithelial sheet, sometimes originating from a hair follicle and always more extensive in the direction of hair slope than on the opposite side, has reached a distance not exceeding 1·0 mm. by the 4th day. During the same period, the graft roof epithelium swells from its normal thickness of about 0·006 mm. to 0·015 ± 0·002 mm. Two or possibly three processes play a demonstrable part in the thickening. The first (Pl. 1, fig. 1) is the swelling of the nuclei from their normal plate-like shape to frank ellipsoids; and this is associated with a swelling of the cytoplasm, a temporary obscuring of distinct cellular interfaces, and a slight reduction in the otherwise strongly basiphilic reaction towards haematoxylin dyes. Secondly, there is an amoeboid upward migration of follicular epithelium towards the surface, the external openings of the hair follicles now shaping themselves into a trumpet-like flare instead of into the familiar goblets (cf. Pl. 1, fig. 6; Pl. 2, fig. 16; Pl. 3, fig. 29; Pl. 4, fig. 45; etc.). Thirdly, mitoses may play a part, though their infrequency suggests that it cannot be an important one.

Between the 4th and 8th days there is a most spectacular outburst of proliferative activity. The graft roof (Pl. 1, fig. 2; Pl. 2, fig. 16; etc.) now thickens up to sevenfold (0·040 ± 0·005 mm.) and acquires the vertical differentiation that is characteristic of thick mammalian epidermis (multilayered cuticle; a granular layer of plate-like cells; an intermediate layer of more or less iso-dimensional cells; and strongly basiphilic columnar cells of the basal layer). The repeated exfoliations (Pl. 1, figs. 3, 6, 7, etc.), accompanied by the persistent upward migration of follicular epithelium, have a characteristic effect: all the original hairs of the graft are thrown off, and the graft roof comes to be crudely indented with cone- or flask-shaped re-

entrants (Pl. 1, figs. 6–9; Pl. 2, fig. 16; Pl. 3, fig. 30; and many others). This throwing off is probably a by-product of the proliferative and amoeboid activities of the epithelium: it may not occur in grafts which are accurately fitted to a raw area of just the right size—grafts in which, therefore, outgrowth and the need for 'paying out' cells does not occur. The upward migration of follicular epithelium leaves a long-persistent mark on the structure of the grafts, for the glandular acini come to lie almost at the base of the indentations of the roof which mark the original positions of the thrown-off hairs (Pl. 1, fig. 6) and the strips of arrector muscle lie right across the dermal papillae.

Proliferative activities extend to all the epithelial elements of the graft. Multiple acini of new glandular epithelium are elaborated—in extreme cases reaching 0·10 mm. in diameter—and primordia of new hairs are laid down (Pl. 1, fig. 10; cf. Pl. 2, fig. 16; Pl. 4, fig. 45). The spread epithelium in the inner ring of new outgrowth thickens to an average of 0·13 mm., and like the epithelium of the graft



Text-fig. 2. The rise and fall of the thickness of autograft roof epithelium, each point representing the mean of six independent determinations.

roof it acquires the vertical differentiation of thick mammalian epidermis (Pl. 1, fig. 4). As the epithelium is traced away from the graft towards its outer ring (Pl. 1, fig. 5) the development of the cuticle is seen to become progressively more feeble. The cells of the extreme margin (which—cf. Pl. 2, fig. 23—is typically blunt and rounded) show the secondary pathological changes already described and figured in some detail by Gibson & Medawar (1943). For some reason they provoke a mild non-specific inflammation (Pl. 1, fig. 5) in the tissue underlying them.

After the 8th day, the graft enters a phase of differentiation accompanied by many retrogressive changes. The graft roof simplifies morphologically and (Text-fig. 2) thins out; by the 12th day a single layer of granular cells remains (Pl. 1, figs. 3, 6); by the 16th day the granular layer is lost (Pl. 1, fig. 7); and by the 24th (Pl. 1, figs. 8, 9) the graft roof is no thicker than the epidermis of normal skin. The largest glandular depots soon 'explode' (Pl. 1, fig. 6); sometimes a thin sheath of glandular cells surrounds an otherwise empty lumen. By the 24th day (Pl. 1, figs. 8, 9) a progressive reduction of their size and number reduces them almost but not quite to the level of normal skin. The differentiation of hairs is, however, progressive; the primordia laid down between the 4th and 8th days and added to thereafter mature rapidly (Pl. 1, figs. 11, 12) and fine new hairs pierce the graft roof by the 12th day, or between the 12th and 16th. Not merely the slope, but also the pattern of the mature hair follicles is controlled by the structure of the collagenous endoskeleton of the original graft. If the graft contained multiple nests of hair follicles, multiple nests are developed when the hairs are newly formed (Pl. 1, fig. 9).

Outward spread of epithelium from the graft is progressive up to the time of coalescence—either with epithelium from another graft, or with that which has spread inwards from the margin of the raw area. The spread epithelium shares in the thinning out and structural simplification of the graft roof, but much less prominently. The average thickness at 24 days is still 0·032 mm., one-quarter of its maximal thickness at 8 days, but still five or six times thicker than the epidermis of normal skin.

The outlying tissue. At the moment of operation the 'raw area' consists of the fascial planes of the deeper layers of the dermis in which the principal vessels of the integument run. Its subsequent evolution, of which a very short account is given below, is familiar from our knowledge of healing by 'second intention'.

The fascial planes become distended by oedema fluid and fibrinous matter, and are 'organized' from below by the upwardly directed growth of capillary vessels. By the 8th day (Pl. 1, fig. 4) a brisk orientated fibroblast reaction has developed, and new collagen is laid down in a characteristic fashion, viz. a loose trellis-work of fibres orientated in the plane of the integument, with bolder mature fibres traversing the trellis-work vertically. Collagenization is progressive; as it proceeds, the fibroblasts become reduced in number and individually attenuated, as if they were squeezed out by the connective tissue they form. Blood vessels and, much more slowly, lymphatics likewise thin out in size and number, and traumatic inflammation becomes reduced to a feeble mobilization of histiocytes in the neighbourhood of the vessels. A capillary bed associated with a characteristic plexus of mature staining collagen fibres is established a little below the ingrowing or outgrowing epithelium, and from this basis capillaries pass upwards and loop round (cf. Pl. 1, fig. 4) in the papillae defined by the digitate or blunt and rounded ingrowths of the surface epithelium.

New hairs are not formed in the fibrous tissue of the outlying field by the 24th day. Evidence will later be given to show that this is because the connective tissue endoskeleton is not of the right pattern, and not because the new epithelium, however 'immature' it may appear to be, lacks any part of its normal capacity to manufacture new hairs.

Summary. The evolution of autografts falls into three principal periods:

(*a*) a period of *primary union and vascularization*, characterized by migratory and amoeboid activities of cells of all types;

(*b*) a period of *generalized hyperplasia*, in which all the cellular elements of the graft participate;

(*c*) a period of *partially retrograde differentiation*, during which the grafts return towards the condition of normal skin.

These three stages will be made the base-line for a general comparison between autografts and homografts.

THE BEHAVIOUR OF HOMOGRAFTS

Introduction

Altogether 121 homograft-bearing animals have been studied. Of these, seventy-eight showed a total breakdown of all the foreign cellular elements of the grafts at the time of examination, or at the last examination of a series; eleven showed breakdown in progress (Pl. 3, figs. 30–32), but not, at the time of examination, complete; and nine showed the specific degenerative changes that are the precursors of breakdown. On the remaining twenty-three, which include the majority of 4-day and 8-day specimens from lower-dosage graft bearers (q.v.), no judgement can be passed.

There is therefore no evidence that homografts can be transplanted with permanent success between rabbits of a genetically heterogeneous population.

The evolution of homografts of all types may, without exception, be resolved into two successive and well-defined time stages:

(i) The *primary cycle*, during which homografts exhibit cellular activities of a type similar to those of autografts. It is the rule with lower-dosage grafts, and the exception with higher, that the primary cycle extends in time beyond the period of generalized hyperplasia (see above) into that of differentiation.

Superimposed upon the otherwise autograft-like events of the primary cycle is the entire range of the phenomena of acute inflammation, one consequence of which is a massive invasion of the grafts by leucocytes of native origin. The primary cycle closes when the inflammation reaches a peak of violent intensity, and every living element in the graft, irrespective of its source of origin, breaks down.

(ii) The *secondary cycle*. The dermis is then at once reinvaded by capillary vessels from the graft bed and populated anew by leucocytes of native origin (the 'secondary native population'). Epithelium sweeps in from the margins of the raw area and either overgrows or undermines the naked collagenous pad which is all that remains of the homograft. Inflammation subsides into the chronic state.

High-dosage homografts

Homografts described as of *high dosage* are those borne by animals which weigh not less than 2 kg. and not more than 2½ kg., and which receive a modal average weight of 0·36–0·44 g. foreign skin in the form of eight large pinch grafts. The operation is thus identical with that upon which the study of autografts was based, except that the grafts are cut from the thigh of some animal other than that which becomes their recipient.

The behaviour of high-dosage homografts has been reconstructed from the serial study of ten animals, from each of which biopsy specimens were removed every 4th day from the 4th to the 20th days, supplemented by the study 'in parallel' of five further animals, from which *all* the grafts were removed at 4, 8, ..., 20 days respectively. (For reasons given later, these 'parallel' graft-bearers received not eight large grafts, but seven large and three small. The total dosage of grafts is effectively the same.)

The parallel series will not be mentioned specifically hereafter: they were done to confirm the impression given by naked-eye inspection, that degenerative changes take place simultaneously in all the grafts transplanted from a given donor to a single raw area on a given recipient. No measure of the intensity of the homograft reaction can be devised, which distinguishes in any way between the members of such a uniform population.

This judgement is reinforced by what may be called the perfect consistency 'in series' of the ten animals from which grafts were removed at 4-day intervals. The biopsy specimen removed from animal 101 at the 8th day, for example, showed the mildest inflammation of all 8-day specimens; alone among the others, the 12-day specimen gave no indication of epithelial breakdown; and the 16-day specimen, again unique, showed persistence of foreign epithelium over the graft roof (Pl. 4, fig. 37). Conversely, 12-day specimens taken from a population of grafts in which breakdown is evidently in progress at the 8th day, invariably show total breakdown of long standing.

There have been no anomalies in the serial record from this or from any other group of experimental animals. The technique of repeated graft sampling may therefore be supposed to give a faithful and accurate picture of the evolution of homografts.

The time-relations of the homograft reaction vary from one pair of animals—donor and recipient—to another; but for convenience the reaction will be described as *violent* when it reaches its peak by the 8th day, and *mild* when it reaches it after the 12th. The gradation is continuous, of course; the majority of animals show a reaction that is intermediate in all respects.

Outward appearance. At the 4th day there is nothing to choose between the outward appearance of autografts and homografts; thereafter it varies with the intensity of the reaction. An intermediate between violent and mild breakdown will be described.

At the 8th day the grafts are ringed with 2½–3½ mm. of more or less symmetrical outgrowth, the cuticle over the surface of which is unwettable and has the dry shiny white appearance of healthy autograft epithelium. No progress

takes place between the 8th and 12th days; at the 12th, the cuticle loses its unwettable character and becomes almost like a film of fibrinous matter or pus. It can now be scraped off with a scalpel, leaving a whitish film beneath; and if the cuticle is thick enough, it can be pulled off in strips which often carry parts of the graft roof with them. The raw area (as with the loss of epithelium it again becomes) granulates afresh. Meanwhile the grafts themselves undergo a constant and characteristic series of changes. By the 8th day they are so turgidly swollen as to stand out like buttons from the level of the outlying tissue; and this swelling persists thereafter. The very delicate pink flush which autografts acquire with primary union and then lose here deepens to brick red, dark brick red, brown, and finally, by the 16th or 20th days, to black. Eventually the roofing epithelium of the graft can be pulled or scraped off, leaving a pitted and leathery collagenous pad behind. If the epithelium is not pulled off, the grafts acquire a characteristic frilled appearance, the frill being formed from dried cuticular debris.

The 'wetting' and loosening of spread epithelium is complete, with *violent* breakdown, by the 8th day. In milder breakdown, outgrowth proceeds beyond the 8th day, so that the grafts coalesce with each other and with native epithelium from the edges of the raw area. The swelling and discoloration of the grafts is correspondingly retarded.

Dermis, blood vessels, and graft bed. The primary healing of homografts is as secure as with autografts, and all, however violent the reaction they elicit, acquire a definitive circulation of small arteries and veins (Pl. 1, fig. 13). New collagen (now to be called *primary* native collagen) is laid down in the same places and in the same way. The vascular response is, however, much more intense. A far higher proportion of the invading capillaries become converted into the dilated and engorged 'wound vessels' which in autografts are formed merely as a short-lived accompaniment to traumatic inflammation; they are, moreover, added to between the 4th and 8th days. Wound vessels, though anatomically capillaries, commonly range from 0·10 to 0·15 mm. in diameter and may exceptionally reach 0·20 mm. Haemorrhage, diffuse yet in the main perivascular, is the rule at the 4th day.

The definitive blood vessels and the lymphatics share in the general proliferation: Pl. 2, fig. 16, illustrates their extraordinary richness.

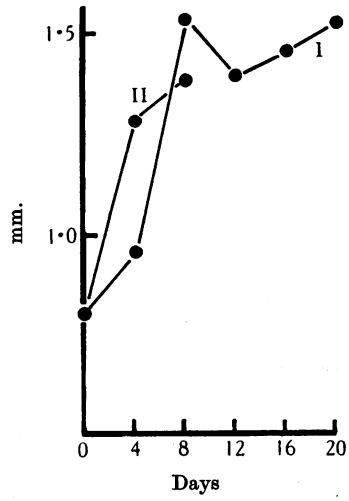
The subsequent behaviour of the vascular system of the graft and the rate of development of the inflammatory reaction vary from one graft-bearer to another. It is convenient to describe a mild reaction first, because the changes to be described are more widely spaced out in point of time.

By the 8th day (Pl. 2, fig. 16) the graft is densely invaded through its vessel walls by a primary native population of lymphocytes and monocytes—the former predominant—so giving rise to the gross cellular halation of the blood vessels that is characteristic of the early stages of acute but (in view of the role of the lymphocyte) atypical inflammation. The cells of the primary population spread outwards and upwards through the dermis, though the perivascular pattern of their distribution is not lost. At this stage (loc. cit.) many pyknotic lymphocytes, together with phagocytes and red cells, are to be found in the abundant and hypertrophied lymphatics. Fluid, as well as cells, passes through the vessel walls: fibrinous matter may be found in the fascial planes of the graft bed, and the graft swells to twice its original thickness or more (Text-fig. 3).

The inflammatory process reaches its climax with the stagnation and with the rupture of the endothelial lining of all the blood (Pl. 1, figs. 14, 15) and lymphatic (Pl. 2, fig. 18) vessels, and the breakdown of the primary native population, the cells of which first become pyknotic, then lose their cytoplasm, and then finally fragment into distorted chromatin droplets. (It is at this stage that the breakdown of the foreign epithelium over the graft roof is complete: breakdown of the spread epithelium usually anticipates it.)

In *violent* breakdown the pathological changes described in the preceding paragraph are greatly accelerated and intensified. In extreme cases, oedema and diapedesis halation of the vessels may be well marked by the 4th day; and the vascular and lymphatic proliferation is more intense and longer sustained. The characteristic feature of violent breakdown is the accumulation of the primary native population in a wide but sharply defined band—the 'black band'—at a medial level of the dermis (Pl. 2, fig. 17*a*). The black band forms by the 8th day, and its formation is accompanied by the total breakdown of the primary vascular system of the graft and its primary native population. All the tissue that lies above it is 'fixed' (Pl. 2, fig. 17*b*); for apart from the obliteration of the spaces of the blood vessels, it persists more or less unchanged until it is rubbed off during changes of dressings, or undermined by native epithelium.

The black band of violent breakdown is easily visible to the naked eye in the stained section. Equally characteristic



Text-fig. 3. The oedematous swelling of the dermis of (I) high-dosage homografts and (II) 2nd-set homografts; each point representing the mean of not less than ten independent determinations. The thicknesses of high-dosage and 2nd-set homografts differ significantly at the 4th day, but not at the 8th. The drop thereafter in (I) lies within the admissible range of sampling error of the estimates.

of violent breakdown is the gross distension of the fascial planes of the graft bed by a band of fibrinous matter which may reach 0·4 mm. in thickness (Pl. 2, fig. 19). This, too, is visible to the naked eye.

With violent as with mild breakdown reactions, the secondary cycle begins with the 'organization' or 'granulation' of the oedematous graft bed. Capillaries originating just above the upper epimysium of the *panniculus carnosus* thrust upwards through the graft bed into the lower reaches of the dermis (Pl. 2, fig. 20). Monocytes and lymphocytes (now sometimes with excentric nuclei and with an abnormally large investment of basiphilic cytoplasm) pass through the vessel walls and establish a secondary population of native cells within the graft (Pl. 2, fig. 21). Fibroblasts accompany the invading vessels, or migrate inwards independently, and the old collagen bundles are thrust apart by the formation of secondary native collagen (Pl. 2, fig. 22). Meanwhile the unlined tissue spaces formerly occupied by the primary blood vessels are obliterated (Pl. 2, fig. 21). A chronic inflammatory condition (loc. cit.) persists in the dermis; accordingly (Text-fig. 3), the oedematous swelling of the dermis does not subside. (Reference to the text-figure suggests that after the initial swelling, which reaches a peak at the 8th day, there is a drop corresponding to the breakdown of the primary vascular system, and a rise thereafter as the secondary system is established. The drop, however, is within the admissible range of random sampling error, and may not perhaps express a genuine difference.)

Epithelium. The vascular and inflammatory reactions of the primary homograft cycle are superimposed upon migratory and hyperplastic activities in the foreign epithelium. Up to a certain point these proceed in autograft-like fashion. The graft roof thickens by the 4th day to $0{\cdot}017 \pm 0{\cdot}002$ mm. (autografts: $0{\cdot}015 \pm 0{\cdot}002$ mm.—virtually identical) and in the same way, i.e. by a swelling of the cells and a general upward migration of follicular epithelium which brings the original hair follicles towards the surface (Pl. 3, fig. 29). The epithelial elements of the graft then enter the period of generalized hyperplasia. By the 8th day, the graft roof thickens with typical vertical differentiation to $0{\cdot}035 \pm 0{\cdot}002$ mm. (autografts: $0{\cdot}040 \pm 0{\cdot}005$ mm.—again virtually identical), rich new depots of glandular epithelium are elaborated, and follicle primordia are laid down (Pl. 2, fig. 16). Outward spread of epithelium (Pl. 2, fig. 23) proceeds up to the 8th day (to the nearest 2-day interval), but not, in grafts which elicit a violent reaction, beyond it.

The tempo of epithelial breakdown in high-dosage homografts is such that they do not usually enter the typical 'differentiation' phase of autograft development. Hairs, for example, have never been found to mature and pierce the graft roof. Nevertheless, the longest high-dosage survivor, animal 101, shows at 16 days the retrograde differentiation of the roofing epithelium which occurs typically in autografts, and which restores the hypertrophied epidermis to somewhere near its normal thickness again (Pl. 4, fig. 37).

With very rare exceptions, the breakdown of foreign epithelium, which is the central feature of the homograft reaction, takes place first in that which has spread outwards from the graft, and extends thereafter, always within the compass of a 4-day sampling interval, to the graft roof. In describing the characteristic train of events, I have drawn upon material offered by lower-dosage homografts as well as that which relates to the high-dosage grafts under consideration here.

Breakdown in the spread epithelium is invariably anticipated by the accumulation of lymphocytes in a narrow zone immediately below it (Pl. 2, fig. 24); by the congestion and engorgement of many of the capillary vessels in the tissue which it overlies; and by the accumulation of oedema fluid in the zone of lymphocytic aggregation (Pl. 2, fig. 25). To outward appearance, the critical stage is marked by the 'wetting' of the cuticle and its ready disengagement upon pulling or scraping. In histological preparations (Pl. 3, figs. 27, 28) it takes the form of a separation of the basal-layer cells of the epidermis from each other and from their underlying attachments. What remains of the epithelium, if it is not accidentally or deliberately scraped off, now forms a pus-like film over the surface of the outlying tissue (Pl. 3, fig. 26). During the process of breakdown, the cytoplasmic reaction to haematoxylin passes from basiphilic to strongly acidophilic.

No specific nuclear or cytoplasmic abnormality is associated with the process of disengagement. All categories of the histopathological classification are exampled, and their variety denies significance to any one. All are doubtless of secondary significance.

'Violent' breakdown of the spread epithelium is just complete by the 8th day, and no spread epithelium has been found to survive so far as the 16th day in any high-dosage homograft.

Breakdown of the epithelial elements in the graft itself takes place simultaneously with—or, since a diffuse 'black band' may develop in grafts eliciting a mild reaction *after* breakdown is complete—very shortly before the disruption of the primary blood vessels and the cell-death of the primary native population. It is not associated with a pronounced *local* mesenchyme cell aggregation, possibly because the vessels stagnate before cellular infiltration of the dermis reaches its papillary layers. But as with the spread epithelium, breakdown is anticipated by capillary proliferation, oedema, and haemorrhage in the dermal papillae. Pl. 3, fig. 30, and in more detail Pl. 3, figs. 31, 32, illustrate a typical 'mild' reaction of breakdown spreading through the epithelium of the graft roof. No one particular cellular abnormality is associated with it. 'Young' epithelium, i.e. that of the hair-follicle primordia, is consistently the most resistant (Pl. 3, fig. 30); or it appears to be so, because its nuclei stain so densely, and the cytoplasm associated with them is so ill-defined that the histological picture of maceration and disengagement is difficult to detect.

Breakdown of the *violent* type—that which is complete within the 8th day after transplantation—is, however, uniform and characteristic: the entire graft roof disengages simultaneously from the blistered papillary layer (Pl. 3, fig. 33), and the basal layer-cell nuclei exhibit a coarse fragmentation (Pl. 3, figs. 34–36). Thereafter little change takes place (cf. Pl. 2, fig. 17*b*), for, as has already been said, the tissue that lies above the black band of violent breakdown becomes arrested in its development.

The entire process of epithelial breakdown in homografts of all types is complete within a time span of 4 days from its beginning to its end. A graft may show no signs of breakdown at one inspection and total breakdown 4 days

later. Correspondingly, if breakdown is in progress at one inspection (e.g. complete in the spread epithelium and partial in the graft roof, as in Pl. 3, fig. 30) it is invariably complete at the next, 4 days afterwards. Breakdown of recent origin or, alternatively, of comparatively long standing, are with experience fairly easy to distinguish from each other. Breakdown of recent origin is indicated by the persistence of traces of 'young' follicular epithelium which is not certifiably 'dead'; by the retention of scraps of endothelium around the vessel spaces; by the mere pyknosis rather than the fine fragmentation of the primary native population; by the merely incipient development of the secondary blood-vessel invasion; and so on. Breakdown of long standing (cf. Pl. 2, fig. 21) is indicated by the occlusion of the primary vessel spaces and the advanced secondary vascularization and collagenization of the graft dermis. Evidence of this type, taken in conjunction with the invariably consistent serial record provided by the 4-day sampling intervals, makes it possible to time the point at which breakdown is *just* complete to the nearest 2 days, without much possibility of error. The estimates so obtained may be used to express the relationship between the survival time of the graft and the intensity of the inflammatory process which accompanies its breakdown. Inflammation itself, a phenomenon compounded of many variables which are only partially interdependent, can hardly be measured (though the oedematous swelling of the dermis (Text-fig. 3) is a transformed measurement of one aspect of it); but the severity of the inflammation can easily be *ranked*, i.e. given an ordinal number. The 8-day biopsy specimens provide the fairest data for such a ranking, because even in the most violent breakdown the necrosis into which inflammation here ultimately develops is of very recent standing. In the accompanying series, inflammation at the 8th day is set in order of decreasing intensity against the estimated survival time of the foreign epithelium:

Inflammation, animal no.	20, 86		96	24, 26		28, 30		106	17	101
Survival time, days	8	8	10	10	12	10	10	12	14	18

Mere inspection shows that there is an almost exact negative ordinal correlation between the two variables: the longer epithelium survives, the milder is the inflammatory reaction at the 8th day.

For precise analysis I have, however, avoided timing the breakdown of the grafts on the basis described above, because it does involve an unduly high subjective element. But comparatively little difficulty arises in stating whether breakdown is complete or not in any one specimen. It is true that this also involves a histological appraisal of the specimens, and that one may wrongly suspect a group of cells of being 'alive'—capable of subsequent amoeboid movement and division—when in fact they are not. The validity of the histological judgement of total breakdown has therefore been subjected to a rigorous experimental test, described in full in the section of this paper dealing with the problem of immunity. This test, which consists in transplanting a homograft back to its original donor and allowing it to continue its development there, has confirmed the 'intuitive' histological judgement and established the following general rule, which may be of interest to other students of the histology of skin: *if there is the slightest suspicion that epidermal cells are not dead, then the cells are alive.* In practice this means that if a cell has a nucleus with a membrane and the cytoplasm surrounding it is not wholly acidophilic, then the cell must be judged living, and capable of resuming normal activity when transplanted to a favourable environment.

Text-fig. 4, II, has been constructed upon these grounds. It is based upon the ten high-dosage homograft-bearing animals for which the serial record from 4 to 20 days is complete, and it plots for each 4-day interval the *number of animals* which still bear surviving foreign skin epithelium. From this table, using the methods of Bliss (1937), the following statistic may be calculated: the time at which the grafts borne by 50% of the experimental animals have just broken down is 10·4 days, with a standard sampling error of 1·1 days. Estimates of this *median survival time* should, in nineteen samples out of twenty in an indefinitely extended series of trials, lie within the range 7·9–12·9 days. The spread in time of the graft mortality distribution is best given (for reasons explained later, when the technique of the analysis is discussed) by the reciprocal of its standard deviation, which is 0·3025 (if corrected for grouping) and 0·2855 (if not so corrected).

The behaviour of the outlying tissue. The fibroblast reaction in the outlying tissue which accompanies the hyperplasia of graft epithelium in autografts occurs in homografts likewise (Pl. 2, figs. 23, 24). In the former it appears to have a purely reparative significance, and it subsides in proportion as the tissue becomes collagenized. But in homografts it persists, and the continued proliferation of fibroblasts, though not their initial mobilization, is one ingredient of the acute inflammatory process that spreads through the whole of the outlying tissue before subsiding into the chronic state. The oedema associated with it appears to maintain the activity of the fibroblasts, and layer upon layer of fibrous tissue is consequently laid down, mature in the deeper tissue and progressively 'younger' in the more superficial. The characteristic zonation that is established is illustrated by Pl. 4, fig. 43. It will be seen that in default of epithelium, or after its disappearance, the outlying tissue is surfaced by a delicate layer of granulations. If epithelial debris remains upon the surface, or is trapped in the remains of an epithelial re-entrant, mononuclear phagocytes are found to be associated with it. Multinucleate 'foreign body' giant cells occur only in association with fragments of fibre from the dressing materials.

The chronic inflammatory state, represented by the general vascular proliferation and the leucocytic halation of the vessels is illustrated by Pl. 4, fig. 42. Here, as in the graft itself, the lymphocyte is the dominant cell. In the layer of granulations, however, the polymorph asserts the ascendancy normal to non-specific inflammatory processes.

The history of homografts carried to the 20th day. Homografts of all types are ultimately reduced to collagenous pads, with or without scraps of epithelial debris adherent to their surfaces. During the whole of their evolution, native epithelium has been growing in from the margins of the raw area, and a generalized contraction of the operation field has brought the grafts to within half their diameter's distance from each other. Grafts lying near the margin of

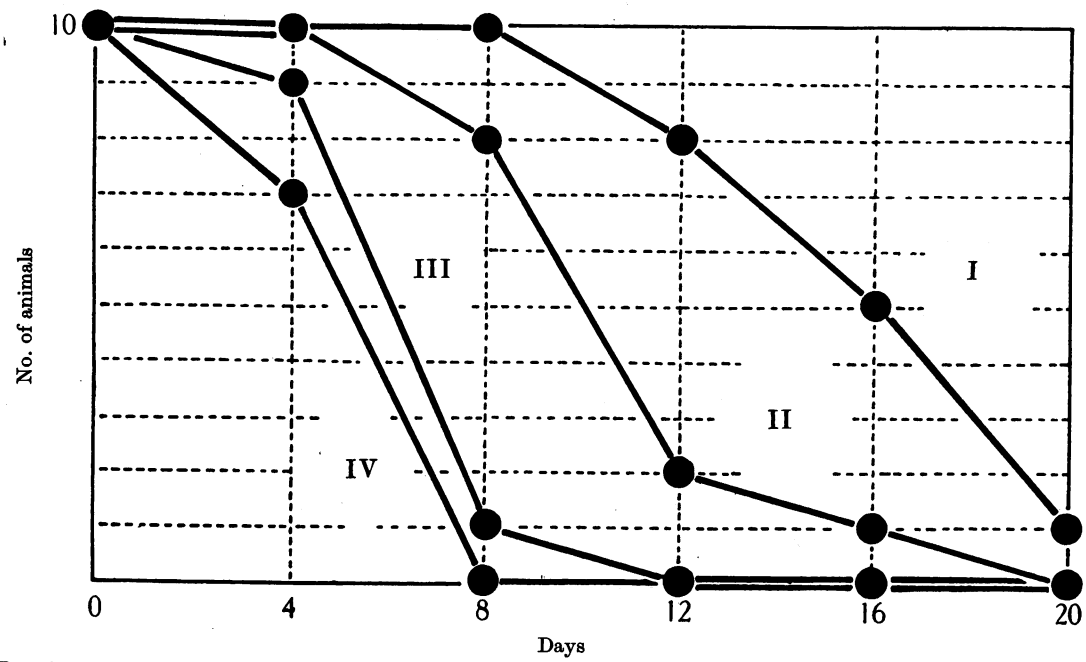
the origina raw area are *undermined* by native epithelium, either at the graft-bed junction, or at the level of the black band, or, apparently, in any plane (Pl. 4, figs. 38, 39). If, however, the graft lies in the centre of a sufficiently large raw area, native epithelium will take longer to reach it, and by the time it has done so, granulation tissue will have grown round the graft and may even have started to grow over it. In such cases, the foreign collagen is *overgrown* (Pl. 4, fig. 41). Whether undermining or overgrowth takes place seems to be merely a matter of time relations. The rabbit is a small animal, and undermining is therefore the general rule. In the larger areas of skin loss which may occur in the human being, overgrowth is to be expected (Gibson & Medawar, 1943).

graft cells have long since disappeared, this collagen must have been manufactured by native cells of the graft's secondary population.

The ultimate fate of overgrown graft collagen cannot be predicted, since this investigation closes when the process is in a very early stage. Recollagenization may be progressive, but the foreign collagen may well maintain the chronic inflammatory state for so long as any of it persists.

THE SIGNIFICANCE OF GRAFT DOSAGE

Any systematic analysis of the homograft reaction must begin with an examination of the degree to which it varies with graft *dosage*. The ten animals



Text-fig. 4. The survival of homograft epithelium as a function of time. Each point defines the number of animals, out of ten for each type of experiment represented, which still bear surviving foreign epithelium at the stated times. I, lower-dosage graft bearers: computed from fifty pairs of animals in five independent groups of ten; II, high-dosage graft bearers: computed from ten pairs of animals, from each of which a graft sample was removed at 4-day intervals from the 4th to the 20th day; III, standard 2nd-set graft bearers: computed from ten pairs of animals, from each of which a graft sample was removed at the 4th and at the 8th days; IV, local 2nd-set graft bearers: computed from ten pairs of animals from each of which a graft sample was removed at the 4th day. Curves III and IV are extrapolated to zero survival at the 12th and 8th days respectively without error. Curve I cannot be extended in this manner to the 24th day, since the 4-day groups of data are here independent (see text).

Almost as soon as native epithelium reaches the foreign dermis, whether it approaches it from above or from below, hair-follicle primordia are laid down (Pl. 4, figs. 40, 41). Evidently skin epithelium, however 'immature', has the power to manufacture hairs provided that it reaches a depot of collagen fibres of the right size and pattern of packing. The primordia and the young glandular acini associated with them are unhealthy in various ways, since a chronic inflammation is in progress all round them. They are, nevertheless, supported and invested by a fine delicate collagenous scaffolding of new formation. Since the original

providing the serial record analysed in the preceding paragraphs received a modal average weight of 0·36–0·44 g. foreign skin in the form of eight large pinch grafts for each animal. In the experiments to be described below, animals weighing between 2 and 2½ kg. as before received *either* one large pinch graft (0·045–0·055 g.) *or* one small (0·006 g.). For most purposes, these 'medium' and 'low' dosage grafts have been grouped into one category, that of *lower* dosage. Since each animal provided only

one biopsy specimen instead of a series, fifty, equally divided between the two types, were operated on in order to provide a record as full as that provided by high-dosage grafts. Lower-dosage graft bearers in general therefore provide ten independent graft samples for each 4-day period from the 4th to the 20th days. The technique of grafting remained unchanged.

Two points of interpretation require special mention at the outset:

(1) The fifty animals which provide the five sets of ten 'readings' for lower-dosage grafts were operated on and later examined in accordance with a prearranged plan—that which fitted in best with the general routine of the laboratory. The *effect* of this prearrangement is to make each group of ten animals a purely random one, for one can have no foreknowledge of the length of time that foreign epithelium is to survive. With certain reservations to be discussed below, the lower-dosage grafts are therefore comparable with the high. No such comparison would have been possible if, for example, biopsy specimens from the fifty lower-dosage bearers had been removed at, or as soon as possible after, the time when breakdown was to outward appearance complete. Had this scheme been adopted, the entire comparison would have been falsified, for the data for each group of ten animals would have been drawn from a richer pool of genetic variance—in the extreme case, that represented by fifty animals instead of ten.

(2) The dosage ratios that have been quoted for animals of the high, medium, and low series relate to the *initial* dosages alone. Save that it is known to stand in this order of rank, the *effective* dosage cannot be computed, because the grafts grow and manufacture new epithelium. If, as proves to be the case, the survival of lower-dosage grafts is relatively prolonged, then the outward spread of thick foreign epithelium, which is progressive, will naturally close the gap between the initial skin dosages. Indeed, the typical lower-dosage graft bears at 12 days more foreign epithelium than all eight high-dosage grafts bear at the outset. Although the differences between high- and lower-dosage grafts are striking, this may account for the fact that they are not more striking still. (A stricter test for dosage effects would be one in which varying numbers of grafts were accurately fitted into raw areas only just large enough to receive them, so that little or no outgrowth took place. Had this test been made, however, many phenomena not related to this particular problem of dosage could not have been observed.)

The behaviour of lower-dosage grafts

The following brief summary gives the typical though not invariable features of grafts of the lower-dosage series.

The evolution of lower-dosage grafts extends *beyond* the proliferative phase, into the period of hair formation and retrograde differentiation of the blood vessels, graft roof, and glandular acini (Pl. 4, figs. 44, 46). It is the rule for lower-dosage grafts to survive beyond the 12th day: in higher dosage grafts it was the exception. Outgrowth is correspondingly richer; 5–6 mm. of spread epithelium usually rings the lower dosage graft at 12 days and, exceptionally, it may persist until the 16th (Pl. 4, fig. 46). Inflammation and vascular proliferation are slower to develop, for at the 8th day inflammation rarely exceeds a feeble and diffuse diapedesis halation of the blood vessels, and in low-dosage grafts (Pl. 4, fig. 45) it may be quite inappreciable. A ranking of the inflammation at 8 days in order of decreasing intensity puts this judgement beyond question:

	Animal no.								
High dosage	20, 86	96	24, 26	28, 30	106	17	101		
Medium dosage			75				76	34, 35, 120	
Low dosage							112		90, 93, 134, 135

All ten high-dosage graft bearers fall within the first thirteen places, a result which—irrespective of the order of the terms within the subgroup—would be expected to occur by chance only once in 626 random drafts. Moreover, the *peak* intensity of the inflammation at the close of the primary cycle is well below that of high-dosage grafts (Pl. 4, fig. 47): a diffuse 'black band' sometimes develops *after* breakdown has occurred, but never before it.

Chart I in Text-fig. 4 has been constructed for lower-dosage grafts exactly as for high (chart II): it represents the number of animals in each of five independent groups of ten which still bear surviving foreign epithelium at 4, 8, ..., 20 days. Reference to it shows that the majority of the 12-day group and half the 16-day group of ten lower-dosage animals bore surviving grafts. The *median survival time*, computed by the methods of Bliss (1935 *a*, *b*), is 15·6 days, with a standard sampling error of 0·9 day. Estimates of the median survival time are to be expected, in nineteen cases out of twenty of an indefinitely extended series of trials, to lie within the range 12·8–18·5 days. The spread of the graft mortality distribution is given (see below) by the slope of the probit-mortality regression straight line when the percentages of mortality are transformed into areas of the normal curve of error. This estimate of the spread is $0{\cdot}2820 \pm 0{\cdot}0668$ unit.

High-dosage and lower-dosage grafts compared

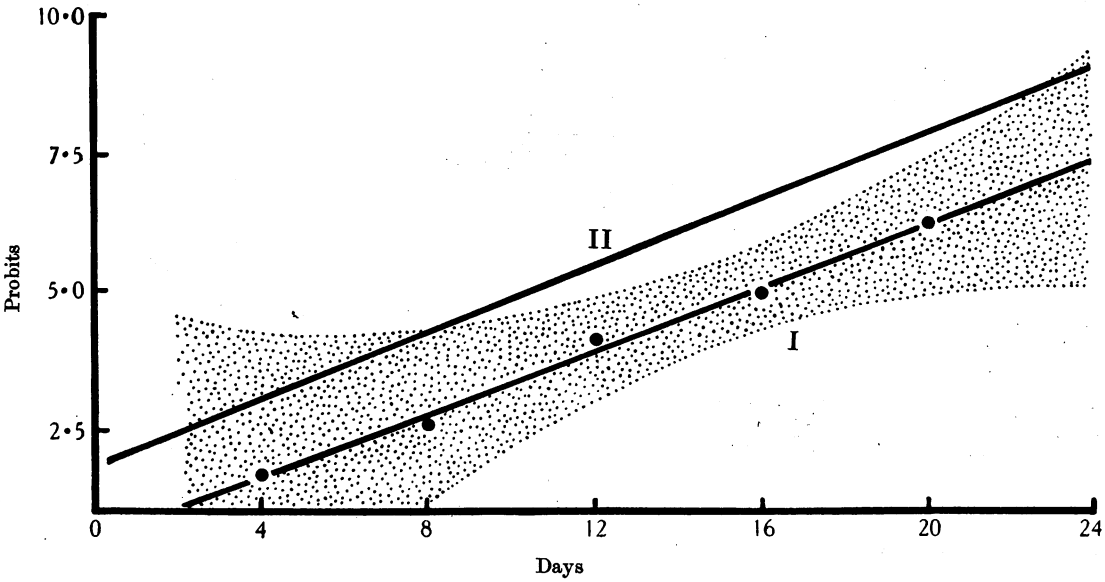
In this section an attempt will be made to validate in detail the generalizations of the preceding paragraphs.

(*a*) *The survival time of epithelium.* Text-fig. 4, charts I and II, shows that the disparity between the survival times of high- and lower-dosage grafts is more strongly marked at the 12th day than at any other 4-day period. The data are here based upon two independent and randomly chosen groups of ten animals, and, neglecting all

other readings, may be set out as a '2 × 2 contingency table' and analysed by Fisher's method for the exact treatment of such data (*Statistical Methods for Research Workers*, 21·02; *Science*, **94**, 210, 1941). The odds are 86:1 against obtaining, by luck of sampling, so extreme a partition in favour of the prolonged survival of lower-dosage grafts, or a more extreme one.* There is therefore little reason to doubt that a higher proportion of lower-dosage than high-dosage recipients bear surviving foreign epithelium at the 12th day.

This test does not make full use of the data, and no readings other than the 12-day need have been taken for it. Nor does it necessarily compare the data at the time which is most favourable for exhibiting a difference between them. Comparison between the two sets of data *as a whole* is complicated by the fact that the lower-dosage set form five independent groups, though the higher-dosage data do not. For example: only one high-dosage homograft recipient bore surviving epithelium at the 16th day; but not more than two could have done so in any case, for only two bore surviving epithelium at the 12th. This progressive narrowing down of the possibilities of survival does not affect animals of the lower-dosage series. Here, the 16-day readings are independent of the 12-day; it is possible, though on empirical grounds highly unlikely, for all the 16-day animals to bear surviving grafts, regardless of the fact that only eight of the 12-day group were found to do so.

The upshot of this is that different treatments must be applied to the high-dosage and lower-dosage data if the number of survivors is in each to be expressed as a function of time and then compared. Charts I and II of Text-fig. 4 express a cumulative, i.e. successively summed, distribution of graft mortality: the number of animals bearing 'dead' grafts at any one time is the number that has accumulated during the whole of the period that precedes it. By subtraction of the survivors at one 4-day period from those of the 4-day period that immediately precedes it, the data for *high-dosage* grafts may be cast into the form of an ordinary non-cumulative frequency histogram, and analysed by the method of Bliss (1937). For the reasons already given, the data for lower-dosage grafts must be analysed in the cumulative form in which they already stand in chart I.



Text-fig. 5. I, the *probit*-mortality regression straight line for lower-dosage graft bearers, with the working values of the probits to which it was arithmetically fitted: computed from the data of Text-fig. 4, chart I. The area which bounds the line symmetrically is that within which 95% of random sampling estimates of the probit mortality corresponding to an arbitrarily chosen time may be expected to lie. II, the corresponding line for *high-dosage* homografts, which differs in position but not in slope from I: computed from the data of Text-fig. 4, chart II.

The essence of the method that has been devised for this purpose (see Bliss, 1935 *a*, *b*) is to express the percentages of graft mortality as areas of the normal curve of error in terms of the normal deviate. To avoid negative values in practice, 5 is added to the appropriate value of the normal deviate. This is the 'probit' of the observed mortality percentage. The probit mortality will be linear in that function of time, about which the mortality frequency is normally distributed. In this case, the probit mortality may be represented as a linear function of time itself with a degree of fidelity measured by $\chi^2 = 0{\cdot}344$, corresponding roughly to a 95% point in its distribution for three degrees of freedom.

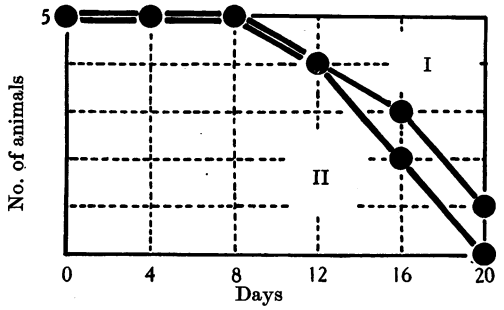
Text-fig. 5 illustrates the regression straight line for lower-dosage graft bearers and the working values of the probits to which it was arithmetically fitted. The best estimate, corresponding to any chosen time, of the percentage of animals bearing grafts which have broken down, may be

* The conventional significance level—that at which the experimenter is supposed to feel himself reasonably exempt from the chances of random sampling—is here represented by odds of 39:1, not 19:1.

read from the line itself or calculated from its equation. The shaded area about the line, symmetrically bounded by the arcs of two hyperbolas, is that within which 95% of random sampling estimates of this variate may be expected to lie.

For comparison, the probit-mortality line for high-dosage graft bearers is also represented in Text-fig. 5. Its slope—the reciprocal of the standard deviation of the graft mortality distribution—is virtually identical with that calculated from the data for lower-dosage grafts. One may infer that the *tempo* at which breakdown proceeds, once the process has started, is the same for both: the difference between them lies in the length of the latent period which must pass before the homograft reaction becomes effective.

If a comparison between the two sets of data as a whole is based upon the respective estimates of the median survival times ($15{\cdot}63 \pm 0{\cdot}89$ days for lower-dosage graft bearers, $10{\cdot}40 \pm 1{\cdot}11$ days for the high), then the probability that these two estimates have been in reality drawn from the same normal population may be expressed by odds of



Text-fig. 6. The fifty lower-dosage graft bearers represented in Text-fig. 4, chart I, are here resolved into: I, twenty-five low-dosage, and II, twenty-five medium-dosage graft bearers. Each chart is thus computed from twenty-five pairs of animals in five independent groups of five.

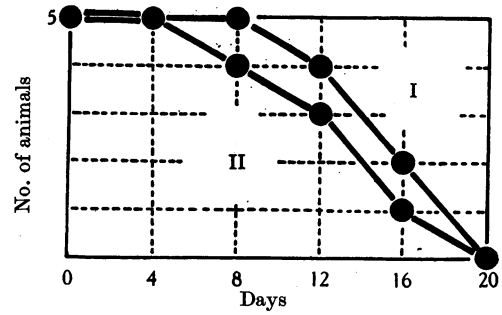
approximately 100:1 against. There can be no reasonable doubt that lower-dosage grafts survive longer than high, and that the difference between them lies predominantly in the latent period that elapses before the homograft reaction reaches a critical operative level.

(Had these experiments been redesigned in the light of evidence from what could then be regarded as a preliminary trial, certain modifications would have been made. The grouping—or graft-sampling—interval of 4 days would be reduced to 3, so that it lay just within instead of just outside the standard deviation of the mortality distributions. This would also have helped to justify what is here a guess, viz. that the mortality distribution of *high-dosage* grafts is a normal one. Since only three empirical probits can be derived from the high-dosage data with the grouping interval so large as it is, a straight line can be fitted to the cumulative frequency diagram (see Bliss, 1937) with only one degree of freedom. As a straight line is not an obvious misfit, and as the normality of the *lower-dosage* distribution is not in doubt, I accordingly chose time itself, rather than some function of it, as the abscissa of the high-dosage distribution.)

(*b*) *The extent of differentiation.* It has been said that lower-dosage grafts usually enter the phase of differentiation that is characteristic of autograft development. The maturation and piercing of newly formed hairs, which may be expected to occur by the 12th day or later, makes a significant measure of this property. Not one of the thirteen independent sets of high-dosage homografts (ten in series, three in parallel) examined at the 12th day or after progressed so far as hair formation, yet seventeen of the thirty lower-dosage grafts examined within this period were found to do so. Yet if in reality hair formation occurred with equal frequency in both groups, so great a discrepancy in favour of the lower-dosage grafts would be expected to occur only once in 3517 such sets of random drafts from them. Obviously there is a systematic difference: differentiation goes farther in lower-dosage grafts than in high.

Medium- and low-dosage homografts compared

Charts I and II of Text-fig. 6 are derived from chart I, Text-fig. 4, by dividing the fifty lower-dosage graft bearers to which it relates into their



Text-fig. 7. Similar to Text-fig. 6 in all respects except that the loss of *spread* epithelium, rather than the tota breakdown of homograft epithelium, is chosen as the criterion of graft survival.

equal subgroups of 'medium' and 'low'. The low-dosage group (I) contains the majority of survivors beyond the 12th day, but the difference cannot be described as other than suggestive. The same applies to charts I and II of Text-fig. 7, in which the loss of *spread* epithelium and not the overall breakdown of the grafts is made the basis of comparison.

In other respects a firmer comparison can be drawn. An ordinal ranking of the intensity of inflammation at the 8th day (see p. 187) shows that all five homografts of the medium-dosage group are present in the first six places of the lower-dosage group as a whole—a result which would be expected to occur by chance only once in 42 trials. Again, no less than twelve grafts of the low-dosage group of fifteen animals examined at the 12th day or after progressed so far as hair formation. The corresponding figure for medium-dosage grafts is 5 in 15, a partition in favour of the former which should occur only once in 79 random trials. There is therefore a strong indication that low-dosage grafts progress farther than medium before the homograft reaction overtakes them.

One objection to this series of comparisons may now be considered. Animals of the high and medium series differed with respect to the number (eight and one respectively) of uniform large grafts that they received; medium and low, however, differ in the *size*—large and small respectively—of the single grafts they bear. Is the somewhat greater resistance of low-dosage grafts due to some factor bound up with the difference of their absolute size, irrespective of dosage? This possibility is refuted by the behaviour of the five high-dosage graft bearers which were studied 'in parallel'. Each animal of this group received not eight large grafts, but seven large and three small. The small grafts thus had an opportunity to display any characteristics associated with their absolute size, though they were in a high-dosage environment. In fact, it was found that the small grafts broke down at precisely the same time and in precisely the same way as their larger contemporaries. Other things being equal, it is evidently the overall *dosage* of grafts, and not their absolute size, that governs the intensity of the homograft reaction.

THE SIGNIFICANCE OF IMMUNITY

The term *immunity* has not hitherto been used to describe the reaction generated in their recipients by homografts, although rabbits are in the literal sense 'immune' to the transplantation of foreign skin. For in practice the term is reserved to describe a reaction which can be expressed in terms of an antigen-antibody relationship.

Immunity in this technical sense is said to be 'innate' when, as with blood antigens of the *A*, *B*, *O* group, the corresponding antibodies are ready-made; 'actively acquired' when the antibodies are manufactured *de novo* in direct response to an antigenic stimulus; and 'passively acquired' when antibodies are transferred in suitable form from one animal to another.

Immune reactions may be demonstrated either directly, by a reaction *in vitro* between antigen and antibody, or indirectly. The most important indirect demonstration of actively acquired immunity is the greatly reduced latent period that precedes the reaction to a *second* series of doses of antigen after immunity has been generated by a first.

The great majority of students of tissue homografting have consistently and systematically denied that immune phenomena play any significant part: they claim that a second dose of homografts, transplanted when the reaction to a first is complete or at its height, provokes a reaction neither more rapid nor more intense than the original. Gibson, however (1942), reversed this judgement in his studies on skin homografting in human beings, and one fully controlled demonstration of accelerated breakdown in '2nd-set' pinch homografts has been described by Gibson & Medawar (1943).

The experiments described in this section form an attempt to settle the problem one way or the other. The principle here adopted is the serial study of the behaviour and fate of homografts in presumptively immune animals—animals which have already reacted to a first dose of grafts from the same donor source. In practice, six large immunizing or '1st-set' are transplanted to a chosen recipient in the manner already described for high-dosage homografts (which, but for the practically insignificant disparity of dosage, their behaviour may be expected to resemble in every respect). Sixteen days later (12 proved to be insufficient) two or three further grafts from the same donor are transplanted to a raw area freshly stripped from the opposite thoracic wall. A biopsy specimen was taken from this second set of grafts at the 4th day and at the 8th. A group of ten (pairs of) animals operated on in this way provides the record upon which the descriptions which follow are based.

The behaviour of '2nd-set' homografts

Outward appearance. Second-set homografts are found to be swollen by the 4th day after transplantation, but do not swell markedly thereafter. Their colour change is variable: the most usual is a rapid transition from the shiny white of the original graft to dirty yellow or yellowish brown. Neither outgrowth of epithelium nor the remains of it are visible to the naked eye either at the 4th day or at the 8th. Primary adhesion is often weak: special care must be taken in removing the dressings, for a moderate shearing force may cause the graft to slide out of its bed. If this happens at the 4th day, the underside of the graft is usually seen to be dead white in colour, except where it is grossly blotched by large wound vessels from which a brownish red serum can be expressed.

Dermis and graft bed. Second-set grafts are vascularized. The great majority of the invading capillaries are, however, converted into 'wound vessels', i.e. grossly dilated and engorged capillaries with a simple endothelial lining, lying in a haemorrhagic field (Pl. 5, figs. 48–50). Wound vessels ranging between 0·2 and 0·3 mm. in diameter of lumen are not uncommon. Lymphatics either do not penetrate the grafts, or do so only at its deepest levels. Nevertheless, the most deep-lying blood vessels may by the 4th day have differentiated into small and ill-defined arteries and veins (Pl. 5, fig. 49).

It is the rule for the primary blood vessels to stagnate by the 4th day, with a partial or more usually a total disruption of their endothelial linings (Pl. 5, fig. 48). By the 8th day (Pl. 5, fig. 52) even the tissue spaces of the primary vessels may be obliterated, and the process of secondary vascularization by capillaries from the granulating tissue of the graft bed is found to be in full swing. Correspondingly, 2nd-set homografts reach their maximum thickness of $1{\cdot}29 \pm 0{\cdot}10$ mm. (Text-fig. 3) by the 4th day; the recorded rise thereafter to $1{\cdot}38 \pm 0{\cdot}11$ mm. is hardly significant.

One striking consequence of the precocious breakdown of the primary vascular system is that no native population of leucocytes invades the grafts by passage through the vessel walls. No more leucocytes enter the graft (Pl. 5, figs. 48–50, 52) than would be expected as an accompaniment to a purely non-specific (traumatic) inflammation. This is by far the most striking superficial difference between 1st-set and 2nd-set grafts: compare the black band of violent breakdown in the former (Pl. 2, figs. 17 *a*, *b*) and the

still massive invasion which occurs in mild breakdown (Pl. 2, fig. 16), with the clear dermis (Pl. 5, fig. 52) of 2nd-set grafts. Yet it will be shown that 2nd-set grafts break down the more rapidly. Only in one 2nd-set homograft did a black band form.

The inflammation elicited by 2nd-set grafts is precocious in time of onset but somewhat variable in its peak intensity. It is confined to the tissue of the graft bed and that which immediately surrounds or underlies it. Qualitatively it resembles the inflammatory process in the bed of 1st-set grafts: a fibrin band may distend the fascial planes of the graft bed by the 4th day, and the capillaries which 'organize it' (Pl. 5, fig. 50) sweep upwards and enter the base of the graft as its secondary vascular system (Pl. 5, fig. 52).

The evolution of the outlying tissue requires no special mention.

Epithelium. Breakdown of the epithelium is usually in progress, though very rarely complete, at the 4th day: epithelium of the graft roof may have disengaged round the rim of the graft, and the nuclei of the epithelium of the hair follicles are either fragmented or are framed as densely pyknotic ovals within an acidophilic cytoplasm (Pl. 5, fig. 50). By the 8th day the graft exhibits the characteristic appearance of 'total breakdown of long standing' (Pl. 5, fig. 52), and the roof epithelium is fragmented (Pl. 5, fig. 53) or lost. Only one graft bore patches of surviving epithelium at the 8th day (Pl. 5, fig. 54), and in one, apparently, breakdown was complete at the 4th. Chart III, Text-fig. 4, is constructed accordingly: extrapolation to the 12th day, in the manner indicated, is fully justified by the fact that breakdown in the one 8-day survivor was very far advanced. The *median survival time* of 2nd-set grafts, computed by Bliss's method (1937), is 6·0 days with a standard sampling error of 0·6 day. In 95% of an indefinitely extended series of random trials, an estimate of the median survival time will be expected to lie within the range 4·7–7·4 days. (The error introduced by the magnitude of the grouping interval, 4 days, is here so great that the sampling error can only be computed roughly: the figure quoted, 0·6 day, is an overestimate.)

General cellular activity in 2nd-set grafts is excessively feeble, though its mere existence is of some importance. As a general rule the greater part of the graft roof epithelium remains almost unthickened, and its cells retain their flattened plate-like character (Pl. 5, fig. 51). Some upward migration of follicular epithelium may cause a purely local thickening of the roof in the neighbourhood of the follicle openings. Pl. 5, fig. 54, taken from the one 8-day survivor, illustrates the most advanced development of the graft roof that has been observed: though upward migration of follicular epithelium has taken place, the vertical differentiation of the graft-roof epidermis characteristic of 1st-set (high-dosage) grafts is no more than incipient. Outgrowth of epithelium from the graft, though it does occur, is confined to a one- or two-layered sheet that can usually be traced to its origin in a hair follicle. Since the spread epithelium neither extends beyond 0·5 mm. from the graft, nor develops a cuticle, outgrowth is not visible to the naked eye. In short: the cellular activities are no greater than may be attributed to localized amoeboid movements without proliferation. New hair-follicle primordia and new depots of glandular epithelium are never manufactured.

High-dosage (1st-set) and 2nd-set grafts compared

The evolution of autografts has been shown to fall into three stages: the period of primary union, the period of generalized hyperplasia, and the period of partially retrograde differentiation. Lower-dosage grafts usually enter the third period before breakdown; high-dosage homografts always enter the second, but rarely the third; and 2nd-set homografts do not even enter the second. The thickening of the roof epithelium has hitherto been used as a consistent measure of cellular activity, and numerical analysis of it confirms the qualitative judgement. The mean roof thickness of surviving epithelium in 2nd-set grafts at 4 days is $0{\cdot}009 \pm 0{\cdot}001$ mm.; of 1st-set grafts, $0{\cdot}017 \pm 0{\cdot}002$ mm. The difference between the means speaks for itself, since it exceeds its standard error, computed from 18 degrees of freedom, more than threefold.

The swelling of the dermis (Text-fig. 3) that accompanies primary vascularization is precocious in 2nd-set grafts. The mean dermal thickness of 2nd-set grafts at the 4th day was found to be $1{\cdot}29 \pm 0{\cdot}10$ mm.; of 1st-set grafts, $0{\cdot}95 \pm 0{\cdot}09$ mm. The disparity between the two means, 0·34 mm., is such as would be expected to occur in substantially less than 5% of random samples drawn from the same normal population, and may therefore be described as 'significant'. By the same token, the recorded increase in the thickness of 2nd-set grafts between the 4th and 8th days—here valued by the methods appropriate to paired data—is insignificant; nor do the thicknesses of 1st-set and 2nd-set grafts differ at the 8th day. This failure to swell between 4th and 8th days is doubtless attributable to the precocious breakdown of the primary vascular system, which in at least five of the ten 2nd-set grafts was apparently functionless at the 4th day. In 1st-set grafts, the primary vascular system is still intact at the 4th day. The chances are 61:1 against the null hypothesis of uniformity.

Mere survival or non-survival of epithelium provides no ground for a comparison between 1st-set and 2nd-set grafts at the 4th day, as reference to chart III, Text-fig. 4, shows; but a more subtle test will be taken into account whether or not breakdown is *in progress* in the graft. Breakdown has never been found to be in progress in 4-day 1st-set grafts; but it proved to be so in at least six 2nd-set grafts of the ten. Here the odds against a hypothesis of uniformity are 184:1. At the 8th day, 1st- and 2nd-set grafts may be compared on lines similar to the comparison between high-dosage and lower-dosage grafts at the 12th. Of ten randomly chosen pairs of animals in each case, *one* 2nd-set and *eight* 1st-set homograft bearers still retained surviving foreign epithelium. The odds are 364:1 against obtaining so great a disparity in favour of the 1st-set grafts by luck of sampling alone.

As with the comparison between high- and lower-dosage grafts, the *median survival times*, computed from the whole of the available data, provide the firmest ground for analysis. The estimates for 1st-set and 2nd-set grafts were found to be $10{\cdot}4 \pm 1{\cdot}1$ days and $6{\cdot}0 \pm 0{\cdot}6$ days respectively. The difference between the means, 4·4 days, is such as would be expected to occur in substantially less than 1% of samples drawn from the same normal population.

A biological test of survival

The foregoing analysis leaves little room for doubting the fact that 2nd-set homografts undergo a relatively accelerated retrogression. Yet the matter is so crucial that it cannot be allowed to rest upon a histological appraisal of biopsy specimens alone. The histological verdict of survival or non-survival has therefore been checked by an independent test which serves to emphasize the significance of the difference between 1st-set and 2nd-set homografts. A homograft is removed from its recipient after a varying period of residence upon it, and transplanted back to its original donor. Eight days later the specimen (now an autograft) is removed and examined histologically. Such epithelium as may have survived in the homograft will by that time have migrated from it in the usual way. The presence or absence of this epithelium therefore makes a consistent and independent test for the survival or non-survival of the graft. (It is true that this test too makes use of a histological judgement; but it is of so elementary a character that it may be supposed to have absolute precision.)

Simple as it is in theory, this test is in practice complicated, though in no way invalidated, by the intervention of non-specific factors. A homograft transplanted back to its donor does not behave thereafter as a normal autograft, for it promptly elicits a violent inflammation in the graft bed which in the majority of cases seriously interferes with the invasion of the graft by primary vessels. The inflammation has most of the characteristics of the lymphocyte-dominated reaction which accompanies the breakdown of homografts; but this is not surprising, when we recollect that the homograft is turgid with exudates which may be supposed (Menkin, 1940) to contain the causal agents of at least some of the ingredients of the inflammatory process. It is, however, surprising that the reaction retains its specific character. Nevertheless, epidermal epithelium has such spectacular powers of overcoming circumstances unfavourable to its growth that this test is if anything more sensitive than the histological; and in any case, once the spread epithelium is clear of the graft from which it arises, it behaves just as native epithelium does, and provokes no inflammation in the tissue of the outlying field.

The foregoing qualification amounts to this: a *positive* result from the test is not subject to error, since dead epithelium cannot by definition have come to life; but a *negative* result—non-survival—is probably subject to a small and systematic but perfectly consistent error.

Autografts such as these, which have spent a proportion of their life as homografts, are called 'autohomografts' for short. Twenty-four independent autohomograft tests on twenty-four pairs of animals have been distributed as follows:

Six 4-day 1st-set autohomografts (i.e. grafts returned to their donors after 4 days' residence on their original recipients as high-dosage homografts);
Six 8-day 1st-set autohomografts;
Six 4-day 2nd-set autohomografts;
Six 8-day 2nd-set autohomografts.

Some of the animals belonging to the 4-day subgroup of twelve pairs were among those which provided the histological data already analysed; but the 8-day subgroup of twelve pairs are wholly independent in the source of the material as in its technique of analysis.

The histological analysis of autohomografts is naturally of the greatest complexity. Therefore the presence or absence of living epithelium in them, or arising from them, has been made the sole basis for comparison.

All twelve 4-day autohomografts, irrespective of their differentiation into 1st-set and 2nd-set subgroups, exhibited survival of epithelium when transplanted back to their donors. A rough division may be made into 'complete', 'partial', and 'trace' survival. Four 4-day 1st-set autohomografts showed complete survival (cf. Pl. 5, fig. 56), and two partial. The corresponding figures for 4-day *2nd-set* autohomografts, one complete survival and five partial, already indicate a 'suggestive' if not a 'significant' difference on just the lines that the histological data (Text-fig. 4) would have led one to expect.

All six 8-day *1st-set* autohomografts likewise bore surviving epithelium, now distributed into one complete (Pl. 5, fig. 56), three partial, and two 'trace' (Pl. 5, fig. 57). On the other hand, four of the six 8-day 2nd-set autohomografts showed no trace of surviving epithelium whatsoever: their histological appearance was that of total necrosis of long standing (Pl. 6, fig. 58). The remaining two showed partial survival, to the accompaniment of violent specific inflammation in the graft bed.

Taking the presence or absence of surviving epithelium as criterion, the partition of values for 8-day 1st-set and 8-day 2nd-set autohomografts becomes 6:0 and 2:4 respectively—one which would be expected to occur only once in thirty-three random trials. The difference as it stands is very strongly suggestive, if not in itself decisive. In conjunction with the *wholly independent* tests based upon a histological appraisal of graft death alone, its effect is to put the hypothesis of accelerated retrogression in 2nd-set homografts upon a thoroughly secure foundation.

How far is the autohomograft test consistent with the histological? Both for 8-day 1st-set and for 8-day 2nd-set autohomografts the number of grafts judged survivors exceeded that which the histological data would have led one to expect. The two tests are quite independent, and the chances against getting this degree of disparity in favour of the autohomograft test in *both* cases are only 9:2 against. There is therefore no evidence of inconsistency between them.

The local immune state

In the operation upon which the preceding analyses were based, the 2nd set of homografts were transplanted to an entirely fresh raw area lying on the side of the body opposite to that which carried the 1st set. In no other way could a *systemic* immune

state be demonstrated. The behaviour of 'standard' 2nd-set grafts, as they will now be called, can be usefully contrasted with the behaviour of 2nd-set grafts which are transplanted to the very places occupied by the 1st set of grafts, after the latter have broken down—'local' 2nd-set grafts. The standard and the local 2nd-set grafting operations thus represent two extremes: in the one case, only systemic factors can be at work; in the other, local immune factors are superimposed upon them.

The first set of immunizing grafts are transplanted in the usual way. Sixteen days afterwards, when breakdown may be supposed to be in the great majority of cases long complete, each 1st-set graft is grasped at an edge with dog-toothed forceps and firmly *stripped* from its bed. Since the primary vascular system has stagnated, there is little more than capillary bleeding; and this may be brought to a standstill by sustained light pressure with an absorbent pad.

Into each of a number (in practice four proved to be convenient) of the graft 'craters' so formed, one *small* graft from the original donor is planted. Dressings are then applied in the usual way. After 4 days the grafts are removed for histological examination and for transplanting back to their donors in accordance with the procedure of the 'autohomograft' test.

Ten such operations on ten independent pairs of animals provide the data upon which the following summary is based.

The local 2nd-set graft differs from the standard only in the degree of its responses, and not very markedly in degree. Vascularization is, however, notably backward, and primary healing correspondingly weak, although (as will be demonstrated in the final section) the richly vascular graft bed supports the growth of autografts particularly well (Pl. 6, fig. 59). Breakdown of the vessels is complete by the 4th day and no primary population of native leucocytes invades the graft through their walls. Inflammation in the graft bed flares up at once, though it may before transplantation have subsided into the chronic state. The epithelium exhibits no discernible activity: there is no thickening of the graft roof, no upward migration of epithelium from the follicles, and no outgrowth from the graft. The graft is 'fixed' in the same sort of way as the standard 2nd-set graft, but rather more promptly.

Breakdown of the epithelium (Pl. 6, figs. 60, 61) is either complete by the 4th day, or if merely in progress, very far advanced. The form of breakdown is similar to that of standard 2nd-set grafts, and requires no special mention.

From the point of view of computing the survival time of local 2nd-set grafts, it is most unfortunate that the period of 4 days appears to lie on its very boundary. In two grafts of the ten, partial survival was unquestionable, and in three, non-survival equally not in doubt (Pl. 6, fig. 60). In the remaining five, traces of mature follicular epithelium and of the more central areas of the graft roof still, to histological appearance, just survived (Pl. 6, fig. 61); for the nuclei of the cells were intact, and the cytoplasm surrounding them not acidophilic. The figure of '99% breakdown' has a purely formal significance—it indicates the order of the degree of survival of these five grafts.

In chart IV, Text-fig. 4, the dubious 99%-breakdown grafts are ranked as survivors, in accordance with the rule that has been consistently adopted throughout. But had a 5-day period been allowed to pass before biopsy, the likelihood is that all the grafts would have been found to have broken down. In this case, therefore, the actual figures for survival are not particularly indicative. Extrapolation to zero survivors at 8 days, as indicated by the chart, can, however, be made without possibility of error, since in every graft breakdown was far advanced.

The autohomograft test has been applied to eight pairs of local 2nd-set graft bearers, with results fully consistent with the histological. One graft showed partial survival, four trace survival of an exemplary type, and three showed total necrosis of long standing. (Here, as before, the *positive* result is not subject to error.)

The accelerated retrogression of local 2nd-set grafts in relation to grafts of the *1st set* requires no special demonstration. In every respect in which a comparison can be drawn, local 2nd-set grafts are slightly more degenerate at the 4th day than standard 2nd-set grafts; in no one taken singly, however, is the difference between them statistically decisive. Evidently the local immune state adds something, but not very much, to the systemic. The essential inference to be drawn from these experiments is that breakdown is far advanced, *but not necessarily complete*, 4 days after the transplantation of a susceptible graft to an environment in which immune processes should be fully operative. Three additional experiments in which the 2nd-set grafts were transplanted not 16 days after the 1st set, but as soon as possible after the 1st set had to outward appearance broken down, do nothing to qualify this judgement.

Since native epithelium grows rapidly inwards across the raw area during the 16-day immunizing period, it may encroach upon and even invade the local 2nd-set graft during its 4 days of residence. Pl. 6, fig. 62, again (cf. Pl. 4, figs. 40, 41) illustrates its power to establish hair-follicle primordia in a foreign dermis within a short time of arrival. The foreign epithelium of the graft roof has in this case been wholly undermined. Evidence for the high specificity of the homograft reaction which this phenomenon provides is considered in greater detail in the final section which follows.

THE SPECIFICITY OF THE IMMUNE REACTION

The immune state eventually generated in response to the transplantation of skin homografts is very sharply specific to foreign tissue as such. As foreign epithelium breaks down, epithelium of native origin often replaces it *pari passu*; and even native epithelium which is in the process of undermining a homograft during or immediately after its breakdown may show no significant pathological changes (Pl. 4, figs. 38–40; Pl. 6, fig. 62). When sheets of native and foreign spread epithelium coalesce before the homograft reaction reaches its peak intensity,

the two components can be distinguished almost to a cellular boundary (Pl. 3, figs. 27, 28). These are indirect demonstrations of the specificity of the reaction.

In each of three independent trials, *autografts* replaced 2nd-set homografts in the standard operation by means of which the systemic immune state is demonstrated. Their behaviour differed in no wise from that of normal autografts. Moreover, each of the ten animals used for experiments on *local* 2nd-set homografts—2nd-set grafts transplanted to the very positions formerly occupied by grafts of the 1st set—received an autograft 'control' in addition to the four small homografts from the original donor. In the extent of their outgrowth and of the thickening and stratification of the graft roof, these autografts were superior at the 4th day to normal ones (Pl. 6, fig. 59); very possibly because they were transplanted to already granulating and highly vascular beds. The point at issue, however, is that they were no worse: the local immune state is likewise highly specific.

Various types of 'mosaic' graft provide an even more convincing demonstration. An autograft surrounded by a ring of homografts in such a way that it is, at the peak of their development, completely surrounded by a tide of foreign epithelium, shows no specific abnormality when the homografts break down. On the contrary, autograft epithelium at once begins to undermine the epithelium of the homografts and proceeds thereafter to undermine the homografts themselves. Moreover (Pl. 6, fig. 63), a small autograft transplanted into a shallow well in the centre of a large homograft (Text-fig. 1*e*) in such a way that it receives its entire vascular supply via a belt of foreign tissue will, provided that the belt is not too thick, override all obstacles and invade the necrotic tissue that surrounds it when breakdown of the homograft element is complete. If, however, the belt of foreign tissue underlying the autograft is as much as 1 mm. thick, the autograft shares the fate of the homograft; which is hardly to be wondered at, since the vessels which supply it stagnate. Evidently the obliteration of blood vessels may contribute to, or accelerate, the 'violent' type of reaction of breakdown which occurs in the graft *roof* of high-dosage homografts. The histological picture of violent breakdown—nuclear fragmentation (Pl. 3, figs. 33–36)—may owe its comparative uniformity to this fact.

The specificity of the reaction towards donor skin. In each of three independent tests, animals operated on according to the technique used for demonstrating the systemic immune state received an immunizing dose of homografts from one rabbit and a second dose *from another*. Biopsy specimens were removed at the 4th day and at the 8th in the usual manner.

The specimens from one of the three animals were in all respects similar to normal, i.e. constant-donor, 2nd-set homografts: breakdown of the vascular system was precocious, hyperplastic changes failed to occur, and no primary population of native leucocytes was established within them. Those from the second anima showed partial breakdown at the 8th day, to the accompaniment of hyperplastic changes never once seen in normal 2nd-set grafts (Pl. 5, fig. 55). Specimens from the third animal exhibited breakdown of the violent type after the hyperplastic changes and the massive invasion of native leucocytes that are characteristic of 1*st-set* high-dosage homografts.

Evidently the immune reaction does not *necessarily* extend with equal vigour to skin from a graft donor other than that which generated the immune state. (These experiments have not been carried beyond the point necessary to establish a qualitative principle: they are of a type for which the use of genetically heterogeneous material is particularly ill-suited.)

DISCUSSION

The evidence presented above is consistent with a hypothesis first put upon a more than speculative foundation by Gibson (1942) and Gibson & Medawar (1943), namely, that the mechanism by means of which foreign skin is eliminated belongs in broad outline to the category of *actively acquired immune reactions*. The accelerated retrogression of 2nd-set homografts, here demonstrated on a scale which makes due allowance for the degree to which genetic variance governs the intensity of the homograft reaction, argues the existence of a systemic immune state. If the immune state were confined to the graft and its immediate neighbourhood, the homograft reaction and the inflammatory processes associated with it should not vary, as they have been shown to do, with graft dosage. The dosage phenomenon also points to a systemic reaction.

The degree to which *innate* immunity may intervene is difficult to assess. The fact that even high-dosage homografts enter into the period of generalized hyperplasia which is characteristic of autograft development, and that lower-dosage grafts proceed beyond it into the period of graft differentiation—in short, the fact that a serologically latent period exists—shows that innate immunity cannot play a decisive part in the mechanism of breakdown. If, moreover, ready-made antibodies were present in the tissue or body fluids, a second set of homografts would not be expected to behave much differently from a first, nor lower-dosage grafts to survive longer than the higher. It is noteworthy in this connexion that natural iso-haemagglutinins are not found in the rabbit (see the review by Wiener, 1939, pp. 231–3); like the *Rhesus* antibody in human beings (Landsteiner & Wiener, 1940; Levine, 1941), they may be called forth by active immunization. Innate immunity is not a *sufficient* explanation of tissue-grafting incompatibility (Wiener, 1939; Brown, 1941; Medawar, 1943).

Much attention has been paid to the inflammatory process which accompanies the breakdown of foreign skin epithelium in rabbits. In rabbits, where the

reaction eventually proceeds to necrosis, it is the predominant histological feature of grafts; in the human being, though it exists, it is altogether of a lower degree of severity (Gibson & Medawar, 1943). The rabbit is known to be peculiarly susceptible to *anaphylactic inflammation* (the Arthus reaction), a phenomenon usually attributed to the meeting of antigen with antibody within the tissues. The inflammation which accompanies the homograft reaction in rabbits is very probably of the anaphylactic type (Medawar, 1943). Yet, though all the ingredients of the inflammatory process are present—vascular and lymphatic proliferation, oedema, and the mobilization and deployment of mesenchyme cells of every type—the reaction is nevertheless atypical; for the lymphocyte takes the place of the polymorph in the 'classic picture'. At no stage in the homograft reaction has the polymorph been found to play an appreciable part (cf. Harris, 1941). It is as yet impossible to judge of the significance of this difference. If the lymphocyte is here directly concerned in the manufacture of antibodies (cf. Ehrich & Harris, 1942), then a particularly high proportion must be manufactured in the immediate neighbourhood of the grafts themselves. The local immune state (p. 192) is certainly more powerful than the systemic; but not as much more powerful as this hypothesis would lead one to predict. One must not, however, forget that a systemic lymphocyte reaction in response to tissue homografts is superimposed upon the local (Blumenthal, 1939, 1941).

In many other ways the immune reaction is 'atypical', i.e. does not conform to the clear-cut pattern defined by, for example, the bacterial antigens. The latent period that passes before the homograft reaction reaches its peak intensity may in high-dosage homografts be as short as 8 days. There are at least three possible explanations for this. One is that innate immune bodies do indeed play an appreciable part in the reaction; a second, that the immune reaction is partly short-circuited by a very rapid manufacture of antibodies in the neighbourhood of the grafts.

A third explanation is that the short latent period may later come to be regarded as the 'typical' one. The classic concepts of immunology have hitherto been based upon such gross serological disparities as those between the bacterial antigens and the organisms from which they elicit an immune reaction. It may in future prove that the more intimate, gene-determined reactions of tissue homografting have an equal claim to be considered 'normal' or basic. There should certainly be no attempt to *force* the homograft reaction into the stereotyped serological mould.

A striking fact is that even when 2nd-set grafts are transplanted to the very positions occupied by 1st-set grafts when their breakdown is complete, a period of 4 days may elapse before they, in their turn, are wholly destroyed. Its interpretation must await the discovery of what exactly, in foreign but homologous skin, *is* the antigen. If the antigen is intranuclear, then the antibody may have no opportunity to exert its effect until the nuclear membrane is dissolved in the late prophase of mitosis; and mitosis—the part played by which was carefully distinguished, in the foregoing account, from mere amoeboid movements and changes of shape in the epithelial cells—contributes little or nothing to the activities of graft epithelium until the 4th day has been reached. At all events, 2nd-set homografts *never* enter the characteristic period of generalized hyperplasia: they are finally destroyed just about when it is due to begin.

The whereabouts of the antibody is equally conjectural. Its free circulation in the blood stream may be doubted. Although the experiments are still in their early stages, I confirm Harris's observation (1943) that cultures of donor tissue (in this case, donor skin) will spread freely and undergo mitosis in presumptively immune serum (Pl. 6, fig. 64). Donor skin incubated for 24 hr. with constant stirring in 'immune serum' will, moreover, resume normal growth and activity when transplanted thereafter as an autograft. Gibson (in unpublished experiments) has likewise failed to demonstrate complement fixation in the reaction between immune serum (from human beings) and cell suspensions of donor-skin epithelium.

The possibility that antibodies may be detected in the serum by biological tests when an intravenous route of immunization is used, has yet to be examined. Donor leucocyte concentrates may, for example, be used as immunizing agents. Technical difficulties may interfere with the use of epidermal cell suspensions for this purpose.

One further experimental result remains to be discussed. Even when the other variables are controlled as carefully as is possible, the survival times of homograft epithelium differ strikingly from one pair of animals (donor and recipient) to another. It is a truism to say that this variation is genetic in origin, and that in accordance with Mendelian principles it has a combinatorial basis. The number of combinatorial types of the hypothetical antigens available to the rabbit is such that in each of a total number of 98 trials the donor had an effective credit balance, and the foreign skin was destroyed. In arbitrary symbols, we may suppose that the antigenic make-up of donor and recipient are related to each other in this sort of overlapping way:

Donor:	*ABCDEFGH*
Recipient:	*EFGHIJ*

The 'credit balance' is here *ABCD*; for the reciprocal transplantation, donor and recipient being interchanged, it would have been *IJ*. A second donor, such as may be used to provide a 2nd set of grafts in place of the original, may share any or no one of the antigens *ABCD* with it. The vigour of the *immune* reaction cannot therefore be greater against grafts from a donor source other than the original; it may be equal to it, or it may be less. The experimental

results upon this score (p. 194) are fully consistent with this straightforward interpretation.

SUMMARY

1. An investigation into the behaviour and fate of skin autografts and skin homografts in rabbits is described.

2. A technique of operation and of post-operative dressing has been employed, by means of which pinch grafts transplanted from the outer aspect of the thigh to the lateral wall of the thorax may be made to undergo full primary healing in 100% of cases.

3. The evolution of autografts falls into a period of primary healing and vascularization; a period of generalized hyperplasia, in which all the cellular elements of the grafts participate; and a period of partially retrograde differentiation during which the grafts return towards the condition of normal skin.

4. Homografts undergo normal primary healing in a latent period during which they provoke no specific reaction from their recipients. At some time thereafter, they are invariably destroyed.

5. The evolution of homografts in *high* graft dosage (0·36–0·44 g. skin per rabbit) embraces the period of generalized hyperplasia of autografts, but does not usually extend into the period of differentiation. New hairs do not mature and pierce the graft roof.

6. The phenomena of acute inflammation are superimposed upon the otherwise autograft-like behaviour of high-dosage homografts in the period of hyperplasia.

7. The inflammatory process includes vascular and lymphatic proliferation; a massive invasion of the grafts by lymphocytes and monocytes of native origin through the walls of the vessels within them; oedema of such severity that the graft bed may be distended by tracts of free fibrinous matter; and a general mobilization of mesenchyme cells.

8. Inflammation reaches its peak, and passes into necrosis, with the stagnation and obliteration of the vascular system of the graft and the death of every cellular element within it. Homografts are then invaded anew by capillary vessels from the graft bed; lymphocytes and monocytes passing through their walls establish a secondary population of native cells within them.

9. The intensity of the inflammation and its rate of development vary inversely with the time of survival of foreign skin epithelium.

10. Disengagement and breakdown of the foreign epithelium begins in that which has spread from the graft, and extends thereafter to epithelium of the graft centre, to the accompaniment of a variety of non-specific pathological changes in the cells. The entire process of breakdown is complete within a compass of 4 days from start to finish.

11. The *median survival time* of homografts is defined as that at which the foreign epithelium borne by 50% of the experimental animals has just broken down.

12. The median survival time of homografts in high graft dosage is $10{\cdot}4 \pm 1{\cdot}1$ days.

13. The survival time of homograft epithelium varies inversely with the dosage of foreign skin which an experimental animal receives.

14. The median survival time of homografts in initial dosage of 0·006–0·06 g. foreign skin per rabbit is $15{\cdot}6 \pm 0{\cdot}9$ days. The evolution of these *lower-dosage* grafts extends into the period of differentiation characteristic of autograft evolution. Newly formed hairs mature and pierce the graft roof.

15. The median survival time of homografts transplanted to a freshly prepared raw area on the opposite thoracic wall 16 days after a rabbit has received a 1st set of homografts in high dosage from the same donor is $6{\cdot}0 \pm 0{\cdot}6$ days. The evolution of such '2nd-set' homografts does not extend into the period of generalized hyperplasia. Inflammation is precocious in time of onset.

16. The accelerated retrogression of 2nd-set homografts has been demonstrated by a wholly independent test: one in which the survival of homograft epithelium is confirmed or refuted by transplanting a graft back to its donor after a varying period of residence on its original recipient as a 1st-set or 2nd-set homograft.

17. The accelerated retrogression of 2nd-set homografts does not necessarily extend with equal vigour to 2nd-set grafts derived from a donor source other than that which provided the 1st set.

18. The breakdown of 2nd-set grafts which (cf. 15) are transplanted to the positions formerly occupied by grafts of the 1st set is far advanced, but not necessarily complete, within 4 days of transplantation. The *local immune state* adds little to the systemic.

19. Analysis has been made of the sampling errors of a variety of numerical estimates of survival time, intensity of inflammation, extent of hyperplasia, and degree of differentiation.

The differences between high-dosage and lower-dosage grafts on the one hand, and between 1st-set and 2nd-set grafts on the other, are in each such respect greater than those for which the chances of random sampling provide a sufficient explanation.

20. The reaction elicited by homografts is sharply and precisely specific to foreign as opposed to native skin.

21. The mechanism by which foreign skin is eliminated belongs to the general category of actively acquired immune reactions.

22. The inflammatory process which accompanies it has in all likelihood the character of a local anaphylaxis.

ACKNOWLEDGEMENT

This work has been done in association with the Department of Plastic Surgery, Oxford University, from which I have received an expenses grant. I am particularly grateful to Prof. T. P. Kilner and Dr Leonard Colebrook for their advice and for their criticisms of this paper.

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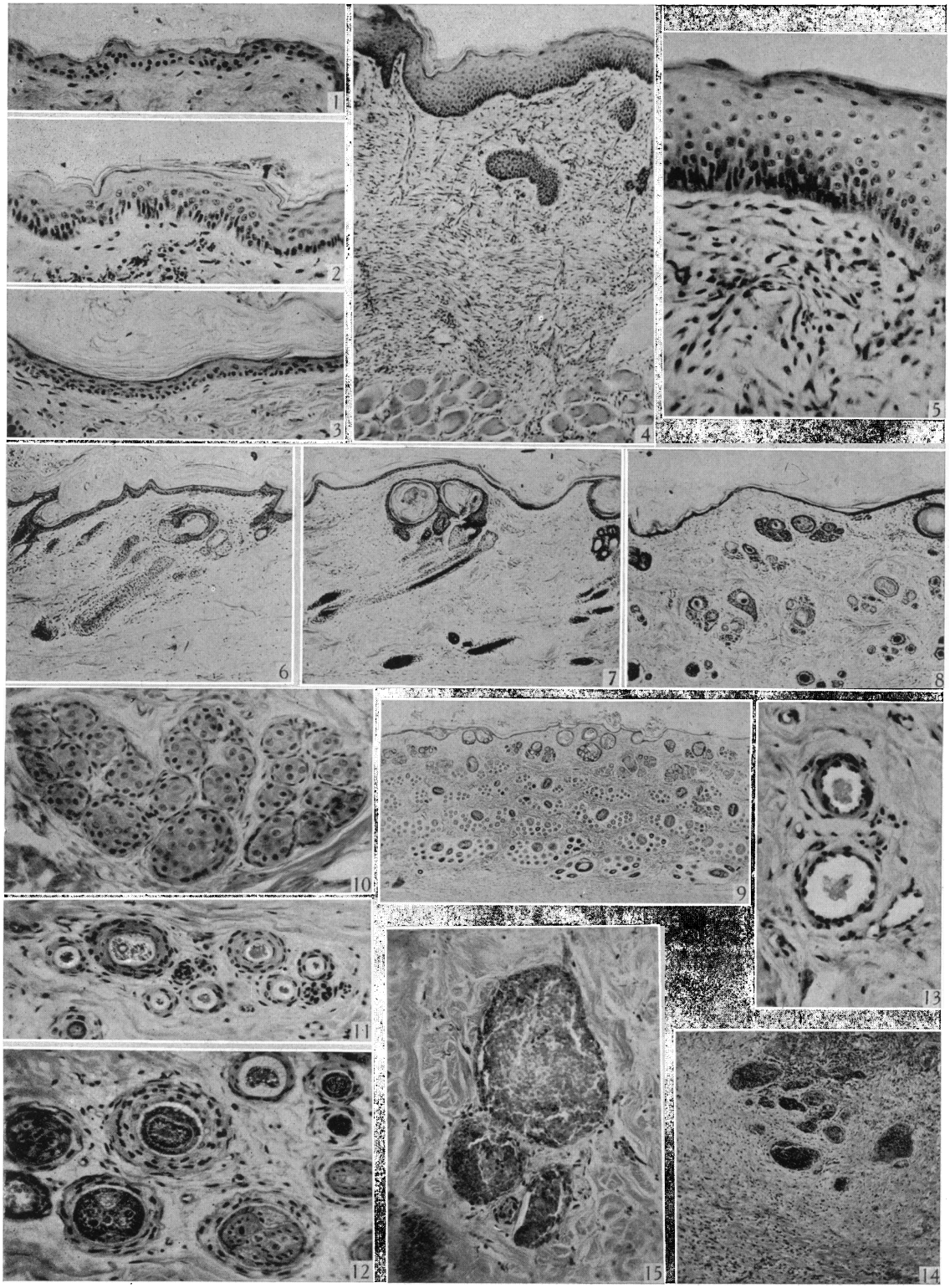
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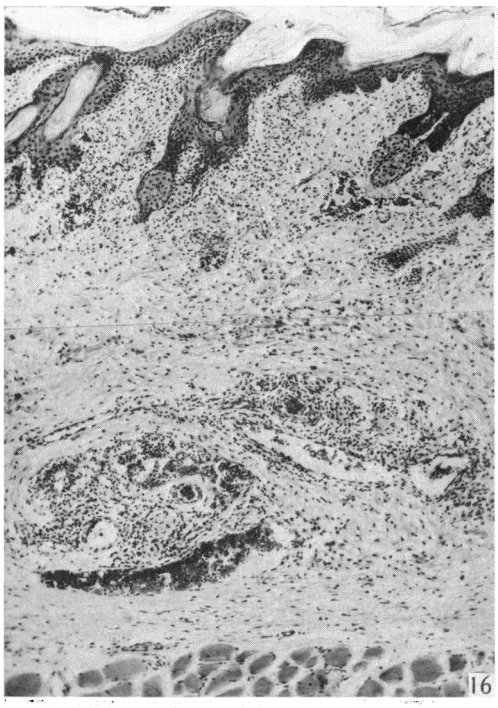

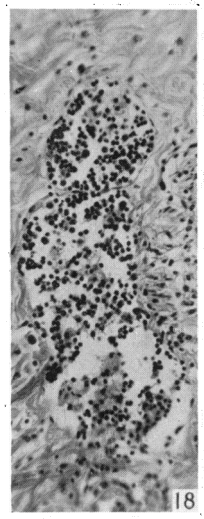

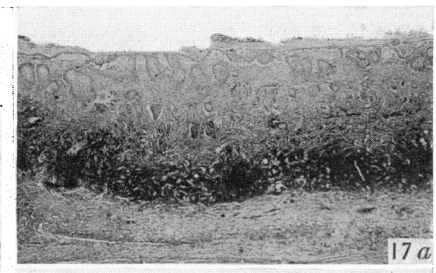

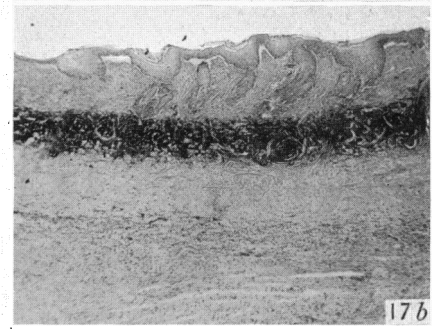

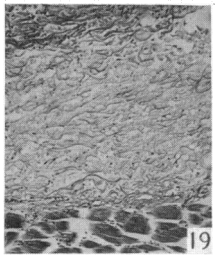

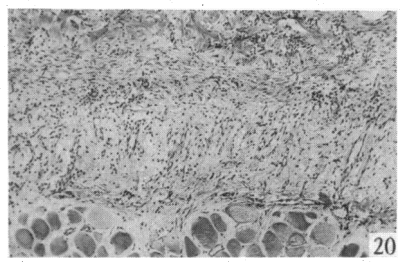

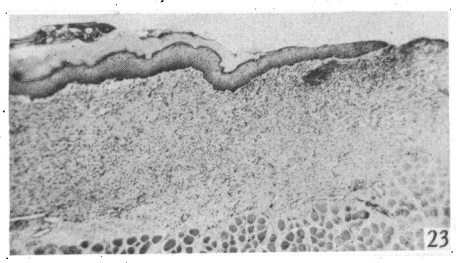

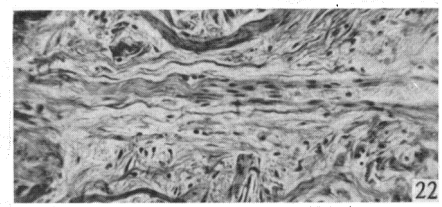

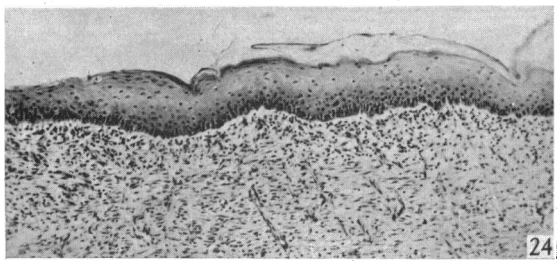

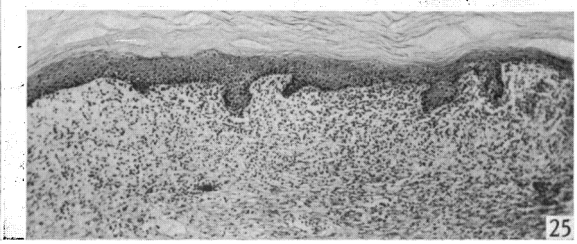

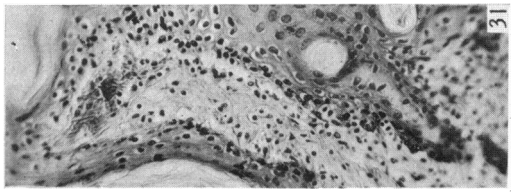
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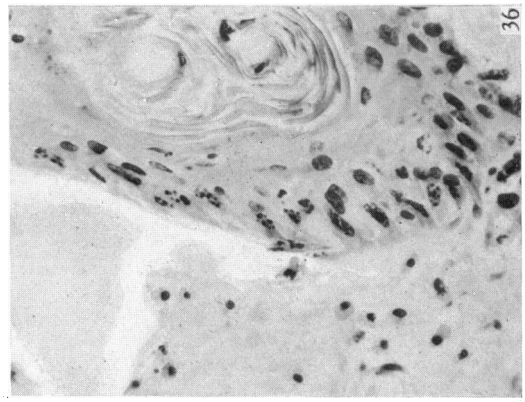
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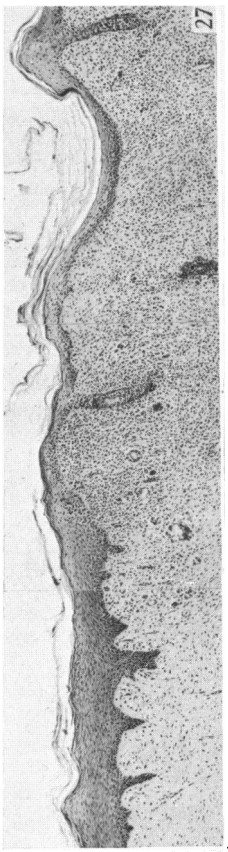
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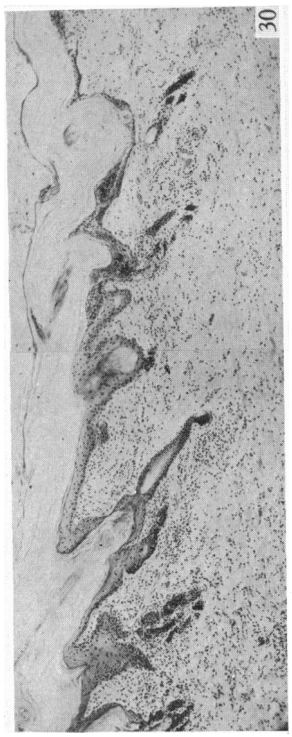
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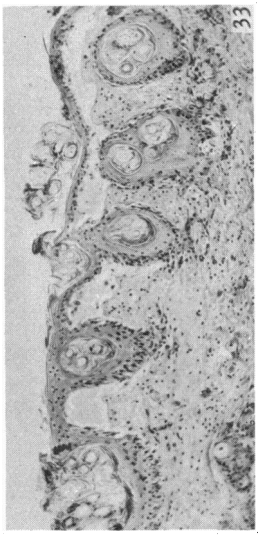
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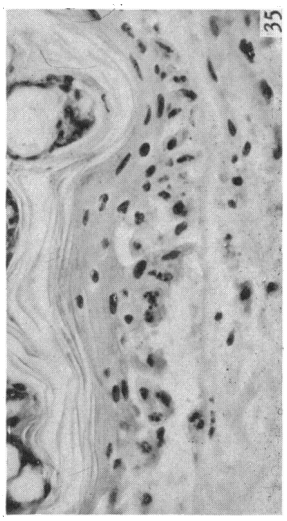
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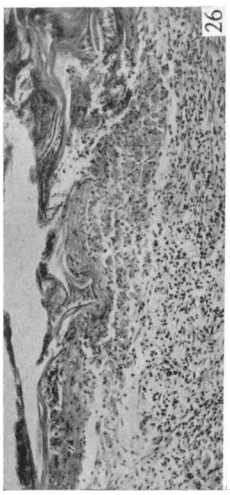
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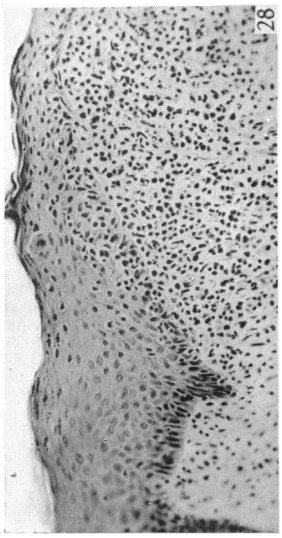
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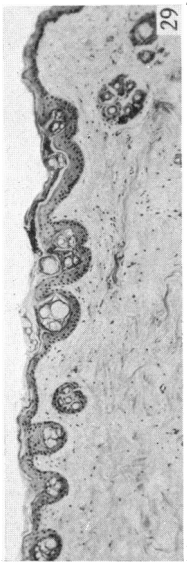
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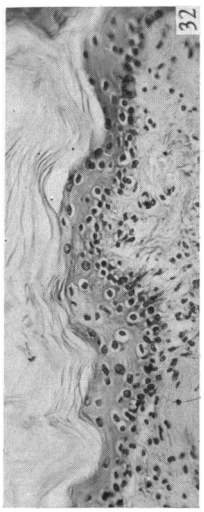
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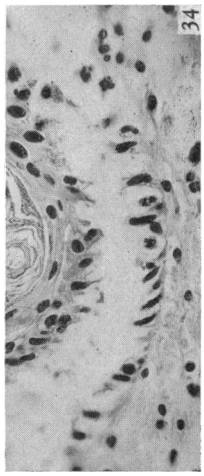
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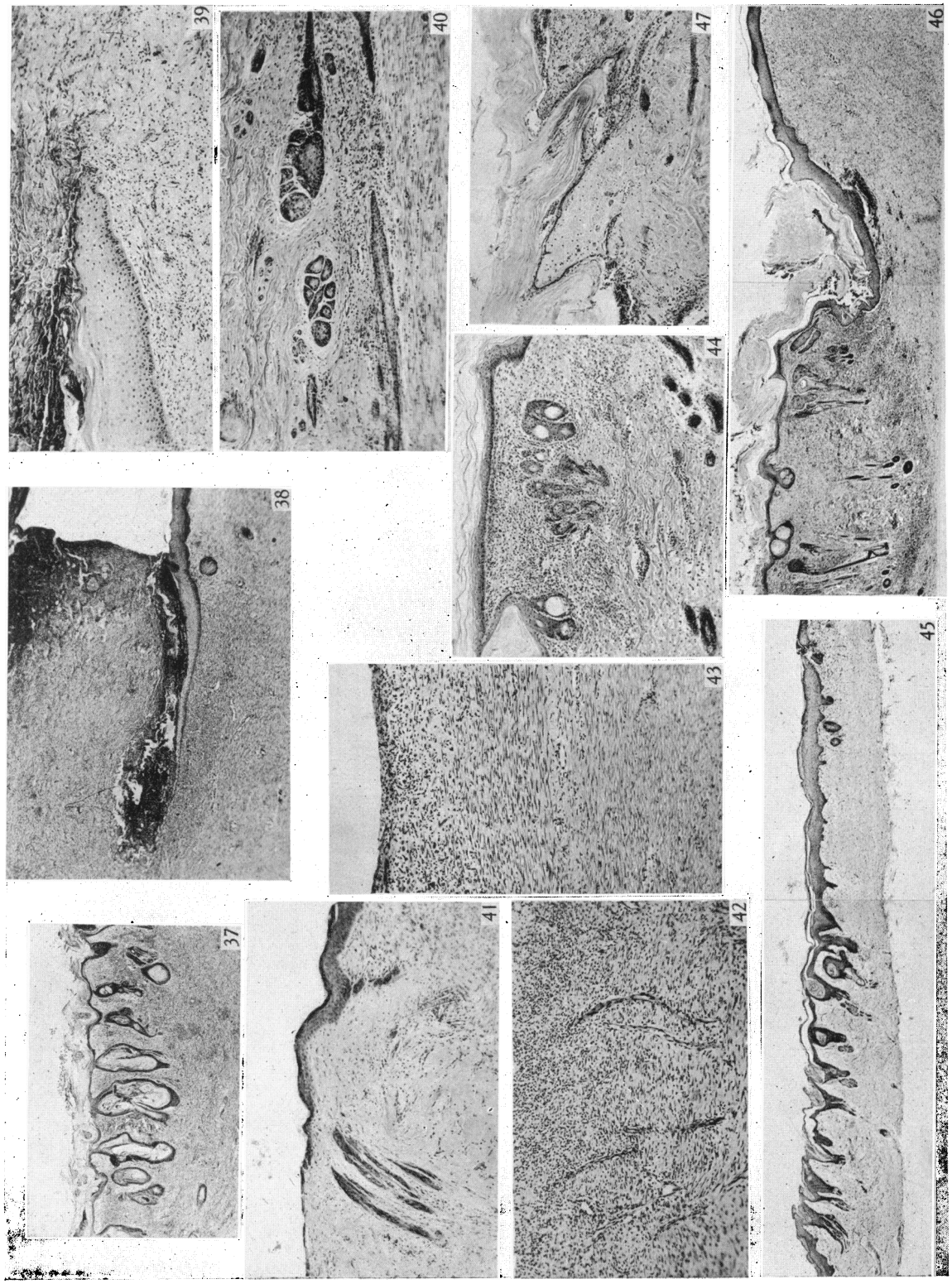
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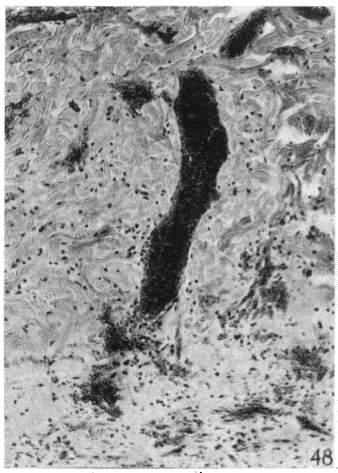
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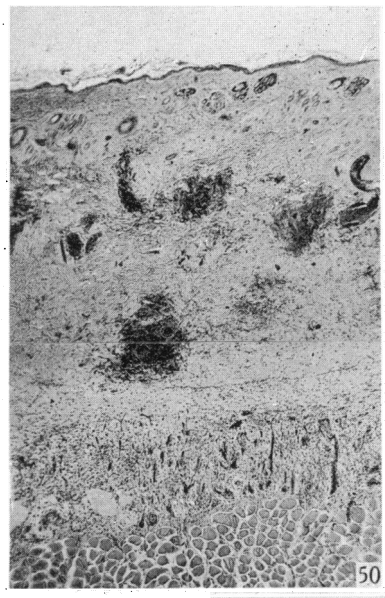
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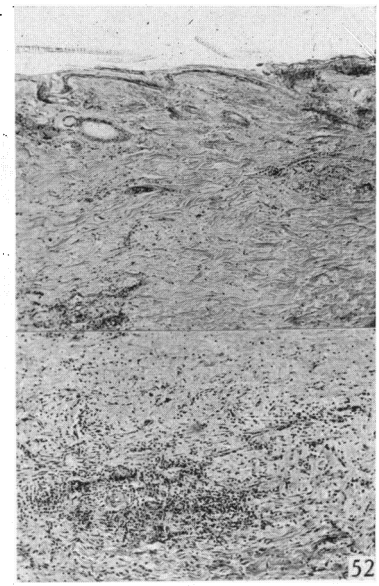
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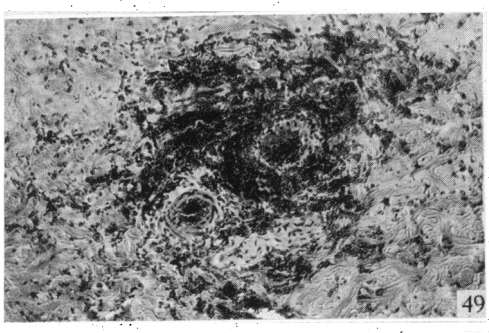
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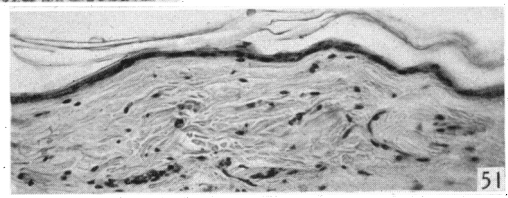
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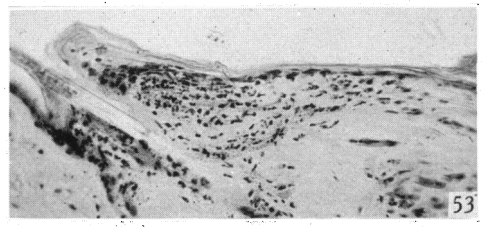
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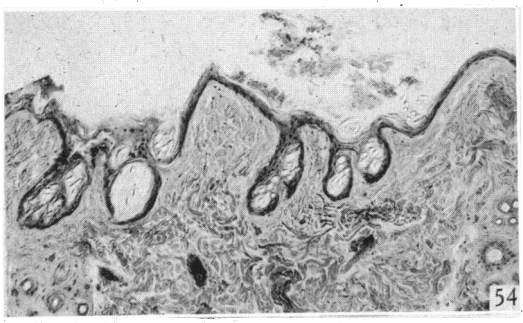
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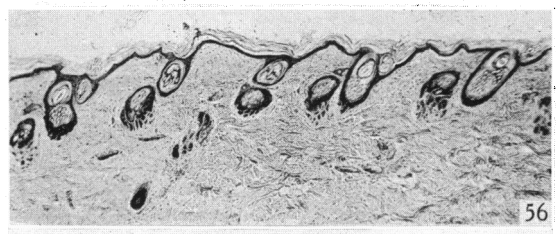
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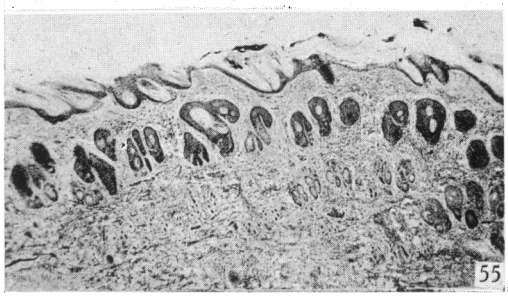
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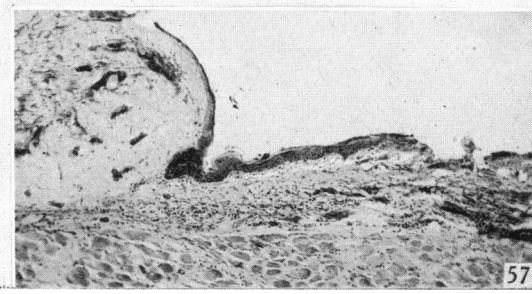
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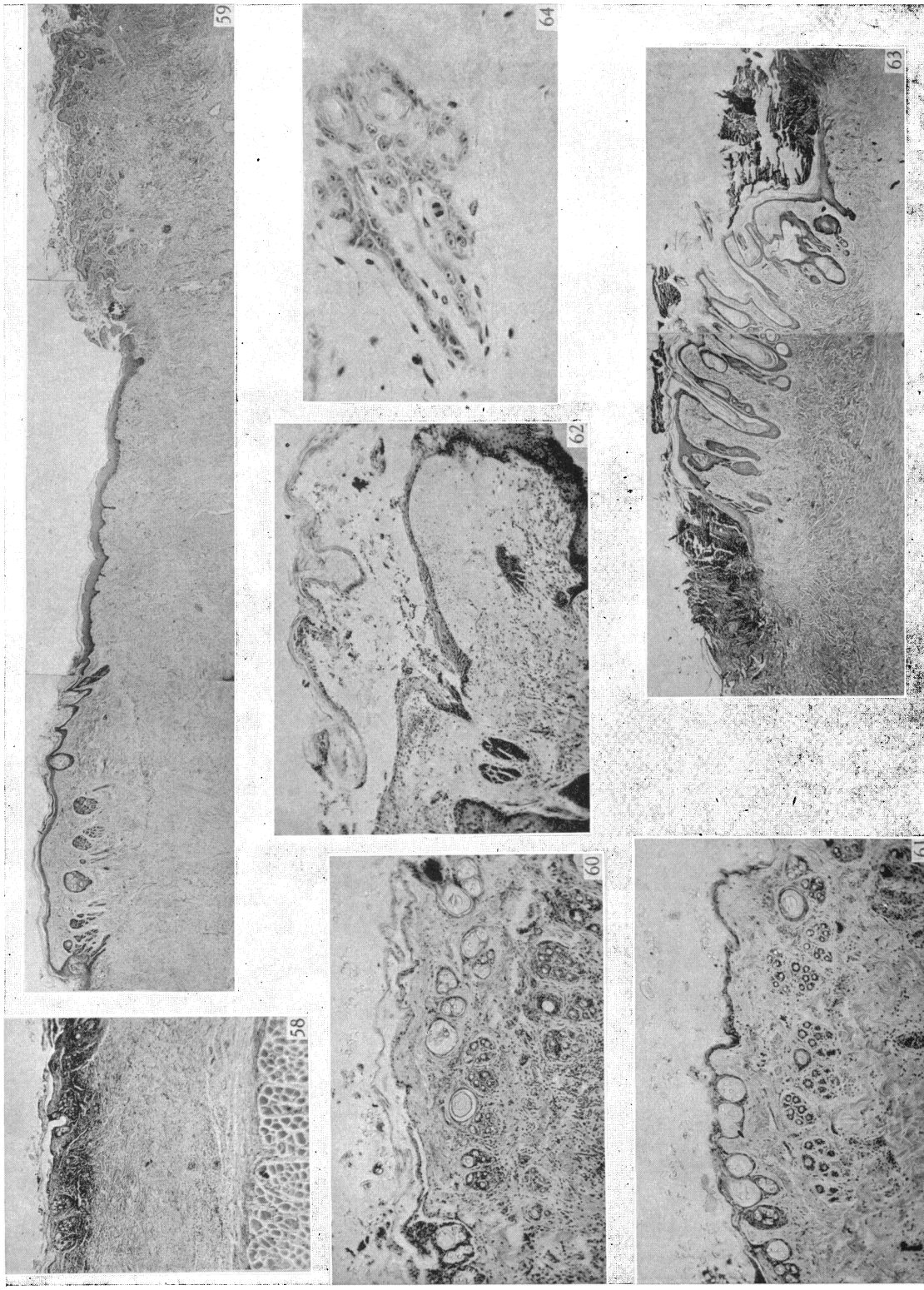
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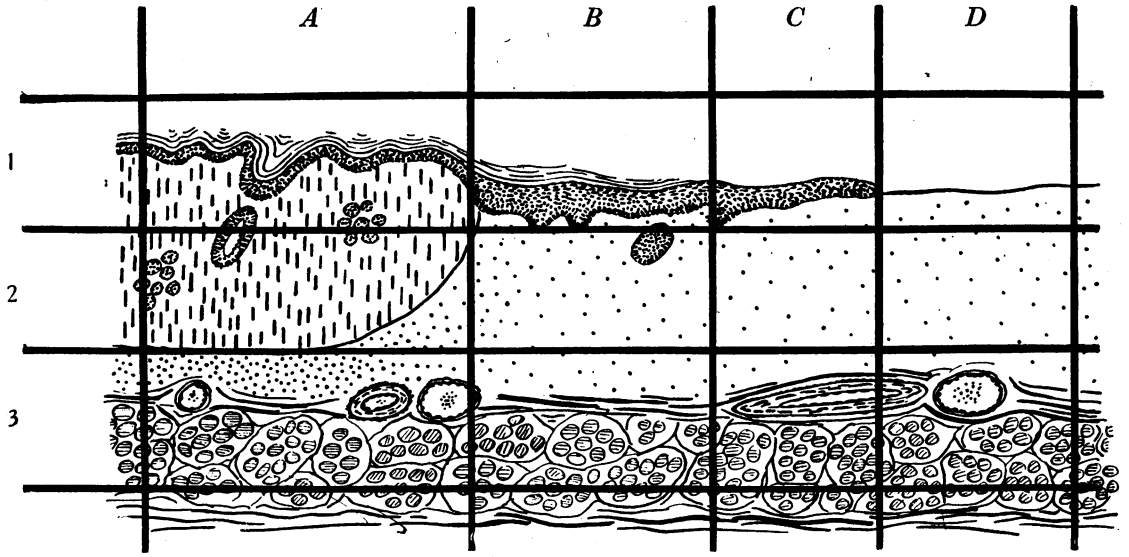
EXPLANATION OF PLATES



Explanation of key-figure. A diagrammatic median vertical section of (half a) pinch graft lying *in situ* upon the graft bed, with epithelium spreading from it over the outlying tissue. The panniculus carnosus bounds the specimen below. The topographical relationships of the sections chosen for illustration can be ascertained by the use of the letters and numbers which identify the rectangles of the superimposed grid. Thus the graft roof is identified by the reference 1 A, the graft bed by 3 A, the inner ring of new outgrowth by 1 B and so on.

All sections have been stained by Ehrlich's haematoxylin, aqueous orange G, and alcoholic eosin, save where other stains are specifically mentioned.

PLATE 1

Figs. 1–3. The roofing epithelium of 4-, 8-, and 12-day autografts respectively, illustrated by successive graft samples from a single animal. Note the swelling of the cells in the 4-day specimen (cf. fig. 29); their multiplication and vertical differentiation in the 8-day, and the retrograde simplification (e.g. to a single granular layer) of the 12-day specimen. Cf. Text-fig. 2 and figs. 6–9. (1 A.) ×160.

Fig. 4. The *inner ring* of epithelial outgrowth from an 8-day autograft, showing the vascular and fibroblastic reaction in the tissue surrounding the graft. (123 B.) ×60.

Fig. 5. The *outer ring* of epithelium spreading from an 8-day autograft: backward cuticularization, and some cellular abnormalities. (1 C.) ×190.

Figs. 6–8. General illustrations of the autograft roof in 12-, 16-, and 24-day specimens successively removed from the same graft bearer. Note the progressive simplification of the epithelium (cf. figs. 1–3), and in particular the maturing follicles and the hypertrophied and now 'exploded' glandular acini of the 12-day specimen, and the matured hairs and the deep indentations (here cut obliquely) of the graft roof in the 16- and 24-day specimens. The thinning of the roof epithelium is accompanied throughout by rich exfoliation of cuticle. (12 A.) ×45.

Fig. 9. Cf. fig. 8. A 24-day autograft showing how the pattern of newly formed follicles conforms with that of the old, long since undermined and thrown off by amoeboid and proliferative activities of the epithelium. (12 A.) ×17.

Fig. 10. The primordia of new hairs and new depots of glandular epithelium in the dermis of an 8-day autograft. Note the delicate collagenous framework of new formation. (2 A.) ×190.

Figs. 11, 12. Matured or maturing hairs in the dermis of a 16-day autograft. Each follicle has its own collagenous sheath, and each is united to its neighbours by delicate new collagen fibres in open packing. (2 A.) ×190.

Fig. 13. Differentiated vessels of the primary circulation in the lower reaches of the dermis of a 4-day homograft. The endothelial swelling is probably without specific significance. (2 A.) ×190.

Fig. 14. Stagnation and endothelial breakdown in the

primary blood vessels of an 8-day homograft which elicits a 'violent' reaction. Note the abundance of the vessels, which include arteries. (2A.) ×55.

Fig. 15. Stagnation of 'wound vessels' in an 8-day homograft. Their endothelial lining has disappeared, and leucocytes arrested within the lumen of the vessel are pyknotic or otherwise degenerate. (2A.) ×160.

PLATE 2

Fig. 16. An 8-day high-dosage homograft exhibiting the preliminary phases of a *mild* reaction of breakdown, the appearance at 12 days being illustrated by fig. 30, q.v. The graft roof has thickened and stratified, glandular epithelium has hypertrophied, and the primordia of new hair follicles are budding off from epithelium at the bases of the old. Note the wedge-shaped indentations of the roof. Superimposed upon this autograft-like activity, note the acute vascular proliferation and inflammation in the lower reaches of the dermis. A 'primary' population of native leucocytes is beginning to invade the graft, and the grossly enlarged lymphatics just below the graft roof already contain many degenerate cells. As yet, the fascial planes of the graft bed are undistended with oedema fluid. (123A.) ×60.

Figs. 17 *a*, *b*. The 'black band' of gross localized cellular infiltration in the dermis of homografts which elicit a violent reaction: (*a*) on its first appearance at the 8th day and (*b*) its appearance thereafter at the 16th. In (*a*) the entire graft roof epithelium has separated simultaneously (see fig. 33), and in (*b*) it is still present, 'fixed'. (12A.) 17*a* ×17, 17*b* ×23.

Fig. 18. The enlarged lymphatic vessels of homografts, now packed with pyknotic lymphocytes and containing mononuclear phagocytes, at a stage somewhat later than illustrated by fig. 16. The endothelium has disappeared, and the drainage of the vessel is presumably at a standstill. (2A.) ×160.

Fig. 19. The fascial planes of the bed of a homograft undergoing violent breakdown, now grossly distended with fibrinous matter: the 'fibrin band'. (3A.) ×45.

Fig. 20. The 'reorganization' of the fibrin band (fig. 19) by the upgrowth of capillary vessels through it. These vessels subsequently invade the underside of the dermis (fig. 21) and create its secondary vascular supply. (3A.) ×45.

Fig. 21. A late (20-day) stage in the evolution of the homograft. The superficial layers of the dermis above the level of the black band have been scraped away by repeated changes of dressings, and the deeper layers have been reinvaded by secondary vessels from the graft bed (fig. 20). Note the chronic inflammatory condition. Even the tissue spaces of the primary vessels are now no longer recognizable. (12A.) ×60.

Fig. 22. The blood vessels which secondarily invade the homograft (fig. 21) have new collagen laid down around them—the 'secondary native collagen'—in fibres which are at first fine and wavy, as they are here. (Celestine blue and picro-fuchsin.) (2A.) ×160.

Fig. 23. Inner and outer rings of the epithelium which has spread outwards from an 8-day homograft which elicits a mild reaction. (The graft centre is illustrated by fig. 16.) No specific abnormality in the epithelium is as yet apparent. (123BCD.) ×23.

Figs. 24, 25 and Pl. 3, fig. 26. Successive stages in the breakdown of homograft spread epithelium: lymphocytic aggregation (fig. 24) accompanied by oedema and vascular congestion becomes more intense (fig. 25) until (cf. fig. 28) the basal layer cells separate from each other and from the substratum (fig. 26). (1B or 1BC.) Fig. 24, ×70; fig. 25, ×55; fig. 26, ×75.

PLATE 3

Fig. 27. A coalesced sheet of autograft and homograft epithelium, at a critical stage in the reaction provoked by the latter. Substantially normal autograft epithelium, lying to the left, grades sharply (fig. 28) into the thinner, acidophilic, homograft epithelium, which is now beginning to macerate. Lymphocytic aggregation is sharply localized beneath the foreign tissue. ×45.

Fig. 28. The margin of coalescence illustrated by fig. 27, here in higher magnification. (Regaud's haematoxylin.) ×145.

Fig. 29. Cf. fig. 1. The roof epithelium of a 4-day high-dosage homograft. Note the thickening, largely brought about by swelling of the cells; and the upward migration of follicular epithelium which brings the more superficial follicles towards the surface. (1A.) ×55.

Fig. 30. A 'mild' reaction of breakdown spreading in the roof of a 12-day high-dosage homograft. Some retrograde thinning-out of the roof epithelium has already occurred (cf. figs. 3, 6). Note the apparently greater resistance of immature follicular epithelium. There is no intense local aggregation of mesenchyme cells. (1A.) ×45. (For the appearance at 8 days of the grafts borne by this animal, see fig. 16.)

Figs. 31, 32. Details of the mild reaction of breakdown. The basal layer cells are beginning to separate from the tissue underlying them; pyknosis is the most prominent nuclear abnormality. (1A.) ×160.

Fig. 33. Contrast with fig. 16 and with figs. 31, 32. A *violent* reaction of breakdown in an 8-day high-dosage homograft, taking place simultaneously in the whole graft roof. (See fig. 17*a*, of part of which this is an enlarged reproduction.) Note the pools of oedema fluid in the crests of the dermal papillae. (1A.) ×75.

Figs. 34–36. Cytological details of the violent breakdown reaction: from the specimen illustrated by fig. 33. Coarse fragmentation of the nuclei of the basal layer cells. (1A.) ×380.

PLATE 4

Fig. 37. A high-dosage homograft surviving to the 16th day. Note the retrograde thinning out of the roof epithelium (as in fig. 7), and the deep pockets which mark where the original hairs have been thrown off. No new hair follicles have matured. The dermal papillae are oedematous, but the inflammation, now reaching peak intensity is otherwise mild. (12A.) ×18.

Fig. 38. The undermining of a homograft by native epithelium at the junction between dermis and graft bed. (12AB.) ×23.

Fig. 39. An advancing wedge of native epithelium undermining a homograft somewhat above the level of the dermis—graft-bed junction. (23A.) ×70.

Fig. 40. A slightly later development of the condition illustrated by fig. 39: the native epithelium has here infiltrated the foreign dermis from below upwards and established hair-follicle primordia within it. The primordia lie in a collagenous framework of new formation. (23 A.) ×60.

Fig. 41. The *overgrowth* of the homograft dermis by native epithelium, here in its very early stages. Note the formation of hair-follicle primordia (cf. fig. 40). (12 AB.) ×50.

Fig. 42. The persistent fibroblastic reaction and the general inflammatory state of the outlying tissue in a homograft operation field. (2 BC.) ×55.

Fig. 43. The characteristic zonation of the outlying tissue in the homograft operation field: densely collagenized tissue, with proportionately reduced fibroblastic activity, below, grading upwards to less collagenized zones where fibroblasts are still active. Delicate granulation tissue on the surface. (123 D.) ×55.

Fig. 44. A 12-day medium-dosage homograft at the critical period before breakdown. Retrograde thinning-out of the graft roof with rich exfoliation of cuticle (exactly as in figs. 3, 6); the maturation of hairs; and the 'explosion' of the larger glandular depots. The lymphatics have now lost their endothelial linings. (12 A.) ×55.

Fig. 45. An 8-day low-dosage homograft, indistinguishable from an autograft. Note the thickening of the graft roof epithelium, and the wedge-shaped openings left by the upward migration of follicular epithelium. (12 ABC.) ×17.

Fig. 46. A 16-day low-dosage homograft. The graft has differentiated fully, and new hairs have matured. Although the greater part of the spread epithelium persists, it is beginning to break down in small patches (e.g. at the junction between the graft and the outlying tissue). The graft is accordingly reaching a critical phase in the development of the homograft reaction, with a characteristically low inflammatory response. (12 AB.) ×17.

Fig. 47. A 20-day low-dosage homograft in which, from histological appearances, breakdown is *just* complete. The inflammatory reaction is characteristically weak, and some of the primary vessels of the graft remain patent. The graft roof has returned to the thickness of normal skin epidermis (cf. figs. 8, 9). (1 A.) ×55.

PLATE 5

Figs. 48, 49. The precocious breakdown of the primary vessels in a 4-day 2nd-set homograft. Small vessels have differentiated to form a 'definitive circulation' in the lower reaches of the dermis (fig. 49). Haemorrhage is prominent in fig. 49; but as fig. 48 shows more clearly, no significant number of native leucocytes have been able to pass through the vessel walls. See fig. 50. (2 A.) ×85.

Fig. 50. A 'typical' 4-day 2nd-set homograft. The epithelium (fig. 51) is inactive; the dermis is swollen, haemorrhagic, and blotched with broken-down primary vessels (fig. 49); and the graft bed is distended with fibrinous matter, now being 'organized' by the upgrowth of capillaries. (123 A.) ×23.

Fig. 51. The surviving roof epithelium of a 4-day 2nd-set homograft. The cells remain plate-like, and (contrast fig. 29) there is little appreciable cellular activity. (1 A.) ×145.

Fig. 52. The typical appearance of an 8-day 2nd-set homograft. No primary population of native leucocytes has invaded it, and even the tissue spaces of the primary vessels are now obliterated. The secondary invasion of capillaries from the graft bed is in progress. The graft roof, now broken down (fig. 53), had undergone no hyperplastic or amoeboid activity. (12 A.) ×50.

Fig. 53. Fragmentation of the epithelium of the 8-day 2nd-set homograft roof (see fig. 52). (1 A.) ×160.

Fig. 54. The most advanced condition found in any 2nd-set homograft: the graft roof epithelium of the one specimen in which breakdown was not wholly complete at the 8th day (Text-fig. 4). The amoeboid activities are fairly clear-cut, but the roof epithelium is little thickened, and vertical differentiation almost negligible. (1 A.) ×85.

Fig. 55. An 8-day 2nd-set homograft from a donor *other than the original*: generalized hyperplasia, to the accompaniment of acute inflammation within the graft, of a type unparalleled by normal (constant donor) 2nd-set homografts. (12 A.) ×23.

Figs. 56, 57 and Pl. 6, fig. 58. 'Autohomografts' showing complete (fig. 56, 12 A, ×23), 'trace' (fig. 57, 123 AB, ×55), and *no* survival (Pl. 6, fig. 58, 123 A, ×23).

PLATE 6

Fig. 59. A 4-day *local* 2nd-set homograft, with its autograft control and the epithelium spreading from it. The homograft is now totally degenerate, though it still provokes violent inflammation in the graft bed (the crater left after removal of the 1st-set graft, in the original position of which it lies). The autograft, planted in a neighbouring crater, is in anything superior to a normal one. Acute inflammation has subsided in its bed. Note that the autograft epithelium has very nearly reached the homograft (cf. fig. 62). ×17.

Fig. 60. Showing the total fragmentation of the epithelium of a 4-day *local* 2nd-set homograft. (1 A.) ×60.

Fig. 61. As fig. 60, save that here some of the basal layer cells of the roof epithelium, which shows no signs of activity, are not certifiably 'dead': '99% breakdown'. (The autohomograft test shows that they are in fact alive.) (1 A.) ×60.

Fig. 62. Overgrowth of a 4-day *local* 2nd-set homograft by native epithelium, within 4 days of planting (cf. fig. 59). The foreign graft roof has been wholly undermined: in the section it appears suspended in mid-air above the graft. Note that the native epithelium at once establishes hair-follicle primordia in the foreign dermis. (12 A.) ×50.

Fig. 63. A 'mosaic' graft (Text-fig. 1*e*) exhibiting very clearly the specificity of the homograft reaction: an autograft wholly surrounded and underlain by a larger homograft, in which breakdown is now complete. The autograft has survived and is beginning to invade and undermine the necrotic tissue around it. (123 A.) ×17.

Fig. 64. An apparently normal mitosis—one of at least twenty counted in this specimen—in the follicular epithelium of a tissue-culture of donor skin in presumptively immune serum, withdrawn from a high-dosage homograft bearer 16 days after operation. ×290.