A SECOND STUDY OF THE BEHAVIOUR AND FATE OF SKIN HOMOGRAFTS IN RABBITS

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

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INTRODUCTION

A first systematic survey of the 'homograft problem', as it relates to the transplantation of rabbits' skin, has been published in this *Journal* by Medawar (1944). The behaviour of autografts and homografts was described; and the intensity of the homograft reaction, measured in terms of the time of survival of homologous grafted skin, was shown to depend upon (*a*) the dosage of grafts borne by the recipient (the 'dosage phenomenon'); (*b*) the recipient's previous experience of grafting from the same donor source (the 'immunity phenomenon'); and (*c*) a measured but causally unanalysed element of genetic diversity. It was, in addition, shown that the homograft reaction is absolutely specific towards foreign as opposed to native skin, and relatively specific towards the skin of the donor.

The following additional descriptive and analytical matter is reported in the present paper:

(1) The history of homografts is followed beyond the time of breakdown of the foreign skin epithelium into the period during which the homograft dermal collagen is attacked and removed.

(2) An enquiry is made into the significance of the *ages* of donor and recipient animals.

(3) Local and systemic components of the homograft reaction are dissociated by the technique of grafting skin in widely different local dosages to widely separated parts of the body.

(4) The element of genetic diversity is analysed in accordance with the hypothesis of immunity, and a statement is made of the number of antigens which govern the grafting reactions of rabbits' skin.

(5) New and wholly independent demonstrations of the phenomena of dosage and of transplantation immunity are described.

(6) A working hypothesis of the mechanism of the immune reaction is put forward for consideration.

Two hundred and one rabbits have been used in these experiments, and like their 181 predecessors of the earlier set, they formed in the genetical sense a completely heterogeneous assembly. In this second paper and the first, homograft operations between 802 distinct pairs of rabbits are described.‡ Of these, 708 grafts showed total breakdown of the foreign epithelial elements at the time of examination, or at the last examination of a series; 39 showed breakdown to be in progress but not, at the time of examination, complete; and 21 showed the specific degenerative changes which anticipate the process of breaking down. On the remaining 34, mostly grafts borne in low dosage by short-term animals, no judgement can be passed. There is still, therefore, no evidence that skin can be transplanted with permanent success between animals of ordinary genetic diversity.

† Working with a part-time personal grant and an expenses grant from the Medical Research Council.

‡ Two animals, A and B, may provide two distinct pairs, A→B and B→A. Three animals may provide six. Such pairs will not be described as 'independent'. (Here and below, an arrow is used to indicate the direction of transplantation between donor and recipient.)

OPERATIVE TECHNIQUES

Four new techniques of transplantation have been used in the experiments described in this paper: (*a*) transplanting grafts into raw areas just sufficiently large to receive them ('fitted grafts'); (*b*) transplanting fitted grafts to the shank; (*c*) grafting to both sides of the body simultaneously; (*d*) operating upon baby rabbits. The terms 'large' and 'small' graft are used in the sense quantitatively defined by Medawar (1944, p. 177). The general principles of the technique of grafting remain unchanged: unless some variant is specifically mentioned, grafts are transplanted to a raw area stripped down to the vascular fascial planes overlying the panniculus carnosus on the right thoracic wall, and are covered with tulle gras, gauze, plain bandage, and plaster-impregnated bandage in the stated order. The prophylactic use of sulphadiazine powder by dusting has been maintained; but experience has shown that it is never necessary to use more than ½ yd. of 4 in. plaster bandage ('Cellona') for winding round the thorax.

(*a*) *The fitted graft.* In the experiments described by Medawar (1944), as in those described by Gibson & Medawar (1943), pinch grafts were so distributed over the surface of a raw area as to be separated by at least 1½ diameter's distance from each other and from the margins of the operation field. Such grafts accordingly 'grow' by symmetrical outward spread of epidermal epithelium. This technique of grafting has one important defect: the dosage of skin that is grafted is an increasing quantity, so that only the initial graft dosage can be specified with precision.

The alternative is to transplant one or more large pinch grafts into separate raw areas which are only just large enough to receive them. The technique amounts to putting one pinch graft into the donor area left by cutting another. When a pinch graft is cut, the graft tends to contract and the donor area to expand; so that if a graft is immediately replaced in its own donor area, it is surrounded by a narrow symmetrical ring of raw tissue. A pinch graft should therefore be cut to rather less than the standard large size if another large graft is to fit perfectly into its donor area. The adjustment is simply a matter of practice.

Grafts transplanted in the manner just described are called 'fitted pinch grafts' or just 'fitted grafts'. Conversely, grafts which are spaced over a continuous raw area are said to be transplanted in 'open style" (The term 'free graft', on other grounds desirable, has another meaning in plastic surgery.) Animals bearing fitted homografts received the same dressings as any others.

(*b*) *Grafting to the shank* is described on pp. 164–5. After clipping the outer aspect of the shank and rubbing the clipped skin with 5% Dettol, a small pinch graft is cut at a point 2½–3½ cm. above the heel, from the skin which lies in the longitudinal valley between the tibial bone and the achilles tendon. A large pinch graft is fitted into the raw area so formed. The operation field is dusted with sulphadiazine powder, covered with a double thickness of tulle gras, a small pad of cotton wool cut to the shape of the longitudinal valley, and then—without intervening bandage—by 8 in. plaster-impregnated bandage 1 in. wide. The double tulle gras layer protects the graft from the exudate of the wetted plaster, which elsewhere sticks firmly to the hair of the shank. The little plaster jacket is quite flexible, and should always be found to ride freely up and down the shank. This movement does not displace the graft, for although the panniculus carnosus is absent, the graft bed consists of fascial planes which move with the integument. The animal is in no way immobilized, and can squat in its usual position if it chooses to do so. In spite of this, it will certainly gnaw away the plaster sheath unless the sheath is impregnated daily with a saturated aqueous solution of picric acid. (The only three grafts which in the writer's entire experience have failed to 'take' were lost from a neglect of this precaution.) In no case has the picric acid been found to percolate through the plaster so deeply as to stain the graft or the skin of the operation field.

(*c*) *Grafting to both sides of the thorax* (pp. 164–5) is simply a matter of grafting to one side by any of the usual methods, turning the animal over, and then grafting to the other side. It is obvious that the greatest care must be taken with the turning and the bandaging of the animal, but no special instructions can be given. Although a few failures were expected, no graft transplanted in the course of this double operation has ever failed to heal securely.

(*d*) *Grafting to baby rabbits* (pp. 161–3) is a variant of the technique used for grafting in open style to the thoracic wall of adult animals (1944, p. 178). The skin of the thoracic wall is clipped and not shaved, soaped and not rubbed with Dettol. In spite of the thinness and delicacy of the skin, the superficial layers 'split' very easily from the fascial planes overlying the panniculus carnosus. The raw area should never exceed 20 mm. in diameter.

The animals are anaesthetized very cautiously with ether in a large desiccator jar. A phase of jumpiness is followed by a period of full anaesthesia, during the course of which the operation must be done. After planting a single small graft to the centre of the raw area, the operation field is dusted as usual with sulphadiazine powder and covered with two 1 in. squares of tulle gras. As with grafting to the shank, no plain bandage is used; instead, the thorax is immediately wound with 8 × 1 in. plaster bandage. As it dries, the plaster sticks firmly to the hair. Baby rabbits should be allowed to recover from the anaesthetic in a 37° incubator; and before returning them to their mothers, whose attention should be momentarily distracted by the offer of food, the plaster bandages should be painted with picric acid.

Thirty baby rabbits have been operated upon, ranging from 280 to 470 g. in weight and from 2½ to 5 weeks in age. Seven of these died, but only one within the first seven days post-operatively. Four of the animals which died belonged to a single litter of eight. The only two animals of this litter which were *not* operated upon also died. There is no evidence, therefore, that the operations significantly increased the mortality. On the contrary, the high mortality in the litter just mentioned, the first to be received, can almost certainly be attributed to faulty diet. Baby rabbits have responded well to a basic diet of dry mixed bran and oats, supple-

mented by a little well washed or scraped carrot daily and an occasional scrap of thoroughly dry stale bread. The animals of the litter in which mortality was high had been fed with crisp lettuce and other green stuff intended for the mother's consumption, and had been given wetted bran.

The data set out in Tables 1 and 2 show that the mortality among experimental baby animals was highest in the older age groups and the heavier weight groups.

THE ULTIMATE FATE OF SKIN HOMOGRAFTS

The behaviour of skin homografts up to and a little beyond the time at which the foreign epidermis is finally destroyed has already been made the subject of a full description (1944, pp. 182–6). The account which follows is continued from this point and makes use of the same terminology. It is in all essentials a description of the fate of homograft dermal collagen.

Method

Native epithelium growing inwards from the sides of the operation field may overgrow or undermine the dead homograft at any level from the graft-bed junction to a plane a little below its naked surface (Pl. 1, figs. 7, 8; Pl. 4, figs. 22*b*, 23*b*; and 1944, Pl. 4, figs. 38–41). Three types of operation have been devised in order to make sure that the second of these possibilities is so far as possible realized, and that a reasonably large quantity of foreign dermal collagen is trapped beneath the native epidermis.

(1) A single large homograft is transplanted into the centre of a square raw area of side 2½ in. The foreign epidermis breaks down well before the ingrowing native epithelium can reach it, and the graft has a chance to settle down in the newly granulating tissue around it before it finally does so. Overgrowth at a high level is the result (Pl. 1, figs. 7, 8).

(2) Homografts are transplanted in fitted style to normal animals. When they break down, native epithelium at once bites into the collagenous remains and nips off the superficial necrotic layers. This second technique avoids certain special drawbacks of the first; but the quantity of collagen that is imprisoned in this way is variable, and almost always small.

(3) By far the most satisfactory method is to plant grafts in fitted style to animals which have been immunized by an earlier grafting from the same donor. A single large fitted homograft is a sufficient immunizing dose. Sixteen days after its transplantation, two further large grafts from the same donor may be fitted to the opposite thoracic wall. Their breakdown is very rapid, and is not accompanied by a massive cellular invasion, nor by any violent internal reaction.† Overgrowth by native epithelium is correspondingly prompt and uncomplicated, and a substantial part of the homograft dermal collagen may be wholly enclosed within 16 days of transplantation (Pl. 4, fig. 23*b*).

† This and other peculiarities of the behaviour of '2nd-set' homografts have been described by Medawar (1944, pp. 190–3).

Twenty animals were operated upon, ten by method (1) and five each by methods (2) and (3). The 26 biopsy specimens they afforded range in time of sampling from 20 to 100 days. In addition to these specimens, it has been possible to draw upon material from the fitted 2nd-set homografts described on pp. 170–1.

Description

The collagenous remains of a broken-down homograft are not normal. In particular, the dermis of a graft transplanted by methods (1) and (2), is vacuolated by the spaces formerly occupied by the primary blood and lymph vessels, and riddled by necrotic cellular remains. The deeper collagen bundles may have been prised apart by still unresolved oedema fluid. However, the spaces of the primary vessels are very soon obliterated (cf. 1944, Pl. 2, fig. 21), the cellular remains disappear, and the grossly necrotic superficial layers of the dermis are selectively nipped off by the invading native epithelium (Pl. 4, fig. 22*b*; 1944, Pl. 4, fig. 39).

Secondary vessels; secondary native population. The 'secondary' vessels which invade the dermis from the granulating tissue of the graft bed are abnormal capillaries which never differentiate into arteries and veins. (Lymphatic capillaries may be common among them, but they are seldom recognizable as such.) The capillaries are widely and irregularly dilated, and follow a tortuous and even 'spiky' course (Pl. 1, figs. 1–3). The appearance of these new dermal vessels has every mark of instability and impermanence; and since the vessel density reaches a peak not greater than that illustrated by Pl. 1, fig. 1, and thereafter slowly declines, it is probable that the capillaries are constantly breaking down and being replaced by new. If this interpretation is correct, the term 'secondary cycle' needs some qualification. The secondary cycle consists of a *series* of little waves of vascularization which become progressively more feeble. At all events, the homograft dermis becomes in the end almost completely avascular.

The secondary native population is a sparse and diffusely distributed assembly of miscellaneous mononuclears, including some plasma-cell like lymphocytes, and of groups of fibroblasts which seem to have entered the dermis independently of the secondary vessels (Pl. 1, figs. 1–3). Both categories were recognized in the earlier and incomplete account of the secondary cycle (1944, p. 184); but it is now clear that the second is by far the more important, for it is the fibroblasts which play the principal part in the recollagenization of the homograft remains. Like the vessels from which they originated, the leucocytes appear to be short-lived. A high proportion is always found to have pyknotic nuclei. At first, replacement more than makes good this loss, so that a cell density equal to that illustrated by Pl. 1, fig. 1 may be reached within 10 days of complete overgrowth; but within 30 days whatever may

remain of the homograft dermis is almost cell-free as well as almost wholly avascular.

Formation of new collagen and the disappearance of the old. The topographical relationship of native epidermis to foreign dermis varies with the method by which the graft has been planted. With animals operated upon by methods (2) and (3) the homograft dermis is *immediately* overlain by the native epidermal cells (cf. Pl. 1, fig. 11; Pl. 4, fig. 23*b*). If method (1) has been used, the dermis comes to be surrounded on all sides, including the outer, by new fibrous tissue of native origin. As soon as the epidermis grows over the naked surface of the graft, a clear narrow oedematous band develops immediately below it, and is very shortly afterwards invaded by capillary sprouts and by fibroblasts orientated in the plane of the integument (Pl. 1, fig. 8). Fine new collagen fibres are laid down, and the homograft dermis becomes farther and farther removed from the superficial epithelium as the band of new collagen thickens (Pl. 1, fig. 9). The whole process is most simply interpreted as a response to the slight infection which must occur when, by the procedure of method (1), the dermis of the homograft has been naked and unprotected for some days before the native epithelium grows over it and seals it off.

The collagen of all homografts slowly disappears, and is replaced pari passu by collagen of new formation. The dermis of grafts transplanted by method (1) disappears within 30–50 days of complete overgrowth, and the new ('secondary') native collagen is laid down by a characteristic process of *internal replacement*. The oedema which accompanies the breakdown of 1st-set homografts has already caused the old dermal fibres to become separated, or at least to lie in abnormally open packing; and into the interstices so formed, the fibroblasts of the secondary population migrate and in due course lay down an internal trelliswork of fine new fibres (Pl. 1, figs. 4–6). Old collagen fibres are easily distinguishable from new, by virtue of their great size, the three-dimensional packing that is characteristic of dermal collagen, and the staining reaction, which is such that the fibres tend to stain yellowish red rather than scarlet with picro-fuchsin, and tend to retain the acid fuchsin component of Masson's three colour stain, notwithstanding differentiation with phosphomolybdic acid. The new fibres are, conversely, one-tenth to one-twentieth of the diameter of the old; their orientation is two-dimensional; and their staining reaction more rapidly specific.† Pl. 1, figs. 4–6 illustrate very clearly the early stages of collagen replacement. At the stage illustrated by Pl. 1, fig. 9, the old fibres are more than half gone; and at that illustrated by Pl. 1, fig. 10, only four or five remain.

The dermis of homografts transplanted by method (3) disappears more slowly, and replacement is 'creeping' and external. A violent fibroblast reaction develops in the graft bed (Pl. 4, fig. 23*b*) and persists during the entire process of removal (Pl. 1, fig. 11). No granulating band develops between the epidermis and the homograft remains. All that can be said of the old collagen is that it disappears as new collagen encroaches upon it. At the stage illustrated by Pl. 1, fig. 11, 40 days after planting and about 20 after complete overgrowth, it has been reduced to a shallow plate only 0·22 mm. in thickness.

Since fibrous scar tissue of native origin does not develop in the neighbourhood of grafts transplanted by method (3), their internal changes may be correlated fairly accurately with their outward appearance. When the necrotic superficial layers of the dermis are nipped off by the completion of the process illustrated by Pl. 4, fig. 22*b*, the overgrown residue of the dermis (cf. Pl. 4, fig. 23*b*) stands out from the surrounding field as a smooth, evenly rounded, dead white prominence covered by shiny and somewhat tissue-papery native epithelium. A ring of very rapidly growing pigmented hairs usually develops round the periphery, and their slope is such that the bases of the follicles abut into the new collagenous tissue *below* the old homograft dermis. From the 30th day of transplantation onwards, the dermal disc becomes progressively smaller and less dense. The growth rate of the marginal hairs falls off and their pigmentation is lost. It is thus obvious, even to outward appearance, that the homograft dermal collagen is being removed. Yet a small fraction was still found to remain in the one 100-day specimen which the experiments by method (3) afforded, though it measured only 1 mm. in the antero-posterior direction and was nowhere more than 0·12 mm. thick.

The mechanism by which the foreign collagen is removed is altogether uncertain. Phagocytosis plays no demonstrable part. It is true that small groups of mononuclear phagocytes may be found between the epidermis and the collagen of grafts transplanted by method (1), but their presence is probably associated with the trapping of necrotic collagenous debris below the epithelial surface. Gibson & Medawar (1943) described the removal of *human* homograft collagen by phagocytosis; and unless the reaction is a peculiarity of the human being, it must be attributed to the complete imbedding of the collagenous remains within soft, 'active' granulation tissue (1943, Pl. 4, figs. 28–33). Phagocytosis was not, however, claimed to be more than 'in part responsible' for the final removal of human homograft collagen: the term 'fading away', used to describe an unknown component of the process, is perfectly appropriate here (Pl. 1, figs. 9, 10 herein).

The behaviour of the epidermis. The epidermis which grows over grafts transplanted by method (1) is at first most insecurely attached. Gentle scraping alone will lay the dermis bare. During the course of overgrowth, the epidermis throws down hair-follicle primordia into the underlying tissue (Pl. 1, fig. 7; 1944, Pl. 4, fig. 41, and compare Pl. 4, fig. 40). Contrary to expectation, such follicles are at least in the great majority of cases aborted: no hair shafts come to lie within a jacket of foreign collagen. In grafts transplanted by method (3), they may lie below it, and in grafts transplanted by method (1), they may form in the new fibrous tissue above it (Pl. 1, fig. 9).

The under surface of the superficial epidermis is characteristically plane, i.e. it does not send down digitate or columnar processes into the underlying tissue in such a way as to form dermal pseudo-papillae between them. The

† The difference between the staining reactions is not one of final quality but of rate. The old dermal collagen responds more slowly to the specific stain but not in the long run less completely. The staining reaction is thus different from that of grossly abnormal (e.g. heat-coagulated) collagen.

histological appearance is just that which would have been expected from the smooth, shiny appearance which the epithelium outwardly presents.

The specificity of the reaction

The collagen of autoplastic pinch grafts, unlike that of homografts, persists unchanged; either indefinitely, or over a period of time of quite a different order of length to that which suffices for the removal of the latter. The most long-lived of a series of control autografts transplanted with reversed orientation of hair slope is at present 140 days old. It is still recognizable as a disc 7 mm. in diameter with the aid of the hairs growing from it in a direction diametrically opposed to the direction of hair slope in the outlying field.

The persistence of the collagen of autografts does not imply that the removal of foreign collagen is a specific process. Would not a naked pad of *autograft* dermal collagen be removed if, in default of epithelium of its own, it had been overgrown in the manner otherwise characteristic of long-term homografts? The answer must await the discovery of a treatment which will kill skin epithelium without so affecting the collagen fibres as to make them behave as foreign bodies.

If, as is at least probable, the removal of homograft collagen *is* a specific reaction, then one must infer either that the collagen is antigenic, or that it is capable of responding to the action of immune bodies generated in the first instance by foreign epidermal epithelium or dermal mesenchyme cells. All that can be said of the former possibility is that the collagen derivative gelatin is known to be immunologically inert; but the evidence presented here is consistent with the last of four possible explanations reviewed by Wormall (1944), namely, that the manner of its preparation is sufficient to destroy any antigenic power that native collagen may originally have had. Pullinger & Pirie (1942) found that semi-purified ox corneal collagen is removed after implantation into the rabbit. They 'do not attribute the reaction which ensues to its foreign quality', presumably because of the known inertness of gelatin. The possibility that the collagen behaved at least in part antigenically, as a 'collagen heterograft', should however be kept in mind.

THE SIGNIFICANCE OF AGE

The problems to be considered under this heading are these: other things being equal,

(*a*) do skin homografts from an adult donor survive longer on a young than on an adult recipient?

(*b*) do skin homografts from a young donor survive longer than grafts from an adult donor?

The first question relates to the power of young animals to respond to an antigenic stimulus; the second, to their power to generate it. It is well known that embryos in general do not react even towards heteroplastic grafts (see Needham, 1942, pp. 348–51), but little is known of the time relations of the development of the immunological specificity of the individual. Harris's analysis (1941) of tissue-grafting specificity in Anuran tadpoles has shown, however, that a homograft reaction towards implanted gastrular fragments is not developed in the period before the differentiation of the hind limbs. Apart from this suggestive result, and the evidence discussed by Wiener (1943) concerning the time of appearance in post-natal development of the mammalian red-cell agglutinins, the questions can be examined without theoretical bias. The qualification 'other things being equal' cannot of course be realized by the use of genetically heterogeneous animals. The 'null hypothesis' which the experiments described below were intended to test therefore centres around the factor of genetic diversity as the unknown variable quantity.

Plan of the experiments and the 'null hypothesis'

The case of 'young versus old recipients' will be considered first.

One large and one small pinch graft were cut from a single mature donor. The large graft was transplanted to a raw area on the thoracic wall of a mature recipient, and the small graft to a raw area on the thoracic wall of a young recipient (see p. 158). The initial graft dosages were approximately the same. The two recipients, 'linked' by the donor they shared in common, were killed on the 12th or on the 16th day, and their grafts removed for microscopical examination. The result of the experiment was marked ' + ' if the graft on the young animal had survived longer than the graft on the mature animal; otherwise it was marked ' − '.

The genetical relationship between the animals of the two linked pairs was quite unknown: on genetical grounds, a longer survival of the graft on the old animal is therefore just as likely as its converse. The 'null hypothesis'—that the age of the recipient is without significant effect—may therefore be tested by a group of experiments of the type outlined above; and the hypothesis to be appraised is simply that pluses and minuses occur with equal frequency within it. One complication of this otherwise straightforward plan is that, on the day of examination, *both* grafts may show either complete survival or complete breakdown. In either case, the result of the experiment is marked ' = ', for there is no means of telling to which category, ' + ' or ' − ', the result would have belonged, had the examination been timed to coincide with the beginnings of breakdown in one graft or the other.

The most obvious way to approach the problem of 'young versus old *donors*' would have been through the converse of the experiments just described; i.e. by grafting simul-

taneously from a young and an old donor to a single mature recipient. The tests described on pp. 165–7 show that quite small antigenic differences could have been shown up in this way, notwithstanding the combined action of such antigenic factors as the two grafts may have shared in common. When the experiments described in this section were done, however, no such presumption could justifiably have been made. They therefore take the form of independent (unpaired) tests, in each one of which a large graft cut from a young animal was transplanted to a raw area on the thoracic wall of a mature animal and removed for examination on the 16th day thereafter. The results were compared *en bloc* with a group of 15 controls in which grafts were transplanted in medium-dosage, open style, from mature donors to mature recipients (Summary Table, row 2, p. 173). ('Large' grafts cut from baby animals are similar in area to large grafts cut from old animals, but very much thinner. The dosage of skin *epithelium* is nevertheless much the same.)

In practice, experiments of the 'young v. old donor' and 'young v. old recipient' types were combined, and performed in a single operating session, in the manner diagrammatized by the following scheme, in which 'O' and 'Y' stand for 'old' and 'young' animals respectively, and arrows indicate the direction of transplantation:

The results of ten such experiments are reported. The scheme itself illustrates an experiment in which *two* young animals were grafted in parallel from one old donor, as a measure of insurance against the death of either one, a circumstance which would otherwise have ruined the experiment. Sometimes the operation plan was adjusted to the use of three baby animals. But the mortality (see p. 158) proved to be lower than was expected; and the result is that each experiment often provides two or three 'readings' or marks (Table 4).

Experimental material

The 'old' donors and recipients were mature rabbits ranging from 2 to 2½ kg. in weight. The 'young' rabbits, on the day of operation, ranged from 2½ to 4½ weeks in age, and from 280 to 470 g. in weight. The distributions of the initial ages and of the initial and final weights of the twenty-three baby rabbits which survived to give a 'reading' (*) and of seven which did not (o) are summarized in the accompanying Tables (1–3). The baby rabbits used in any one experiment were litter mates.

Experimental results

The reaction elicited from young rabbits by homografts of 'old' skin does not differ in any important respect from the reaction generated by skin which has been transplanted between mature animals. There is the same hyperplastic phase, accompanied by outward growth of epidermal epithelium, and the same division of the vascular invasion and breakdown into two sharply defined cycles (1944, p. 182). The leucocytes which invade the homografts on young recipients are, however, predominantly of the type which in older animals would have been given the non-committal name of 'mononuclears': they have a richer cytoplasm than is usual with lymphocytes.

Table 1. *Distribution of the weights of baby rabbits on operation day. Median weight* 330 *g., mean weight* 360 *g., principal modal class* 295–315 *g.*

Wt. class (g.)	No. of animals
275–295	**
295–315	*******
315–335	****
335–355	**o
355–375	**oo
375–395	*o
395–415	*
415–435	**o
435–455	*o
455–475	*o

Table 2. *Distribution of the ages of baby rabbits on operation day. Modal age class* 3–4 *weeks; mean age of those which survived,* 3½ *weeks*

Age (weeks)	No. of animals
2½	*****
3	*******o
3½	***
4	*****ooo
4½	***o
5	oo

Table 3. *Distribution of the weights of baby rabbits at biopsy on the* 12*th and on the* 16*th days. (One animal from each group unweighed)*

Wt. class (g.)	12 days	16 days
350–399	*	*
400–449	***	**
450–499	***	
500–549	**	****
550–600	**	***

The experimental results, so far as they relate to the length of survival, are most easily summarized in tabular form (Table 4). Each animal is identified by its laboratory number, followed (in brackets) by a number which represents the order of the degree of survival of homograft epithelium at the time of biopsy. The symbol (1) stands for complete survival, and (0) for none. Intermediate grades are symbolized by (¾), (½), and (¼). The marks '+', '−', or '=' which are allotted to each experiment relate only to the problem of 'young v. old recipients', and they are based upon a collation of the degree of survival of the 'old' recipient with that (or those) of the young re-

cipient(s). The most important objection to grading a continuous series, such as that represented by the degree of survival, into the disjointed levels represented by such symbols as (½), (¼), is that one may not always be able to keep in mind a clear picture of the absolute standards to which these levels are to be referred. This difficulty does not affect the present experiments, in which only the *relative* value of the intermediate degrees of survival have been taken into account in marking. In Exp. 2, for example, animal 258 (¼) certainly bears a smaller proportion of surviving homograft epithelium than animal 259 (½); so that the mark '+' is correct, even if the degree of survival in 258 does not tally exactly with, for example, that in animal 272 (¼), Exp. 4.

Table 4. *Summarizing the results of ten experiments on the problem of 'young v. old' recipients. For explanation of the symbols, see text*

Exp.	Donor	'Old' recipient	'Young' recipient(s)	Mark	Duration of exp. (days)
1	243	244 (0)	245 (0)	=	16
2	257	258 (¼)	259 (½)	+	16
			260 (0)	−	
			261 (0)	−	
3	262	263 (0)	264 (0)	=	16
			265 (0)	=	
			266 (0)	=	
4	271	272 (¼)	273 (¼)	=	16
			274 (0)	−	
5	275	276 (½)	277 (0)	−	16
			278 (0)	−	
6	280	281 (¾)	282 (0)	−	12
			284 (1)	+	
7	285	286 (¾)	287 (1)	+	12
			288 (¼)	−	
			289 (0)	−	
8	290	291 (0)	292 (1)	+	12
			293 (0)	=	
9	321	322 (0)	324 (¾)	+	12
			325 (0)	=	
10	326	327 (½)	328 (¾)	+	12
			329 (0)	−	
			330 (¼)	−	

The results of Exp. 7 are illustrated by Pl. 2, figs. 12 *a–d*; of Exp. 8, by Pl. 2, figs. 13 *a–c*; and of Exp. 10 by Pl. 2. figs. 14 *a–c*.

The donor rabbit, as the diagram of the operation procedure shows, is itself the recipient of a homograft from one of the young rabbits registered in the same row of the table. The 'young v. old donor' experiments therefore comprise the ten independent tests set out in the second column. To avoid unnecessary confusion, the degree of survival of the 'young' grafts borne by the adult donor animals is not entered in the table.

Conclusions

The case of 'young v. old recipients'. The data set out in Table 4 may be treated either as a whole, or separately by 12- and 16-day biopsy age groups; and either in the 'paired' form in which they are there set out, or by the block-control method, making use of the extra data entered, with reference to their sources, in the Summary Table, row 2, p. 173. Another possibility is to compute by probit transformation the median survival time of the grafts borne by young rabbits, and to compare it with the corresponding estimate for grafts borne in medium dosage by adult animals (loc. cit.). The analysis of the marks allotted to the experiments when treated as pairs is complicated by the equalities ('='); one method of giving them their due weight is to divide them equally among the '+'s' and '−'s', which should, according to the null hypothesis, occur with equal frequency. Whichever of these methods is used, the result is the same: the null hypothesis is not invalidated, and there is no evidence that baby rabbits react less strongly to skin homografts than do the old.

The case of 'young v. old donors'. The ten adult rabbits entered as donors in Table 4 received one standard large homograft from ten separate young donors respectively, and in all cases the graft was inspected and removed on the 16th day after transplantation. One of the ten grafts showed a complete survival of homograft epithelium, and another, a degree of survival very little removed from the point of complete breakdown. The remaining eight grafts showed the typical and uncomplicated picture of total breakdown of long standing.

For comparison, one may refer to the ten independent 16-day 'medium dosage, open style' recipients of skin homografts from *adult* donors, entered in row 2 of the Summary Table, p. 173. Five of these ten animals still bore surviving homograft epithelium at the 16th day. The ratio of survivors to non-survivors among the recorded samples is therefore 2 : 8 in the one case and 5 : 5 in the other. If these were samples from a population in which the ratio of survivors to non-survivors was the same, then a disparity as great or greater than that actually observed might be expected to occur as often as once in every seven trials. The experiments do not therefore indicate that 'young' skin is any more or any less powerfully antigenic than old. They do, however, suggest that the former possibility is worth further investigation.

THE SIGNIFICANCE OF POSITION

It has been shown by Medawar (1944, pp. 186–90), and it is again shown here (pp. 168–70), that the time of survival of homograft epithelium varies inversely with the quantity of skin that is grafted. Other things being equal, a graft in low dosage will always survive longer than a graft in high dosage. The reality of the dosage phenomenon is a powerful, but

not a decisive argument in favour of the hypothesis that the reaction generated by skin homografts is predominantly *systemic* in nature. This is the problem which receives further investigation here. In all the experiments on graft dosage described elsewhere, the grafts, although varying in size, number, and manner of transplantation, were always made to lie within a single localized operation field. No distinction could therefore be made between a local and a systemic component of the homograft reaction, because local and systemic graft dosages were the same. The experiments described here make use of a new variant of the dosage operation: skin from a single donor is transplanted in a single operation session to widely separated parts of a single recipient; in very high dosage to the one part, and in very low dosage to the other.† The systemic dosage, the sum of the local dosages, will of course be high.

Two extreme possibilities may be considered. If the systemic dosage is the only operative factor, then *all* the grafts of a uniform population will break down simultaneously and in identical fashion, irrespective of their local dosages. If, on the other hand, the homograft reaction is mostly generated in the immediate neighbourhood of the grafts, then the graft in local low dosage will be expected to behave like a graft in systemic low dosage, and therefore to survive in all cases longer than the grafts transplanted in high dosage elsewhere. In general, any disparity between the survival times of homografts of a uniform population in different local dosages will provide a measure of the degree to which a local component of the homograft reaction is superimposed upon a systemic.

There is already one theoretical indication for a choice between the two extreme possibilities: 2nd-set homografts which have been transplanted to the very positions formerly occupied by immunizing grafts of a first set do not break down very much more rapidly than 2nd-set grafts which have been transplanted to the opposite side of an immunized animal (1944, pp. 192–3). A systemic immune state therefore certainly exists, and 'evidently the local immune state adds something, but not very much, to the systemic'. The results of these experiments will be re-appraised in the light of the new evidence presented here.

Experimental method. Grafts of a uniform population transplanted to opposite sides of the thorax may be supposed to form independent groups, so far as local dosage is concerned. In the majority of experiments, therefore, the grafts have been transplanted in local high dosage to the right side of the thorax and in local low dosage to the left. Any possibility of the venous anastomoses across the dorsal midline becoming transiently effective was overridden, in a smaller subgroup of the experiments, by grafting in high dosage to the right thoracic wall and in local low dosage to the lower extremity of the left shank.

† A set of homografts will hereafter be said to form a *uniform* population if, whatever their sizes, positions, manners of grafting, or systemic and local dosages, its members are genetically uniform and have been transplanted simultaneously to a single recipient animal.

The exact subdivision of the experiments was as follows. Twenty adult recipients were operated upon, and each one received a set of seven large grafts transplanted in open style, local high dosage, to the integument overlying the right thoracic wall. Ten of these animals received in addition a single large graft transplanted in open style to the left thoracic wall; five, a single large graft in fitted style to the left thoracic wall; and five, a single large graft in fitted style to the outer aspect of the left shank. The grafts in local low dosage were always uniform with the grafts in high dosage on the thoracic wall. Biopsies were in all cases taken on the 12th day post-operatively, a time chosen because the majority of systemic low dosage grafts survive twelve days and the majority of high dosage graft do not. Moreover, the data available for 'block' controls are particularly rich on the 12th day (see Summary Table, p. 173).

Each recipient animal provided a pair of biopsy specimens, which, after being most carefully marked, were fixed, dehydrated, and embedded together, cut in the same block, and mounted on the same slide, on which the two series of sections lay side by side.

Experimental results. The grounds upon which a comparison between the two members of each pair of grafts may be made are, in summary:

Outward appearance: extent of outgrowth, if any; degree and quality of the oedematous swelling (hard, 'pneumatic'); colour of the graft, whether white, pink, freshly haemorrhagic or dark red and blotchy, or dirty yellow, brown, black; firmness and 'dryness' of the superficial epithelium; extent of exfoliation, response to gentle scraping; the degree of firmness of the attachment of the rim of the graft to the graft bed.

Internally: the general structure of the graft roof; the manner and degree to which it is indented by the cystic re-entrants marking the follicles of thrown-off hairs; the quantity and distribution (e.g. predominantly follicular, superficial) of surviving epithelium, if any; the manner of its disengagement and the cytological changes (acidophily of cytoplasm, pyknosis or rhexis of nuclei) associated with it; extent of hyperplastic changes and of retrograde or progressive differentiation (e.g. thickening and later thinning of graft roof, extent of exfoliation, elaboration of glandular acini, formation of new hairs, etc.); the extent, thickness, and pathological changes of the spread epithelium, if any remains.

The inflammatory reaction: thickness of the graft, quantity and disposition of free oedematous matter, particularly in the dermal papillae; the degree of differentiation or hypertrophy of the primary or secondary blood vessels as the case might be, with special reference to: engorgement by red cells, arrest of leucocytes within the

lumen, condition—intact, pyknotic, broken down—of the endothelial lining and its nuclei, and the pathological changes of later stages, viz. thrombosis or partial obliteration of the lumen; the size, distribution, and pathological condition of the lymphatic vessels (active, stagnant, with or without living or pyknotic or fragmented leucocytes within them). The composition, density, and distribution of the primary or secondary native populations of leucocytes; e.g. whether densely aggregated into a 'black band' or merely perivascular or diffuse; the proportion of pyknotic or fragmented cells in the population.

The state of the graft bed, with special reference to: vascular proliferation; the degree of distension of the fascial planes with oedema fluid; the maturity of the fibroblastic reaction and general granulation of the bed, or of the collagenization which follows it.

A complete series of point-for-point comparisons between the members of each pair of grafts has been founded upon these criteria. They lead to the following conclusion, which requires qualification in no particular: the grafts of a uniform population are quantitatively and qualitatively *identical*, regardless of their local dosages. The grafts are so similar in every fine detail of their appearance that there is not even a theoretical, let alone a practical possibility of making any distinction between them. It follows then (*a*) that the reaction provoked by skin homografts is systemic in nature; (*b*) that any local component of the reaction that may exist is wholly overridden by the systemic; and (*c*), a corollary of (*a*), that the effective dosage of a uniform population of grafts is the systemic dosage alone.

The complete similarity of the behaviour of the grafts of a uniform population over the entire range between complete survival and total breakdown, is illustrated by the four pairs of photographs in Pl. 3. It is hard to believe that the photographs in each pair are not in reality of sections at different levels through one and the same graft.

It is now possible to appraise the significance of the 'local immune state' described by Medawar (1944, pp. 192–3). The clue is given by the behaviour of the autograft controls described on p. 194—autografts transplanted to the craters left by 1st-set homografts after their removal in accordance with the procedure of the 'local 2nd-set' grafting operation. '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; very possibly because they were transplanted to already granulating and highly vascular beds.' A similar explanation will account, *mutatis mutandis*, for the slightly accelerated retrogression of local 2nd-set grafts as compared with those which are transplanted to the opposite thoracic wall. Vascularization is more rapid, and the local 2nd-set graft therefore reaches more quickly that stage—the threshold of the period of generalized hyperplasia—at which 2nd-set grafts are characteristically destroyed. The differences between local and standard 2nd-set grafts, at the most only slight, do not therefore argue the existence of a local immune state.

Data relating to the degree of survival. A system of marking the degree of survival of epithelium in homografts has already been used in Table 4. The five marks which may be allotted are:

(1) Complete survival; or breakdown not further advanced than is represented by slight 'weakness', i.e. tendency towards maceration, of the epithelium at the bases of the follicles or at the tops of oedematous dermal papillae.

($\frac{3}{4}$) Breakdown of the epithelium in progress, but the greater part still surviving.†

($\frac{1}{2}$) The epithelium about half broken down.

($\frac{1}{4}$) The greater part of the epithelium broken down.

(0) Total breakdown.

The degrees of epithelial survival in the twenty independent pairs of homografts reported upon above were:

[Ten animals in which the local low-dosage graft was transplanted to the left thoracic wall in open style] 196 (0), 198 ($\frac{1}{2}$), 201 (0), 211 (0), 219 (0), 225 ($\frac{1}{4}$), 228 (0), 230 (0), 232 (0), 240 ($\frac{1}{4}$).

[Five animals in which the local low-dosage graft was transplanted to the left thoracic wall in fitted style] 252 (0), 254 (0), 268 (0), 295 (0), 304 (1).

[Five animals in which the local low-dosage graft was transplanted in fitted style to the left shank] 340 ($\frac{3}{4}$), 341 ($\frac{1}{4}$), 342 ($\frac{1}{2}$), 346 (0), 349 ($\frac{1}{4}$).

The surprisingly high proportion of grafts showing some degree of epithelial survival in the third of these subdivisions can only be attributed to the luck of sampling. It goes without saying that the two grafts which each animal provides are identical in respect of degree of survival. The pairs chosen for illustration are: Pl. 3, figs. 15*a*, *b* = 304 (1); fig. 16*a*, *b* = 349 ($\frac{1}{4}$); fig. 17*a*, *b* = 341 ($\frac{1}{4}$); fig. 18*a*, *b* = 295 (0).

Less than twenty animals would have sufficed even for a rigorous demonstration of the absence of any 'position effect'. As it happens, however, the majority were in the first place intended for use with serological tests, and the position tests described above were done with little extra labour.

THE NUMBER OF SKIN ANTIGENS

The time of survival of foreign skin epithelium has been shown to vary with the systemic dosage of the grafted skin; with the 'history' of the recipient, in regard to its previous experience of grafting from the same donor source, or from a source having antigens in common with the new donor; and with the antigenic relationship between donor and recipient.

This third factor, the variance introduced by the element of genetic diversity, may outweigh all others. It may outweigh a disparity of initial graft dosage as great as that between eight large grafts transplanted in open style (which sometimes survive beyond the 12th day) and a single large graft exactly fitted to its recipient area (which may, in a minority of cases, break down before the 12th day). It may even override the immune reaction; for in one case out of ten (1944, p. 186, text-fig. 4, chart III) a 2nd-set high-

† The validity of such a judgment has been discussed by Medawar (1944, p. 185).

dosage homograft survived until the 8th day, and in two cases out of ten (loc. cit. chart II) high-dosage 1st-set homografts failed to do so. These facts, taken in conjunction with the demonstrably wide 'spread' of the graft mortality distribution in time, and with the total failure of any homograft so far reported upon to survive beyond a very limited period, suggest that a very large number of *distinct combinations* of antigens are concerned in the reaction provoked by the transplantation of foreign homologous skin. The temptation is to suppose that a very large number of distinct antigens come into operation, and by a further step in the analysis to argue that all the genes, or all the dominant genes of the organism are capable, under the appropriate circumstances, of determining an antigenic stimulus. Both guesses may be right, but critical evidence for them is wanting. 'One need not... assume the existence of very many different substances in the cells of one species, since even a moderate number of serologically defined characters would furnish a number of combinations (2^n for n independent factors) sufficient to explain the results [of grafting incompatibility]' (Landsteiner, 1936).

The primary object of the experiment described in this section was to attach a certain least number to the antigens which govern the skin grafting reactions of rabbits. (A subsidiary object was to contrast the behaviour of non-uniform with uniform populations of grafts; the latter having been dealt with in the preceding section of this paper.) The only assumptions made with regard to the antigenic basis of the reactions are (*a*) that it is indeed particulate and combinatorial in nature, and (*b*) that a skin homograft will fail if it contains at least one antigen that is not represented in the tissues of its recipient. The second assumption follows from the definition of the word 'antigen': if it is not true of some particular factor, then that factor is simply not a skin antigen.

The reasoning is as follows. Suppose that four distinct antigens are concerned: *A*, *B*, *C* and *D*. The maximum number of distinct combinations that may be determined by four antigens, no antigen being represented twice in any one, is $2^4 - 1 = 15$, and the types are

A, B, C, D; AB, AC, AD, BC, BD, CD; ABC, ABD, ACD, BCD; ABCD.

These combinations have been set out in four subgroups, each subgroup having the property that either member of any pair of combinations chosen from it contains *at least one antigen not present in the other member of the pair*. The largest of these will be called the 'principal subgroup': its members, where four antigens are concerned, are *AB*, *AC*, *AD*, *BC*, *BD*, *CD*. The existence of this principal subgroup makes it possible to attach a certain least number to the skin graft antigens. For suppose that six animals are so operated upon that each one receives a skin graft from each one of the others, making $6 \times 5 = 30$ distinct homografting operations in all. Such a procedure may be called a 'complete cross-grafting test with six animals'. If all the homografts fail to survive, one may reason that *at least* four antigens are in operation, because four antigens is the smallest number that will yield six mutually incompatible combinations.

The following table gives, for small values of the number n of antigens, the total number $(2^n - 1)$ of combinatorial types or 'skin groups', and the size of the principal subgroup of combinations which are incompatible one with another (^nC_r, where $r = \frac{1}{2}n$ when n is even and $(n/2) \pm \frac{1}{2}$ when n is odd):

No. of antigens, n	2	3	4	5	6	7	8
No. of distinct combinatorial types	3	7	15	31	63	127	255
No. of combinations in principal subgroup	2	3	6	10	20	35	70

In order to examine the possibility that (say) seven distinct antigens are in operation, it is not necessary to do a cross-grafting test with thirty-five animals. Twenty-one are necessary—one more than the number of combinations in the principal subgroup entered under the column heading for one antigen *less*. The reason is that six antigens are only *just* sufficient to account for the failure of a cross-grafting test between twenty animals. If the test fails with twenty-one animals, then the existence of one antigen more must be invoked. No extra information is gained by using in the test any number of animals greater than twenty-one and less than thirty-six. Twenty-one animals is, in the usual formula, necessary *and* sufficient to investigate the possibility that seven antigens are in play. It can be shown, moreover, that the 21st animal should be a donor to and a recipient from each one of the other twenty. (In theory, something a little short of this *complete* cross-grafting will suffice; but the elimination of a few of the 21×20 possible homograft pairs would only complicate the performance of the experiment.)

Inspection of the table shows that a cross-grafting test with the next significant number of animals above twenty-one, namely thirty-six, is on the threshold of the physically impossible. A demonstration of the existence of seven distinct antigens is the upper workable limit of experimental proof. Such a demonstration is provided below.

Technique of the experiment

The cross-grafting test makes use of no new experimental principle, save that the population of grafts borne by any one recipient is wholly non-uniform. Twenty-five animals were used. No more information is provided than with the use of twenty-one; but the extra four animals were treated as 'insurance', since it would be a disaster to have the experiment fail as a result of the death of one animal alone. Of these twenty-five animals, all were graft donors; but one, having had grafts cut from it, was judged to be in too poor a condition to receive them; a second died 2 days and a third

7 days after transplantation. The remaining twenty-two—one animal more than the theoretical requirement—lived until the conclusion of the experiment.

For receiving, sorting and storing the grafts, filter-papers were fitted into five large (150 mm.) and twenty-five small (95 mm.) pyrex Petri dishes. Each of the former were marked into five equal sectors, numbered 1–5 in the first dish, 6–10 in the second, and so on. Each of the smaller Petri dishes was marked into a square grid of twenty-five numbered cells. Before use, the filter papers were wetted with sterile Ringer's solution.

Two days having been allotted to clipping the outer aspect of the right thigh and shaving the skin over the right thoracic wall, twenty-four small (1944, p. 177) and one large graft were cut from the thigh of each animal in turn and placed in the appropriate numbered sector of one of the large Petri dishes. At the end of the day, the members of each uniform set of twenty-five grafts were distributed one to each small Petri dish, each graft occupying a numbered square of the grid. Any one small Petri dish therefore contained one distinct and identified graft from each one of the twenty-five donors. The grafts were stored in the refrigerator before planting. (Grafts will survive at least three weeks under these conditions; here, they were expected to survive not more than 24 hr.)

On the following day, the contents of each small Petri dish were transplanted in open style, and in definite and known arrangement, to each of the twenty-five donors in turn. In order not to complicate the interpretation of the grafts, the one large autograft 'control' was transplanted either to the margin of the raw area, or in fitted style to its own recipient area just outside. All the autografts healed perfectly: they will not be mentioned again. Dressings were applied in the usual way, viz. sulphadiazine, tulle gras, 2 yd. of 3 in. bandage, and ½ yd. of Cellona plaster-impregnated bandage.

Results of the experiment

The first examination was made on the 15th day after transplantation. With the exception of those individually mentioned below, all the $22 \times 24 = 528$ homografts showed the outward appearances of complete epithelial breakdown. Total breakdown is indicated with more than sufficient precision by the sum of the following characters, of which one or two are in themselves completely diagnostic:† swelling and induration of the graft; its dirty yellow or brown colour; the disengagement of the rim of the graft from the surface of the raw area; the total loss of the spread epithelium; the wet, loose condition of the epithelium of the graft roof, which can be scraped off in strips to discover a naked pitted collagenous pad below; and the fact that the dermal collagen so revealed contains only stagnant blood vessels, which do not bleed on cutting. Had any doubt arisen about the validity of these criteria, which have been founded upon a correlation between the naked-eye and histological appearances of some hundreds of distinct grafts, it could have been set at rest by the later inspection at the 20th day.

In twenty-three grafts, breakdown could not be judged complete. One graft, that transplanted from animal 19 to animal 1 (19 → 1), was not even markedly abnormal. The appearance of the remainder ranged between a condition representing the early stages of a specific reaction—'pneumatic' or soft swelling, with fresh (dark pink) internal haemorrhage, either diffuse or punctate, and a wet rather than shiny dry appearance in the spread epithelium—to a condition distinguished from total breakdown only by the partial resistance to stripping of the epithelium of the graft roof, the still reddish colour of the graft, and the fact that the graft edge was still confluent with the surface of the raw area. The surviving grafts were, individually, these:

4 → 1, 7 → 1, 13 → 1*, 15 → 1*, 19 → 1*;
8 → 2*;
7 → 9, 23 → 9;
18 → 11, 24 → 11;
1 → 12, 5 → 12*, 8 → 12*, 13 → 12*, 15 → 12*, 18 → 12*, 19 → 12*;
18 → 17, 22 → 17;
8 → 18, 22 → 18;
3 → 23*;
3 → 25*.

The grafts still surrounded by firm spread epithelium are marked with an asterisk. Animals 1 and 12 are evidently 'weak' recipients.

By the 20th day, all the grafts had broken down except 8 → 12* and 13 → 12*. These two had developed fine new hairs, which had pierced the surface. By the 24th day they in turn were found to have broken down; and nothing remained of the homograft population but a series of dry blackened necrotic scabs lying within an operation field that had contracted to one-quarter or less of its original area.

In accordance with the reasoning of the preamble, this not-unexpected but still astonishing result entitles one to draw a principal and a subsidiary conclusion. The principal conclusion is that *at least* seven independently combined antigens govern the grafting reactions of rabbits' skin; with the corollary, that a rabbit may belong to one of *at least* 127 skin transplantation groups. The subsidiary conclusion is that the homografts of a *non*-uniform population are very boldly distinguished by the varieties and intensities of the reactions which they elicit from the same recipient, notwithstanding the cumulative dosage-effect of the antigens which they doubtless share in common. This observation, contrasted with the complete similarity of the behaviour of grafts in a uniform population (pp. 163–5) suggests a method by means of which the antigenic composition of the different tissues of the same body may in the future be compared.

† It is degrees of *partial* breakdown which are so difficult to identify from naked-eye appearances.

Seven antigens are necessary to account for the transplantation reactions of rabbits in general, and sufficient to account for the results of grafting between the twenty-two considered here. That they are sufficient to account for the grafting reactions of rabbits in general is in the highest degree unlikely. If that were indeed the case, one would have been particularly lucky in choosing, from limited stock, twenty-two animals which were wholly incompatible one with another.

'FITTED GRAFTS' IN THE STUDY OF DOSAGE AND IMMUNITY

Dosage

The inverse relationship between the dosage of grafted skin and the time of its survival is not very boldly defined. Other things being equal, a single large graft will survive longer, but not much longer, than one of a uniform set of eight. One explanation for this must be that the outgrowth of thick proliferating epidermal epithelium from the grafts tends very rapidly to close the gap between their initial dosages. An animal to which a single small graft has been transplanted in 'open style' will, as a rule, carry more foreign epithelium on the 12th day than an animal grafted in high dosage carries on the first. The differences between the survival times of 'medium' and 'low' dosage homografts (1944, pp. 186–90)—though not between their degrees of differentiation—were for a similar reason inappreciable. The cumulative increase of graft dosage which the outgrowth of epithelium brings about has, moreover, the unfortunate effect of creating a progressively more severe bias against grafts which, had the dosage remained uniform, might have had a fairly long expectation of life. An open-style graft which has managed to survive until the 12th day has built up a high dosage of foreign epithelium *because* of its survival and outgrowth; and so it expedites its own destruction.

The *fitted graft* (p. 158) was designed to overcome these difficulties. A fitted graft is one which has been transplanted to a raw area only just large enough to receive it. It does not therefore grow by outward spread of epithelium. Contrary to expectation (1944, p. 181), the fitted graft is not immune from the general thickening and proliferation of the graft roof epithelium which begins by the 6th day of transplantation. The graft dosage does increase; but much less rapidly than with grafts of the same initial dosage transplanted in open style.†

† It has been pointed out (1944, pp. 180–1) that the throwing off of the graft's original hairs is a necessary consequence of the general proliferation of the graft roof and of the migratory and 'amoeboid' movements of the epithelium that precede it. Barker (1941) believes that the degree of preservation of these original hairs is a measure of the efficiency of grafting; of which belief it may be observed (*a*) that his first inspections were made 18 days after transplantation, by which time newly formed hairs have had time to mature and to pierce the graft roof; and (*b*) that the old hairs are very often trapped in a life-like position within their old follicles, which only microscopical investigation can show to be cystic and dedifferentiated.

The object of the experiments described in this sub-section is to reinforce the 'dosage theorem' by a second and final independent analysis: the survival time of fitted grafts will be compared with that of grafts in open style of the same *initial* dosage.

Unless the luck of random sampling tells strongly against the observer, a single fitted large graft (initial dosage 0·045–0·055 g. modal average weight) may be expected to survive until the 8th day of transplantation in the very great majority of trials. The record constructed from the experiments now to be described therefore begins with the 12th day. Forty distinct pairs of animals were operated upon, the recipient animal of each pair having a single large graft accurately fitted to the right or left thoracic walls. The grafts were removed from twenty of these animals, randomly chosen, on the 12th day; from ten on the 16th day; and from the remaining ten on the 20th day. Each graft was examined histologically, and the degree of survival of homograft epithelium duly put on record. Using the already defined notation for degrees of survival, the results were:

12 *days:* 202 (1); 205 (1); 297 (1); 305 (1); 306 (¼); 309 (1); 310 (0); 314 (1); 315 (1); 319 (0); 320 (1); 333 (1); 334 (1); 338 (1); 348 (0); 376 (0); 377 (¾); 378 (0); 380 (0); 381 (0). Thirteen grafts out of twenty show some degree of survival.

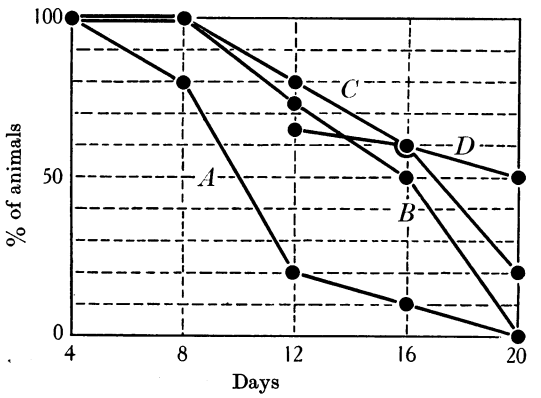
16 *days:* 212 (1); 220 (0); 221 (¼); 226 (0); 233 (1); 241 (0); 242 (0); 255 (¼); 256 (1); 269 (1). Six grafts out of ten show some degree of survival.

20 *days:* 195 (0); 199 (1); 200 (0); 203 (1); 204 (¾); 206 (¼); 207 (0); 208 (¼); 209 (0); 213 (0). Five grafts out of ten show some degree of survival.

In Text-fig. 1 the mortality distribution (*D*) is set in comparison with: (*A*) the mortality distribution for high-dosage homografts transplanted in open style (initial dosage 0·36–0·44 g. modal average weight: from the data of Medawar, 1944, pp. 185–6 and Text-fig. 4, II); (*B*) the newly enriched data for 'medium dosage' homografts transplanted in open style (initial dosage 0·045–0·055 g. modal average weight: from the data of the Summary Table, p. 173); and (*C*) the data for 'low-dosage' homografts transplanted in open style (initial dosage 0·006 g. mean weight: from the data of Medawar, 1944, pp. 186–9 and Text-fig. 6, I). In constructing this chart, a graft is judged a 'survivor'

if it carries *any* surviving epithelium, the grades ($\frac{1}{4}$), ($\frac{1}{2}$), ($\frac{3}{4}$), and (1) being assimilated for the purpose. The same principle was used in constructing Text-figs. 4–6 in Medawar (1944).

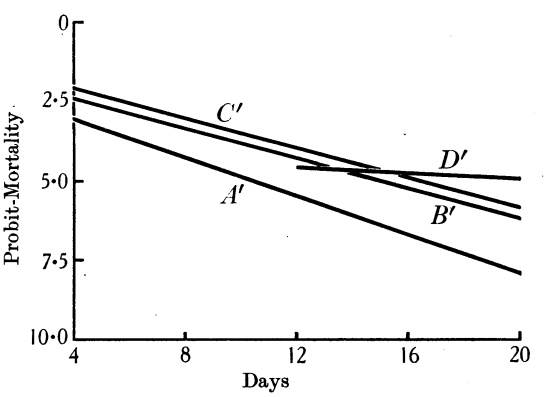
An examination of Text-fig. 1 shows that the disparity between the mortalities defined by curve (*A*) on the one hand and curves (*B*), (*C*) or (*D*) on the other is greatest at the 12th day. This fact may be made the basis of a preliminary comparison. To the data for the 12th day on curve (*A*) may be added the 23 independent 12-day high-dosage recipients entered in the Summary Table—all but three derived from the 'position' experiments earlier described. The ratio of survivors to non-survivors at the 12th day is 10 : 23 in the case of high dosage homografts and 11 : 4 in the case of



Text-fig. 1. The survival of homograft epithelium as a function of time: the chart constructed from the data set out in the Summary Table, p. 173. Each point defines that *percentage* of animals in each experimental group which still bears any fraction of surviving homograft epithelium at the recorded times. *A*, high-dosage graft bearers: computed from ten recipient animals, from each one of which a graft sample was removed at 4-day intervals from the 4th to the 20th day; *B*, bearers of medium-dosage homografts in open style: computed from forty recipient animals in independent groups of fifteen animals for the 12th day, ten for the 16th, and five each for the remainder; *C*, bearers of low-dosage homografts in open style: computed from twenty-five recipient animals in five independent groups of five; *D*, bearers of medium-dosage homografts in fitted style: computed from forty animals in independent groups of twenty for the 12th day and ten each for the 16th and 20th days.

medium-dosage homografts (*B*). A disparity of proportions as great as or more extreme than this would be expected to occur only once in 153 random drafts of this size from a population in which the proportions were in fact uniform. The sampling odds for the completely independent comparison between the 12-day readings for curves (*A*) and (*D*) are of the same order of magnitude: but the luck of sampling has made the proportion of survivors in the fitted grafts (*D*) rather smaller at the 12th day than in the open style grafts of the same initial dosage (*B*).

It may now be useful to make a specific comparison between curves (*B*) and (*D*). There is clearly nothing to choose between them either at the 12th or at the 16th days. Thereafter the curves diverge, (*D*) giving the higher proportion of survivors. This initial similarity followed by a later divergence could have been anticipated on theoretical grounds. The reaction to foreign skin epithelium is preceded by a latent period; and there is also a latent period before, in open style grafts, the dosage begins to increase in consequence of epidermal proliferation. Since the grafts defined by (*B*) and (*D*) are of the same initial dosage, and since the dosage of the former will not start to increase



Text-fig. 2. The mortality distributions defined by Text-fig. 1 *A–D*, cast into linear forms (*A′–D′*) by probit transformation. The median survival times entered in the Summary Table, p. 173, are defined by the points at which the ordinate 5 probits (=50% mortality) transects the transformed mortality curves.

before about the 6th day, we should not expect to find a striking difference between the two populations so early as the 12th. Beyond the 12th day, the outgrowth from open style grafts brings them into the high-dosage category; whereas the dosage of 'fitted' grafts will actually decrease a little, as a result of the typical retrograde thinning out of the superficial epidermis. The result is that an open-style graft which is 'non-antigenic' enough to survive 12 days will very soon build up a sufficient dosage to accelerate its own destruction. A fitted graft which lives to see the 12th day is very much more likely to live beyond it to see the 20th. The experimental results bear out this prediction in full. Five of ten fitted grafts bore some quantity of surviving epithelium at the 20th day; but not one of five open style grafts of the same initial dosage. Moreover, two of the five survivors in the former category are completely intact—199 (1) and 203 (1) (Pl. 4, figs. 19, 20); and their fully differentiated primary blood vessels are evidently still functional (Pl. 4, fig. 21). In both respects such behaviour is unparalleled by open-style grafts. Only one open-style

graft has been found to bear surviving epithelium at the 20th day; and then only a trace (see the Summary Table). These differences cannot be given quantitative expression, but they are significant none the less.

If mere survival or non-survival is to be made the basis of comparison between curves (*B*) and (*D*), they are best cast—by 'probit transformation' (1944, pp. 188–9)—into a form in which their mortality distribution is made linear (Text-fig. 2). Analysis shows that the *slopes* of the transformed and now linear mortality distributions (*B'*) and (*D'*) are indeed 'significantly' different: the mortality distributions are differently spread out in time. The disparity of the estimated median survival times—the times at which the grafts borne by 50% of the experimental animals have just broken down—is itself noteworthy: $20{\cdot}4 \pm 8{\cdot}0$ days for the fitted grafts (*D'*) and $15{\cdot}1 \pm 1{\cdot}0$ days for the open style grafts (*B'*) of the same initial dosage.

Unless the theoretical reasoning of the last paragraph but one is at fault, a case may be made for considering the 20-day reading from curve (*D*) apart from the 16-day and 12-day readings. The chances are about 20 : 1 against this 20-day reading, individually treated, being a member of the population defined by the mortality distribution of curve (*B'*) in Text-fig. 2 (following the 'direct χ^2 test' of Bliss, 1935*b*, p. 315: there are reasons for treating the population defined by curve (*B'*) as 'homogeneous' in his sense).

The various analyses that have been described constitute the second independent demonstration of the reality of the dosage phenomenon; and since, in sum, the hypothesis is made plausible by sampling odds of some thousands to one in its favour, no further demonstration should be required.

Immunity

The fitted graft has lent itself well to a third demonstration of the immunity phenomenon which is independent of its predecessors not merely with regard to the animals used for it, but with regard also to the nature of the experiments to which the animals were subjected. The two earlier tests were:

(*a*) comparisons *en bloc* between groups of data for 1st-set and 2nd-set grafts respectively, separately compiled by the technique of repetitive graft sampling (1944, pp. 190–3);

(*b*) the 'autohomograft' test (1944, p. 192) in which the degree of survival of 1st-set and 2nd-set grafts were compared by means of grafting them back to their original donors.

The new tests, based upon the material offered by the experiments described in the subsection on Dosage, make use not of block but of *paired* controls. Instead of comparing 2nd-set grafts as a whole with 1st-set grafts as a whole, each 2nd-set graft is compared, in a series of trials, with a 1st-set graft transplanted in the same manner and in the same dosage from the same donor to the same recipient. One may therefore determine with added precision whether 2nd-set grafts do or do not survive as long as their predecessors. It is upon this question that the genuineness of the immunity phenomenon turns.

In each test, a single large graft was transplanted in fitted style to the left thoracic wall of a randomly chosen recipient. After either 12 or 16 days, the graft was wholly extirpated. The animal was then turned upon its other side, and a second large graft from the same donor was fitted to the right thoracic wall. The graft of second planting—it is convenient sometimes to call it a '2nd-set graft' although the set contains but one member—was removed for examination in its turn, either on the 12th or on the 16th day of residence. The paired grafts were cut in the same block and mounted in series for examination side by side. Such a test was done on ten of the twenty 12-day animals and all ten of the 16-day animals reported upon in the preceding sub-section. Making use again of the standard notation for degree of survival, the results may be summarized as follows:

12-day exps.			16-day exps.		
Animal no.	1st set	2nd set	Animal no.	1st set	2nd set
305	1	0	212	1	0
306	¼	0	220	0	0
309	1	0	221	¼	0
310	0	0	226	0	0
319	0	0	233	1	0
320	1	1	241	0	0
348	0	0	242	0	0
377	¾	0	255	¼	0
378	0	0	256	1	0
380	0	0	269	1	0

Each 1st-set graft resembled the 2nd-set graft with which it was paired in the genetic relationship between its donor and recipient, in its local and systemic dosage, its relative position in the body, its size and manner of planting, and in all variable characters, significant or not significant, uniquely determined by the pair of animals under consideration. *Age* is included in this last category. The fact that donor and recipient were either 12 or 16 days older on the occasion of second planting obviously comes under the subheading 'Insignificant': see the data on pp. 161–3.

The 'null hypothesis' relating to grafts of second planting is that immune factors play no part in their breakdown; from which it follows that each 2nd-set graft should be effectively *uniform* with its predecessor (footnote, p. 164), and should therefore be distinguishable from it only by the written label attached at biopsy (Pl. 3, figs. 15–18 *a*, *b*). According to the null hypothesis, therefore, the expectation of survival in (say) the 16-day 2nd-set grafts is not represented by a *probability* that 0·6, 6 in 10, or 60% of the 2nd-set grafts will show some degree of survival, but by the certainty that six stated grafts

will survive in a particular manner and degree, and that four stated grafts will have broken down completely. The result actually found, not one survivor in ten 2nd-set grafts, is therefore the most complete refutation of the null hypothesis that the design of the experiment allows. (Even if the expectation of survival in the 2nd-set grafts *were* represented by a probability of 0·6, the sampling odds against finding no survivors in ten trials would be overwhelmingly high.)

The same considerations apply to the 12-day experiment, though as it happens the 10 (of 20) 12-day animals show a smaller proportion of survival in the 1st-set grafts than do the 16-day. It will be noted that one defaulter, 320 (1), appears in the 12-day 2nd-set group. Although the marking of survival cannot express the difference between them, the 1st-set and 2nd-set grafts were far from similar. All hyperplastic activity in the 2nd-set graft had been suppressed: it resembled a 4-day 1st-set homograft, before the onset of generalized hyperplasia. And whenever the state of preservation of a 2nd-set graft has allowed any such judgement to be made, a 2nd-set graft has never been found to exhibit proliferative activities, nor to be invaded by a dense primary population of native leucocytes. The descriptions of the earlier paper (1944, pp. 190–1) are thus fully confirmed.

It is possible to reason that at least some part of the difference between paired 1st-set and 2nd-set grafts is to be attributed to a disparity of dosage, the 2nd-set graft being the second of two carried by the recipient animal. It cannot, however, be a dosage effect in any ordinary sense, because the 1st-set graft was wholly extirpated before the grafting of the second. The disparity of dosage is in any case totally insufficient to account for the precocious breakdown of 2nd-set grafts, of which only one of twenty could have survived until the 16th day. That the dosage and the immunity phenomena are different aspects of the same thing cannot, however, be denied.

The two extreme possibilities of survival, viz. complete survival in the 1st-set graft with complete breakdown in the 2nd-set, and complete breakdown in both, are illustrated by Pl. 4, figs. 22*a*, *b* and 23*a*, *b* respectively.

It will be noted that breakdown has been longer complete in the 2nd-set graft illustrated by Pl. 4, fig. 23*b* than in that illustrated by fig. 22*b*, for the process of mid-level undermining has not gone very far in the latter. The distinction is not recognized in the marks allotted for degree of survival, '0' in both cases. The pair illustrated by figs. 23*a* and 23*b* illustrates even more clearly how precison must be sacrificed to the requirements of arbitrary marking. According to the marks allotted to them, the 1st-set and 2nd-set grafts borne by animal 242 are identical: foreign epithelium survived in neither, and both are accordingly marked '0'. But the 1st-set graft, fig. 23*a*, has only *just* broken down. One may see in it the still open spaces of the primary blood vessels, the twisted chromatic remains of the primary native population, and the still naked and indented surface of the dermal collagen. In the 2nd-set graft, on the contrary, breakdown is of such long standing that the process of overgrowth seen in fig. 22*b* to be beginning is here complete. Its degree of survival should more justly be represented by a negative number, by way of indicating that the process of breakdown is long since complete. In practice, it is virtually impossible to 'mark' finely separated degrees of breakdown in such a way as would carry conviction to anyone but the observer.

Specificity of the immune reaction (cf. 1944, pp. 193–4). Only ten of the twenty available 12-day animals with single fitted grafts were used for the group of experiments just described. The results obtained from a further five, in which the grafts of second planting came not from the original donor, but from a third animal, provide an interesting contrast:

12-day exps.

Animal no.	1st set	2nd set
315	1	½
333	1	0
334	1	1
376	0	0
381	0	½

Thus, when the 2nd-set graft came from the original donor, only one of ten survived until the 12th day (see above). In this experiment with changed donors, three out of five were found to bear surviving foreign epithelium. Notice in particular the result from animal 381: the 1st-set graft had broken down completely by the 12th day, whereas some fraction of the epithelium of the 2nd-set graft survived.

Since the grafts of 1st and 2nd planting were genetically different, the 'null hypothesis' that relates to immunity here takes a special form. Instead of demanding the survival in specified degree of some stated grafts, and the breakdown of others, one must postulate that the proportion of survivors in the 2nd-set group as a whole should be the same as the proportion in the 1st-set group as a whole; i.e. that grafts of second planting from a new donor are quite unaffected by, and therefore just as likely to survive until the 12th day, as grafts of first planting. The proportion, 3 : 5, is in fact the same in both cases. The experiment is not large enough to do more than reinforce the conclusion of Medawar (1944), namely, that an immune reaction does not *necessarily* extend with equal vigour towards a graft of 2nd-planting derived from a donor other than the original. The result from animal 381 is pretty well decisive by itself.

As the experiments described in this sub-section as a whole constitute the third independent demonstration of the 'immune phenomenon', there should be no need to investigate the immune state further, so far as the mere empirical fact of its existence is concerned.

A WORKING HYPOTHESIS OF THE IMMUNE REACTION

It is now possible to make a general statement about the variables which govern the behaviour of skin homografts in rabbits.

Skin homografts always heal securely during the course of an immunologically latent period; but they are not known to survive beyond a period of time of the order of weeks. The survival time of homologous grafted skin epithelium is a direct measure of the intensity of the reaction which ultimately leads to its destruction. It is the same for all homografts which have been transplanted in a single operating session from one donor to one recipient, irrespective of the positions of the grafts or of their local dosages in any position; but it varies

(*a*) with the *systemic* graft dosage: the more skin that is grafted, the more rapidly it breaks down; and

(*b*) with the rabbit's previous experience of grafting from a source having antigens in common with a new donor. Grafts of a second planting break down more rapidly than their predecessors. Above all, the time of survival varies with

(*c*) the antigenic relationship between donor and recipient; which, if it can be assumed to have a particulate and combinatorial basis, is under the control of at least seven antigens freely distributed among at least 127 skin transplantation groups.

Resistance to homologous grafted skin therefore belongs to the general category of actively acquired immune reactions. The greater part of what is known about such reactions has been learnt from the study of immune systems exhibiting a gross serological disparity between stimulant and responding tissue, and there is little likelihood that the homograft immune system, the most delicately differentiated of any that can be imagined, will fit into the bold and crude framework so defined. The following combination of experimental facts may therefore be appraised with an open mind:

(1) Homografts of a first planting—i.e. those borne by a non-immunized animal—never break down before the epidermal cells start dividing in the period of generalized hyperplasia that is characteristic of autograft and 1st-set homograft development (1944, p. 182).

(2) The breakdown of the epithelium of such grafts is never, from the standpoint of the cell population as a whole, an instantaneous, all-or-nothing reaction. The death of the cell population is spread out in time over a period which may reach, though it is not known to exceed, 4 days in length† (1944, pp. 184–5). Consequently, a homograft will very often be caught at biopsy with some of its cells alive and others dead. (See the tables on pp. 163, 165, 168; many illustrations in 1944 and herein; and the high proportion of only partial survivors given by the autohomograft test, 1944, p. 192.)

(3) Grafts of a second planting—i.e. those borne by an animal adequately immunized beforehand—never enter the period of generalized hyperplasia, and mitosis contributes nothing that is appreciable to their cellular activities (1944, pp. 191, 195; and p. 171 herein).

(4) On the other hand, it has been proved by tests which are hardly subject to error in this respect (1944, p. 192), that 2nd-set grafts are not destroyed within several days of planting. They survive just until the outburst of cellular proliferation would be expected, from the study of autografts and 1st-set homografts, to begin (1944, pp. 192, 195).

Item (2) suggests that there is a phase or period in some cycle of the activity of each epidermal cell at which alone it becomes susceptible to the action of an immune body. Items (3) and (4) suggest that this cycle is the mitotic-intermitotic cycle, and that an epidermal cell will not be destroyed until it begins to divide. Item (4) in particularly shows that the failure of mitosis in the epithelium of 2nd-set homografts is not just a non-specific consequence of an earlier cell death or debilitation, for the 'vegetative'—non-mitotic—activities of the cell are not obviously, or at least not rapidly affected. (One possible interpretation of (4) is that the condition of vegetative survival is made possible only by the latent period of the graft's vascularization. This cannot be so, because new blood vessels have riddled the grafts by the 4th day of transplantation, whereas the median survival time of 2nd-set homografts is 6 days. In any case, tissue-culture and a number of semi-*in vitro* tests yet to be reported upon have shown that this interpretation is untenable.)

There is therefore some ground for supposing that the antigen-antibody reaction is a nuclear event, in the cytological sense; and that its consequence is to upset the nucleus in such a way that cell division cannot be brought to completion. This interpretation may be wholly incorrect; but it represents a working hypothesis which lends itself without very great difficulty to experimental refutation or proof.

The appearance of mitotic figures (1944, Pl. 6, fig. 64) in tissue cultures of donor skin in immune serum suggests that the antibody is not present in serum itself.

Fully documented evidence for the complete suppression of mitosis in '2nd-set' grafts will be given in a later paper.

SUMMARY TABLE

This table summarizes the greater part of the data upon which the purely numerical analyses of this paper and its predecessor (1944) have been founded. 'Autohomografts'

† This spread-out in time of the process of dying has nothing to do with spread of the graft mortality distribution in time, which expresses a relationship between distinct pairs of animals; a sampling error, in fact; *or* with the dying of an individual epidermal cell, a process of which nothing is known. It is a property of the cell population of individual grafts, or of the cell population of the grafts of a uniform set.

	Days					Median survival time ± standard error (days)
	4	8	12	16	20	
1. High-dosage homografts, open style	**10** (10)	[**10** (8)]	[**10** (2)]	[**10** (1)]	[**10** (0)]	10·4±1·1
	1 (1)	**2** (0)	**23** (8)	**1** (0)	**1** (0)	
	a. ***10*** *(9)*	[***10*** *(1)*]	[***10*** *(0)*]*	[***10*** *(0)*]*	[***10*** *(0)*]*	*6·0±0·6*
	b. ***10*** *(7)*	[***10*** *(0)*]*	[***10*** *(0)*]*	[***10*** *(0)*]*	[***10*** *(0)*]*	
2. Medium-dosage homografts, open style	**5** (5)	**5** (5)	**15** (11)	**10** (5)	**5** (0)	15·1±1·0
3. Low-dosage homografts, open style	**5** (5)	**5** (5)	**5** (4)	**5** (3)	**5** (1)	16·5±1·5
4. Medium-dosage homografts, fitted style	—	—	**20** (13)	**10** (6)	**10** (5)	20·4±8·0
			[***10*** *(1)*]	[***10*** *(0)*]		
5. 'Young v. old recipients'	—	—	**12** (7)	**11** (2)	—	12·8±1·1
6. 'Young v. old donors'	—	—	—	**10** (1)	—	—

1. High-dosage homografts, open style. Cumulative initial dosage: 0·36–0·44 g. skin (eight large pinch grafts). Donor and recipient: 2–2½ kg., adult. *References:* for the ten animals studied by repetitive graft sampling, 1944, pp. 182–7; one independent reading for each day from the 'parallel' series, 1944, p. 182; twenty independent readings for the 12th day, herein pp. 163–5; together with two further animals not previously reported upon. Data for standard 2nd-set grafts (*a*), 1944, pp. 190–1; for local 2nd-set grafts (*b*), 1944, pp. 192–3.

2. Medium-dosage homografts, open style. Cumulative initial dosage: 0·045–0·055 g. skin (one large pinch graft). Donor and recipient: 2–2½ kg., adult. *References:* five readings for each day from 1944, pp. 186–90; of the ten extra readings for the 12th day, five come from pp. 162–3 herein, with five not previously reported upon. The five extra readings from the 16th day, also from pp. 162–3 herein.

3. Low-dosage homografts, open style. Cumulative initial dosage: 0·006 g. skin (one small pinch graft). Donor and recipient: 2–2½ kg., adult. *References:* all data from 1944, pp. 186–90.

4. Fitted medium-dosage homografts. *Non*-cumulative initial dosage: 0·045–0·055 g. skin (one large pinch graft): contrast (2). Donor and recipient: 2–2½ kg., adult. *References:* 1st-set grafts, pp. 168–9 herein; paired 2nd-set grafts, pp. 170–1.

5. 'Young v. old recipients.' Cumulative initial dosage: 0·006 g. (one small pinch graft). Donor: 2–2½ kg., adult. Recipient: 280–470 g. (Table 1), 2½–4½ weeks in age (Table 2). *References:* see (6).

6. 'Young v. old donors.' Cumulative initial epithelial dosage equivalent to 0·045–0·055 g. adult skin (one large pinch graft). Donor: as recipient under (5). Recipient: as donor under (5). *References:* pp. 161–3.

(1944, p. 192), 2nd-set homografts from changed donors (1944, pp. 193–4, and herein p. 171), and the results of the antigen test (pp. 165–8) are not included in it.

Each numerical entry in bold type defines the total number of recipient animals allotted to the experiment named at the beginning of its row for the day entered at the head of its column. The number which follows it (in brackets) represents the number of animals which still bore surviving foreign epithelium on that day. The entry for fitted homografts, row 4, on the 16th day is for example **10** (6), meaning that six of the ten animals grafted in that style still bore some fraction of surviving epithelium. The entries in *italics* refer to 2nd-set grafts carried by animals immunized beforehand by grafting in the manner and dosage indicated by the heading of the row in which the italicized readings are entered.

The entries in bold type, italicized or not, define separate and independent groups of animals, save where they are enclosed within square brackets. In row (1), enclosure within square brackets means that the animals which provided the stated number of 'readings' were also those which provided the readings entered beforehand in the same row; in other words, that the mortality distribution has been computed by repetitive graft sampling from the same group of animals. When both independent and dependent readings are available for the same day, the former are entered below the latter; but the median survival times (see below) are computed from the dependent data alone. In row (4), the italicized entries in square brackets define groups of animals which also provided readings for the 1st-set grafts entered immediately above them.

The readings marked with an asterisk have been determined by a legitimate process of extrapolation (see 1944, Text-fig. 4 and pp. 191, 193). Apart from these, every graft taken from every animal is represented by a complete histological record.

Grafts which have been transplanted to a raw area across which the epithelium arising from them can grow are said to be in 'open style'. Their dosage is cumulative. Grafts exactly fitted to recipient areas only just large enough to receive them are said to be in 'fitted style'. Their dosage is non-cumulative. The terms 'large' and 'small' graft have been defined by Medawar (1944, p. 177).

The *median survival time* is the estimated time at which the grafts borne by 50% of the experimental animals have *just* broken down. The estimates are based upon fuller or more finely subdivided data than those of Medawar (1944), which they accordingly supersede. The estimates in row (1) were computed by the method of Bliss (1937); the remainder by the method of Bliss (1935*a*).

SUMMARY

1. The systematic survey of the behaviour and fate of skin homografts in rabbits, begun by Medawar (1944), has been continued.

2. The collagenous remains of the dermis of a skin homograft are in due course wholly removed and replaced by new collagen fibres of native origin.

3. If the removal of foreign collagen is the consequence of a specific reaction, it implies either that homograft collagen is antigenic, or that it is capable of responding to the action of immune bodies generated by the cellular elements of the graft of which it was a part.

4. There are, however, reasons for supposing that the reaction is non-specific.

5. The power of resistance to skin homografts is fully developed in rabbits ranging between 2½ and 4½ weeks in age and between 280 and 470 g. in weight on the day of operation.

6. Skin from baby rabbits of the age- and weight-classes just defined elicits at least as strong a reaction as adult skin after transplantation to an adult recipient.

7. A population of grafts is said to be *uniform* if its members have been transplanted in one operating session from the same donor to the same recipient.

8. Grafts of a uniform population, whether they lie in the same operation field or are variously distributed about the body, break down simultaneously and in histologically identical fashion, irrespective of the positions they occupy, their local dosages in those positions, their sizes, numbers, or manners of grafting.

9. It is inferred that the homograft reaction is systemic in nature, and that the systemic component is the only significant component of graft dosage.

10. Grafts of a population non-uniform with regard to their donors break down at widely differing times, to the accompaniment of cellular reactions of very various intensities.

11. When the other variables which govern the homograft reaction have been given a constant value, the residual variation between the survival times of two distinct homografts is an expression of the antigenic relationship between donor and recipient of each pair.

12. The homograft reaction is governed by the operation of *at least* seven antigens freely combined among *at least* 127 skin transplantation groups.

13. A 'fitted' skin homograft is one transplanted to a raw area only just large enough to receive it. Since such grafts do not grow by outward spread and proliferation of epithelium, their initial graft dosage remains approximately constant.

14. Fitted grafts survive longer than grafts of the same initial dosage which have been transplanted in such a manner that their dosage increases cumulatively by epithelial outgrowth. The reality of the 'dosage phenomenon' is thus for the second time independently demonstrated.

15. Strictly paired tests show with a high degree of precision that a second fitted graft, transplanted from the same donor to the opposite side of the same recipient 12 or 16 days after the transplantation of a first, undergoes a highly accelerated retrogression. The reality of the 'immune phenomenon' is thus for the third time independently demonstrated.

16. The immune reaction so generated does not necessarily extend with equal vigour to a graft of 2nd planting derived from a donor other than that which provided the graft of first planting.

17. The median survival times, with their standard errors, of grafts of various dosages and in various immune environments are set out in a comprehensive Summary Table. The data upon which these estimates are founded amplify or further subdivide the data of Medawar (1944), which they accordingly supersede.

18. The following working hypothesis of the homograft reaction has been founded upon a new general survey of the available evidence: the antibody generated by skin homografts is such that it specifically prevents the completion of nuclear division in the cells of homologous grafted skin.

I again wish to thank Dr Leonard Colebrook particularly for his advice and encouragement in all stages of this work. The work has been done in association with the Department of Plastic Surgery, Oxford University (Prof. T. P. Kilner), which has made a grant towards the expenses of research apparatus.

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EXPLANATION OF PLATES

All the photographed sections have been stained with Ehrlich's haematoxylin, aqueous orange G, and alcoholic eosin, save where other stains are specifically mentioned. The reference letters and numbers (e.g. 12 A) relate to the key-figure, 1944, p. 197. The key is applicable to figs. 7–9, 11, and 23*b* when allowance is made for the fact that in these specimens the epidermal epithelium which bounds the homograft dermis above is of *native* origin.

Plate 1. The ultimate fate of homograft dermal collagen

Figs. 1–3. Illustrating the highest level of development of the secondary blood vessels and secondary native population in the dermis of a homograft which has been overgrown by native epithelium. The capillaries are widely and irregularly dilated and follow a characteristically tortuous pathway. No trace of the primary vessels remains. The nuclei of a high proportion of the secondary population are pyknotic. Notice that in addition to the cells which have presumably entered the dermis by passage through the vessel walls, there are a fair number of 'independent' fibroblasts. (2 A.) Fig. 1 ×73; figs. 2, 3 ×165.

Figs. 4–6. The early stages of the internal replacement of old homograft dermal collagen by fine new fibres of native origin. Notice the three-dimensional and now very 'open' packing of the stout, dark-staining old fibres. Fine new fibres now occupy the spaces between them. Fig. 4 includes the superficial part of the newly collagenized graft bed: in the dermis above it, somewhat to the left side, may be seen the open space formerly occupied by a dilated primary blood vessel. Haematoxylin and picrofuchsin. Fig. 4 (23 A) ×73; figs. 5, 6 (2 A) ×165.

Fig. 7. The earliest stages of the overgrowth of homograft dermal collagen by native epithelium. The epidermis throws down hair primordia into the underlying foreign dermis; but these do not as a rule mature. (1 A) ×52.

Fig. 8. To illustrate the clear oedematous band that develops between native epithelium and the homograft dermis which it has been induced to overgrow by the first of the three operative procedures described in the text. Fibroblasts have already made their appearance in the oedematous band. See fig. 9 for a later stage in its development. (1 A) ×52.

Fig. 9. A development of the condition illustrated by fig. 8: about 25 days after the completion of overgrowth by native epithelium. The homograft collagen which in fig. 8 is seen to occupy the entire sub-epidermal zone is here reduced to a tract of coarse, dark-staining and widely separated individual fibres. Fine new collagen fibres of native origin occupy the spaces between them (cf. figs. 4–6). Between this tract of old fibres and the native epidermis is the collagenized zone which develops from the condition illustrated by fig. 8 (12 A) ×58.

Fig. 10. The very last stages in the removal of homograft dermal collagen: a development of the process seen in figs. 4–6 to be beginning and in fig. 9 to be more than half complete. About 40 days after the completion of overgrowth. It is not absolutely certain that the coarse collagen fibres here shown are truly of foreign origin. Their size, staining properties, orientation and position suggest that they are. If they are not, then the homograft collagen has disappeared completely; for no other fibres of this type are to be found. Masson. (2 A) ×165.

Fig. 11. Showing the manner in which homograft collagen disappears when native epithelium has been induced to grow over it by the third of the three operative procedures described in the text. About 20 days after complete overgrowth: for an earlier stage, see fig. 23*b*. The epidermis, with a smooth lower surface, lies immediately over the band of still compact foreign collagen, which is in turn underlain by a zone of very vigorous fibroblastic proliferation. Notice that the old collagen fibres are being replaced not internally, but at the marginal surface alone. (123 A) ×95.

Plate 2. The significance of age

Three groups of photographs, each one of a distinct experiment, illustrating the reactions to skin homografts of adult and baby rabbits. The animals within each group received skin in the equal initial dosages from the same donor; see Table 4 in the text. All ×18, 12 A or 123 A.

Fig. 12 (Exp. 7). (*a*) The adult control, 286 ($\frac{3}{4}$). Breakdown is just beginning with the maceration of the deep follicular epithelium. Notice the grossly oedematous condition of the dermal papillae, and the rather densely packed cells of the primary population of the dermis, the majority with pyknotic nuclei. The cracks in the dermis are artificial. (*b*) First baby recipient, 287 (1). A specific cellular reaction is evident, but breakdown has not begun. (*c*) Second baby recipient, 288 ($\frac{1}{4}$). A 'trace' survival of deep follicular epithelium, which is not obvious from the photograph. The roofing epithelium has clearly disengaged. (*d*) Third baby recipient, 289 (0), showing the typical picture of total breakdown. The graft dermis is bare even of epithelial remains.

Fig. 13 (Exp. 8). (*a*) The adult control, 291 (0): total breakdown of the violent type. (*b*) First baby recipient, 292 (1). The graft has reached a critical stage, but although some of the cell nuclei of the roofing epithelium are pyknotic, breakdown cannot yet be said to be in progress. (*c*) Second baby recipient, 293 (0). Total breakdown of the violent type.

Fig. 14 (Exp. 10). (*a*) The adult control, 327 ($\frac{1}{2}$). Breakdown is only about half complete, for the greater part of the mature follicular epithelium survives. (*b*) First baby recipient, 328 ($\frac{3}{4}$): breakdown in its early stages. (The photograph gives the impression of complete survival.) (*c*) Second baby recipient, 329 (0): total breakdown.

Plate 3. The significance of position

Four 'uniform' (see text) pairs of homografts illustrating conditions which range from complete survival to complete

breakdown. The members of each pair can be distinguished only on completely trivial grounds, e.g. the plane of the section in relation to the direction of slope of the hair follicles. One member of each pair is nevertheless in local high dosage, the other in local low dosage. A key to their identities is given at the foot of the explanations of Pl. 4.

The graft in local high dosage is in all cases a member of a population of seven grafted in open style to the right thoracic wall. The biopsy specimens are in all cases of 12-day grafts. All 1 A ×32.

Fig. 15 *a*, *b*. Animal 304 (1). The graft in local low dosage was fitted to the left thoracic wall. Generalized hyperplasia.

Fig. 16 *a*, *b*. Animal 349 (¼). The graft in local low dosage was fitted to the left shank. Trace survival of mature follicular epithelium.

Fig. 17 *a*, *b*. Animal 341 (¼). As fig. 16 *a*, *b*, except that there is here slight difference in the structure of the graft roof, faithfully reproduced in both members of the pair.

Fig. 18 *a*, *b*. Animal 295 (0). The graft in local low dosage was fitted to the left thoracic wall. Total breakdown.

PLATE 4. The use of fitted grafts in the study of dosage and immunity

Fig. 19. Animal 199 (1). A 20-day fitted homograft showing complete survival of homograft epithelium, with maturation of new hairs. Notice the fascial organization of the connective tissue of the graft bed, which is undistended by oedema fluid. (123 A) ×30.

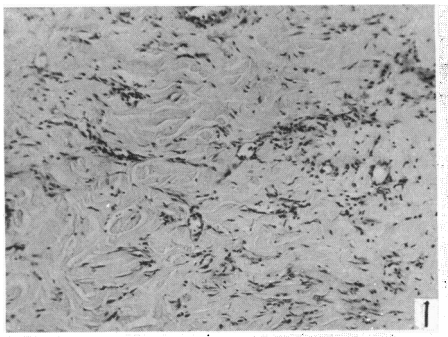
Fig. 20. An enlarged reproduction of the roof of the graft illustrated by fig. 19, showing that specific degenerative changes have in fact just begun. The dermal papillae are oedematous, and the primary native population (many members of which show pyknotic nuclei) is moderately dense. The lymphatic capillaries (*L*) are somewhat dilated and contain degenerate leucocytes. There are also abnormalities in the cells of the roof epithelium. (1 A) ×95.

Fig. 21. Animal 203 (1). A group comprising artery, vein, and lymphatic, in the lower reaches of the dermis of a 20-day fitted homograft. The vessels belong to the primary system of the graft, which is thus shown to be fully differentiated and functional. Pathological changes are slight. (2 A) ×200.

Fig. 22. Animal 212. (*a*) A 16-day fitted homograft showing complete survival (1) of epithelium. Any appearance of pyknosis or lysis in the epidermal nuclei is a photographic artefact. (*b*) The 16-day graft of second planting from the same donor to the same recipient. Breakdown (0) is of such long standing that native epithelium is well advanced in the process of undermining the necrotic superficial layers of the dermis. (12 A) ×35.

Fig. 23. Animal 242. (*a*) A 16-day fitted homograft showing complete breakdown (0); evidently of recent origin, since the roof has not begun to be undermined by native epithelium, the spaces formerly occupied by the primary vessels are still well defined, and the secondary system of vessels is little developed. (12 A.) (*b*) The 16-day graft of second planting from the same donor to the same recipient. Although still awarded the mark '0', breakdown is of such long standing that the process illustrated by fig. 22*b* has now passed to completion, and the lower reaches of the homograft dermis are fully roofed by native epithelium. Notice the beginnings of the fibroblastic reaction in the graft bed (cf. Pl. 1, fig. 11). (123 A) ×35.

In Pl 3, the right-hand member (*b*) of each pair is the graft in local low dosage.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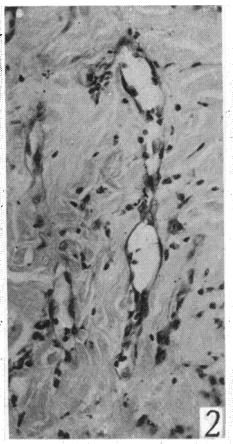
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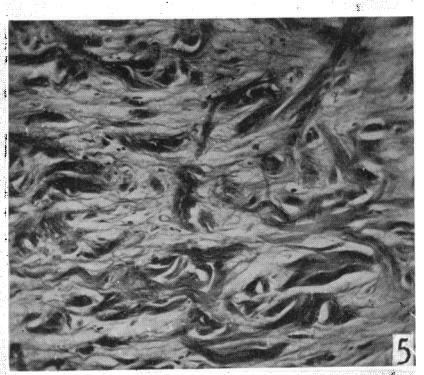
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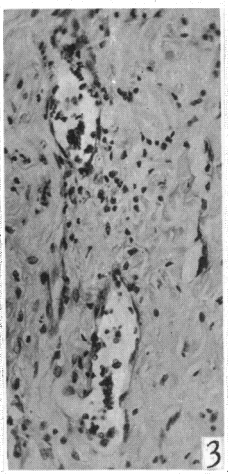
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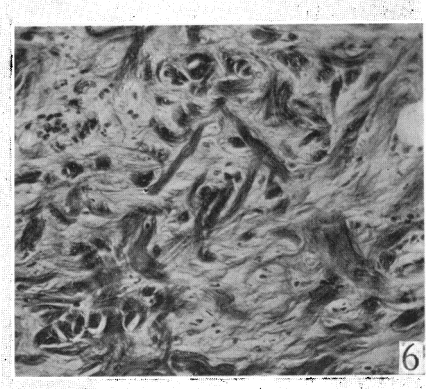
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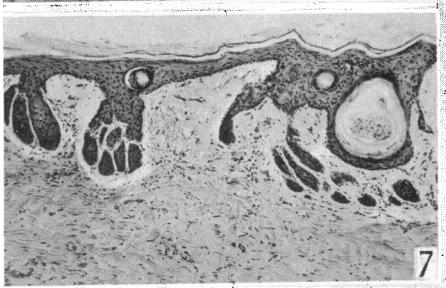
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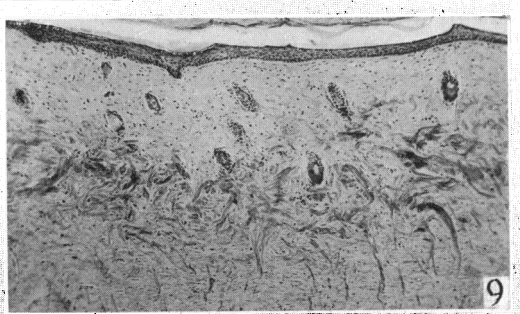
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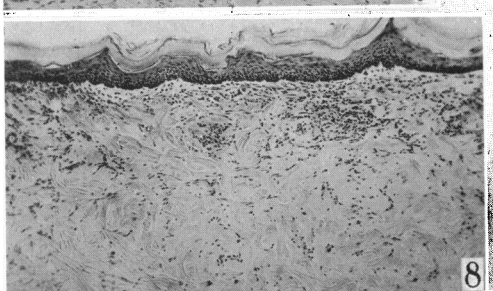
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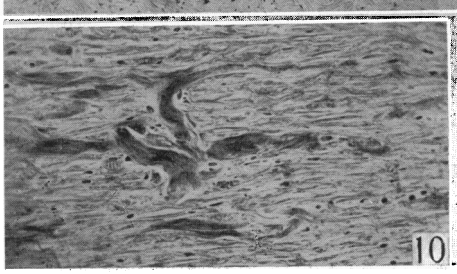
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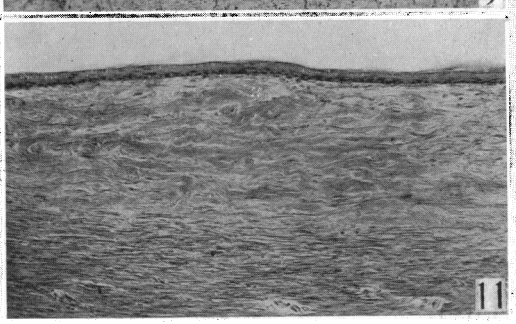
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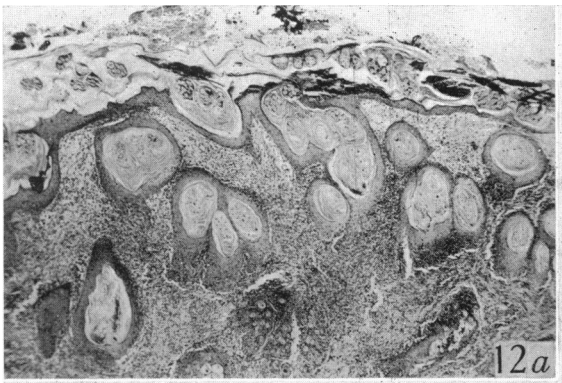
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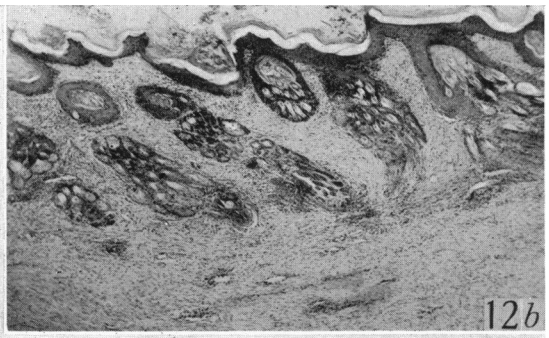
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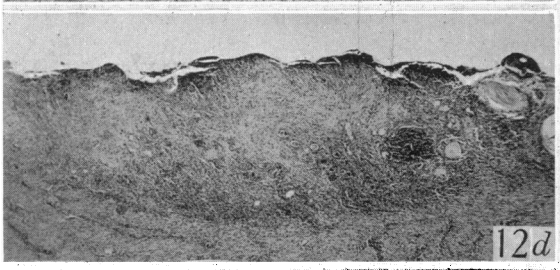
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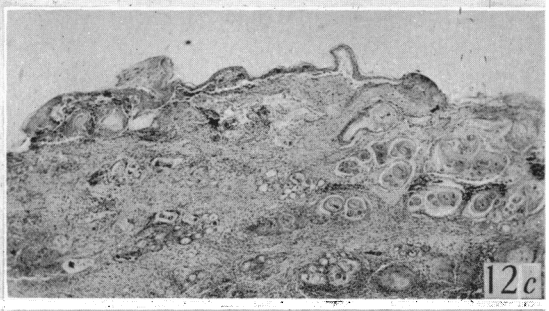
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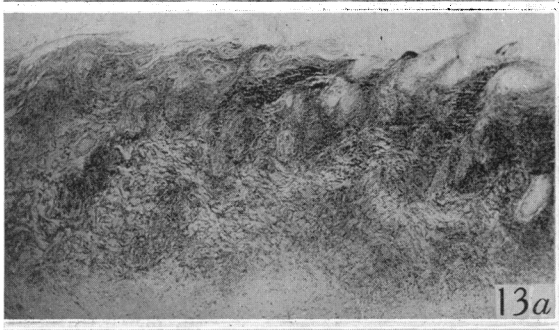
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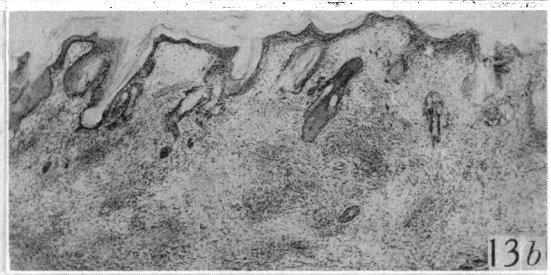
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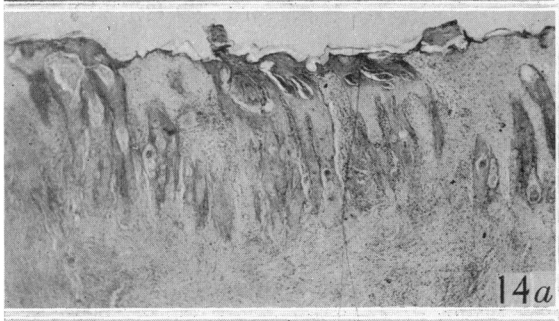
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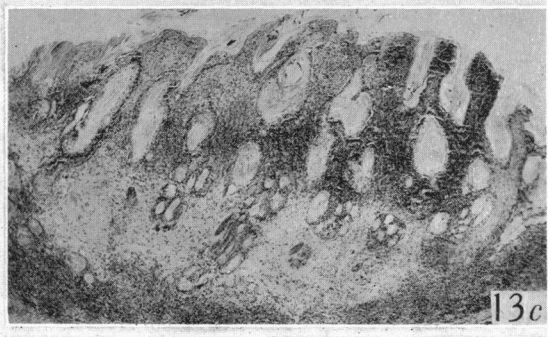
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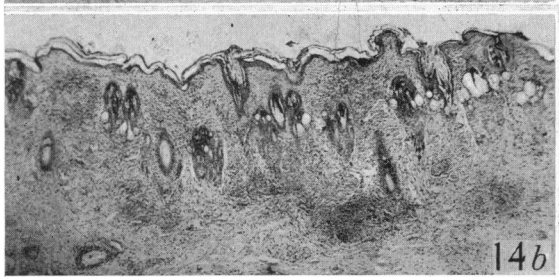
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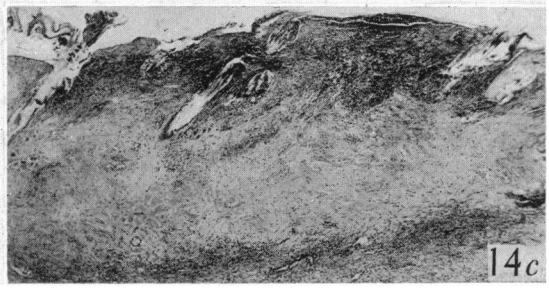
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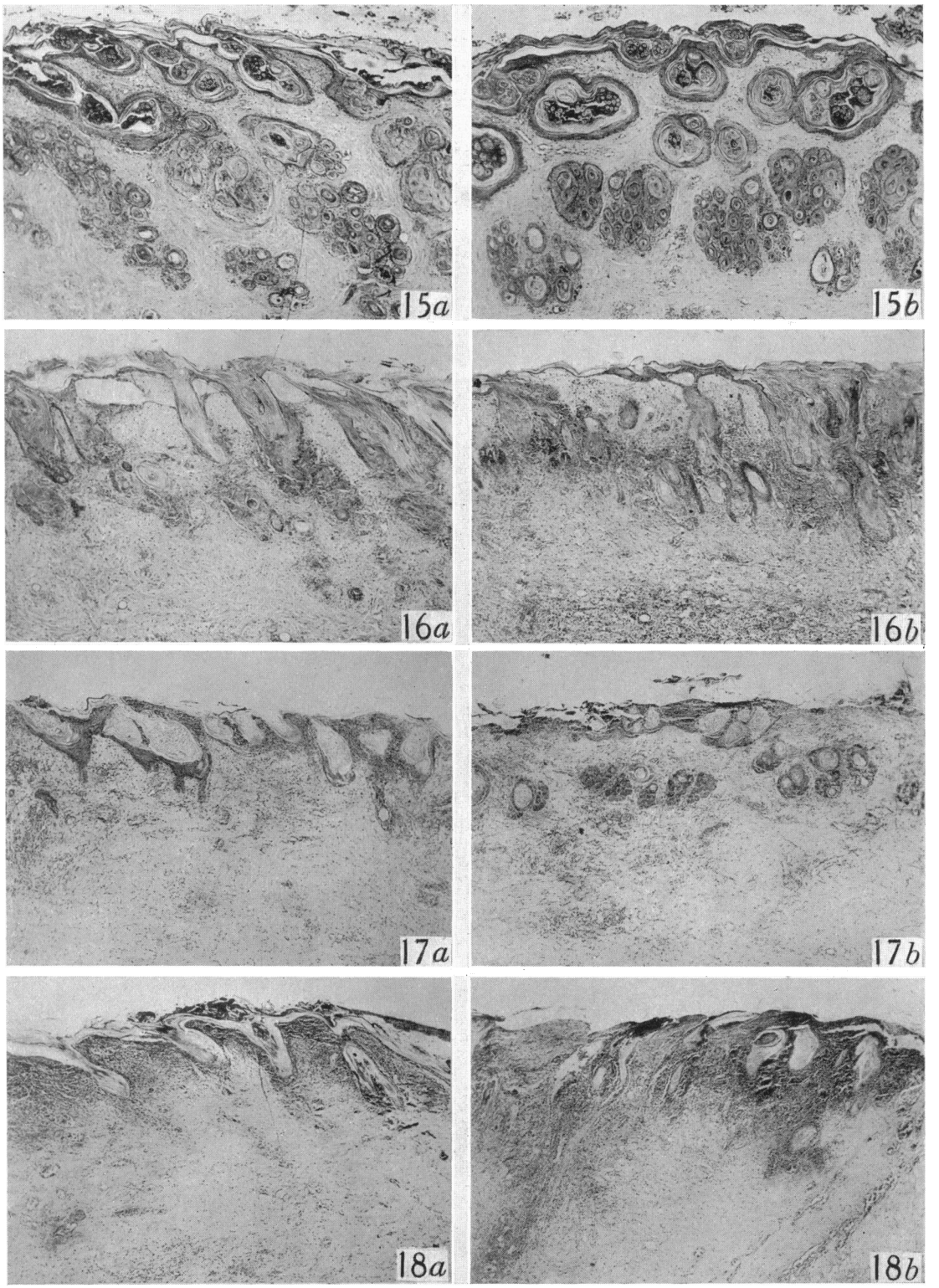
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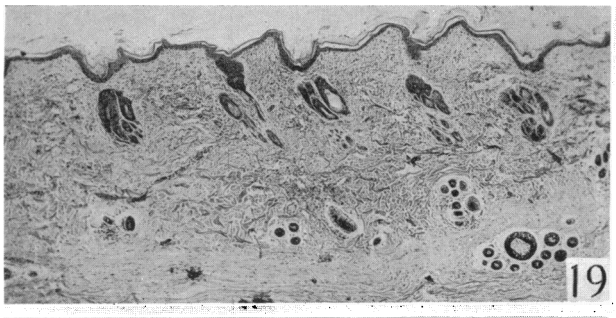
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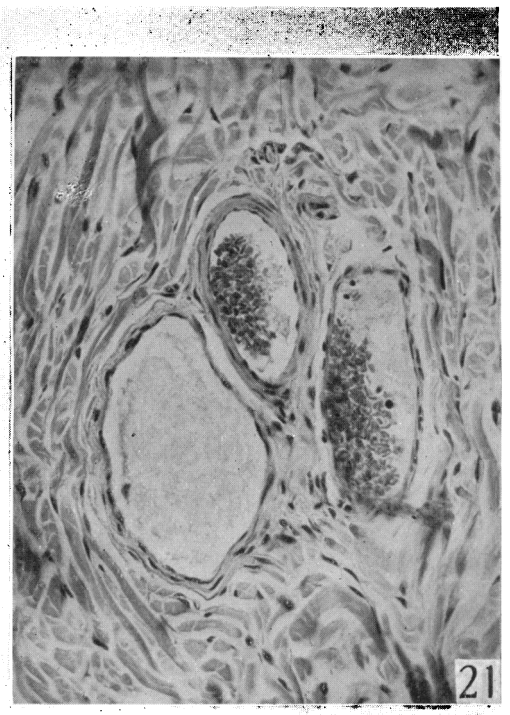
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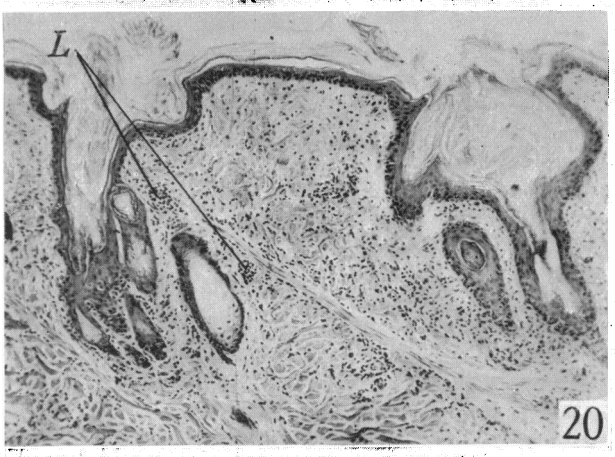
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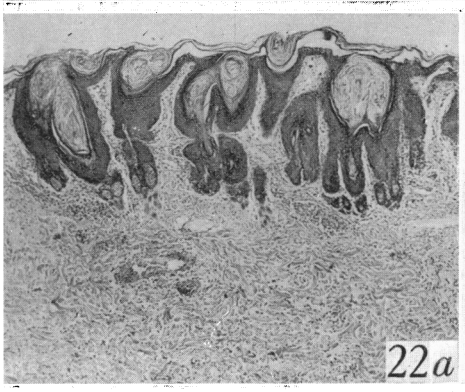
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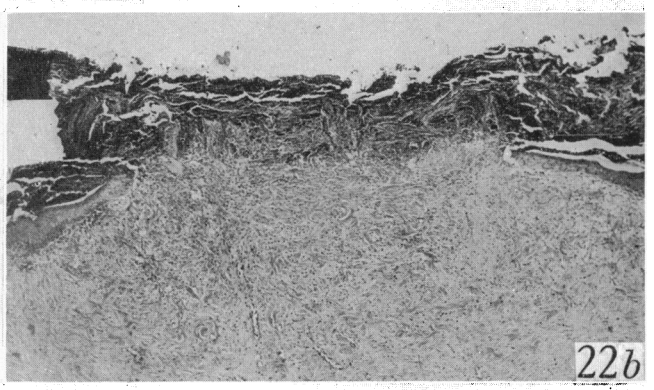
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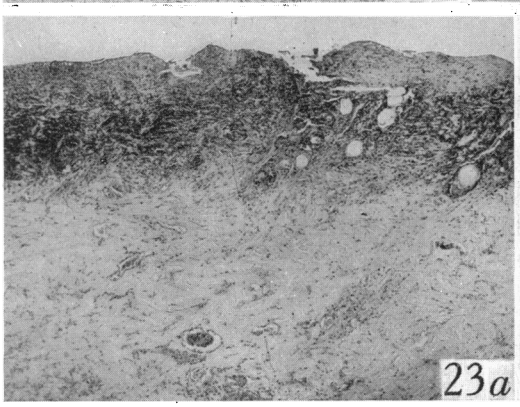
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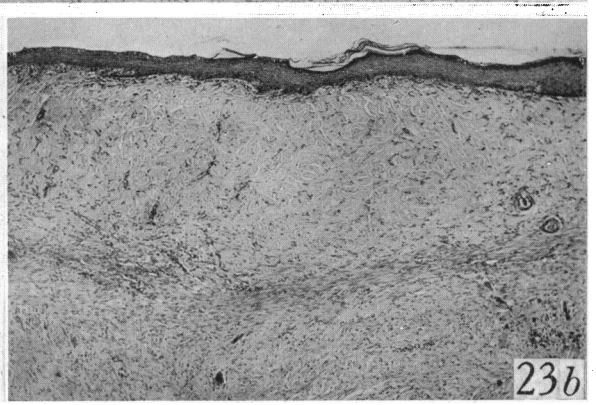
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