STUDIES ON THE NATURE OF IMMUNITY TO HOMOLOGOUS GRAFTED SKIN, WITH SPECIAL REFERENCE TO THE USE OF PURE EPIDERMAL GRAFTS

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(With Plates 1 and 2)

INTRODUCTION

It is now generally accepted that skin does not long survive orthotopic transplantation between animals of ordinary genetic diversity; skin homografts soon break down and slough off as a consequence of an actively acquired immunity reaction on the part of the host (Medawar, 1944, 1945; Dempster, 1951). So far, all attempts to demonstrate antibodies to skin homografts by serological methods have been unsuccessful, and likewise the careful in vitro tests designed to reveal the existence of a specific cytotoxic activity in serum or tissue fluids against explants of the donor's skin (see Medawar, 1948a; Allgöwer, Blocker & Engley, 1952) have been negative. Recently, techniques have been devised for the preparation of viable pure epidermal 'grafts' comprising the superficial epidermis completely free from all dermal elements and, more particularly, of epidermal cell suspensions (Billingham & Medawar, 1951; Billingham & Reynolds, 1952). The present study represents an application of these methods to determine, firstly, whether homografts of pure epidermis can elicit and succumb to a transplantation immunity or homograft reaction like ordinary skin homografts (Part I), and secondly, as this was found to be the case, to make use of pure epidermal grafts, especially suspensions of isolated epidermal cells, in in vitro tests carried out in an attempt to demonstrate a specific cytotoxic activity towards a donor's cells in the blood, serum, or other derivatives from animals which had previously reacted against homologous skin from that donor (Part II). It was hoped that by using these pure epidermal preparations the tests would be made more sensitive since the generative cells of the basal or Malpighian layer would be exposed directly to the medium under test. In previous studies of this type, where thin shavings of skin have been used, a layer of mesenchymal tissue has been unavoidably interposed between the cells whose viability was being challenged and the medium whose specific activity was under examination. A shortcoming common to all in vitro tests so far described has been that the cells under test were in no sense isolated; thus many cells were not completely exposed to the medium.

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MATERIAL AND METHODS

Adult male rabbits and guinea-pigs from dealers' mixed stocks were used throughout. In all experiments involving rabbits the paired donors and recipients were deliberately selected from different breeds whenever possible, in the hope of achieving the greatest possible genetic diversity.

Preparation of pure epidermal grafts. Viable sheets of pure epidermis and epidermal cell suspensions were prepared by a trypsin digestion process and handled by techniques fully described elsewhere (Billingham & Medawar, 1951; Billingham & Reynolds, 1952).

Immunization of recipients. Each test made use of an independent pair of animals, a donor (D) and a recipient (R). The initial immunizing operation as used for rabbits was essentially that described by Medawar (1944, 1945, 1948a) except that in the present study ear skin has been used in preference to the somewhat thicker skin of the flank. Nine pinch grafts—thin disks of skin approximately 9 mm. in diameter and comprising the epidermis and the full thickness of the corium—were cut from the shaved skin of D's ear and transplanted to separate recipient areas of appropriate size cut in the shaved skin of the right side of R's chest. When the outward appearance of these homografts was such that breakdown was undoubtedly complete and of long standing—but in no case sooner than the 15th day after grafting —this immunization operation was repeated in the hope that the immunity elicited by the first set of homografts would be augmented; a further set of nine ear-skin homografts from D being transplanted to the unoperated left side of R's chest. Not until at least 12 days had elapsed since it had received the second set of immunizing grafts was an animal used for experiment. Breakdown of these grafts was by then of long standing. Since there is as yet no information as to the duration of immunity against normal tissue homografts, care was taken not to allow more than 20 days to elapse after transplantation of the final immunizing grafts before carrying out a test.

The immunizing procedure for the guinea-pig was essentially as described for the rabbit, except that the grafts were cut from the shaved skin of the donor's flank. In the majority of cases two successive sets of homografts were transplanted as for the rabbit.

Details of the various operative procedures have been fully described elsewhere (Billingham & Medawar, 1951) and will be presented here only in outline.

PART I. STUDIES ON THE FATE OF PURE EPIDERMAL HOMOGRAFTS IN RABBITS

(a) The fate of grafts transplanted to non-immunized recipients

The experiments to be described in this section comprise seven independent tests, each making use of a paired donor and recipient. The superficial epidermis was isolated as an intact sheet by the trypsin method from each of four to six rectangular shavings of skin, about 1.25 x 0.75 cm. in lengths of side, cut from the dorsum of D's ear. These 'pure epidermal homografts' were then spaced out over a rectangular full-thickness recipient area—about 6 x 7 cm. in lengths of side—prepared on the
side of R's chest by stripping the skin down to the level of the vascular fascial plane that overlies the panniculus carnosus muscle. These grafts were thus transplanted in 'open style'; that is, in such a manner that each could enlarge its epithelial surface by outgrowth over the raw area separating it from its neighbour, or from the margin of the operation field.

The grafts were first examined on the 9th day post-operatively, and thereafter at 2-day intervals when biopsy specimens, which included the epithelium and some of the bed underlying it, were taken as necessary. After fixation in Formol-mercuric chloride paraffin sections were cut at 10μ and stained with Ehrlich's haematoxylin and eosin.

Results

At the first inspection, on the 9th day post-operatively, the epidermal homografts were firmly united to the underlying graft bed in all animals, and had proliferated to such an extent that a thin dry cuticular sheet could be peeled away from the surface to reveal the pinkish white, waxy surface of healthy, viable epidermis. As a consequence of the migratory outgrowth of epithelium over the developing granulation tissue, the grafts had extended considerably in area (Table 1, column 3), and had frequently lost their identity as a result of the confluence of outgrowing epithelium from adjacent grafts to produce a single epithelial sheet (Pl. 1, fig. 1). Only in one animal at this stage (Sr-110) did the appearance of the pure epidermal homografts differ significantly from that of pure epidermal autografts which Billingham & Reynolds (1952) have described in detail. In this animal the histological appearance of the biopsy specimens showed that breakdown of the grafts was far advanced.

Subsequent inspections established that this apparently healthy hyperplastic epithelium did not survive for very long. Soon its delicate pink colour deepened, becoming reddish then purple, indicating inflammatory changes in the graft bed beneath, and culminating in vascular congestion. The texture of the epithelium became increasingly delicate as it failed to make good the layers of dry cuticle which could be easily peeled off from its surface, and its attachment to the graft bed progressively weakened so that it tended to come away at inspections with the immediate graft dressings. Finally, only the smooth moist surface of the granulation tissue, which had formed the graft bed, remained on an area which a day or so previously had been surfaced by an extensive and apparently robust sheet of homologous epithelium. This sequence of changes in the outward appearance of the epidermal homografts was very constant, the only variable being the time of onset of the sequence of changes leading to the loss of the epithelium which was of the order of a few days.

Histological examination of biopsy specimens removed at 2-day intervals, commencing on the 9th day, indicated that after primary healing the grafted epithelium went through a phase of violent hyperplasia, in the course of which its thickness increased many-fold and a multi-layered cuticle was formed. Though transplanted initially to the vascular fascial layer that overlies the panniculus carnosus muscle, the grafted epidermis became separated from it by the progressive building up of an interposed layer of granulation tissue—highly vascular tissue in which young
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collagen fibres differentiated (Pl. 1, fig. 2). By the 9th day, in some animals, the immediate graft bed had already been infiltrated by leucocytes, mainly lymphocytes. This infiltration was rapidly followed by dilatation of the numerous small vessels and capillaries, and distension of the young collagenous fibres by oedema fluid. There were widespread superficial haemorrhages and eventually breakdown of the vessel walls and of the native leucocytes. In other animals the sequence of events was delayed in onset. Cellular erosion took place along the interface where the Malpighian cells of the grafts abutted on to the graft bed so that this interface became obscured and lost its former histological distinctiveness. The epidermal cells became vacuolated in appearance and lost the characteristic basiphilia of their cytoplasm which became eosinophilic. Finally, as a consequence of a process of blistering, the entire necrotic remains of the epithelium became separated from the graft bed. This sequence of changes is illustrated in Pl. 1, figs. 3–5.

Though completion of the breakdown process of these grafts was conspicuous to the naked eye, the accurate timing of their survival was based on the microscopical evidence provided by the biopsy specimens. A 2-day serial sampling interval was adopted, and the chosen measure of ‘survival time’ (see Table 1,

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Maximum area of homologous epithelium (cm.²)</th>
<th>Homograft survival time (days)</th>
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<td>Sr-189</td>
<td>Sr-201</td>
<td>14.0</td>
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Median survival time 13.4 ± 1.4 days.

Table 1. The survival of pure epidermal homografts transplanted to raw areas prepared in the skin of the side of rabbits’ chests

column 4) was taken as the time at which breakdown of the homologous epithelium was just complete (Medawar, 1944). The results, summarized in Table 1, show that the transplanted epithelium survived for at least 10 days in all animals, but in none of them did it survive beyond the 15th day. The median survival time of the pure epidermal homografts as computed from the data in Table 1 is 13.4 ± 1.4 days.

For full thickness skin homografts exchanged between rabbits of a randomly chosen heterogeneous stock in a very much higher dosage (both on the basis of the aggregate area of their superficial epithelium, and of their weight) Medawar (1944) gave a median survival time of 10.4 ± 1.1 days. This figure compares not unfavourably with the results of the present investigation, and suggests very strongly therefore that the epidermal component of a skin homograft plays a more important role than the dermis in eliciting immunity.
(b) The fate of grafts transplanted to immune recipients

Full thickness skin homografts, even when transplanted to specifically immunized recipients, do not undergo immediate destruction, although their breakdown is greatly accelerated. As Medawar (1944) has shown, the median survival time of such grafts in rabbits is about 6 days. It has been suggested that this delay in the fulfillment of the immune state is due to the time taken for blood vessels in the graft bed, conveying the presumptive cytotoxic agent or antibody, to penetrate the dermal portion of the graft and so gain access to its epithelial component. If this is so, then one might reasonably anticipate that pure epidermal homografts transplanted to immune animals would be acted against almost at once, since their Malpighian cells are immediately exposed to copious fluid exudates containing the presumptive antibody or cytotoxin. Moreover, a very rich capillary bed rapidly develops beneath these grafts.

Our intention was to transplant a series of small rectangular sheets of epidermis, 4-5 mm. in length side, to a standard rectangular full-thickness bed prepared in the skin of the side of an immunized recipient's chest, and then to remove a pair of these grafts each day, commencing on the 2nd day after transplantation. One of these was for histological examination, and the other was to be grafted back to the original donor as a biological test of its continued viability. Unfortunately, preliminary trials indicated that pure epidermal homografts prepared from the skin of the donor's ear, lacking a robust cuticle, were far too delicate for this purpose, which necessarily involved repeated inspections commencing soon after operation. This difficulty was overcome by first transplanting a series of pure epidermal grafts prepared from the skin of D's ear to a standard operation field cut in the skin of its own chest. By about the 15th-17th day after operation these grafts had given rise to a continuous sheet of heavily keratinized epithelium covering the central area of the operation field. From this a series of suitable pure epidermal grafts was easily obtained by defining rectangles of appropriate size by shallow incisions with a no. 15 scalpel, and then simply peeling the epidermis free from its bed with fine forceps. It may be added that although initially this epithelium was firmly united to its bed, and showed characteristic rete peg-like re-entrants into it, the union soon became very weak and the interface between the two tissues quite even, thus allowing the epidermis to be stripped off completely free from mesenchymal elements (Pl. 1, fig. 6).

In the majority of the present series of tests, instead of using paired donors and recipients, pairs of recipients shared a donor in common (see Table 2).

From the second post-operative day onwards until the 6th day, the outer dressings of R's chest were removed daily and the immediate graft dressing—fine meshed tulle gras—was deflected just sufficiently to enable two grafts, including the superficial layers of the bed underlying them, to be excised. One of these specimens was fixed for histology, and the other was wrapped in a piece of sterile gauze moistened with Ringer's solution and sealed off in a small empty sterile glass vial and stored in the refrigerator at 5° C., pending the biological test of survival. This test merely
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entailed transplanting the series of stored grafts in known dispositions on a standard full-thickness bed prepared on the original donor's chest. Survival of a graft was established by the appearance of epithelial outgrowth from its margins.

Results

In all animals the outward appearance of the grafts at the daily inspections proved to be completely misleading. Quite frequently, even at the first inspection on the 2nd day, their cuticles appeared damp and messy, suggesting that the grafts were already necrotic. However, the histological appearance of biopsy specimens and the results of the biological test of survival established that this was not so. There was no difficulty in assessing the survival time of the transplanted epidermal grafts: the results obtained by the grafting test completely confirmed those obtained by the histological method. In the majority of animals the grafts lived longer than 4 days, though no graft survived longer than 7 days (Table 2, column 3). It may be added that since no specimens were removed after the 6th day the assessment of a survival time of 7 days in animal Sr-156 was computed from the histological appearance of the 6-day biopsy specimen.

Table 2. The survival of pure epidermal homografts transplanted to raw areas prepared in the skin of the side of the chest of specifically immunized rabbits

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<th>Homograft survival time (days)</th>
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<td>Sr-208</td>
<td>Sr-209</td>
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</table>

Histologically, the problem of interpreting what took place in the grafts and in the underlying host tissue was more difficult. Sections of biopsy specimens removed on the 2nd or 3rd day after grafting showed that the thick stratified cuticular layers had become grossly distended and blistered by fluid exudates, and a heavy infiltration by polymorphs had taken place. Below this necrotic mass and very feebly united to it could be seen small patches of indolent, vegetative, feebly basophilic epidermal cells which were intimately united to the now highly vascular bed afforded by the developing granulation tissue. Though these epithelial cells were unquestionably viable, their appearance suggested a gloomy prognosis (Pl. 1, figs. 7, 8). Contrary to expectation, however, the appearance of biopsy specimens removed subsequently suggested that these indolent strands of epithelial cells were not necessarily doomed. In some cases they took on a new lease of life, their cytoplasm becoming more strongly basophilic. By their proliferative activity they frequently regenerated anew a more or less well-differentiated epithelium beneath
the necrotic remnants of the old one (Pl. 2, figs. 9, 10). The histological evidence strongly suggested that the grafted epithelium underwent one or more cycles of indolence during which many of the cells died, becoming eosinophilic, followed by partial regeneration of the survivors when necrotic areas of the graft became undermined by the proliferating epithelium. In some grafts there was extreme variability—patches of thick, hyperplastic epithelium being juxtaposed to areas of more or less complete epithelial breakdown. It was our impression that the condition of the grafted epithelium was a direct consequence of events in the graft bed. Though in all cases the latter had become exceedingly vascular by the 2nd or 3rd days after operation, areas of it beneath the homologous epithelium frequently showed signs of vascular stagnation in the abundant fine capillaries with an indolent phase of the epithelium above it. Frequently this apparent ischaemic phase was followed by one of activity in the capillaries in the graft bed which in turn led to a proliferative phase in the grafted epithelium. Sooner or later, however, the balance between the vascular and ischaemic states of the graft bed was tipped too far in the adverse direction, and all the cells succumbed.

Whatever the precise significance of the histological changes seen in these grafts may be, the results obtained show quite clearly that the survival of pure epidermal homografts transplanted to raw areas on immune animals is comparable with the survival of homografts of more orthodox anatomical constitution transplanted to immune animals. Thus the tissue exudates present on these graft beds have no immediate cytotoxic action. Moreover, on the basis of the present results it does not seem justifiable to attribute the survival of orthodox skin homografts transplanted to specifically immunized recipients merely to the time taken for blood vessels to penetrate them from the graft bed.

The demonstration that pure epidermal homografts both elicit and succumb to an immune reaction provided the necessary justification for making use of epidermal cell suspensions in the tests for cytotoxic activity to be described below.

PART II. ATTEMPTS TO DEMONSTRATE CYTOTOXIC ACTIVITY IN SERUM AND VARIOUS TISSUE PREPARATIONS FROM IMMUNE ANIMALS

Principle of the methods employed

In the majority of tests to be described suspensions of isolated epidermal cells prepared from a donor's skin were treated in vitro under various conditions with serum and various tissue preparations from the immunized recipient, and then recovered and transplanted back to the animal from which they had originally been taken as a biological test of their viability. Controls were carried out concomitantly in all tests; these entailed treatment of a similar preparation of D's epidermal cells with the serum or other preparation from an animal which had not received skin homografts. It may be added that in planning the various tests it was hoped that all-or-none results would be obtained, because the techniques employed could not distinguish with certainty between different percentages of surviving cells in an epithelial population.
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Two separate techniques have been employed, these being applicable to the guinea-pig and to the rabbit respectively.

(a) The seeding technique in guinea-pigs

There is some evidence that the melanocytes (pigmentary dendritic cells) of the mammalian epidermis (Billingham & Medawar, 1953) are more sensitive to a variety of physical and other stimuli than the neighbouring and more abundant Malpighian cells (see Taylor, 1949; Billingham & Medawar, 1952). Consequently, it seemed possible that these cells might also prove more susceptible to the action of a cytotoxic agent than their neighbours. In spotted black and white guinea-pigs with pigmented ears a readily available source of melanocytes can be obtained in the form of epidermal cell suspensions prepared from the intensely black skin of the ear—the fact that melanocytes so obtained are mixed with Malpighian cells is immaterial. After subjecting these cells to various in vitro treatments their continued viability can be tested simply by transferring them to a half-thickness 'graft bed' prepared in white skin of the donor's chest by the removal of a series of skin shavings cut so thin that the bases of the hair follicles are left intact. Areas of this sort, unlike full-thickness defects, heal rapidly by the multiple upward migration of epithelial cells accompanied by non-pigmentary melanocytes from the follicle remnants. Resurfacing of such an operation field is normally complete within 7–10 days. Survival of the grafted melanocytes is revealed by the appearance on the resurfaced bed of a number of superficial leaden-blue spots, which between about the 10th–20th days deepen in colour and extend in area (Pl. 2, fig. 11). Eventually the several expanding foci of pigmentation coalesce giving rise to a more or less continuous patch of epidermal pigmentation over the initial operation field (Pl. 2, fig. 12). Quite frequently a number of fully pigmented hairs pierce the surface since the grafting technique employed gives the melanocytes direct access to the hair follicles. A full description of the functional anatomy of the melanocyte, the 'seeding' technique for grafting these cells, and an account of the phenomenon of pigment spread in guinea-pigs' skin has been given by Billingham & Medawar (1948, 1950, 1953).

From what has already been said about the seeding technique it will be apparent that when selecting the donors it is essential that they must be recessively spotted guinea-pigs with at least one ear black and the skin of at least one side of their chest white. The colour of the recipient is of no consequence.

(b) The seeding technique in the rabbit

In the rabbit, unlike the guinea-pig, the skin splits naturally at the level where the corium is united to the vascular fascial plane that overlies the panniculus carnosus muscle, so that extensive full thickness graft beds free from all traces of follicular epithelium can easily be prepared. These have been referred to repeatedly in the previous section. When viable epidermal cell suspensions are transplanted to such graft beds by the method described by Billingham & Reynolds (1952) they give rise to small scattered foci of epithelium which proliferate and expand in area,
eventually coalescing to produce a more or less continuous sheet. In the present experiments two such areas of approximately equal size were prepared in the skin of the right side of the donor's chest; one of these was to receive the experimentally treated epidermal cells and the other their controls—cells treated in a similar manner with material from a non-immune rabbit. It may be added that the dressings applied both in the guinea-pig and in the rabbit were such as to preclude any possibility of cells being accidentally transferred from one area to the other. To meet the possible objection that one of the paired operation fields might be more favourable to grafted cells than the other, possibly through variation in the anatomical distribution of blood vessels etc., in each consecutive test in a series the upper and lower sites were made alternately the beds for the experimentally-treated and control cells respectively. (This precaution was also adopted in the melanocyte seeding experiments in the guinea-pig.) Primary inspections were carried out on the 9th day post-operatively, and subsequent inspections at 2-day intervals.

(c) Withdrawal of blood

In the rabbit 15–20 ml. of blood was withdrawn from the dilated median ear artery into a glass centrifuge tube fitted with a hard glass cannula. In the guinea-pig about 10 ml. of blood was obtained by cardiac puncture under ether anaesthesia. The blood was incubated at 37°C in a water-bath, for not longer than 15 min., to facilitate clotting, after which it was spun for serum. All samples of blood, serum, and tissue suspensions used in the experiments to be described, were freshly prepared, full aseptic precautions being observed throughout.

(d) Method of treatment of the epidermal cell suspensions in vitro

In this paper the word 'treatment' has been employed non-committally; although it was found that the cells survived many of the in vitro procedures to which they were subjected, there were no grounds for supposing that any proliferation took place in vitro so that the word 'culture' was clearly inadmissible.

In the experiments to be described, whether designed to demonstrate the presence of cytotoxic agents by their effect on epidermal melanocytes in the guinea-pig, or on Malpighian cells in the rabbit, the method adopted for the in vitro treatment was the same. The cells were treated in small stoppered glass weighing bottles. In each individual test the epidermal cell suspension from D, suspended in not more than 1 ml. of saline containing sodium citrate to a final concentration of not more than 0.8%, was divided equally between two of these glass vessels containing respectively a known volume of the preparation from R whose specific cytotoxic effect was under test, and an equal volume of a similar preparation from an unimmunized animal to provide a control. The cells were treated in vitro for various lengths of time, the temperature being maintained constant at either 37°C. in an incubator or at 5°C. in the refrigerator; in a few experiments room temperature, approximately 20°C., was adopted. The effect of the lower temperature of treatment was investigated in case the cytotoxic system that it was hoped to reveal was thermolabile. It may be added that a temperature of 5°C. is known not to be
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harmful to the cells of skin, this being the storage temperature normally adopted for maintaining or 'banking' this tissue in a viable state for future clinical application (see Matthews, 1945). After addition of the serum or other media to the epidermal cells in the glass weighing bottles, their contents were thoroughly mixed with the aid of a pipette. It was noted that the suspended epidermal cells soon settled out on the bottom though they did not agglutinate.

In tests conducted at 37°C the glass weighing bottles were placed on a mechanical rocker of the type illustrated by Medawar (1948b). Its action, though insufficient to maintain the cells in suspension, was considered to be advantageous in promoting gaseous exchange with the medium enclosed. At the lower temperatures, continuous agitation was considered to be unnecessary in view of the presumed lower level of metabolic activity of the cells. Irrespective of the particular treatment under test and the temperature at which it was being carried out, the contents of the weighing bottles were vigorously agitated manually, on at least four separate occasions during the treatment, to resuspend the cells completely.

As the period of in vitro treatment was in all cases very short compared with those normally involved in tissue-culture experiments, antibiotics were not added in the majority of the experiments, and infection was exceedingly rare. In one test to be described in Exp. 2 (section 2), following Medawar (1948a), 9 vol. of the serum used were diluted with 1 vol. of sterile streptomycin, 200 u./ml. in Ringer, to bring the final concentration of this substance to 20 u./ml.

On completion of treatment in vitro the cells were resuspended, transferred to a centrifuge tube and spun down. Almost all the supernatant was then discarded, just sufficient being left to enable the cells to be taken up in a pipette in the form of a thick 'soup' for transplantation.

SECTION 1. Attempts to demonstrate cytotoxic activity directed against the melanocytes of the epidermis and other cells in guinea-pigs

Exp. 1. Treatment of melanocytes with serum

The four independent tests that comprise this experiment simply entailed the treatment in vitro of a standard suspension of D's epidermal melanocytes in citrated saline with an excess of freshly prepared serum from R, followed by their recovery and transplantation to an appropriate bed cut in the white skin of D's chest. As a control a similar suspension of D's cells was treated in a similar manner with serum prepared from an unimmunized animal.

In each test the melanocytes were suspended in about 50 ml. of serum so that the latter was in very great excess. The in vitro treatment was maintained for a period of 25 hr., the temperature being maintained at 5°C. in three of the tests, while in the fourth it was held at 37°C.

In all four tests the result of the 'seeding' test of melanocyte survival (see Table 3) left no doubt that a high proportion of the cells had survived the treatment, and in no animal did the appearance of the area which had received the experimentally treated melanocytes differ from that to which their controls had been grafted.
Exp. 2. Treatment of melanocytes with serum plus lymphocytes

In each of the five tests that constitute this experiment D's melanocytes in suspension were divided equally between two weighing bottles to which were added respectively (a) 4-5 ml. of R's serum containing lymphocytes obtained by snipping up its right axillary lymph nodes in autologous serum—i.e. the nodes draining the skin of the side of the chest to which the immunizing skin homografts were transplanted; and (b) as a control—a similar volume of serum with lymphocytes prepared from a non-immunized animal.

Table 3. The survival (+) or non-survival (o) of epidermal melanocytes treated with serum from immune animals

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<th>Donor</th>
<th>Recipient</th>
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Table 4. The survival (+) or non-survival (o) of epidermal melanocytes treated with serum plus lymphocytes from immune animals

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</table>

The cells were then maintained at constant temperatures for periods ranging from 6 to 28 hr. On completion of this treatment in vitro all the cells, i.e. a mixture of both the melanocytes and the lymphocytes, were recovered and the standard transplantation test of survival carried out.

The results of these tests are summarized in Table 4. In all five tests on both the experimental and control recipient areas the number of separate foci of pigmented activity which developed was such as to indicate a very high percentage of survival of the treated melanocytes. In none of the tests was there any significant difference between the appearance of the experimental and control sites.

The results obtained in this experiment and the previous one indicate that epidermal melanocytes will withstand prolonged treatment in vitro for considerable periods at a variety of temperatures.
Studies on the nature of immunity to homologous grafted skin

Exp. 3. Treatment of melanocytes with serum containing spleen cells

A preliminary series of five tests was carried out, each making use of the trio of animals as before—i.e. the donor (D), the immune recipient (R), and an unimmunized animal (C). After the withdrawal of blood for serum, R and C were destroyed and their spleens removed and chopped up as finely as possible with scissors in about 1 ml. of autologous serum. A further 3–4 ml. of the autologous serum was added to each and thoroughly triturated with the dissociated spleen. After the very coarse particles had been allowed to settle, the supernatants, rich in isolated cells, were taken up and added to two small weighing bottles each containing an epidermal melanocyte suspension from D as before. These were then placed in the 37°C incubator, and rocked mechanically for periods ranging from 17 to 22 hr. On completion of this treatment, the melanocytes, mixed with the spleen cells, were recovered in the form of a thick brei and subjected to the 'seeding' test of viability on D. It was observed that in all cases the contents of the weighing bottles darkened considerably in colour during their incubation, changing from pink to brown.

Table 5. The survival (+) or non-survival (o) of epidermal melanocytes treated with serum plus a suspension of spleen cells from immune animals

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Duration of treatment (hr.)</th>
<th>Temperature of treatment (°C.)</th>
<th>Results</th>
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<td></td>
<td></td>
<td>28</td>
<td>18</td>
<td>+</td>
</tr>
</tbody>
</table>

In none of the animals in this series of tests did any trace of melanogenesis appear on either the 'control' or the experimental sites, a result in striking contrast to that obtained when the melanocytes were treated in a similar manner with lymphocyte suspensions. One possibility was that under the conditions of our tests, e.g. at the particular temperature employed, spleen cell suspensions are toxic. Accordingly, a few control experiments were carried out in which melanocytes from a donor were treated for 27 hr. at 5° C. or 18° C., with similarly prepared spleen cell suspensions prepared from unimmunized animals. After treatment at either of these temperatures a high degree of survival of the melanocytes was obtained, as evidenced by the intense blackening of the seeded areas after three weeks.

After the conditions had been determined under which melanocytes would survive treatment with spleen cell suspensions, the series of tests summarized in Table 5 were carried out. It will be noted that controls were done only in the first two. In the remainder, by deliberate omission of the controls, it was possible to carry out two separate tests, each at a different temperature. In all cases the results
obtained indicated a very high degree of melanocyte survival and there was little to
choose between the appearance of the various grafted areas 3–4 weeks after the
operation.

Exp. 4. Treatment of melanocytes with whole blood

In the experiments so far described epidermal melanocytes from a donor have
been shown to survive treatment with serum, with or without the addition of various
cellular derivatives, from a recipient specifically immunized against the donor's
skin. The present experiment was designed to test the effect of R's whole blood on
D's melanocytes. The use of melanocyte suspensions was obviously impracticable
because of the difficulty of recovering these cells from the blood clots. This difficulty
was surmounted by treating the melanocytes in situ in sheets of pigmented epidermis
prepared from the pigmented ear skin of the donor. After treating the epidermal
sheets with whole blood they were transplanted to half-thickness beds cut in
white skin areas on their donors' chests as before. The development of patches of
epidermal pigmentation on areas so grafted was ample evidence of the survival
of the melanocytes.

Paired donors and their immunized recipients were used as before. Four thin
skin shavings were removed from a lightly vaselined area of pigmented skin on D's
ear and each was then gummed out flat, raw side outermost, on a piece of glass
cover-slip made adhesive by the application of a layer of paraffin/rubber stopcock
grease. Thus mounted, the shavings were incubated with the buffered trypsin
solution, rinsed in Ringer's solution and the dermis removed, leaving the delicate
sheet of epidermis behind mechanically supported on the cover-slip. A pair of pure
epidermal grafts, so supported, was then placed on the bottom of each of two
deep Petri dishes of small diameter, the epidermal sheets with their exposed basal
layers being uppermost. About 5 ml. of freshly withdrawn blood from R was then
poured over the grafts in one Petri dish, and a similar volume of blood from an
animal which had not received homografts was added to the other. The Petri dishes
were then placed in a moist chamber to prevent desiccation and maintained at
37° C. for periods of 24 hr. or longer. On completion of this treatment, one of each
pair of epidermal sheets was carefully removed from the clot to which it was
intimately united and used for the transplantation test, while the remaining graft,
together with the portion of the clot to which it was attached, was cut out and fixed
for histological examination.

In the three independent tests in which the cells had been incubated in contact
with the blood for 24 hr., evidence of melanocyte survival was obtained in the case
of both the experimentally treated cells and their controls, though the degree of
survival was considerably lower than anticipated. There was no significant difference
between the pigmentation appearing on the operation field to which the experimentally
treated cells had been transplanted and that on which their controls had
been grafted. In further experiments in which treatment was prolonged for periods
up to 48 hr. and beyond, the degree of survival fell off rapidly in both the experimentally
treated cells and their controls.

Histological examination of sections through treated epidermal grafts with their
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attached clots left no doubt as to the intimacy of the contact between the basal layer cells, including the melanocytes, and the clot. There was no evidence of any tendency on the part of leucocytes present in the clots to migrate and concentrate in the vicinity of the homologous tissue comprising the epidermal grafts.

Exp. 5. Treatment of ciliated tracheal cells and leucocytes with serum and tissue preparations

The ease with which suspensions of ciliated tracheal cells may be prepared, and the fact that ciliary activity persists for a considerable period in suspensions of these cells in physiological saline solutions, suggested to us the possibility that such material might provide a sensitive indicator capable of revealing the existence of cytotoxic activity in serum and other preparations from an immune recipient, directed against a donor's ciliated cells. Cessation of ciliary activity could easily be recognized by direct microscopy. There is a wealth of evidence that immunization by homografts is not tissue specific, and there was no reason to doubt that any antibodies acting against skin epithelium would act against tracheal epithelium.

Three independent trials were carried out, using guinea-pigs. A segment of trachea, 1.5–2 cm. in length, was dissected out aseptically from D and opened up by means of a longitudinal incision. Light scraping of the exposed mucosal surface detached masses of ciliated cells which were collected and suspended in Ringer's solution. A drop of this suspension of D's ciliated cells was placed in each of three cavity slides. To each of these was then added several drops of one or other of the following preparations from R: (a) serum, (b) serum containing suspended lymphocytes, prepared by snipping up the animal's draining axillary nodes in serum, (c) serum containing a suspension of chopped spleen cells. As controls a similar series of cavity slide preparations was set up in which D's tracheal cells were treated in a similar manner with material from an unimmunized animal. By duplicating the series of experimental slides and their controls, one series of tests was carried out at 37° C. and another at room temperature. The slides were sealed off in moist chambers to prevent evaporation. At intervals up to 48 hr. they were removed from the chambers and examined microscopically to assess the degree of ciliary activity. In nearly all of the preparations there was considerable activity for periods up to 24 hr., irrespective of the origin of the medium in which the cells were bathed and the temperature. No significant difference was demonstrable between the experimental series and their controls. In both, ciliary activity declined after 24 hr., there being no trace after 48 hr.

A somewhat similar series of controlled tests has also been carried out in which a carpet of D's leucocytes, prepared on a slide by the method fully described by Wright & Colebrook (1921), were treated for periods up to 1 hr. with presumptively immune serum with or without addition of lymphocytes. On completion of the treatments fine particulate material was added in the form of bacteria (Staphylococcus albus), collodion, or melanin granules prepared from a mouse melanoma to test the phagocytic capability of the treated cells. There was no evidence that phagocytosis was inhibited in any of the numerous preparations examined.
SECTION 2. ATTEMPTS TO DEMONSTRATE CYTOTOXIC ACTIVITY DIRECTED AGAINST THE MALPIGHIAN CELLS OF THE EPIDERMIS IN RABBITS

In the experiments so far described it has only been possible to consider the viability of the donor's melanocytes, tracheal cells and leucocytes after their treatment with serum and other preparations from the specifically immune recipient. Though Malpighian cells were concomitantly treated with the melanocytes and subsequently grafted back to the donor with them, a serious shortcoming of the technique employed to demonstrate the viability of the melanocytes was its inability to reveal the viability of Malpighian cells. Only by transplanting Malpighian cells, whether in the form of suspensions or as intact epidermal sheets, in open style to areas completely freed from all traces of native epidermis, can the viability of these cells be established, according to whether or not they give rise to centres of epithelial outgrowth over the denuded bed. In the guinea-pig certain anatomical peculiarities of its skin preclude the preparation of the requisite type of graft bed (see Billingham & Medawar, 1952). The rabbit, however, does not suffer from this shortcoming and for this reason has been utilized in the following experiments.

Exp. 1. Treatment of pure epidermal grafts with whole blood from an immune recipient

Essentially the tests now to be described are similar to those described above for melanocytes Exp. 4 (Section 1). Paired donors and their immunized recipients were used as before. Pure epidermal grafts in the form of small rectangles of hyperplastic epidermis were prepared by the indirect method described in detail on p. 20. Three of these grafts were gummed out directly, raw side uppermost, on the bottom of each of a series of small deep Petri dishes. The grafts in half the number of Petri dishes were covered with 3-4 ml. of freshly drawn blood from R, while those in the remainder provided controls and were covered by an equal volume of freshly drawn blood from an unimmunized animal. Pairs of Petri dishes, containing experimentally treated grafts and their controls respectively, were then placed in moist chambers and maintained at 5° C., 20° C. or 37° C. for 24 hr. On completion of these treatments the clots were carefully removed and the epidermal grafts recovered and transplanted in known dispositions on an extensive full-thickness bed prepared in the skin of the side of D's chest. Survival of the treated Malpighian cells was established by the appearance of annuli of epithelial outgrowth from the grafts. On each donor-recipient pair two separate tests, with their controls, each at a different temperature, were carried out simultaneously. Twelve independent tests were thereby carried out on six donor-recipient pairs, the results being summarized in Table 6. It was found that treatment of the grafts at 5° C. and 20° C. in no way impaired their survival and there was no perceptible difference between the experimentally treated grafts and their controls.

Of the four independent tests carried out at 37° C. no survival was obtained from the experimentally treated grafts in three, but in two of the latter their controls also failed to give evidence of survival.
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Table 6. The survival (+) or non-survival (o) of pure epidermal grafts treated with whole blood from immune animals

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Duration of treatment (hr.)</th>
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<td>20</td>
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</table>

Exp. 2. Treatment of Malpighian cell suspensions with sera from specifically immunized recipients

In the extensive series of tests comprising this experiment a suspension of D's epidermal cells contained in about 1 ml. of citrated saline was divided equally between two small stoppered weighing bottles containing, respectively, freshly prepared serum from R, and a similar volume of serum from an unimmunized animal to provide a control. Although the serum in which the cells were suspended in each individual test was always in excess, compared with the minute volume occupied by the cells themselves, its volume was deliberately varied within a lower limit of 2 ml. and an upper one of 9.5 ml. in the hope of discovering whether the amount of serum used influenced the result. The remote possibility existed that the presumed cytotoxic agent was present in very low concentrations so that by having a great excess of serum there might be a better chance of revealing its existence if it had a specific affinity for the donor's cells. The tests were carried out either at 37° C. or at 5° C., a single one being carried out at 20° C. On completion of the tests the experimentally-treated cells and their controls were recovered and distributed as evenly as possible over the central areas of two extensive full-thickness beds of approximately similar dimensions—about 6 × 4 cm.—prepared on the side of D's chest. Both sites were liberally dusted with sterile sulphadiazine powder before the dressings were applied.

Inspection of the grafted areas was first carried out on the 9th day post-operatively, and thereafter at 2-day intervals until the 15th day. Further inspections were not made after this time since it gave ample opportunity for all epidermal cells which had survived the in vitro treatment to reveal this fact by producing foci of epithelial outgrowth.

Although it was hoped that all-or-none results would be obtained, the outcome
of the experiments already described indicated that this was most unlikely. Preliminary trials conducted with untreated epidermal cell suspensions in rabbits had established that after these had been 'seeded' over an extensive full-thickness bed they gave rise to numerous small scattered foci of keratinized epithelium, easily visible to the naked eye by about the 9th day post-operatively. These, as a result of their proliferative and migratory activity, eventually coalesced to produce a large central patch of epithelium of somewhat irregular outline, which in turn became united to the ingrowing native epithelium from the margins of the bed and so lost its individuality.

Since the number of individual foci of epithelial outgrowth which appeared on the bed, and the extent of the epithelial surface which they had given rise to by the 13th day must have been roughly proportional to the number of surviving cells or clumps of cells applied initially, this was made the basis of the method of scoring adopted in Table 7. Every attempt was made to keep the amount of cell suspension used in each test constant by removing approximately the same aggregate area of skin shavings for its preparation. Once such a suspension had been prepared its exact division into two equal portions for an individual test with its control presented no difficulty.

A total of twenty-seven independent tests, each with its own control, was carried out. In two of the tests the donors died before the results could be assessed, and in four both the experimentally treated sites and their controls were found to be infected at the first inspection, and the animals were destroyed. The results of the twenty-one remaining tests with their controls, in which both areas were perfectly clean and healthy from the primary inspection onwards, are summarized in Table 7.

In the first six tests, instead of using paired donors and recipients as before, pairs of animals were used in such a way that each stood as both donor and recipient to the other. In these tests conducted at 37°C, and in which treatment with the serum was not prolonged beyond 3½ hr., both the treated cells and their controls gave rise to extensive areas of epithelium when transplanted back to D, and in no case was there any significant difference between the appearance of epithelium arising from experimentally treated cells and that from their controls. However, in a series of five tests at the same temperature, but in which treatment was maintained for not less than 23 hr., there was a very obvious difference between the experimental sites and their controls in three, and in two of these no trace of epithelium was obtained from the experimentally treated cells. The single test conducted at 20°C also revealed a striking difference between the appearance of the two grafted areas. Of the nine tests carried out at 5°C, involving treatment of the cells for periods ranging from 22 to 45 hr., only one failed to reveal a significant difference between the areas to which the experimentally treated cells and their controls had been grafted respectively. In four of these tests the experimentally treated cells completely failed to give rise to a trace of epithelium. It may be emphasized that in not a single test of the twenty-one summarized in Table 7 was the outcome such that the epithelium arising from the experimentally treated cells was superior to that arising from their controls; any differences that could be distinguished were always
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in the opposite direction, the controls giving evidence of a higher degree of survival than the experimentally treated cells (see Pl. 2, fogs. 13, 14). Examination of the data presented in Table 7 reveals that prolongation of the in vitro treatment beyond about 23 hr. did not result in an improvement of the result. For example, in tests nos. 15 and 19 at least some cells survived after treatment with the immune sera for periods of 30 and 45 hr. respectively, yet in tests 11 and 13 there was no evidence that cells survived after the much shorter treatments of 23 and 22 hr. respectively.

Table 7. The fate of epidermal cell suspensions after treatment with immune serum

<table>
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<tr>
<th>Test</th>
<th>Donor</th>
<th>Recipient</th>
<th>Volume of serum (ml.)</th>
<th>Duration of treatment (hr.)</th>
<th>Temperature of treatment (°C.)</th>
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* Scoring of cell survival: o = no trace of epithelial survival on operation field 15 days after 'seeding' operation; + = only a very low degree of epithelial survival—as from a single minute focus of outgrowth; ++ = intermediate degree of survival—less than that normally obtained from untreated cells; +++ = high degree of survival, such as that normally obtained from untreated cells.

† Contained streptomycin.

The influence of temperature has not been conclusively established owing to the small number of tests carried out for 23 hr. or longer at 37° C. Certainly it can safely be stated that the results obtained with treatment at 37° C. are no better than with treatment at 5° C.; if anything there is a slight indication that the lower temperature may be the better of the two. Variation in the volume of the serum was clearly without effect, small volumes of immune serum appearing to be just as effective as double or even quadruple these volumes in inhibiting wholly or partially the capacity of the experimentally treated cells to give rise to epithelium on transplantation.

One observation that may yet prove to be of some significance was that in the majority of those tests where there was a marked difference between the amount of

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epithelium resulting from the experimentally treated cells and that from their controls, the first outward indication of surviving epithelium on the experimental site was nearly always delayed to some extent compared with that on the control. Frequently there was ample evidence of cellular survival on the control site at the 9-day inspection, whereas evidence of a small degree of survival from the experimentally treated cells was not obtained until either the 11th or 13th day inspections.

DISCUSSION AND CONCLUSIONS

The evidence presented in Part I of this paper shows that when homografts of pure epidermis, free from all trace of dermis, are transplanted orthotopically between unrelated rabbits they incite and succumb to an immunity reaction in the same way as skin homografts of more familiar anatomical constitution. Rogers (1950) has suggested that pure epidermal grafts which resemble cornea and cartilage in having no direct or inherent blood supply—their nutritional requirements being met by diffusion from the blood supply in adjacent tissue—might be expected to survive homotransplantation as grafts of these other tissues do; this view is clearly erroneous. It is of some interest that the distinctive and characteristic sequence of pathological changes in their dermis that accompany the process of breakdown of ordinary skin homografts, also take place in the differentiating granulation tissue of host origin that forms the bed of a pure epidermal homograft. This tissue becomes heavily infiltrated with round cells, mainly lymphocytes; the young differentiating collagenous fibres become separated by oedema fluid; the contents of the vessels stagnate and eventually there is breakdown of their endothelium; the leucocytes also die. The median survival time of these pure epidermal homografts is only very slightly greater than that of orthodox skin homografts of comparable surface area, a finding that lends strong support to the belief that the epidermal component plays a far greater role than the dermis in eliciting an immunity reaction. Billingham & Boswell (1953) have presented evidence that strongly suggests that the epithelial component of corneal homografts in rabbits is antigenically more effective than the stroma when such grafts are transplanted heterotopically to sites normally occupied by skin.

When pure epidermal homografts were transplanted to vascular beds prepared in recipients already immunized against their donor's skin, their survival was found to be curtailed, but not to the extent that might have been anticipated on the grounds that in such grafts the basal layer cells were directly and intimately exposed to the action of the host's serum. This suggests that tissue exudates and serum present in the graft bed are not in themselves immediately inimical to potentially susceptible homologous cells exposed to them; indeed there was frequently evidence that at least some proliferative activity took place in such grafts before their breakdown was complete.

Of the various experiments described in Part II which have been carried out in the hope of demonstrating, on the basis of a transplantation test of survival after prolonged in vitro treatment, the existence of a specific inimical activity in whole blood or serum from a specifically immunized recipient, with or without the
addition of cellular components from the reticulo-endothelial system, only one has been successful. The remainder have been described in detail because it is felt that the negative result is of genuine significance for the understanding of the nature of the immunity reaction. It has been found that treatment of a suspension of a donor’s epidermal cells for a period of 22 hr. or longer with excess serum from a rabbit specifically immunized against that donor’s skin, in the majority of tests partially or completely suppressed their capacity to produce epithelium when they were recovered and transplanted back to an appropriate bed on the animal from which they were removed. There was no evidence that this treatment was any more effective when carried out at 37°C than at 5°C, nor did prolongation of the treatment beyond 24 hr. enhance the effect. Controls were provided by treatment of a similar quantity of D’s epidermal cells with normal homologous serum from an animal which had been immunized neither against D’s cells nor any others. Controls of this type must surely have been adequate to reveal the existence of any non-specific activity in rabbit’s serum. It may be added that a second type of control, the treatment of a rabbit’s epidermal cell suspension with serum derived from a recipient specifically immunized against a donor other than itself, is not acceptable, as Medawar (1948a) has emphasized, for although the skin immunity reaction is strongly donor-specific (see Medawar, 1946a) the second donor might well happen to possess at least some antigens in common with the first, but not present in the recipient. In that event some degree of immunity would be directed against its own cells.

The apparent non-susceptibility exhibited by suspensions of epidermal melanocytes in the guinea-pig when treated with immune serum under similar conditions is surprising. One possibility is that these cells, contrary to our initial assumption, are in fact more resistant than Malpighian cells. An alternative but less probable possibility is that melanocytes are susceptible but that after transplantation to an appropriate bed cut in white skin to test their survival they succeed in ‘infec-tively’ inoculating the non-pigmentary melanocytes of the white hair bulbs with some cytoplasmic ingredient, so conferring upon them the faculty of melanogenesis (see Billingham & Medawar, 1948, 1950) before their eventual destruction or inactivation.

The question as to the nature and mode of action of the factor demonstrable in a specifically immune serum by the combined in vitro—in vivo procedure described must now be discussed. It may be stated at the outset that exhaustive attempts to differentiate microscopically between cells, which had been treated in vitro with immune sera and their controls, were without success and the application of suppli-vital staining was to no avail. That some subtle constitutional change had been wrought in the experimentally treated cells could only be inferred from the fact that their capacity to produce epithelium on grafting to D was impaired or destroyed. One possibility, the simplest and probably that which embodies a major portion of the truth, is that we have succeeded in demonstrating the existence of a protective iso-antibody of the type that Gorer (1942), using an essentially similar technique as well as immunological ones, has shown to be produced in response to transplants.
of homologous leukaemic cells in mice. It may be supposed that this protective antibody is at least partially responsible for the regression of skin homografts in vivo. Strictly speaking, the use of the term 'cytotoxin' is at present unjustified, since the method we have used can by its nature yield no information as to whether the susceptible cells were actually killed or not.

The failure of previous workers to demonstrate the regression of skin explants from a donor rabbit when cultivated in a medium composed of serum with or without the addition of tissue extracts or mesenchymal tissues from a highly immunized recipient may be explicable on the grounds that the exposure of the donor's cells to the presumed antibody was of insufficient intimacy to affect the well-being of the majority of cells. Another possibility, suggested by Medawar (1948a) and certainly not excluded by the results of our own experiments, is that immune media affect skin epithelium in vitro only in such a way as to expedite its breakdown on subsequent grafting; i.e. that tissue breakdown requires the co-operation of a specific antibody with vascular-inflammatory changes only demonstrable in vivo. Woglom's (1933, 1937) studies with transplanted rat sarcomata support this view, as do Kidd's (1946) findings that a preliminary incubation of Brown-Pearce rabbit carcinoma tissue in a medium containing its specific antibody reduced or suppressed its growth on transplantation but did not effect any alteration in the appearance of the cells when incubated in vitro with them.

The finding that 'second set' pure epidermal homografts survive transplantation to heavily immunized recipients for as long as 4–6 days is perfectly consistent with the existence of the circulating antibody-like agent. The latter only exerted a demonstrable effect on even dissociated epidermal cells after they had been exposed to excess 'immune' serum for 22 hr. or longer.

A serious shortcoming of any attempt to explain the breakdown of skin homografts in vivo as a consequence of a simple antigen-antibody reaction is that this does not take into account the fact that regression of homografts is accompanied by the destruction of such native lymphocytes and other leucocytes as may have penetrated them, as well as the native cells comprising the vessels that grow into the grafts. Our own observations (see p. 19) demonstrate this important point very clearly.

Medawar (1946b) and Burnet & Fenner (1949) have drawn attention to certain features of tissue transplantation immunity that qualify it for admission into the general category of tissue sensitization phenomena. Burnet & Fenner have suggested that as a working hypothesis the mechanism involved in immunity to skin homografts may be similar to that postulated for tuberculin hypersensitivity. Mitchison's (1953, 1954) recent demonstration that passive transfer of immunity to a lymphosarcoma in mice can be achieved by the transfer of lymph nodes from immune animals but not by means of their serum or peritoneal exudates is particularly relevant.

In addition to the circulating antibody-like agent demonstrated in the present study, there may be a cell-bound component, possibly the antibody itself, as in the Arthus reaction, whose contact with antigens liberated from the donor's cells in a homograft in some way brings about the local liberation of non-specific
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pharmacologically active substances which are responsible for the necrosis of the
graft. It has been suggested (Gorer, 1947) that other factors than antibodies may
be of importance in determining the fate of transplanted tumours.

Certain types of sensitization reaction can be inhibited by the administration of
anti-histamine agents (Halpern, 1950), and at least one inconclusive clinical report
has appeared of an attempt to prolong the life of a skin homograft by administration
of pyribenzamine (Foster & Hanrahan, 1948). These facts prompted us to investi-
gate the effect of phenergan (promethazine hydrochloride) on the fate of skin homo-
grafts in the rabbit and in the guinea-pig. In neither of these species did the
administration by intramuscular or subcutaneous injection of dosages as high as
50 mg./kg. daily, subdivided into two equally spaced injections, with or without
the concomitant local application of phenergan cream (containing promethazine
hydrochloride 2 %, w/w) to the grafted area, have the slightest detectable effect in
prolonging the expectation of survival of the homografts. Marconi (1950) reported
failure to obtain significant prolongation of the survival of skin homografts in
guinea-pigs by the administration of neo-antergan.

SUMMARY

1. Homografts of pure epidermis, i.e. of skin freed from all dermal elements,
survive their orthotopic transplantation between unrelated rabbits for 10–15 days.
They elicit and succumb to a transplantation immunity reaction just as ordinary
skin homografts do.

2. The distinctive sequence of histological changes that take place in the dermis
of a skin homograft during the course of its breakdown take place in the host’s
own young fibrous tissue when this forms the bed underlying pure epidermal
homografts.

3. Although the breakdown of pure epidermal homografts is accelerated when
they are transplanted to specifically immunized recipients, they normally survive
for from 4–6 days. It is inferred that the serum and tissue exudates present in the
bed to which such grafts are transplanted have no immediate inimical effect on the
susceptible Malpighian cells when these are intimately exposed to them.

4. The possible application of pure epidermal grafts, and more particularly of
dissociated epidermal cells, has been investigated in attempts to demonstrate
a specific toxic action against a donor’s cells of the blood, serum, and other deriva-
tives of specifically immunized animals. After treatment in vitro, the ‘vitality’ of
the epidermal grafts or cells was tested by transplanting them back to the animal
from which they originated.

5. It was found that epidermal melanocytes of guinea-pig’s skin are unaffected
by prolonged treatment in vitro with ‘immune’ blood and serum, with or without
the addition of lymphocytes or spleen cells.

6. The capacity of dissociated Malpighian cells in the rabbit to give rise to
epithelium after grafting was completely or partially inhibited by treating them in
vitro with excess immune serum for 22 hr. or longer at either 5° C. or 37° C.
7. It is suggested that this effect indicates the presence of a protective iso-antibody in the serum of rabbits which have reacted against skin homografts. The probable role of this antibody in the breakdown of skin homografts in vivo is discussed.

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REFERENCES


BILLINGHAM AND SPARROW—STUDIES ON THE NATURE OF IMMUNITY TO HOMOLOGOUS Grafted SKIN
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Studies on the nature of immunity to homologous grafted skin


EXPLANATION OF PLATES

PLATE 1

Fig. 1. Animal Sr-201. An extensive sheet of well-keratinized epithelium originating from six pure epidermal homografts transplanted 11 days previously. At this stage the epithelium is completely surviving. x 0.8.

Fig. 2. Animal Sr-121. Transverse section through a biopsy specimen of a pure epidermal homograft of 9 days standing. The epidermis is grossly hyperplastic and overlies an exceedingly rich vascular bed. There is no histological indication of the onset of a specific reaction. x 100.

Fig. 3. Animal Sr-121. Vertical section through a pure epidermal homograft on the 11th day after transplantation. The hyperplastic epithelium which is now covered by a very thick multi-layered cuticle is completely viable. The differentiating native granulation tissue comprising the graft bed is now becoming infiltrated by lymphocytes whose distribution is still mainly perivascular. x 100.

Fig. 4. Animal Sr-121. Vertical section through a pure epidermal homograft of 13 days standing. Cellular erosion of the basal layer of the graft epidermis is taking place and the graft bed, which is inflated with oedema fluid, is heavily infiltrated by leucocytes. Breakdown of the homologous epithelial cells is clearly far advanced. x 100.

Fig. 5. Animal Sr-121. Complete breakdown of the homologous epidermis. This biopsy specimen was removed on the 15th day after transplantation. The necrotic remains of the epithelium are now separating from the bed. Note that the native leucocytes below the graft remnants have nearly all broken down. x 100.

Fig. 6. Vertical section through a sheet of pure epidermis obtained indirectly by first transplanting a series of sheets of delicate pure epithelium prepared from the skin of its ear to a raw area cut in the skin of the side of a rabbit's chest. A pure epidermal sheet as figured could easily be stripped from the bed 17 days after transplantation. It is completely free from all mesenchymal elements and is mechanically supported by its thick adherent cuticle. x 100.

Figs. 7, 8. Vertical sections through pure epidermal homografts of 2 and 3 days standing respectively transplanted to specifically immunized animals. Both grafts are intimately united to their beds which are highly vascular. There is only trace survival of the epithelium which is only weakly basiphilic. Note that the stratified cuticular layers of the grafts are grossly distended and blistered by fluid exudates. x 100.

PLATE 2

Figs. 9, 10. Sections through pure epidermal homografts which have been in residence on specifically immunized animals for a period of 5 days. Note that the degree of epithelial survival is considerably higher than that illustrated in Pl. 1, figs. 7, 8. x 100.

Figs. 11, 12. Illustrating the 'seeding' test for melanocyte survival in the guinea-pig. Both of the sites illustrated in fig. 11 were 'seeded' with epidermal melanocyte suspensions 17 days previously, while those in fig. 12 were seeded 22 days previously. In each case, one site has received experimentally treated melanocytes, while the other site received the appropriate 'controls'. x 1.

Fig. 13. Animal Sr-182. The result of the seeding test of viability of Malpighian cells after treatment in vitro for 29 hr. at 5°C with immune serum. The upper site was grafted with experimentally treated cells and the lower site with their controls. Only a trace of epithelium has arisen from the experimentally treated cells; there is an extensive sheet of epithelium on the control site. Photographed on the 15th day after grafting.

Fig. 14. Animal Sr-215. The very conspicuous difference between the appearance of the areas grafted with Malpighian cells incubated with immune serum and control serum respectively for 23 hr. at 37°C. The lower site was grafted with the experimentally treated cells and the upper with their controls. No trace of epithelium has appeared on the lower site. Photographed on the 13th day after grafting.