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PIGMENT SPREAD IN MAMMALIAN SKIN : SERIAL PROPAGATION AND IMMUNITY REACTIONS

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I. INTRODUCTION

WHEN a spotted black and white guinea-pig is born, the areas of black skin bearing black hairs are incisively distinct from the areas of white skin bearing white hairs ; but as it grows up, the superficial epidermis at the boundaries of the white areas is slowly, evenly, and progressively encroached upon by pigment. The white hairs are unaffected. In an adult spotted guinea-pig, therefore, the areas of black skin bearing black hairs are separated from the areas of white skin bearing white hairs by a transitional band of black skin bearing white hairs—a band which may reach about one cm. in breadth by a combination of ordinary growth and " pigment spread."

Pigment spread occurs in several other animals : in the body skin of Friesian cattle (see below) and spotted pigs, and in the tails of recessively spotted mice (Reynolds, unpub.) ; moreover, as we shall show below, red, chocolate, and doubtless other pigments will likewise encroach upon white skin, though one pigmented area in a parti-coloured animal will only encroach upon another at an altogether lower order of rate. In all such cases, pigment spread is accelerated and made accessible to experimental analysis by grafting operations : for example, by the grafting of black skin into white or white into black.

In our first analysis (Billingham and Medawar, 1948*a*), we considered four hypotheses that might account for the phenomenon of pigment spread, and dismissed two of them—that spread is the consequence of an invasive replacement of a white by a pigmented epithelium, or of a mere leakage or diffusion from black skin into white of a factor required for the initiation or maintenance of pigmentary activity. Two further possibilities remained on probation.

According to the hypothesis of *melanophore migration*, pigment spread is the outcome of a differential migration from black skin into white of the melanophores (pigmentary dendritic cells) * that are the acknowledged origin and seat of the pigmentary activity of

* The term " dendritic cell " is wider in scope than " melanophore " since it may refer to a non-pigmentary cell.

skin. (By " differential migration " is meant the migration of melanophores independently of the epidermal cells to which they normally supply pigment.) This explanation, though not disproved, required the acceptance of a variety of auxiliary hypotheses to sustain it.

The authors' observations and experimental results were, however, directly and adequately accounted for by a fourth hypothesis : that pigment spread involves an *infective cellular transformation.* Gold impregnation methods (Billingham, 1948) had revealed in white skin a system of dendritic cells similar to the melanophores of pigmented skin in anatomy, density, and disposition : these " white " dendritic cells contain no melanin and lack the enzymic equipment required for making it. The entire system of epidermal dendritic cells—the " epidermal glial system " of the skin—forms a partly anastomising and therefore in some degree syncytial reticulum. It is moreover a perhaps unique property of the pigmentary dendritic cell that in some manner it " inoculates " formed pigment granules into the Malpighian cells to which its branches are intimately applied—a type of activity that Masson (1948) has called *cytocrine.*

The fourth hypothesis under discussion accordingly asserts that pigment spread depends upon the inoculation or infection of white dendritic cells by a cytoplasmic ingredient derived from their pigmentary neighbours at the boundary zone between black and white skin where dendritic cells of these two types may come into anatomical contact. The transformation of a white into a pigmentary dendritic cell is permanent in cellular heredity, and a white dendritic cell so transformed may in turn transform its white neighbours. Pigmentation therefore spreads in an evenly advancing front in a manner formally equivalent to a virus infection.

The rate and pattern of pigment spread depend upon the number and distribution of the dendritic cells in the skin that is undergoing infection. The fact that tongue epithelium when transplanted to black body skin remained permanently unblackened was adequately accounted for by the fact that gold impregnation methods had wholly failed to reveal a system of dendritic cells within it ; and we have since shown that a black skin graft transplanted to the tongue, while retaining its full specificity of histological type, fails completely to initiate pigment spread for the same reason (plate I, figs. 1 and 2). Moreover, the epidermal glial system, though continuous in the basal layer of the epidermis, is sharply interrupted at the necks of the hair follicles ; and it is common knowledge that the hair bulbs and hairs may have a colour system phenotypically different from that of the surface of the skin. The fact that white hairs are normally uninfluenced by pigment spread in the epidermis above them is thus adequately explained by absence from the follicle neck of the dendritic cells required to mediate its passage. These facts, and certain others of smaller weight, are more directly explained by the hypothesis of an infective cellular transformation than by that of melanophore migration.

Section 2 of the present paper deals briefly with the inter-relationships between the pigmentary systems of black, red and white skins. Section 3 contains an experimental analysis of the propagation of pigmentary " infection " from one guinea-pig to another. Although nothing in either section can be regarded as an outright refutation of the melanophore migration hypothesis, both describe phenomena that are all but impossible to reconcile with it. Since the majority of our experiments were done with the object of discriminating between the migration and infection hypotheses, and may seem pointless without this background in mind, the " Discussion " of results that is usually relegated to the end of a paper has been incorporated in the body of the text.

2. PIGMENTARY RELATIONSHIPS BETWEEN BLACK, RED, AND WHITE SKINS

Studies by the " skin-splitting " method of Billingham and Medawar (1948*a*) have shown that black, red and chocolate skins differ from each other neither in the number nor the mode of distribution of their melanophores, but only in the pigmentary activity of the individual cells. (The same is true of the difference between negro and white human skin : Billingham, 1949.) Melanophores of each such type are " true breeding " and each is a member of a distinct somatic cell lineage.

Red and chocolate pigments both spread into white skin, whether from grafts or across natural colour boundaries. No particular study has been made of chocolate-coloured skin, but with each of five red-coloured autografts transplanted to white skin areas and watched for from 350 to 550 days, it has been found that pigment spread takes longer to start than with a black-in-white graft and thereafter proceeds at only about two-thirds of its rate (plate I, figs. 3 and 4). Experiments have now been begun which will make it possible to compute the difference more accurately and to correlate the rate of spread from red-in-white grafts with their depth of pigmentation. It is clear that if pigment spread is due to melanophore migration, then red melanophores must be thought to be in some way physiologically feebler than black. If it is due to a spreading infection process, then one possibility is that the infective pigmentary enzyme complex of the cytoplasm is less abundant in red skins. The positive correlation between pigment volume and colour intensity in mouse skins (Russell, 1948) lends somewhat indirect support to this view.

Three methods have been used to investigate the capacity of black pigment to spread into red-coloured skins elsewhere on the same tri-coloured guinea-pig.

(a) *Black to red transplantation*

Of five animals bearing black-in-red grafts (plate II, figs. 6, 7 and 8), one died on the 421st day after operation and the remaining

four are still under observation at the 620th day. Only one (plate II, fig. 8), that in which the red skin surrounding the black graft was conspicuously the palest, gave evidence of pigment spread. There had been some inconclusive evidence of the blurring of what should normally be an incisive graft outline after 56 days ; and a 1 mm.-wide annulus of spread recorded at 295 days had increased to a width of about 2½ mm. by 620 days. In this one animal, therefore, pigment spread has been occurring at the mean rate of about 1½ mm. per annum—a rate hitherto without precedent.

(*b*) *Red to black transplantation*

In one guinea-pig, a very pale red-coloured ear-skin graft was transplanted from the dorsum of the left ear to an area of black body skin on the chest. Inward spread of pigment was just perceptible by the 65th day after operation and conspicuous by the 233rd : it went on at a mean rate of about 2 mm. per annum until by the 555th day after operation only an irregular patch of pale red skin about 1 mm. in width in the centre of the graft remained to be encroached upon (plate II, fig. 5). The rest of the graft was identical in colour with an originally black graft cut from the right ear of the same animal on the same occasion and transplanted to an area of white skin.

The epidermis from four samples of skin was at this stage removed and examined microscopically in the living state and after a short treatment with 1 : 1000 dihydroxyphenylalanine (" Dopa ") in phosphate buffer at *p*H 7·4. These were : the original red-in-black graft, now all but wholly black ; the original black-in-white graft ; a sample from the dorsum of the pale red-coloured left ear from which the red graft had been cut 555 days beforehand ; and a sample from the dorsum of the black right ear, from which the black graft had been cut 555 days beforehand. (The purpose of the " Dopa " treatment was only to render the very faint melanophores of the red skin sharply distinct.)

The epidermal patterns and the number and distribution of the melanophores were the same in all four specimens. But the originally weak red-coloured melanophores from that part of the red-in-black graft which had been encroached upon by black pigment were now fully black (plate II, figs. 9, 10 and 11), and each one was surrounded by Malpighian cells heavily inoculated with pigment granules. In this area, *no* red melanophores were present ; and the melanophores that were present were in no way distinguishable, whether in number or pigmentary activity, from the black melanophores of the black-in-white graft or of the right ear. The small central patch of the red-in-black graft that had still to be encroached upon by the advancing front of black pigment contained weak red-coloured melanophores *only* (plate II, figs. 12 and 13), and these were indistinguishable in any way from the melanophores of the skin of the left ear. Nowhere in the specimen were black and red melanophores found to be mixed.

These results seem to admit of only one interpretation : that the originally red-coloured melanophores of the red-in-black graft had been serially transformed into black melanophores from the periphery of the graft inwards. No single observation we have made seems so damaging to the hypothesis that pigment invasion was on this occasion the consequence of a migration of black melanophores into red skin.

A second guinea-pig, operated upon in exactly the same way, was lost 214 days after operation, but not before giving clear evidence of the beginnings of the process which in the preceding guinea-pig had passed almost to completion. In this guinea-pig, too, the red-in-black graft was originally very pale in colour.

(c) *Black and red to white transplantation*

Five guinea-pigs in which red and black grafts had been transplanted close enough together on white skin to allow the areas of pigmentation spreading from them to coalesce (plate I, figs. 3 and 4 ; plate II, fig. 5) have not so far, after 552 days, given any evidence of interaction between pigments of the two types. On the contrary, the pattern of spread is such that the more rapidly advancing black pigment gives the appearance of avoiding the zones earlier tenanted by red pigment spreading from the red grafts (plate II, fig. 5).

The experimental results outlined above show that black pigmentation may indeed spread into red skins ; and they suggest, at present only very roughly, that the rate of spread of a dark pigment into a white or lightly pigmented skin varies directly with the difference between their degrees of colouration. A discussion of the relevance of these facts to the infection hypothesis will be deferred until more exact quantitative information has become available.

3. THE PROPAGATION OF PIGMENTARY " INFECTION " FROM ANIMAL TO ANIMAL

In our earlier paper on the phenomenon of pigment spread, we reported seven trials in each of which even a very small graft of black skin taken from one guinea-pig failed to initiate pigment spread after transplantation immediately below the white skin of another ; and although attention was drawn to certain technical shortcomings of these trials, we felt that our failure could more properly be attributed to the immunological disparity between genetically heterogeneous guinea-pigs than to inadequacy of technique. In this section it will be shown that our failure to transmit pigmentation from one guinea-pig to another was wholly technical in origin, though the *persistence* of foreign pigmentation will at the same time be shown to be entirely dependent upon the immunological relationship between donor and recipient.

The guinea-pigs used in the experiments described below came from dealers' miscellaneous stock ; supplies were replenished from

time to time by new purchases or by the offspring of pen breeding. From the genetical standpoint they can be regarded as a very heterogeneous assembly.

(i) *Operative methods : the seeding graft*

The cells of skin and of many other tissues do not survive transplantation between individuals of ordinary heterogeneous stocks : cells so transplanted provoke an unusual type of immunity reaction and survive for a length of time that varies inversely with the degree of genetic diversity between donor and recipient and the quantity of foreign tissue that is grafted.* Attempts to transmit the factor responsible for pigment spread by dead cells or cell-free extracts have, however, so far failed (see section 4), and pigmentation that is to be propagated from one guinea-pig to another must therefore be initiated by living foreign cells. (Whether after its initiation pigment spread is *maintained* by surviving foreign cells is a crucial problem to be discussed in full below.)

If melanophores from one guinea-pig are to initiate pigment spread in the white skin of another, two technical conditions must be satisfied : (*a*) the " dosage " of foreign cells transplanted from donor to recipient must be exceedingly small, and (*b*) the foreign pigmentary dendritic cells must be caused to enter very rapidly into intimate contact with their white analogues in the recipient's skin. The success of experiments which satisfy these two conditions is, in our interpretation, to be attributed to the fact that, being in very low dosage, the foreign cells have time to " infect " the host cells before their eventual destruction.

In practice, the " grafts " we have used are suspensions of basal-layer epidermal cells in Ringer's solution, handled and applied by pipette. A pigmented ear is by far the most easily workable source of skin for this purpose. A thin vaselined shaving, about 0·5 cm.^{2} in area, is incubated in sterile trypsin solution (see Billingham and Medawar, 1948*a*) until the dermis may be cleanly peeled away, so leaving the epidermis behind as a single intact sheet. The epidermal sheet is smoothed down on its vaselined cuticular surface and its basal-layer then scraped off by smooth strokes with the blade of a cataract knife. Keratinised cells and the cells of all the more superficial epidermal strata are left behind. The basal-layer scrapings are coarsely broken up and suspended in Ringer's solution by sucking them in and out of a fine pipette. In this condition the preparation awaits use.

The recipient area or " graft bed " to which such an emulsion may be transferred is essentially that area which is left behind after the removal of a Thiersch graft ; *i.e.* the surface of the skin is removed by one or two clean scalpel strokes to such a depth as will expose

* For the behaviour of skin homografts, see Medawar (1944, 1945, 1946*a*, *b*, 1948*a*, *b*). Evidence that many other tissues do not survive homoplastic transplantation is to be found in Loeb (1945).

the bases of the hair follicles (fig. 1). Areas of this sort (unlike full-thickness defects of skin) heal very rapidly, without contracture, and with cosmetically admirable results by the multiple upward emigration of epidermal epithelium accompanied by dendritic cells from the hair follicles. In doing so, the native cells mix rapidly and intimately with such foreign cells as may have been transferred to the surface of the raw area by pipette. Moreover, the rapid epithelial closure of the graft bed to a large extent prevents the seeded cells from increasing their initial dosage by proliferation.

We have come to describe such an operation as a *seeding graft.* If the cells seeded are of native (*i.e.* autologous) origin, there are only technical limits to the area of the recipient bed and the number of cells that may be seeded on to it. When the seeds are of foreign origin, the area of the bed should not exceed 0·5 cm.^{2}, and the quantity of epidermal matter transferred to it should not exceed that equivalent to 2-3mm.^{2} of skin. In theory, this may be too high a dosage, but in practice a sufficiently high proportion of the seeded

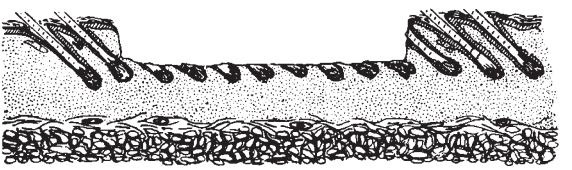

FIG. 1.—Sectional diagram illustrating the depth to which the skin must be cut in the preparation of a seeding bed.

FIG. 2.—Sectional diagram illustrating the area and depth of the smaller type of seeding bed.

cells die. The preferred site for a seeding bed, as for orthodox grafts, is the skin of the side of the chest. After the excess of Ringer's solution has been carefully drained or pipetted away to leave the epidermal seeds thinly and evenly scattered over its surface, the seeding bed is covered by two or three thicknesses of extra fine-mesh vaselined muslin. The thorax is thereupon directly wound with two or three turns of plaster bandage, which is left on until the first post-operative inspection 7-10 days later.

Another type of bed is preferred when for any reason the quantity of cells to be grafted is very small : this is in effect the raw area left behind when a very small " pinch " graft (as opposed to a Thiersch graft) is cut from the skin of the recipient. Fig. 2 makes its structure clear.

It is sometimes obligatory to suspend the basal-layer cells of pigmented *body* skin for propagation, as when an area of pigment spread induced in a second animal by cells from a first is to be transmitted to a third, and then in turn to a fourth, and so on— " serial propagation." The epidermis of body skin is thinner than that of ear skin, and the elasticity of the dermis makes it much more

difficult to cut grafts of even thickness. With increasing experience we have come to succeed with it regularly, provided that the body-skin shaving is fully stretched and then gummed on to a coverslip with rubber-paraffin grease before immersion in trypsin solution for the skin splitting operation. Body skin is more sensitive than ear skin to over-digestion with trypsin, which causes the basal-layer cells to macerate. An accurate timing of the digestion process is therefore particularly necessary.

Many spotted guinea-pigs do not have white skin conveniently placed on the chest region (*cf.* plate IV, figs. 25 and 26) for the seeding operation. By using rubber puncture-patches or cellophane dressings sealed down with " Portex " plastic skin, seeding operations may, however, be done on the sacrum, thigh or abdomen. On four occasions, with successful results on three, seeding homografts have been transplanted to the sole of the foot (plate IV, fig. 31) by using a plaster " sock " as a temporary dressing. But for reasons that will be clear later the abundance of its hair follicles makes body skin superior to any other as an epidermal seed bed.

The latest analysis of our experimental results shows that seeding operations have failed for technical reasons in less than 8 per cent. of trials, but there are a good many reasons for supposing that some of the negative results attributed to technical failures were in reality due to immunological reactions (see below).

(ii) *Inspections and dressings*

Plasters and other dressings may be removed between the 7th and 10th days after operation, by which time the originally raw area of the seed bed has been fully resurfaced by skin epithelium from the hair bases. A second dressing to last until the 15th or 20th day is desirable only inasmuch as it prevents the guinea-pig from scratching the skin of the operation field.

A successful primary " take " is revealed by the presence in the healed graft bed of a variable number of small, rounded, and pale leaden-blue spots with indistinct outlines, but a seeding operation has in some cases proved to be successful even when such spots were not visible at the first inspection, since the rapid cell division associated with the later stages of healing entails a high degree of pigment dilution. Between the 10th and 20th day the spots deepen in colour and coalesce more or less completely to form a tracery of pigmentation over the surface of the original graft bed ; and with very rare exceptions a variable number of stout, fully pigmented hairs will be found to have pierced the skin surface, since this method of transplantation gives the seeded melanophores the direct access to hair follicles which is denied them by ordinary methods of grafting.

Since seedings with foreign cells may fail for immunological as well as technical reasons, we have, however, thought it right to use

more exacting criteria of primary " take " than those described above. A homograft seeding is admitted to be technically successful only if at least one of the following three criteria is satisfied : (*a*) the darkening between first and second inspections of the spots that represent the primary foci of pigmentation ; (*b*) the formation of at least one black hair ; or (*c*) the initiation of pigment spread. The only homograft seedings admitted into the Survival Table computed in section (iii) are those which have satisfied these more exacting criteria of primary take.

Once begun, pigment spread proceeds just as if it had been initiated by a large and cosmetically perfect graft (for a full description, see our article, 1948*a*). A seeded area may be distinguished from a grafted area because the centre of origin of pigment spread is represented only by a few streaks of hyperpigmented skin bearing black hairs—the remains of the primary foci of pigmentation. The spread of pigmentation into normal undamaged skin round the seeding bed leaves the white hair follicles untouched. The peculiarities of spread initiated by seeding operations are better illustrated than described : *cf.* plate II, fig. 14 ; plate III, figs. 15, 16 and 21, etc.

We have made fully annotated inspections of animals carrying seeding grafts every 10th day up to the 50th or 70th day, and thereafter at 20 or 30 day intervals.

(iii) *The fate of seeding grafts of foreign origin*

A certain proportion of pigmented patches induced in one guinea-pig by pigmented cells from another do not long survive their first establishment on foreign soil : such patches undergo a more or less prolonged process of progressive bleaching, the first signs of which (*cf.* plate III, fig. 20) can be detected with some precision. The end result of a complete bleaching process—not all pass to completion—is the re-establishment of normal white skin in an area which had earlier been deeply pigmented. The " expectation of survival " of a homograft seeding has been computed by ordinary actuarial methods from the data which immediately follow. Needless to say, these data exclude animals in which bleaching was artificially brought about by immunological methods (section iv).

Of 79 animals which, after seeding with foreign pigmented skin cells, satisfied our accepted criteria of primary take, only 48 still bore perfectly normal patches of pigmentation by the 50th day. The remaining 31 had either lost their induced pigmentation by that time, or had shown the beginnings of a bleaching process which later inspections proved to pass to completion.

Of 48 animals which still bore patches of normal pigmentation at the 50th day, four were used for immunisation experiments which involved a deliberate bleaching out of foreign pigmentation, three were accidentally killed, and one died, leaving 40. Of these 40, 33 continued normal to the 100th day after operation, and the remaining 7 began to undergo bleaching.

Of 33 animals which still bore patches of normal pigmentation at the

100th day, six had not reached the age of 150 days after operation when this record was compiled on 1st September 1949, and two died, leaving 25. Of these 25, 23 continued normal until the 150th day after operation and the remaining two began to undergo bleaching.

Of 23 animals which still bore patches of normal pigmentation at the 150th day, two had not reached the age of 200 days after operation when this record was compiled, three were used for immunisation experiments, and one died, leaving 17. Of these 17, 16 continued normal until the 200th day after operation and one began to undergo bleaching.

Of 16 animals which still bore patches of normal pigmentation at the 200th day, two had not reached the age of 250 days after operation when this record was compiled, one was used for immunisation experiments, and one died. The remaining 12 continued normal until the 250th day.

Of 12 animals which still bore patches of normal pigmentation at the 250th day, five have not reached the age of 300 days after operation, and one has died. The remaining six have continued normal until the 300th day. (At present two animals still bear pigment of foreign origin at 350 days and one at 400 days after operation.)

From these data we may directly compute the " specific mortality " of patches of induced pigmentation of foreign origin, *i.e.* the proportion of homograft seedings found to have *begun* to fade out by 50, 100, 150, . . . 300 days expressed as a percentage of those that had been perfectly normal 50 days earlier. (Since the bleaching process may take 100 days or even more from start to finish, the tables which follow have been based, not on the total survival time of pigmentation of foreign origin, but on its survival in a state of perfect normality. This is analagous to computing actuarial statistics, not from the ages of death, but from the ages of onset of diseases which sooner or later proved fatal.)

TABLE 1

50	days	39	per	cent.	based	on	79	animals
100	,,	16	,,	,,	,,	,,	40	,,
150	,,	8	,,	,,	,,	,,	25	,,
200	,,	6	,,	,,	,,	,,	17	,,
250	,,	0	,,	,,	,,	,,	12	,,
300	,,	0	,,	,,	,,	,,	6	,,

From these in turn may be directly computed the " expectation of survival at birth " of pigmentation of foreign origin, *i.e.* the percentage of homograft seedings which, having given clear evidence of sound primary healing, may be expected to remain perfectly normal for 0, 50, 100, . . . 300 days (see fig. 3) :—

TABLE 2

0	days	.	.	.	.	.	.	100	per cent.
50	,,	.	.	.	.	.	.	61	,, ,,
100	,,	.	.	.	.	.	.	50	,, ,,
150	,,	.	.	.	.	.	.	46	,, ,,
200	,,	.	.	.	.	.	.	43	,, ,,
250	,,	.	.	.	.	.	.	43	,, ,,
300	,,	.	.	.	.	.	.	43	,, ,,

Finally (fig. 4) we may compute the expectation of survival to (say) 300 days of homograft seedings which have survived in a state of perfect normality to 0, 50, 150, . . . 300 days :—

TABLE 3

0 days	43 per cent.
50 ,,	71 ,, ,,
100 ,,	86 ,, ,,
150 ,,	94 ,, ,,
200 ,,	100 ,, ,,
250 ,,	100 ,, ,,
300 ,,	100 ,, ,,

Two theoretically important inferences may be drawn from the preceding table : (*a*) that the expectation of survival of pigmentation of foreign origin increases with its age from inception, and (*b*) that

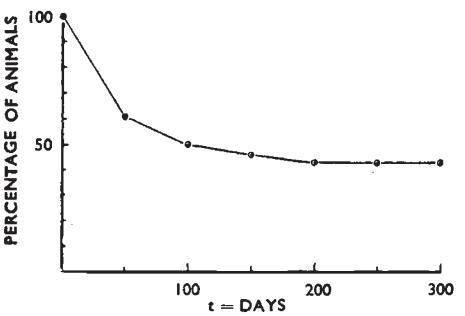

FIG. 3.—The "survival curve" of induced pigmentation of foreign origin : showing the percentage of animals in which pigmentation of foreign origin persisted in a state of perfect normality for t = 0, 50, 100, . . . 300 days after its inception.

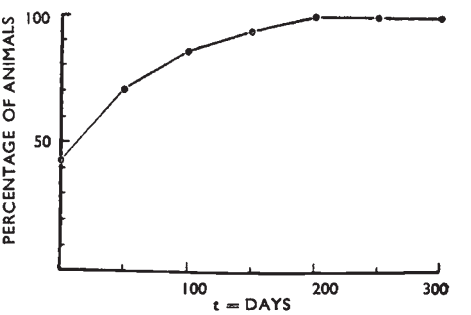

FIG. 4.—The "expectation of survival" of induced pigmentation of foreign origin : showing the percentage of animals in which foreign pigmentation of t = 0, 50, 100, . . . 300 days' standing proved to survive in a state of perfect normality for 300 days after its inception.

the expectation of survival increases in spite of the fact that during the whole period of its residence *the quantity of foreign pigmentary matter is itself steadily increasing*. A successful homograft seeding begins as two or three minute pigment foci representing the survivors of the foreign epidermal cells taken from 2 mm.^{2} of skin or less. After 250 days the area of a successful homograft seeding may well have increased by natural pigment spread and artificial expansion (see below) to an area between 250 and 350 mm.^{2} Yet the former stands a much lower chance of surviving a further 50 days than the latter. It is extremely difficult to reconcile these facts with the hypothesis that pigmentation of foreign origin is caused by the survival, proliferation and outward migration of pigmentary dendritic cells. This interpretation requires us to believe not only that foreign dendritic cells are in high degree exempt from the ordinary exactions of tissue transplantation immunity, but also that their degree of exemption rises as they increase in number. Both these subsidiary hypotheses are possible, but neither is likely.

It might be that the foreign cells progressively desensitize their hosts, as guinea-pigs may be desensitized to foreign protein, but the evidence of the next section shows that they do not.

It is obviously a matter of some difficulty to appraise the significance of a mortality table (table 1) in which each successive entry is of necessity computed only from that fraction of the original fully heterogeneous population which still bore normal pigmentation of foreign origin 50 days beforehand. It is, however, possible to work out what form the survival curve (fig. 3) would take if the foreign pigmentation were indeed due to the survival and proliferation of foreign cells. This may be done in two stages : (*a*) computing its form if the foreign cells remained in constant dosage throughout their period of residence, and then, (*b*) superimposing upon this the effect of a steadily increasing dosage of foreign cells.

(*a*) Other things being equal, the expectation of survival of foreign cells depends upon the antigenic disparity, and therefore the genetical relationship, between donor and recipient. The successive entries in the mortality table would then be a straightforward record of the selection from the original fully heterogeneous stock of those recipients most tolerant to the survival of foreign cells, and fig. 3 would illustrate a transformed tolerance-distribution curve for foreign cells in guinea-pigs of mixed stock. What form would this distribution take ? It is agreed by all investigators that the genetic control of tissue grafting compatibility is multifactorial in type. (It would be more correct to describe it as polygenic.) If this is so, then the distribution through a heterogeneous stock of the lengths of time for which the recipients can just tolerate the survival of foreign cells transplanted from their donors should approximate to the normal, or should at least be bell-shaped ; *i.e.* the proportion of animals which can just tolerate the survival of a uniform dosage of foreign cells for a mean length of time x days will be higher than that which can tolerate it for a shorter time $x-y$ days or a longer time $x+y$ days. If this assumption is correct, then it follows directly that the survival curve which in fig. 3 is seen to fall uninflected at a steadily diminishing rate must be sigmoid, and must fall at a steadily increasing rate down to a point of inflexion beyond which it falls at a decreasing rate. The evidence of Medawar (1944) shows that the underlying assumption is indeed correct : the survival curves of skin homografts transplanted in uniform dosage between members of a fully heterogeneous stock of rabbits are invariably sigmoid in just this way.

(*b*) Other things being equal, the survival time of foreign cells varies inversely with the dosage in which they are transplanted (Medawar, 1944, 1945). The effect of a steadily increasing dosage of foreign cells would therefore be to expedite their breakdown and so to exaggerate and prolong the tendency of the sigmoid survival curve to fall at an increasing rate. In the extreme case, if the dosage factor came to outweigh the effect of the genetic disparity between donor and recipient, the curve would be uninflected and would be such that it fell throughout at a steadily increasing rate. In other words, its properties would be exactly the opposite to those of the asymptotically declining survival curve (fig. 3) actually computed from the data.

In summary, then, the hypothesis that pigmentation of foreign origin is due to the survival and proliferation of foreign cells is very difficult to reconcile with the numerical properties of the mortality table.

A seeding of foreign origin that may be described as " perfectly normal " is one which differs in no respect, outwardly or to histological examination, from one of autologous origin (see plate III, figs. 15-19). Nevertheless the majority of homograft seedings which survive for 100 days or more go through a fairly well defined " bad period " between 30 and 50 days after their initiation. During this period, the heavily pigmented epithelium of the primary seeding foci tends to become weaker in colour or even smokily translucent, and the pigmentation in the area of new spread into normal skin becomes more dilute in colour and less crisply reticular in pattern. Histologically, this period is associated with a mild inflammation under the seeding centres, with dilatation of blood vessels and lymphatics and some infiltration of the dermis by round cells. Our belief, technically almost impossible to verify, is that this transient bleaching period corresponds with the completion of the homograft reaction provoked by the originally minute dosage of surviving foreign cells, and that the pigmentation which survives this transient bleaching phase is caused by a foreign melanogenic system within dendritic cells of native origin.

In five out of about 100 experiments in which foreign pigmentation was initiated by epidermal suspensions prepared from black ear skin, a small crop of bold red-coloured or straw-yellow hairs were found to have pierced the epidermis of the primary seeding foci between the 20th and 50th days. The lack of a comparable number of autograft seeding controls studied with this phenomenon in mind, and the possibility that the seeding material was contaminated by small numbers of red melanophores, makes it impossible to attach much significance to this observation at present.

The process of bleaching in a well-established seeding begins with a general paling or browning of the surface pigment associated with the development of curious and characteristic vacuolar defects, so that the area of pigment spread becomes coarsely patchy instead of sharply reticular (plate III, fig. 20). The rather thicker epidermis over the primary seeding foci fades out progressively, becoming at first smoky in colour and then quite translucent, so that the shafts of pigmented hairs may be seen through it. Except for a long-persistent surface smuttiness that is due solely to tattooing, *i.e.* to the retention within the dermis of histiocytes containing ingested melanin granules, the bleaching of the surface epithelium takes place within 15-30 days (*cf.* plate III, fig. 22). Pigmentation in the hairs persists very much longer—in some cases for well over 100 days, though during this period both the number and the stoutness of the hairs progressively diminishes. It is a matter of empirical fact that the melanophores

within the hair bulbs are for some reason in an immunologically privileged position.

In a small proportion of animals the immunity reaction which starts the bleaching process is just so balanced that it does not pass to completion, and a reduced number of black hairs continues to grow through a perfectly white surface epithelium. In three such cases, after periods ranging from 110 to 144 days after the beginning of the reaction that led to bleaching elsewhere than in a few hair bases, the surface epithelium was caused to be re-colonised by melanophores merely by shaving off the surface of the skin to enforce an upward migration of epithelium from the follicles. Pigment spread thereupon began anew and continues to the present time, not less than 60 days after its second inception. (This experiment, taken in conjunction with the consequences of the seeding operation itself and a variety of other evidence, shows that the dendritic cells of hair and superficial epidermis are mutually interchangeable.) In two other such cases, however, the same operation merely expedited the total loss of pigmentation, presumably because the recipient's immune state was still in being when the melanophores were caused to emerge from their follicles.

The preceding paragraphs are concerned with the course of events during the fading-out of a well-established seeding of foreign origin. Early fade-out, such as may be in progress as soon as the 20th day after operation, requires no special comment, save to point out that here too the pigmentation of the hairs long outlives that of the surface epithelium.

No form of trauma or operative interference will bring about depigmentation in an established normal homograft seeding. It has often been necessary to remove the whole or the greater part of the surface epidermis from such a seeding, either for histological examination or to propagate the induced pigmentation to another animal. Pigment spread is thereupon re-established by the upward migration of pigmented epithelium from the hair bases or from the residual surface pigmentation, or both. The operation we have called "expansion" takes advantage of this property: its object is to increase the total area of foreign pigmentation by quicker means than waiting for natural pigment spread. The operation involves no more than removing a number of Thiersch or pinch grafts from within the perimeter of an established area of pigment spread and then transplanting them in a ring or in some other convenient position in the normal white skin around it (plate III, fig. 23; plate IV, figs. 24 and 25). Each secondary graft acts as a centre of origin of pigment spread which unites with that arising from the parent seeding and thus rapidly and greatly increases the total quantity of foreign pigmentary matter. On two animals expansion operations were done twice with intervals of 50 or 60 days between each, and we have no evidence that the process cannot be repeated indefinitely. In this

manner the area of foreign pigmentation has so far been raised to 350 mm.^{2} or more.

Another method of expediting pigment spread, used with success on each of three trials, is to cause the foreign pigmentation spreading in body skin to " infect " white ear or sole-of-foot skin autografts transplanted to its neighbourhood, for pigment spread, as we found, is more rapid in ear and sole skin than in body skin (1948*a*). It is noteworthy that pigmentation induced by this means in, for example, ear skin, is very much more intense than in the body skin from which it arose in the first instance. This is another of those individually trivial but cumulatively impressive facts that argue against the idea that melanophore migration is the cause of pigment spread.

Although black pigmentation to be propagated from one spotted guinea-pig to another must for all practical purposes be induced in a white skin area on its intended recipient, it is not necessary that the pigmented areas of the recipient should themselves be black. Widespread and (to the present time) enduring pigmentation has been induced in guinea-pigs of the phenotypes red-and-white (plate IV, fig. 26) and chocolate-and-white (plate IV, fig. 24). Pigment spread that was on one occasion propagated to an animal of phenotype agouti-and-white spread abnormally slowly, but progressively nevertheless.

We have now begun a series of experiments making use of guinea-pigs of the so-called " albino " phenotype but coloured genotype.* On each of eight trials, an epidermal suspension prepared from black ear skin was seeded to recipient areas cut from the chest skin of albinos. In five, the seeding foci were perceptibly discoloured by the first inspection at the 9th day ; in none did any trace of pigmentation whatsoever survive until the 29th day. This disappearance of pigment is more rapid than can be plausibly accounted for by the destruction of the very small quantity of foreign cells in the ordinary course of the homograft reaction. Its physiological mechanism is now under investigation. Five strictly controlled experiments failed to reveal that the seeding of albino epidermal cells to black areas of chest skin has any effect on the native pigmentation.

One further experimental result provides a transition between this section and the next. An animal which has proved to be resistant to inoculation by foreign cells on one occasion, in the sense that the induced foreign pigmentation has more or less rapidly faded out, is in the same sense totally resistant to inoculation by foreign cells from the same donor source on a later occasion. Ten spotted guinea-pigs in which foreign pigmentation had faded out so rapidly that in some there was reasonable doubt about the technical success of the

* As is well known, the type of guinea-pig commonly called " albino " is a pink-eyed animal of extremely dilute pigmentation whose points darken after prolonged exposure to cold weather. Its dendritic cells are dopa-positive throughout and, like the weakly pigmentary dendritic cells of white human skin (Billingham, 1948), stain supravitally with methylene blue.

seeding operation were for a second time seeded from their original donors after periods ranging from 91 to 312 days. In every case the foreign pigmentation faded out just as rapidly after this second inoculation from the same donor as it had done after the first inoculation.

(iv) *The immunity phenomenon*

Any pigmentation of foreign origin on any animal may at any stage be bleached out at will by grafting skin in adequate dosage from the animal whose cells initiated the pigmentation to the animal on which it has become established (plate III, figs. 21 and 22). All that this operation does is to bring about deliberately and under experimental control the bleaching process which in a certain proportion of animals has already been seen to occur "spontaneously." The process is distantly analagous to the cure of a bacterial or virus infection by immunological means: in the sense of this analogy, guinea-pigs are "cured" of an attack of foreign pigmentation. Immunisation may also be used prophylactically. If an orthodox skin graft is transplanted from one guinea-pig to another at the same time as a suspension of its epidermal cells is seeded on another area of the same recipient, pigmentation is bleached out almost immediately after its inception. The failure of orthodox skin homografts themselves to initiate pigment spread, and the regular failure of pigment spread in animals in which the process was initiated by too high a concentration of foreign cells, all point to foreign cell *dosage* as a factor critical for the successful initiation of pigment spread. It should be added that the guinea-pigs used throughout all the experiments recorded in this paper are of such genetic diversity that all orthodox skin homografts invariably submit to the immunity reaction they provoke and break down completely.* We have no record of any such graft surviving longer than 25 days. The fact that skin homografts break down no less promptly when transplanted to guinea-pigs already carrying widespread areas of pigmentation initiated by cells from the same donor shows that the recipients are not in any degree desensitized to foreign cells from the same source.

There is one highly qualified exception to the rule that an immunising graft from the same source will invariably bleach out pigmentation of foreign origin. This is when pigmentation initiated in a guinea-pig R by cells from a guinea-pig D is transferred back to a white area on D by a seeding operation in the reverse direction. Massive immunising grafts transplanted from R to D then fail to influence the pigmentation of D in any way. These experiments merely show that the foreign pigmentary matter on R, in whatever form it may be, retains the antigenic specificity of its original donor.

* Unpublished studies on skin homografts in adult mice by McDonald and Medawar show that the degree of genetic uniformity required before skin begins to survive homologous transplantation in certain combinations between mice is roughly that achieved by 10 successive generations of brother-sister mating.

Because of their distinctness and ease of handling, we normally use square black ear-skin Thiersch grafts (area 6-16 mm.^{2}) for immunisation, and transplant them in the orthodox way (Billingham and Medawar, 1948*a*). With one exception their survival time, as judged by outward inspection, has ranged from 8 to 18 days. Breakdown entails the disengagement and degeneration of the epidermis preceded and accompanied by a violent inflammatory reaction in the homograft dermis which is characteristically associated with a massive invasion of the dermal collagen by round cells and the stagnation and rupture of its blood and lymph vessels (plate IV, fig. 27). The bleaching of the foreign pigmentation elsewhere on the same animal is first perceptible when this reaction is at its height, and is associated with a round-cell infiltration of a much milder character (plate IV, figs. 28 and 29). Bleaching so induced (plate III, figs. 21 and 22) does not differ from that which in some animals takes place "spontaneously," and a steadily decreasing number of black hairs may survive the complete depigmentation of the surface epidermis by as long as 80 days.

Immunisation is systemic in effect: an immunising graft on one side of the chest will cause foreign pigmentation to disappear from the skin of the other side of the chest or from the sole of the foot. It had already been found that foreign pigmentation simultaneously induced in two different positions on the same animal—on both sides of the chest in one trial, and on the thigh and the nape of the neck in another—underwent a bleaching process of the same type and tempo in both positions.

Because of the very high degree of individual specificity shown by the homograft reaction (Medawar, 1946*a*), the use of an immunising graft from a donor *other* than that responsible for establishing the foreign pigmentation is theoretically unsound, and its results are unpredictable. A single experiment has shown that the antigenic specificity of the individual is no less conspicuous in seeding operations than in orthodox grafting. Four distinct areas on the chest of a single recipient were seeded with pigmented epidermal cells from four different donors, and pigment spread arose from each one. Bleaching began at 20, 30, 80 and 90 days respectively, after the establishment of correspondingly large areas of spread. In one area the persistence of pigmented hairs 120 days after the beginnings of bleaching prompted us to re-establish the surface pigmentation by the operation already described in section (ii). Active melanogenesis and pigment spread still continues at this one site.

Areas of induced foreign pigmentation have been removed at 12, 15, 16, 26, 42, 56 and 74 days after immunisation for orthodox histological examination (plate IV, figs. 28, 29 and 32) and for treatment by Gairns' gold-impregnation method for dendritic cells (Billingham, 1948)—*i.e.* after periods extending from just before the beginning of the induced bleaching to somewhat after its visible

completion. A normal complement of " white " dendritic cells has been revealed in each instance. It has, moreover, been shown on each of four occasions that a fully-bleached area of foreign pigmentation is " re-infectable " by pigmentation of native origin in the ordinary course of pigment spread. This has been demonstrated either by transplanting a small black ear-skin autograft to the centre of the bleached area, in which case pigment spreads progressively outwards, or by ringing the bleached area with black ear-skin autografts which in due course obliterate the bleached area enclosed within them (plate IV, fig. 30). There is therefore indirect evidence that the native dendritic cells of the artificially pigmented white skin are at least not wholly destroyed by the immunisation process, and that bleaching primarily involves the reconversion of pigmentary dendritic cells into their non-pigmentary analogues. Any more confident inference must be deferred until better histological methods have been devised for studying the dendritic cells of white skin. Orthodox histological study by microtome sections has proved to be totally inadequate.

It is a matter of some theoretical importance that bleaching by immunisation can be achieved not only by the transplantation from the original donor of black skin, which contains pigmentary dendritic cells, but also of white skin, which contains no pigmentary dendritic cells, and of tongue grafts, which contain no dendritic cells at all. Experiments making use of subcutaneous and intraperitoneal immunising grafts of spleen and lymph node, neither of which contains any epithelial ingredient, are not yet complete. This lack of tissue specificity conforms exactly with the immunological behaviour of ordinary skin homografts : a rabbit for example, may be immunised against skin homografts by an earlier intradermal injection of leucocytes from the same donor (Medawar, 1946*b*).

The purely immunological observations so far reported are equally consistent with two alternative interpretations of the nature of induced foreign pigmentation : (*a*) that the foreign pigmentation is maintained throughout by the survival, proliferation and outward migration of surviving foreign pigmentary dendritic cells, and (*b*) that though the foreign pigmentation is quite certainly initiated by surviving foreign cells from the donor, it is maintained by the propagated infection of native dendritic cells with an antigenically foreign cytoplasmic ingredient. The observations which follow go a little way towards resolving this antithesis.

If the continued melanogenic activity of well-established patches of foreign pigmentation is due to the survival of living donor cells, it is clear that they must enjoy an exemption from the homograft immunity reaction which is denied them when transplanted *in situ* in orthodox skin homografts (which invariably break down) and in that percentage of transplantations done by the seeding method in which pigmentation " spontaneously " fades out after a variable length of time. (In

operations of this second type, foreign mesenchymal cells and collagen fibres are not transplanted : the destruction of melanophores when transplanted in whole skin cannot therefore be a mere secondary consequence of the violent inflammatory changes that occur in the homograft dermis.) Yet the fact that *any* area of foreign pigmentation may be bleached out by active immunisation suggests that the hypothetically foreign melanophores of the pigmented area are *not* exempt.

This inconsistency is aggravated by the following fact : that the total quantity of viable foreign cells transplanted in an immunising operation which leads to bleaching of a large area of foreign pigmentation is *very much smaller than the total number of foreign cells which, according to the terms of the melanophore survival hypothesis, must be supposed to be already in residence.* A skin homograft 6 mm.^{2} in area or less will promptly bleach out a pigment patch 300 mm.^{2} in area or more. If this pigment patch contains one foreign melanophore to twenty native Malpighian cells of the epidermis, it follows that there are already more than twice as many foreign cells in residence as are provided in addition by the immunising graft. It can hardly be that the dosage of foreign cells in any area of pigment spread is always so adjusted that the addition of half as many again, or less, is just what is required to start a bleaching reaction.

According to the alternative hypothesis, there is a clear distinction between the wholly foreign pigmentary dendritic cells which start pigment spread and then break down, and native dendritic cells which, carrying an antigenically foreign cytoplasmic ingredient, are responsible for its maintenance. If this distinction is valid it should be possible so to adjust the dosage of an immunising graft that when the foreign cells in it are manifestly destroyed the cells of the patch of foreign pigmentation either survive unchanged or submit to a merely transient bleaching. Both possibilities have been realised, though only by luck, for in a planned experiment the dosage of an immunising graft would have to be adjusted in relation to a quite unknown quantity, the antigenic disparity between donor and recipient, and the small size of the guinea-pig makes it in any case difficult to work accurately with low-dosage ratios.

It is otherwise with the cow, in which low-dosage ratios are obviously easy to achieve. Pigment spread takes place in the spotted Friesian cow as it does in the guinea-pig (plate IV, fig. 33). In the course of skin-grafting operations on Friesian cattle, done with other ends in view, four black ear-skin homografts were reciprocally interchanged between a pair of young dizygotic twins, the grafts being transplanted to a white skin area on the outer aspect of the foreleg. Pigment spread of intense colour around these grafts began within a few weeks of transplantation and continues to the present time ; the grafts themselves, after prolonged chronic inflammatory changes associated with a " weak " homograft reaction, eventually broke down. At the stage

illustrated by plate IV, fig. 34, 69 days after transplantation, the resurfaced remains of the originally black homografts are dead white, though each is framed within a bold black ring of "infective" pigmentation. Grafts on several other cows underwent similar changes.

Guinea-pig 139, carrying a patch of surviving and normal foreign pigmentation, was immunised by a skin graft from the same donor 51 days after its inception. The immunising graft, of which all traces have long since disappeared, broke down about 25 days after its transplantation, and 10 days later the surface pigmentation of the foreign pigment patch began to weaken and disappear. The surface pigmentation never quite vanished: 150 days after immunisation 10 stout black hairs and some smutty traces of genuine epidermal pigmentation still survived. The pigmented area thereafter very slowly returned to normal and pigment is now spreading at the periphery of a 150 mm.^{2} area 400 days after its first inception.

It has already been argued that the "seeding" operation owes its success partly to the fact that the initial dosage of foreign cells is so small that the immunity they provoke in the course of their supposed destruction offers no more than a slight temporary setback to the progress of pigment spread. This interpretation is, of course, guesswork; but the fact that partial immunisation causes only partial and temporary bleaching is directly demonstrable. On two occasions immunising grafts were removed very shortly before their final destruction: one such graft, grossly œdematous and infiltrated by round cells, is illustrated by plate IV, fig. 27. In both cases the foreign pigmentation underwent a transient incomplete bleaching and then returned to normal. Spreading patches surviving to the present time are now of 330 and 250 days' standing respectively.

A pronounced "spontaneous" bleaching that proves to be incomplete and merely temporary is exactly similar in principle. One instance is so extreme as to deserve special mention. Guinea-pig 204 was subjected to a homograft seeding operation on the sole of its right foot (*cf.* plate IV, fig. 31). Weak diffuse pigmentation recorded at 26 and 33-day inspections proved to have become weaker by the 50th day, and nothing remained to be seen at inspection on the 70th and 120th days. A routine inspection at the 288th day, however, revealed a weak patch of pigmentation, and this has gained in colour and has been slowly spreading until the present time, 311 days after operation.

There are, then, definite grounds for making an immunological distinction between genuinely foreign melanophores and those which may be supposed merely to contain a foreign cytoplasmic element. The interpretation of our immunological data in the terms of an infection theory has, however, special difficulties of its own. These will be dealt with in section 4.

(v) *The serial propagation of pigmentary activity*

In theory, there is no reason why pigmentation induced in a second animal by cells from a first should not itself be transmitted to a third and then in turn to a fourth, and so on. Having overcome the technical difficulties of " splitting " and preparing epidermal cell suspensions from body skin, as this experiment requires, we have so far carried such serial propagations to the fourth stage in two lines and to a third stage in a third. (The fourth stage represents the fifth animal of the sequence, counting the original donor as one.) The time interval between successive propagations has so far ranged between 80 and 131 days ; in future maintenance it will be not less than 100. At each stage we now propagate pigment from one donor to not less than four recipients, in order to insure against the dangers of technical or immunological failure ; and as an additional safeguard against the latter, a particularly low dosage of foreign cells is transferred to only one of the smaller type of seeding bed (fig. 2) on each recipient.

4. A DISCUSSION OF THE " INFECTIVE " THEORY OF PIGMENT SPREAD

The majority of the observations recorded in this paper have made it possible to discriminate between two alternative and mutually inconsistent interpretations of pigment spread ; those of melanophore migration and infective cellular transformation. The inadequacy of the former has already been made clear, and certain shortcomings of the infection hypothesis may now be dealt with.

Repeated attempts have been made, using a variety of techniques, to initiate pigment spread by extracts of pigmentary dendritic cells injected into or otherwise applied to white skin. All such attempts have so far failed. The reasons which we have already discussed in a more general context (1948*b*) may now be enlarged upon. A pigmentary dendritic cell can infect its non-pigmentary neighbours because, as a " cytocrine " cell whose normal activity leads to the inoculation of pigment granules into the Malpighian cells around it, it is specially adapted to perform just that sort of function. Moreover, when two dendritic cells enter into anastomosis, the cytoplasmic bridge between them is a cell process that may be as much as 2μ in diameter. The hypothetical infective agent might therefore be much larger than a virus-like particle—a " plastogene," in Darlington's terminology, though its infective properties require it to be provisionally classified as a provirus (Darlington and Mather, 1949). There are good grounds for supposing that the infective agent is associated with the melanin granules, for the researches of Herrmann and Boss (1945), Riley *et al.* (1949) and DuBuy *et. al.* (1949*a* and *b*) have shown that the complex of enzymes associated with the formation of melanin in the ciliary body and in transplantable melanomata is recoverable from the pigment granules themselves. (DuBuy *et. al.* have impressive

evidence that melanin granules are variant forms of mitochondria. See also Reyni, 1924.) The analogy between melanin granules, as the " elementary bodies " of an infective agent, and the Borrel bodies of fowl-pox virus or the Paschen bodies of vaccinia need hardly be pressed, and it is noteworthy that Sonneborn's kappa factor in *Paramecium aurelia* has proved to be of this order of size (*cf.* Preer, 1948). That the infective agent is highly complex cannot be doubted, since it carries the antigenic specificity of the individual and is destroyed by active immunisation with grafts of white skin as well as black. Its complexity is perhaps analogous to that of the Rous fowl sarcoma virus, which is known to contain an immunologically chicken-specific component (Amies and Carr, 1939) ; this type of association between viruses and the ingredients of normal cells is by no means rare (*cf.* the reviews of Pirie, 1946, and Kidd, 1946).

Another objection to the infection hypothesis turns upon its immunological behaviour when propagated from one individual to another : it appears to be non-antigenic, or only feebly antigenic, when residing in the dendritic cells of the host, but at the same time it is capable of being destroyed by an immunising graft of living foreign cells. Even this type of behaviour, however, is not without precedent. Although it is widely thought that viruses within cells are exempt from the action of circulating antibodies, Kidd (1942) has summarised evidence which shows that the cells of transplantable tumours may be freed from extrinsic (" passenger ") viruses by passage through specifically virus-immune hosts.

We have no explanation for the fact that, after natural or induced bleaching, foreign pigmentation endures longer in the hair follicles than in the superficial skin.

It has already been suggested that the slow rate of spread of red pigmentation into white skin may be due to the presence of a more dilute or attenuated pigmentary system in red skin than in black. The very slow rate of spread of black pigmentation into red skin might be due to an antagonism between two closely related cytoplasmic pigmentary systems, analogous to that between related viruses (Luria and Delbruck, 1942 ; Henle and Henle, 1945). (We are indebted to Dr G. Pontecorvo for suggesting this interpretation.) Both black and red pigmentary systems may be present in " infected " red dendritic cells : the former is " dominant " only in the sense that it is darker in colour and so takes the credit for the cell's phenotypic appearance. A red pigmentary system in a black dendritic cell would give no outward evidence of its presence.

5. SUMMARY

The phenomenon of pigment spread in spotted guinea-pigs has been the subject of a further investigation. Red pigmentation is found to spread into white skins more slowly than black pigmentation, and black pigmentation encroaches upon red skin more slowly still.

Different degrees of attenuation of the pigmentary factors, and competition between different but closely related pigmentary systems, may be responsible for these differences of rate. When red skin is transformed into black, the red dendritic cells are apparently transformed into black dendritic cells.

Pigmentation may be initiated in one guinea-pig by foreign pigmentary dendritic cells transplanted in very low dosage from another, and may thereafter be serially propagated from animal to animal. New techniques have been devised to bring this about: the "grafting" of dilute suspensions of epidermal cells in Ringer's solution to recipient areas cut to such a depth that the transplanted cells are given direct access to the bases of the hair follicles.

Once started, pigmentation of foreign origin increases in area by pigment spread, and in a high proportion of animals it may persist for long periods or perhaps indefinitely. Such pigmentation may at any time be bleached out by transplanting a small immunising graft from the guinea-pig whose cells initiated the pigmentation to the recipient in which it is spreading.

The quantitative and experimental analysis of these phenomena seems to show that pigment spread is caused by a serially propagable transformation of non-pigmentary into pigmentary dendritic cells by a cytoplasmic ingredient of the latter which is capable of behaving "infectively." The transformation has not yet been brought about by cell-free extracts, possibly because the infective agent is physically associated with the melanin granules themselves.

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6. REFERENCES

AMIES, C. R., AND CARR, J. G. 1939.
J. Path. Bact., *49*, 497.

BILLINGHAM, R. E. 1948.
J. Anat., Lond., *82*, 93.

BILLINGHAM, R. E. 1949.
J. Anat., Lond., *83*, 109.

BILLINGHAM, R. E., AND MEDAWAR, P. B. 1948*a*.
Heredity, *2*, 29.

BILLINGHAM, R. E., AND MEDAWAR, P. B. 1948*b*.
Brit. J. Cancer, *2*, 126.

DARLINGTON, C. D., AND MATHER, K. 1949.
The Elements of Genetics. London.

DUBUY, H. G., WOODS, M. W., BURK, D., AND LACKEY, M. D. 1949*a*.
J. Nat. Cancer Inst., *9*, 311.

DUBUY, H. G., WOODS, M. W., BURK, D., AND LACKEY, M. D. 1949*b*.
J. Nat. Cancer Inst., *9*, 325.

HENLE, G., AND HENLE, W. 1945.
Amer. J. med. Sci., *210*, 369.

HERRMANN, H., AND BOSS, M. B. 1945.
J. cell. comp. Physiol., *26*, 131.

KIDD, J. G. 1942.
J. exp. Med., *75*, 7.

KIDD, J. G. 1946.
Cold Spring Harbor Symp. Quant. Biol., *11*, 94.

LOEB, L. 1945.
The Biological Basis of Individuality. Springfield.

LURIA, S. E., AND DELBRUCK, M. 1942.
Arch. Biochem., *1*, 207.

MASSON, P. 1948.
Sp. Pub. N.Y. Acad. Sci., *4*, 15.

MEDAWAR, P. B. 1944.
J. Anat., Lond., *78*, 176.

MEDAWAR, P. B. 1945.
J. Anat., Lond., *79*, 157.

MEDAWAR, P. B. 1946*a*.
Brit. J. exp. Path., *27*, 9.

MEDAWAR, P. B. 1946*b*.
Brit. J. exp. Path., *27*, 15.

MEDAWAR, P. B. 1948*a*.
Brit. J. exp. Path., *29*, 58.

MEDAWAR, P. B. 1948*b*.
Quart. J. micr. Sci., *89*, 239.

PIRIE, N. W. 1946.
Cold Spring Harbor Symp. Quant. Biol., *11*, 184.

PREER, J. R. 1948.
Amer. Nat., *82*, 35.

REYNI, G. S. 1924.
J. Morphol., *39*, 415.

RILEY, V. T., HESSELBACH, M. L., FIALA, S., WOODS, M. W., AND BURK, D. 1949.
Science, *109*, 361.

RUSSELL, E. S. 1948.
Genetics, *33*, 228.

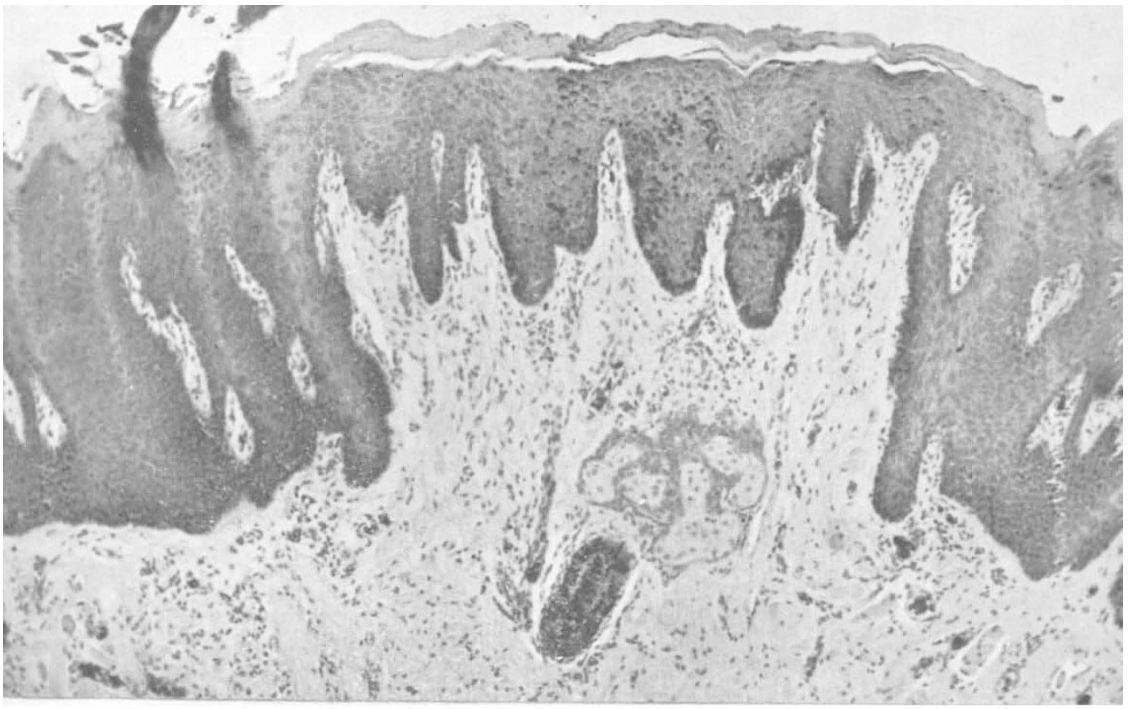
Plate I

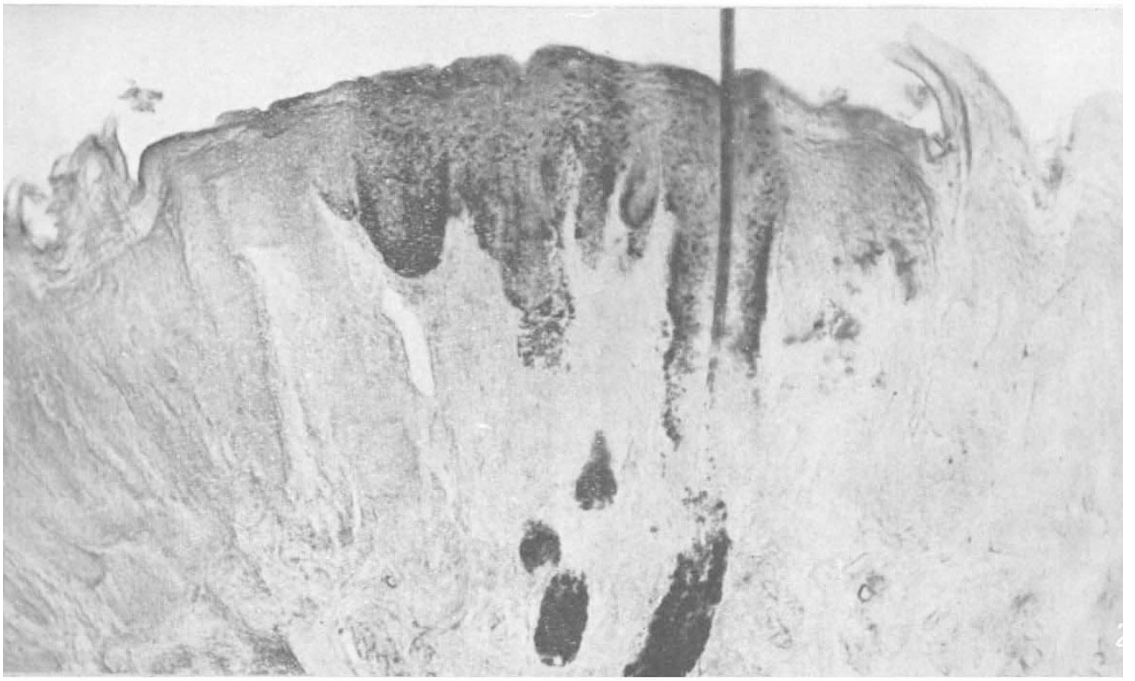
FIG. 1.—Vertical section through a black ear-skin graft 280 days after transplantation to the dorsum of the tongue : Ehrlich's hæmatoxylin and eosin. Note the form of the cuticle, the sebaceous glands, and other evidence that the ear skin epithelium has conserved its exact specificity of histological type. No pigment whatsoever has spread into the neighbouring tongue epidermis, from which dendritic cells are absent (*cf.* fig. 2). × 110.

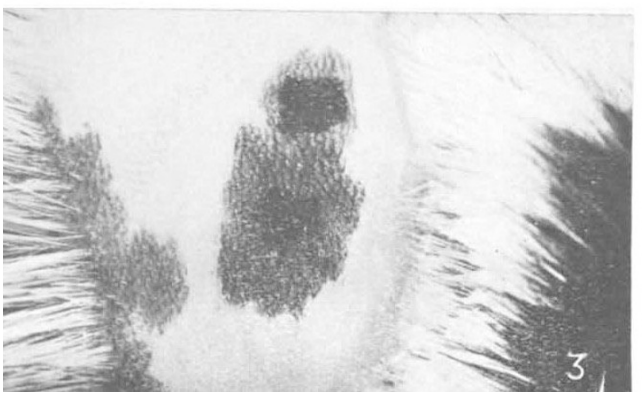
FIG. 2.—See fig. 1 : a vertical section at a different level, stained with carmalum alone. Note the hair follicles and the complete failure of pigment from the ear-skin graft to infect the neighbouring tongue epidermis. × 110.

FIG. 3.—Black (lower) and dark red (upper) ear-skin autografts 220 days after transplantation to white chest skin. Pigment spread has been much more rapid from the former (*cf.* figs. 4 and 5). × 1¾.

FIG. 4.—Black (lower and dark red (upper) ear-skin autografts 232 days after transplantation to white chest skin. Pigment spread has been more rapid from the former (*cf.* figs. 3 and 5). × 1¾.



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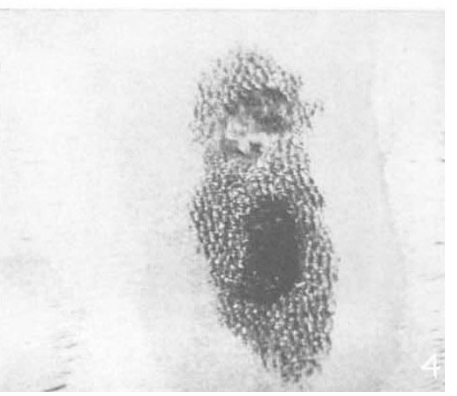
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Plate II

FIG. 5.—A series of ear-skin autografts 555 days after transplantation to the chest. (*a*) An originally pale red graft lying within the black skin in the left half of the photograph, and now almost wholly encroached upon by black pigment. Only a very small irregular central patch remains light red in colour. (*b*) A black skin graft (lower right), pigment spread from which has met, but seems to be moving round and avoiding, the pale red pigment which has spread more slowly from (*c*) a pale red graft transplanted to white skin (upper right). Grafts (*a*) and (*c*) were originally of the same colour; now, after 555 days, grafts (*a*) and (*b*) are of the same colour. For views in higher power, see figs. 9-13. $\times 1\frac{3}{4}$.

FIG. 6.—A black ear-skin graft 301 days after transplantation to dark red chest skin. There has been no trace of pigment spread (*cf.* figs. 7 and 8). $\times 1\frac{3}{4}$.

FIG. 7.—Another black ear-skin graft 301 days after transplantation to red chest skin of medium colour. The edges of the graft are indistinct, but pigment spread is not yet visible (*cf.* figs. 6 and 8). $\times 1\frac{3}{4}$.

FIG. 8.—A black ear-skin graft 623 days after transplantation to pale red-coloured chest skin. The graft is surrounded by a feebly developed annulus of pigment spread (*cf.* figs. 6 and 7). $\times 1\frac{3}{4}$.

FIG. 9.—The epidermis of an originally pale red ear-skin graft 555 days after transplantation to black chest skin (see fig. 5). The epidermis is seen from the under side after weak Dopa treatment, and is taken from that part of the graft wholly encroached upon by black pigment. The epidermal pattern characteristic of ear skin has been fully conserved and a normal number of fully black melanophores may just be discerned (see figs. 10 and 11). $\times 120$.

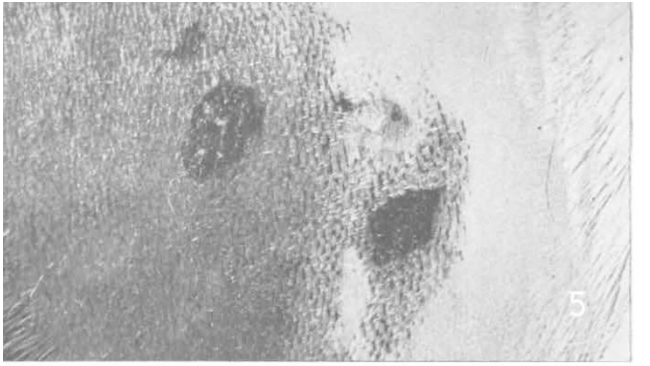
FIG. 10.—A portion of fig. 9 seen in higher power (see also fig. 11) and showing the normal complement of fully black melanophores only, where formerly only red melanophores were present (figs. 12 and 13). $\times 300$.

FIG. 11.—As fig. 10 : another field. $\times 300$.

FIG. 12.—Red melanophores from the central " uninfected " patch of a pale red ear-skin graft 555 days after transplantation to black chest skin (see fig. 5). The processes of the red dendritic cells, almost impossible to discern in living skin, have been rendered visible by weak Dopa treatment. Contrast figs. 10 and 11, in which red melanophores are seen after transformation to the black type, and see fig. 13. $\times 300$.

FIG. 13.—As fig. 12 : another field. $\times 300$.

FIG. 14.—Illustrating the characteristics of pigment spread initiated by a seeding operation : a homograft seeding 127 days after its initiation by a very low dosage suspension in Ringer's solution of black ear-skin cells from another guinea-pig. No graft centre is visible. A sectional view of this graft is illustrated by fig. 17. $\times 3\frac{3}{4}$.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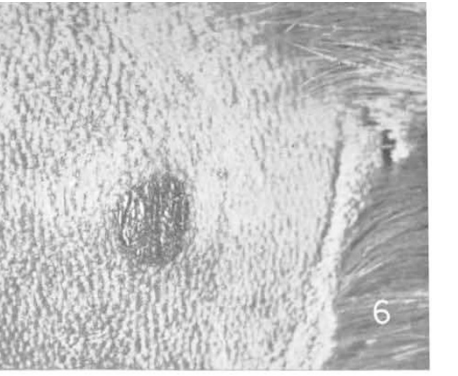
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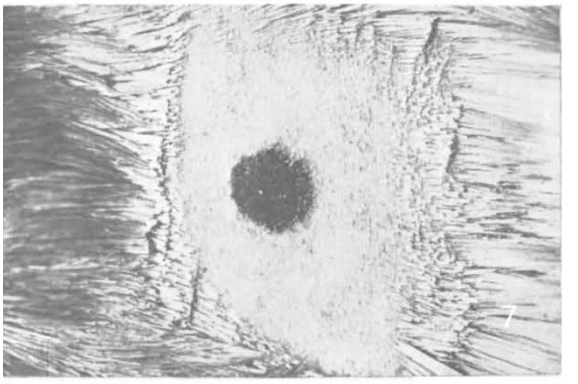
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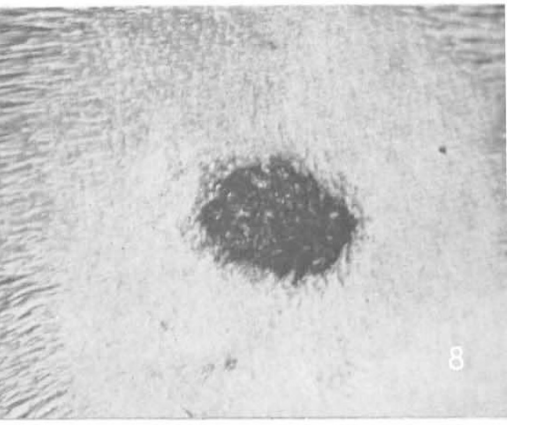
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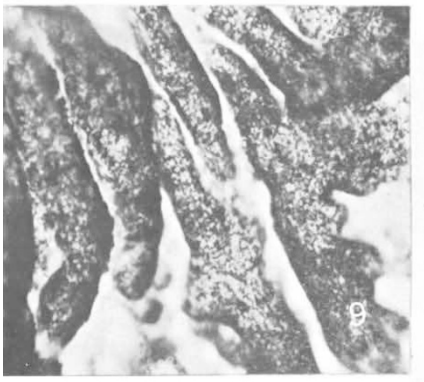
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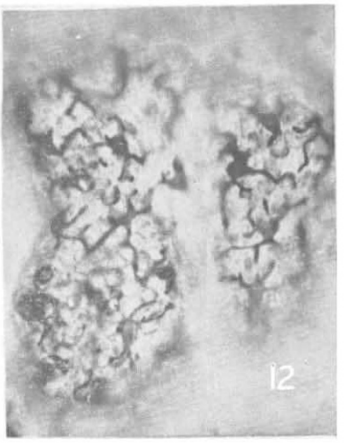
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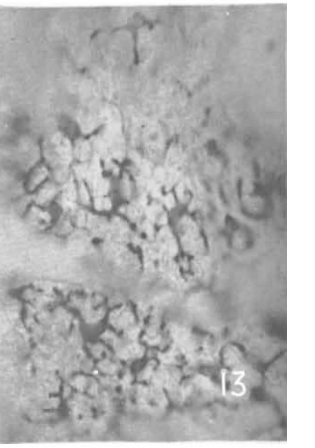
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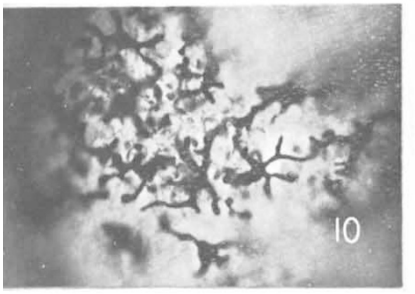
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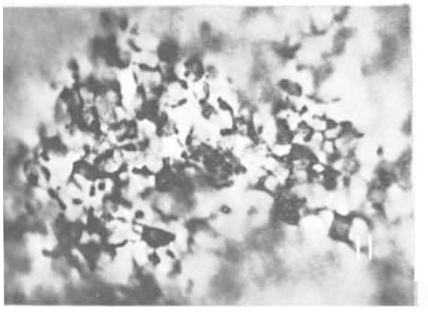
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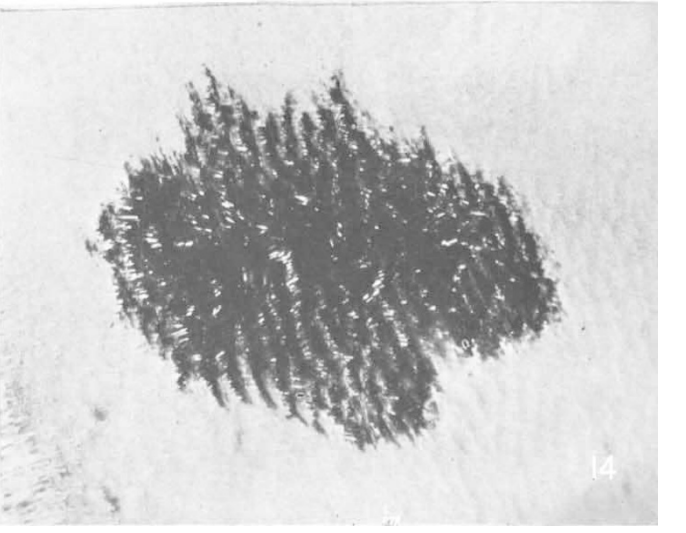
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Plate III

FIG. 15.—Patches of native and of foreign pigmentation 86 days after their initiation by seeding operations making use of autologous and homologous cell suspensions respectively. The two are indistinguishable (*cf.* fig. 16). The pigment patches are shown in sectional view by figs. 18 and 19. ×2¼.

FIG. 16.—As fig. 15: autograft and homograft seedings 86 days after their initiation in another guinea-pig. ×2¼.

FIG. 17.—A sectional view of the homograft seeding of 127 days' standing illustrated by fig. 14. The preparation can be distinguished from normal black chest skin only by a slight persistent hyperkeratosis. ×68.

FIG. 18 (compare fig. 19).—A vertical section through the perfectly normal homograft seeding of 86 days' standing illustrated by fig. 15. Although the mesenchyme cell population of the dermis is slightly denser than in normal skin, it is no more dense than in the control, fig. 19. ×68.

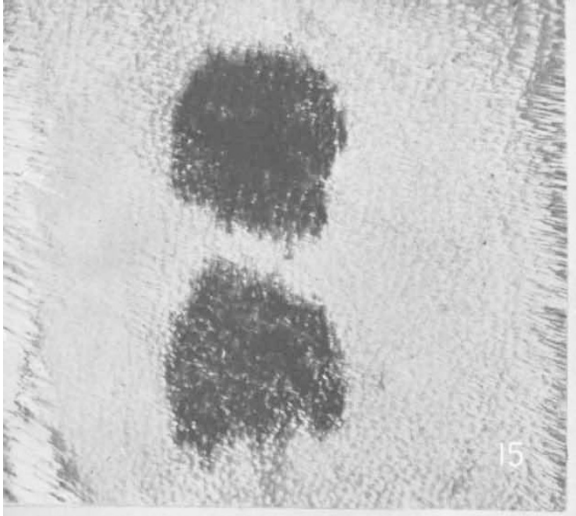
FIG. 19. (compare fig. 18.—A vertical section through the autograft seeding of 86 days' standing illustrated by fig. 15. Its condition is virtually identical with that of the homograft seeding, fig. 18. ×68.

FIG. 20.—Illustrating the characteristics of an early stage of the bleaching process in induced pigmentation of foreign origin: a homograft seeding of 72 days' standing shown 26 days after the transplantation of an immunising graft from its original donor. The epithelium over the primary seeding centre has become smokily translucent and the peripheral pigmentation has weakened in colour and lost its crisply reticular pattern. ×2½.

FIG. 21.—A perfectly normal homograft seeding of 50 days' standing, immediately before the transplantation of an immunising graft from the animal whose cells initiated the pigmentation. See fig. 22. ×1¾.

FIG. 22.—The homograft seeding illustrated by fig. 21, here seen 20 days after the transplantation of an immunising graft. Weak pigmentation is still visible in the primary seeding centres, but has almost disappeared from the peripheral area of pigment spread. ×1¾.

FIG. 23.—Illustrating the operation of "expansion" by which the total area of pigmentation of foreign origin may be rapidly raised. Two Thiersch grafts were cut from within the perimeter of the large primary homograft seeding (to the left) 146 days after its inception, and transplanted to the normal white skin on one side of it. The present photograph, taken 103 days later, shows spread from the secondary grafts about to coalesce with the primary seeding (*cf.* figs. 24 and 25). ×1½.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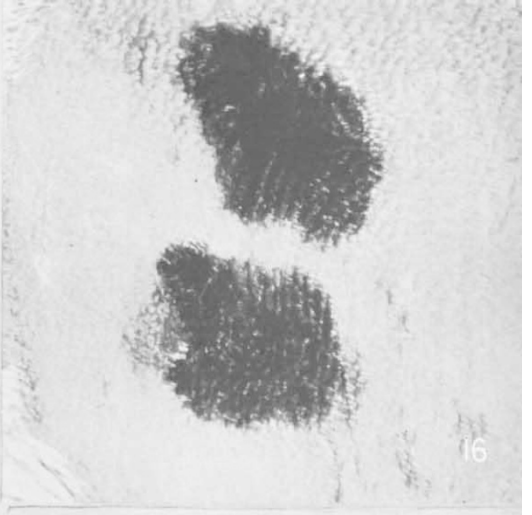
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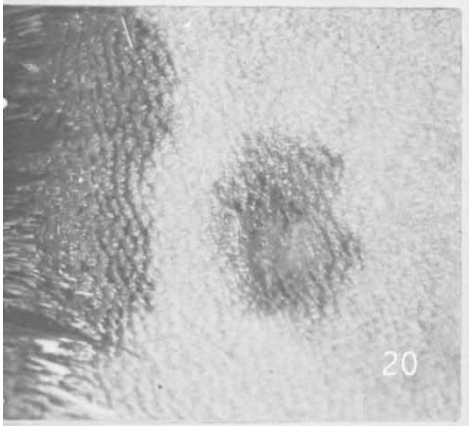
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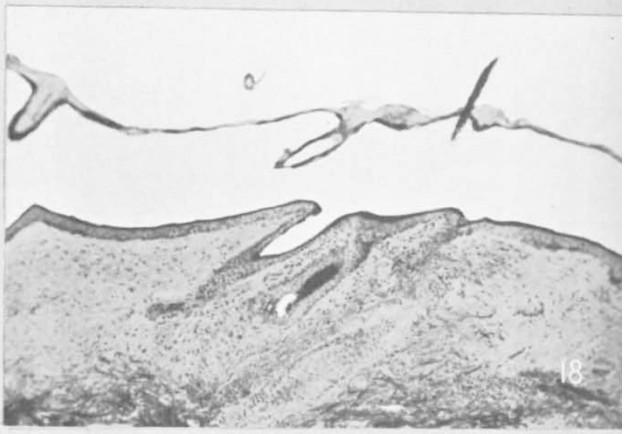
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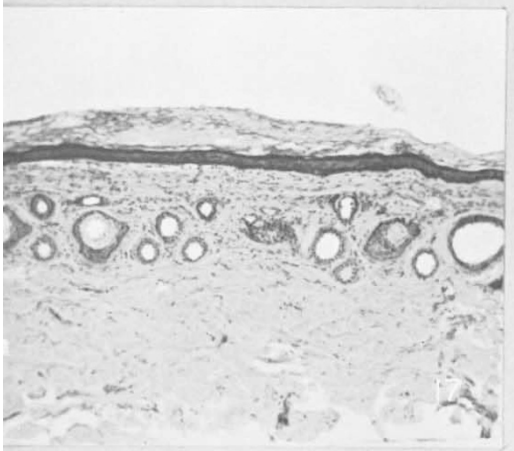
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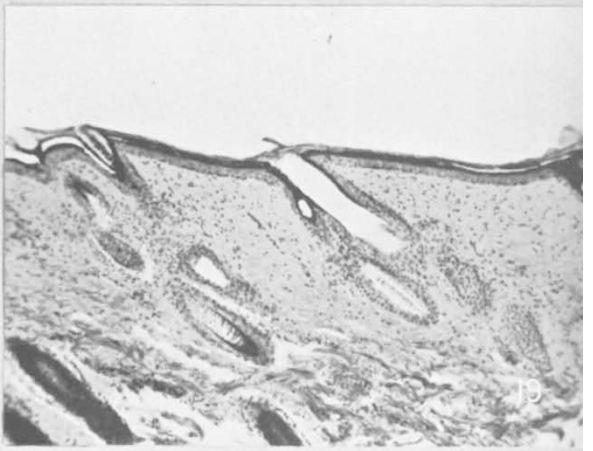
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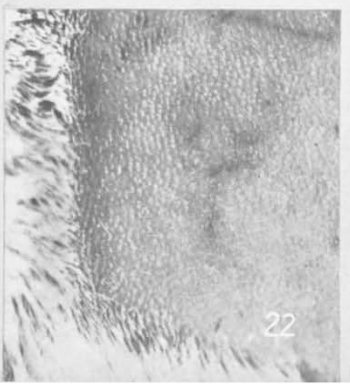
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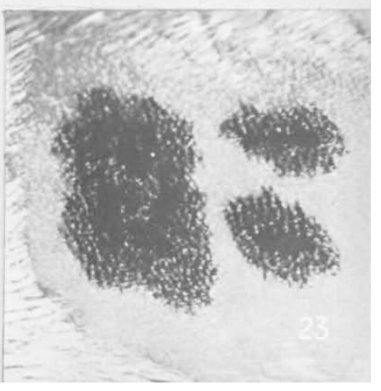
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Plate IV

FIG. 24. (*cf.* fig. 23).—Showing the results of an expansion operation carried out in two stages. A Thiersch graft was cut from within the perimeter of the black primary seeding (shown uppermost) 164 days after its inception in the white chest skin of a spotted chocolate-and-white guinea-pig, and transplanted to the lowermost position. Fifty days later a second graft cut from within the primary seeding was fitted into the white skin still remaining between it and the pigment spreading from the first expansion graft. The photograph was taken 113 days after the second expansion, when the foreign pigmentation was of 327 days' standing in all. $\times 1\frac{3}{4}$.

FIG. 25.—Illustrating the size that a patch of pigmentation of foreign origin may reach by spread combined with expansion : shown 118 days after the expansion of a primary homograft seeding at 148 days.

FIG. 26.—Demonstrating that black pigmentation of foreign origin may spread in the white skin of a *red*-and-white guinea-pig. The foreign pigmentation is shown 267 days after its origin from a number of closely-spaced seeding beds of the smaller (" pinch graft ") type (see Text-fig. 2).

FIG. 27.—An orthodox black ear-skin homograft 16 days after transplantation as an immunising graft to a guinea-pig carrying a patch of pigmentation initiated by cells from the same donor 162 days beforehand. The graft dermis is œdematous and grossly infiltrated with round cells, but breakdown of the epidermis has not quite begun. The immunising graft having been removed, the homograft seeding (fig. 28) did not undergo permanent bleaching and is at present still normal 330 days from its inception. $\times 50$.

FIG. 28.—Biopsy specimen from the homograft seeding referred to in the legend to fig. 27, removed 16 days after the transplantation of an immunising graft. Compare the relatively mild lymphocytic reaction in the dermis with the violent reaction taking place simultaneously within the immunising graft (fig. 27). For a view in higher power, see fig. 29. $\times 50$.

FIG. 29.—A view in higher power of part of the biopsy specimen illustrated by fig. 28. $\times 250$.

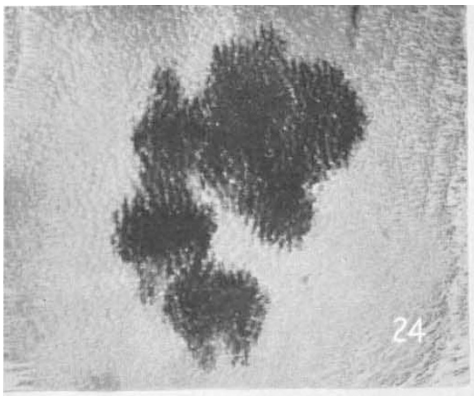
FIG. 30.—Showing that, after being completely bleached out by immunisation, the area formerly occupied by pigmentation of foreign origin is fully " reinfectable." The area was symmetrically ringed by nine small ear-skin autografts which 106 days later had completely obliterated the bleached skin within them by pigment spread. $\times 1\frac{3}{4}$.

FIG. 31.—Pigmentation of foreign origin 162 days after its initiation by a seeding operation on the sole of the foot. $\times 2$.

FIG. 32.—Transverse section through a normal homograft seeding of 154 days' standing 26 days after the transplantation of immunising tongue grafts from the original donor. By this stage, pigmentation had been almost completely lost from the superficial epidermis. The inflammatory reaction is nevertheless of trivial intensity (*cf.* also figs. 27-29). $\times 68$.

FIG. 33.—White skin of the foreleg of a Friesian cow 153 days after the transplantation of a single large " pinch " graft from the black skin of the saddle region. The graft is symetrically surrounded by a wide ring of typical pigment spread.

FIG. 34.—White skin of the foreleg of a Friesian cow 69 days after the transplantation of four black ear-skin homografts from its dizygotic twin. The annuli of pigment spread around the grafts have not been affected by the weak and prolonged immunity reaction, which has, however, led to the destruction of the homografts themselves, at this stage represented by white central patches.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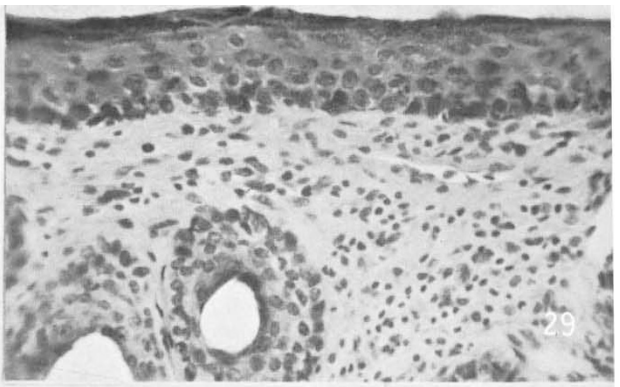
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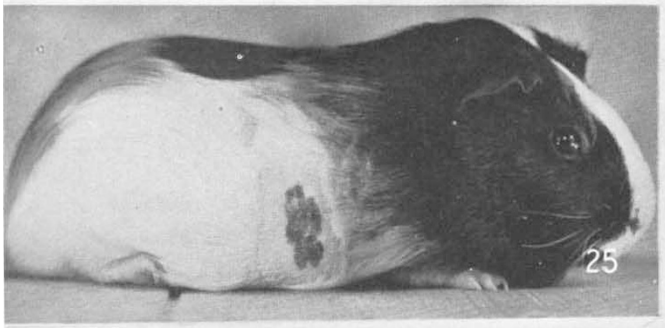
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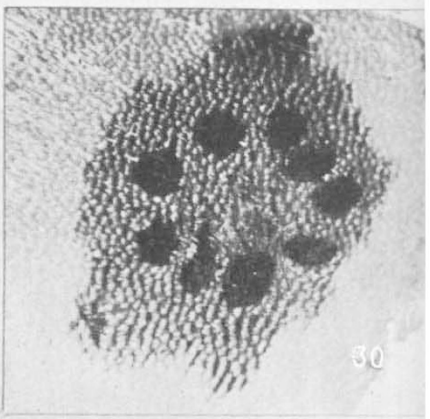
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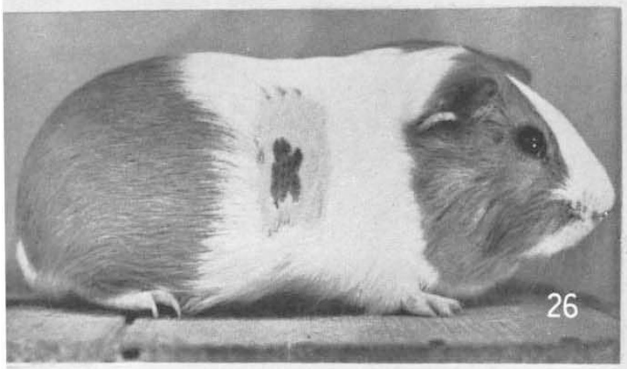
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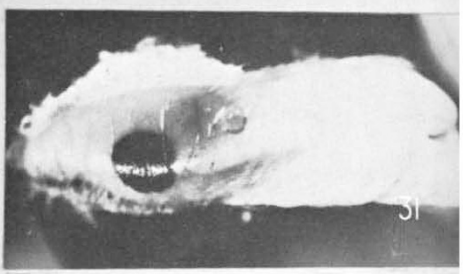
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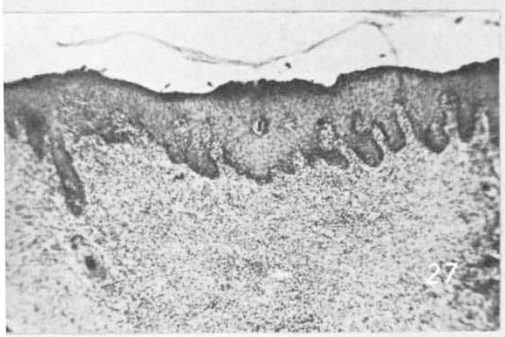
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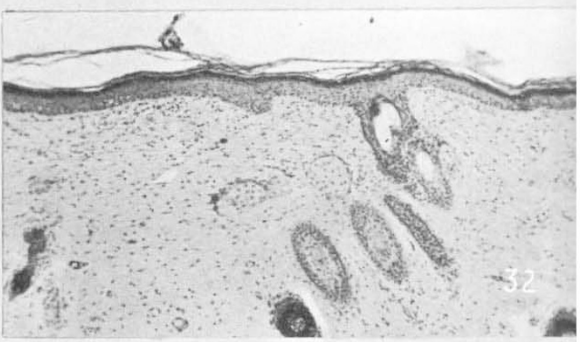
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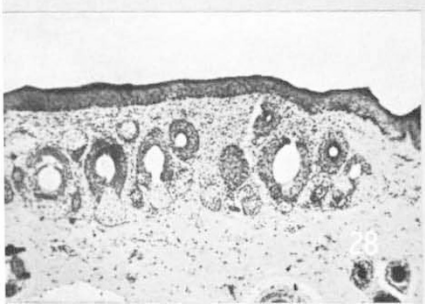
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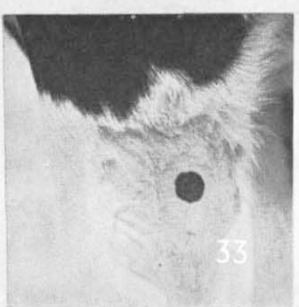
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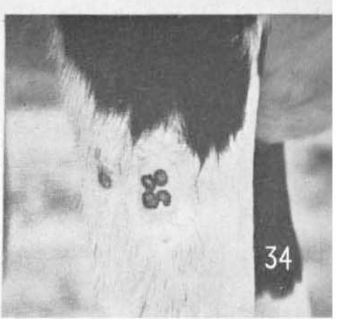
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