A STUDY OF THE BRANCHED CELLS OF THE MAMMALIAN EPIDERMIS WITH SPECIAL REFERENCE TO THE FATE OF THEIR DIVISION PRODUCTS

By R. E. BILLINGHAM* and P. B. MEDAWAR, F.R.S.
Department of Zoology, University College, University of London

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The branched cells of the superficial epidermis of mammals are divisible into two classes: those that occupy a position in the basal layer, and those that occur in more superficial layers. The first class comprises melanocytes visible in the living epidermis after its enzymatic fission from the corium; cells that blacken upon exposure to dihydroxyphenylalanine; cells that maintain quinone-imine dyes in the oxidized state in living skin; and clear cells. It is shown that these are merely different preparation images of the same cell, the melanocyte. The second class comprises cells that may be more or less specifically impregnated by metallic gold (Langerhans' cells); cells stainable in living skin by quinone-imine dyes; and the 'clear cells' of superficial strata. It is shown that these, too, are so many preparation images of the same cell.

It is argued that the branched cells of superficial strata, which have never been seen to divide, represent effete melanocytes which, having discharged or otherwise lost their pigment, participate in the general outward movement of epidermal cells to be cast off at the skin surface. This argument is supported by evidence of their similarity of structure, mode of branching, and relationship to neighbouring Malpighian cells; by their position in the epidermis; by their one-to-one correspondence of number; and by their coincidence of distribution.

* Research Fellow of the British Empire Cancer Campaign.

Introduction

It is now generally agreed that the pigmentation of mammalian skin is conferred upon it by the activity of cells belonging to a lineage quite distinct from that which gives rise to the greater part of the Malpighian layer of the epidermis. Epidermal pigment is formed only within melanocytes or pigmentary dendritic cells, which arise in development, not from definitive epidermis, but from the neural crest (Rawles 1940, 1947, 1948). Epidermal melanocytes are highly branched cells, the perikarya of which lie in the basal layer of the epidermis; their branches weave between the Malpighian cells in their neighbourhood and end upon them in characteristic caps. Pigment formed within the melanocytes is in some manner caused to enter the Malpighian cells to which their branches are applied, so giving rise to the mistaken impression that pigmentary activity is a property of Malpighian cells as well as melanocytes. This mode of activity has been described by Masson (1948*a*) as 'cytocrine'.

The epidermis of mammals is therefore composed of cells of at least two distinct division lineages or histological genera: Malpighian cells and epidermal melanocytes.* The fate of Malpighian cells is, at least in outline, well understood. The great majority of divisions take place in the layer that bounds the dermo-epidermal interface; the study of many thousands of skin preparations has convinced us that their occurrence at more superficial levels is very rare.† Some proportion of the division products of Malpighian cells remains in the basal layer and may divide again. The remainder pass towards the skin surface at a rate which may be computed by ^{14}C autoradiography (Leblond 1951). In doing so, they undergo the regular sequence of changes that gives the epidermis its distinctive pattern of stratification.

The fate of the melanocyte is at present obscure, but two possibilities may be envisaged: either (*a*) all the division products of melanocytes may retain their proliferative power and their position in the basal layer, division occurring only so often as will maintain a fairly constant average number; or (*b*) some proportion of the division products, becoming physiologically effete, takes part in the general outward movement of epidermal cells to be cast off at the skin surface. Masson (1948*a*) has argued in favour of this second possibility, and he has identified the effete melanocytes of the more superficial strata with the 'Langerhans cells' revealed by the treatment of skin with gold chloride.

A third possibility, (*c*), is that melanocytes are not indigenous residents of the epidermis, but that their number is regularly made good by the immigration of cells from the corium. There is no ground for supposing this to be the case. Billingham & Reynolds (1952) and Billingham & Sparrow (unpublished) have prepared pure epidermal cell suspensions from the pigmented epidermis of the ears of spotted black and white (Dutch) rabbits, and have 'seeded' them upon large raw areas cut from unpigmented skin elsewhere on the same

* Citations of the literature embodying this opinion will be found in the papers of Zimmermann & Cornbleet (1948), Billingham (1948) and Masson (1948*a*, *b*).

† In orthodox vertical sections of a thick epidermis, such as that of the guinea-pig's ear or sole-of-foot, mitoses may often be seen in what appears to be a relatively superficial stratum. The study of serial sections shows that this is because, in certain patches, the plane of the section will be tangential to the walls of the steeply rising dermal papillae so characteristic of a thick epidermis. In such patches the section, though vertical to the skin surface, is anatomically horizontal, since it lies in the plane of the dermo-epidermal interface.

animal. They found that the transplanted epidermal cells reassociated themselves and proliferated to form a continuous sheet (cf. figure 1, plate 9) of pigmented epithelium, thus showing that the epidermis, comprising both Malpighian cells and melanocytes, is a reproductively self-sufficient system, in the sense that it is maintained by the division of cells already resident in the epidermis.

Melanocytes and Malpighian cells are not the only constituents of the epithelial layer of skin, but it is probable that cells of all other types are of purely adventitious origin. Lymphocytes, polymorphs and histiocytes are sometimes found within the epidermis, particularly over inflamed areas; cells that look like fibroblasts prising their way into the basal layer in a direction perpendicular to the skin surface are not uncommon in a rapidly proliferating epithelium, such as that which grows out from a skin graft over granulation tissue.

Andrew & Andrew (1949) believe that the number of epidermal cells seen in microtome sections of skin to be in mitosis is insufficient to account for the self-maintenance of the epidermal population, and they argue that the population number is regularly made good by the immigration of lymphocytes into the epidermis. Unfortunately, the cells they describe as lymphocytes are almost beyond doubt the perikarya of melanocytes ('clear cells' of the basal layer; see below, p. 160). An argument that turns upon the apparent inadequacy of epidermal mitoses is meaningless unless attention is paid to the duration of mitosis in the individual cell and the length of the intermitotic interval. The work of Billingham & Reynolds (1952) shows that it is in any event invalid, for areas of skin from which all epithelial elements have been removed become re-epithelialized only if more epidermal cells are added in the form of grafts.

Part 1 of this paper, containing information that is essential for an understanding of the two later parts, deals with the number and distribution of melanocytes. Part 2 comprises a description and reasoned classification of the branched cells that may be revealed by one technique or another within the epidermis, and part 3 contains an assembly of circumstantial and experimental evidence that the melanocyte, as Masson has supposed, is indeed a squamous cell. Our principal object has been to study the pigmentary system of *human* skin, which has accordingly been used whenever possible. The epidermis of guinea-pig's ear skin has been substituted on other occasions, partly because of its ease of supply, and partly because it is available in many more pigmentary variants than human skin. The trunk skin of the guinea-pig has been used for special purposes, but its structure differs from that of human skin, and of guinea-pig's ear skin, in a variety of significant and sometimes revealing ways that will be made clear by the text.

It has been thought best to describe the details of our technical methods in the sections of the text to which they refer. In general, all our analyses have been founded upon examinations of the living epidermis in whole-mount preparations, and then extended to the appearance of skin in orthodox vertical sections. Much use has been made of the preparation described as 'split skin', i.e. a sheet comprising the entire living epidermis disengaged from the corium by mild digestion in crude trypsin solutions. The preparation of split skin has been described in detail by Billingham & Medawar (1951), and it has repeatedly been shown (Billingham & Medawar 1950; Billingham & Reynolds 1952) that the epidermis so prepared is alive and may be used for grafting.

Part 1. The functional anatomy of the melanocyte system

Distribution

The melanocyte system of the skins of man, guinea-pig, pig and cattle consists of two anatomically and (in some degree) physiologically distinct parts: that belonging to the hair bulbs and responsible for the pigmentation of hair, and that belonging to the basal layer of the superficial epidermis. The two sub-systems are separated by that region in the necks of the hair follicles from which melanocytes are absent (figure 2, plate 9; cf. Billingham & Medawar 1948).

It is a commonplace observation that the pigmentary activity of the hair bulbs may differ from that of the superficial epidermis. In white human beings, dark hairs issue through an all but colourless skin, and in patched guinea-pigs of certain colour patterns, black hairs may issue through red skin, or vice versa. In pigs, white bristles may pierce pigmented spots on the surface of the skin. The spread of pigmentation from coloured areas into white in recessively spotted guinea-pigs affects the superficial epidermis but not the hairs that penetrate it (Billingham & Medawar 1948, 1950). Yet, in spite of this distinction, melanocytes of the two anatomical sub-systems are fully interchangeable. If the superficial epidermis of the skin of a pigmented guinea-pig is wholly removed from an area 3 to 4 cm^2 in such a way as to leave the bases of the hair follicles intact—e.g. by removing a shaving of 'Thiersch graft' thickness (Billingham & Medawar 1951)—the defect heals without appreciable contracture by the upward migration of epithelium from the hair follicles. In this manner, a normally pigmented surface epithelium is formed anew, and its melanocytes can only have been derived from those originally resident in the hair bulbs. Conversely, if the superficial epidermis is removed in exactly the same way from the skin of a *white* area in a recessively spotted guinea-pig, and the defect so formed is 'seeded' with a suspension in Ringer's solution of basal-layer epidermal cells prepared from pigmented skin elsewhere on the same animal (Billingham & Medawar 1950, 1951), many of the hairs that grow anew are black, instead of white, and remain so (figures 3, 4, plate 9). The hair bulbs must therefore have derived their pigmentary activity from melanocytes originally resident in the superficial epidermis—either wholly as a result of the colonization of white hair bulbs by pigmentary melanocytes of extraneous origin, or at least in part (so Billingham & Medawar have argued) by the 'infective' inoculation of the non-pigmentary melanocytes of the white-hair bulb with some cytoplasmic ingredient derived from the pigmentary melanocytes which have been given access to them. Dr F. J. Pepper has confirmed these findings in a full analysis of the interchangeability of hair bulb and superficial melanocytes, and the results of her work will be published elsewhere.

In coloured rabbits, rats and mice the superficial epidermis of the skin of the trunk (but not the epidermis of the ear or tail) is unpigmented. The pigmentation of the epidermis of the mouse has been made the subject of a full investigation by Reynolds (1953).

Not all epidermal epithelia are equipped with melanocytes. They are absent not only from the neck of hair follicles around the level at which the sebaceous glands open, but also from the cornea and (at least in the guinea-pig) from the tongue. It will be shown later that their hypothetical degeneration products are also absent from the superficial strata of these epithelia, as was to be expected if Masson's hypothesis is true.

Number in relation to pigmentary activity in the guinea-pig

Melanocytes, like Malpighian cells (Billingham & Medawar 1948), may exist in a single individual in a variety of cellular genetic 'species', i.e. variant forms which preserve their distinctions of character indefinitely even after transplantation to anatomically and physiologically foreign environments. 'Black' and 'red' melanocytes, for example, may form conspicuously distinct species in a single guinea-pig. Such differences of pigmentation *might* be due (amongst other things) to characteristic differences between the concentrations of melanocytes; red skin, for example, might have fewer melanocytes per unit of area than black skin. Billingham (1949) showed from the limited material at his disposal that the melanocytes of negro skin were no more abundant than those of white skin, and the evidence presented below suggests that this conclusion may be generally valid. We now propose to give estimates of the density of melanocytes in guinea-pig's ear skin and general body skin, and to show that differences of density are not correlated with differences of colour. The subjects of these observations were patched red and black or red, white and black guinea-pigs of a variety of genotypes.

The melanocyte population of ear skin

Guinea-pigs were chosen in which one ear was red and the other black, or in which red and black areas were to be found on a single ear. Samples were removed from skins of both colours and handled side by side through all the procedures now to be described.

The dorsum (on one occasion only the ventrum) of the ear was cleaned, shaved and greased with a thin film of vaseline. The thinnest possible skin shaving or 'Thiersch graft' (see Billingham & Medawar 1951), about 1 cm^2 in area and of even thickness, was then sliced off and gummed cuticle-side downwards to a cover-slip thinly smeared with rubber-vaseline stopcock grease. The thin slice was digested with crude trypsin solution for 15 to 20 min in the way described in full by Billingham & Medawar (1951), and the dermis thereupon peeled away from the epidermis, which remained stuck to the cover-slip. The epidermis was fixed for not less than 3 h and not more than 24 h in 2 % formaldehyde in normal saline, washed for 20 min in normal saline, and incubated for 3 to 4 h at 37° C in two changes of the 'Dopa' reagent, viz. 7 ml. 0·1 % L-dihydroxyphenylalanine mixed before use with 3 ml. M/15 Sørensen phosphate buffer at pH 7·4. The cytoplasm of the melanocytes being thus blackened by the formation of 'dopa melanin', the epidermis was again fixed, dehydrated, cleared, and mounted in balsam for inspection from its inner (dermal) side.

The melanocytes were counted by projecting the image of a microscopical field (cf. figure 6, plate 9) of known area on to the ground-glass screen of a standard photomicrographic apparatus. Ink spots were used to mark the positions of the melanocytes; eight to twelve randomly chosen areas of 0·0668 mm^2 were counted on both the red and the black skin samples from each individual. Black skin was less easy to count than red because the abundance of melanin granules made it the more opaque; but neither was difficult, and the individual counts may be assumed to be very precise.

The results are set out in table 1. The mean number of melanocytes per mm^2 of the basal layer of ear-skin epidermis in plane projection ranges between 515 ± 23 and 1751 ± 51 (overall mean 893 ± 85 standard error). In only two of the eight observations was the

difference in cell concentration between the two differently coloured areas of a single individual greater than might be attributed to luck of sampling. In general, the correlation ($r = 0{\cdot}84$) between the melanocyte concentrations of the two coloured areas was very high. If red and black areas contained on the average the same concentration of melanocytes, then the mean of the differences set out in the last column of table 1 should clearly be zero. In practice, it differs from zero only by a gap that might appear by luck as often as once in every three such sets of random trials. It may be concluded, then, that although the concentration of melanocytes varies over a wide range between corresponding areas on different individuals, it differs little, if at all, between corresponding areas on the same individual; and the differences, such as they are, cannot be associated with differences of colour.

TABLE 1

The numbers of melanocytes in red and black guinea-pig's ear skin: the entries in italics are those that relate to *red* skin. Each counted field was 0·0668 mm² in area. Except in animal 444, where both dorsum and ventrum were used, all counts were made upon skin from the dorsum of the ear; in animals 444 and 457, red and black areas occurred on a single ear; in all others, one ear was red and the other black. Entries under the column headed *P* represent the likelihood that the counts relating to red and black areas represent different random samples from the *same* normal population; a value of *P* below 5% may be taken to represent a genuine difference of number.

animal no.	colour and side	no. of counted fields	mean no. of melanocytes ± S.E.	*P*	mean no. of melanocytes per mm² ± S.E.	diff.
437	*red (R)*	*8*	*69·3 ± 2·5*	>40%	*1037 ± 37*	+ *40*
	black (L)	8	66·6 ± 2·5		997 ± 37	
445	*red (R)*	*8*	*91·1 ± 7·0*	<0·1%	*1364 ± 105*	−387
	black (L)	8	117·0 ± 3·4		1751 ± 51	
481	*red (R)*	*10*	*49·7 ± 1·6*	30%	*744 ± 24*	+ *9*
	black (R)	10	49·1 ± 1·7		735 ± 25	
444	*red (L)*	*9*	*35·2 ± 1·3*	>5%	*527 ± 19*	− *52*
	black (L)	9	38·7 ± 1·4		579 ± 21	
444	*red (R)*	*10*	*36·9 ± 2·2*	>30%	*552 ± 32*	+ *37*
	black (R)	10	34·4 ± 1·5		515 ± 23	
457	*red (R)*	*12*	*47·2 ± 4·3*	>30%	*706 ± 64*	+ *82*
	black (R)	12	41·7 ± 3·7		624 ± 56	
482	*red (R)*	*10*	*68·6 ± 2·7*	>60%	*1027 ± 41*	+ *24*
	black (L)	10	67·0 ± 1·6		1003 ± 24	
443	*red (R)*	*8*	*65·0 ± 3·6*	<0·1%	*973 ± 53*	−183
	black (L)	8	77·3 ± 1·1		1156 ± 17	

Overall mean: 893 ± 85 melanocytes/mm².

The melanocyte population of the skin of the trunk

Guinea-pigs were used in which red and black skin could be chosen for sampling from symmetrically opposite areas on either side of the trunk.

The epidermis of body skin is thinner than that of ear skin, and, being densely haired, more difficult to peel away from the dermis after enzymic digestion without running some risk of macerating the cells of the basal layer. This difficulty was overcome by making no attempt to remove the epidermis at all. The thinnest possible Thiersch shavings, cut from tightly stretched skin, were mounted cuticle-side downwards on cover-slips and fixed and washed in the way that has just been described. Each pair of specimens was incubated with the Dopa reagent for 4 to 6 h, and then fixed, dehydrated, cleared, and so mounted as to be inspected from the outer, not the dermal surface.

Eight to twenty randomly chosen areas of either 0·1328 or 0·0471 mm² were counted on both the red and the black skin samples from each of eight individuals.

One important qualification should now be introduced. The epidermis of guinea-pig's trunk skin as it is seen in a whole mount examined from the inner side (figure 5, plate 9) has a broad 'hill and valley' pattern bearing a regular relationship to the rows of hair follicles; the hills are elongated epidermal thickenings which run at right angles to the direction of hair slope and which occasionally unite or bifurcate at acute angles to give Y-shaped junctions (Billingham & Medawar 1948). Living melanocytes are absent from the valleys, where the epidermis is thinnest; they are therefore absent from an area which comprises between one-third and two-thirds of the skin surface. More will be said of this distribution later; for the present, it should be stated that the melanocyte counts on body skin relate to the 'hill' regions only, i.e. to the areas in which melanocytes are actually present, with no allowance in the final figures for areas in which they are not to be found.

The results are set out in table 2, and they confirm those derived from ear skin in every particular. The average number of melanocytes per mm² of the epidermal hills may be as low as 297 ± 6 and as high as 638 ± 25 (overall mean 406 ± 26 standard error). The variation is principally between one individual and another, but in four of the eight pairs of observations the difference between the concentrations of melanocytes on the two sides of a single individual is rather greater than can reasonably be attributed to luck of sampling. Sometimes the 'red' melanocytes are more abundant, sometimes the black. In general, the cell counts in red and black areas are strongly correlated ($r = 0{\cdot}86$), and the mean of the differences between them (last column of table 2) departs from zero by a gap that might be expected to occur by luck as often as once in every five such sets of

TABLE 2

The numbers of melanocytes in red and black guinea-pig's trunk skin: the entries in italics are those that relate to *red* skin. In animals 432, 462 and 483 each counted area was 0·0471 mm²; in the remainder, 0·1328 mm². In each animal the red skin came from one side of the body and the black skin from a symmetrically opposite position on the other side. For entries under the column headed *P*, see legend to table 1.

animal no.	colour and side	no. of counted fields	mean no. of melanocytes ± S.E.	*P*	mean no. of melanocytes per mm² ± S.E.	diff.
182	*red (L)*	*8*	*64·3 ± 1·7*	> 20%	*484 ± 13*	− 20
	black (R)	8	66·9 ± 1·6		504 ± 12	
386	*red (L)*	*10*	*43·1 ± 2·9*	> 80%	*324 ± 22*	+ 5
	black (R)	10	42·4 ± 1·9		319 ± 14	
414	*red (R)*	*8*	*47·3 ± 1·6*	> 50%	*356 ± 12*	− 9
	black (L)	8	48·5 ± 1·2		365 ± 9	
431	*red (L)*	*10*	*58·6 ± 2·6*	< 1%	*441 ± 20*	+ 74
	black (R)	10	48·8 ± 1·8		367 ± 14	
432	*red (L)*	*10*	*24·2 ± 0·5*	< 0·1%	*513 ± 11*	−125
	black (R)	10	30·1 ± 1·2		638 ± 25	
462	*red (L)*	*20*	*21·2 ± 0·7*	< 2%	*449 ± 15*	− 55
	black (R)	20	23·8 ± 0·8		504 ± 17	
483	*red (L)*	*15*	*14·0 ± 0·3*	< 0·1%	*297 ± 6*	− 92
	black (R)	15	18·8 ± 0·5		389 ± 11	
484	*red (R)*	*12*	*36·5 ± 1·4*	> 80%	*275 ± 11*	− 2
	black (L)	12	36·8 ± 1·4		277 ± 11	

Overall mean: 406 ± 26 melanocytes/mm².

trials. Differences of colour within a single individual cannot therefore be attributed to differences in the concentrations of the melanocytes that are responsible for it. It is not the number of melanocytes, but their mode of pigmentary activity that is responsible for differences of colour in the animals we have studied.

Part 2. The branched cells of the epidermis

The gist of the argument to be presented in parts 2 and 3 of this paper is that certain unpigmented branched cells to be seen in the more superficial strata of the epidermis are the histological degradation products of the melanocytes of the lowermost stratum; that they are, in fact, effete melanocytes in course of desquamation. The evidence is circumstantial, for melanocytes have not been *seen* to discharge their pigment and to pass outwards to be flaked off at the skin surface.

In the present part, we shall deal one after another with the various cells believed to be associated with pigmentary function in the epidermis, as they are revealed by various histological techniques. These cells are (*a*) melanocytes which, being visible in living unstained preparations of the epidermis, form the base-line for later comparisons; (*b*) 'dye-positive' cells, those which are stained in the *living* epidermis by dilute and weakly alkaline solutions of quinone-imine dyes (Conn, 1946) such as methylene blue, toluidine blue, brilliant cresyl blue, and nile blue; (*c*) dopa-positive cells in the epidermis, i.e. those which blacken after impregnation with L-dihydroxyphenylalanine in weakly alkaline solution; and (*d*) 'clear cells' (cf. Cowdry 1944; Billingham 1948; Masson 1948*b*), visible in the *basal* layer of the epidermis in orthodox vertical sections after careful fixation. It will be proved that cells of categories *a*, *b* and *c* are identical and that the clear cells of category *d* represent their perikarya.

The branched cells visible in the more superficial epidermal strata (and, for reasons that will be discussed in detail, in the basal layers of the thinner and mitotically inactive 'valley' regions of guinea-pig's trunk skin) are (*e*) highly branched dendritic cells that are stained in the *living* epidermis by dilute and weakly alkaline solutions of quinone-imine dyes; (*f*) highly branched dendritic cells visible in the epidermis after treatment with gold chloride; and (*g*) 'clear cells' visible in the more superficial layers of the epidermis in orthodox vertical sections after careful fixation. It will be proved that cells of categories *e* and *f* are identical and that the clear cells of category *g* represent their perikarya.

Finally, in part 3, grounds will be given for supposing that cells of types *e*, *f* and *g* represent stages in the desquamation of cells of types *a*, *b*, *c* and *d*, much as cells of the stratum granulosum represent stages in the desquamation of the Malpighian cells of the basal layer.

Cells visible in the basal layer of the epidermis

Living melanocytes, dopa-positive cells, and quinone-imine dye positive cells (types a, b, c)

If the epidermis is removed from pigmented human or guinea-pig's skin, mounted in a drop of Ringer's solution, and inspected from the inner side, melanocytes stand out boldly. If the same specimen (preferably from a rather weakly pigmented skin) is immersed for 1 to 3 h at room temperature in about 5 ml. of a 0·01 % solution of methylene blue in Ringer's solution containing 0·05 to 0·1 % $NaHCO_3$, the cytoplasm of the perikarya and branches of the melanocytes becomes bright blue. (Toluidine blue and brilliant

cresyl blue will also serve.) The Malpighian cells remain colourless, presumably because the dye within them is in the reduced state, for they colour promptly upon fixation.

The same preparation may now be fixed for a few hours in 2 % formaldehyde in Ringer's solution, washed, and incubated in the dopa reagent as described in part 1. The perikarya and processes of the melanocytes, formerly blue, now blacken with the formation of 'dopa melanin' (figures 6, 7, plate 9). No other cell blackens. There is therefore no doubt that the dopa-positive and quinone-imine dye-positive cells of the basal layer of the epidermis are melanocytes, and that melanocytes are specifically so revealed.

These results, in so far as they relate to the 'dopa reaction', merely confirm the opinions of all experienced histologists. It should be added that the tyrosinase reaction will also reveal melanocytes in the epidermis (Fitzpatrick, Becker, Lerner & Montgomery 1950), and although the dopa and tyrosine reactions do not always go hand in hand (Foster 1952), there are excellent grounds for believing that the dopa reaction is mediated by tyrosinases and not, as was formerly believed, by enzymes specific to the dopa substrate (Fitzpatrick *et al.* 1950; Lerner & Fitzpatrick 1950; Foster 1951). The fact that quinone-imine dyes are kept in the oxidized state in living melanocytes, though they are reduced in cells of most other types, may perhaps also be a consequence of their oxidase content.

The dopa and methylene-blue reactions may be used to reveal melanocytes so weakly pigmented (e.g. in white human skin; Billingham 1948) as to be indistinguishable in living preparations of split skin. Neither reaction reveals dendritic cells in the basal layer of the epidermis of white skin areas on recessively spotted guinea-pigs (cf. Ginsberg 1944).

Dopa-positive cells are found *only* in the basal layer of the epidermis. Their appearance in vertical sections in more superficial layers is a consequence of the optical illusion referred to in the footnote to p. 152. It will be clear later, however, that dye-positive cells appear in superficial strata as well as in the basal layer.

Cells of type d *in the basal layer* (*clear cells*)

Clear cells in the basal layer of the epidermis have been described and figured by a number of authors; Masson (1948*b*), Billingham (1948) and Zimmermann & Cornbleet (1948) believe that they represent the perikarya of melanocytes. Clear cells that live up to their name are visible only in weakly pigmented skins, though they are not visible in the white skin areas of recessively spotted rats (Taylor 1949) or guinea-pigs. They are peculiarly vulnerable to fixation artifacts, notably the collapse of the cytoplasm around the nucleus to leave an empty space, but in our experience Altmann's fixative (osmium tetroxide-potassium dichromate) can be relied on for good preservation.

Clear cells are distinguished from Malpighian cells by the abundance of their cytoplasm and by the fact that the cytoplasm is optically almost clear and not basiphilic; unlike Malpighian cells, they are not united to their neighbours by structures having the appearance of cytoplasmic bridges (figure 8, plate 9). The grounds upon which they have been assumed to represent the perikarya of melanocytes are (a) a correspondence of position and, by rough computations, number; and (b) the fact that clear processes can sometimes be seen to issue from them and pass between neighbouring Malpighian cells. (In orthodox vertical sections, these processes cannot be traced very far, and as a general rule only their proximal stumps can be distinguished.) That this plausible interpretation

is correct has been demonstrated in the following way. Clear cells are easily visible in vertical sections of white human skin. A Thiersch graft of white human thigh skin was fixed overnight in 2 % formaldehyde in normal saline, washed, incubated for 6 h in two changes of the dopa reagent, fixed in formol-saline, and then dehydrated, embedded in paraffin wax and sectioned in the usual way. Only the cytoplasm of the clear cells was dopa-positive (figures 9, 10, 11, plate 10). From what has been said above, it follows that clear cells are indeed the perikarya of melanocytes.

Clear cells as such are not visible in vertical sections of heavily pigmented skins, because what would otherwise be clear cytoplasm is occupied by an abundance of pigment granules. If the pigment is removed by bleaching with potassium chlorate in 70 % alcohol acidified with HCl, clear cells stand out boldly after staining with Erhlich's haemotoxylin (figure 12, plate 10).

Cells visible in superficial layers of the epidermis

Branched quinone-imine dye-positive cells (type e)

If living preparations of white human 'split skin' are treated with methylene blue in exactly the way that has already been described, and examined from time to time from the inner surface, i.e. that formerly united to the corium, the dye-positive melanocytes of the basal layer begin to make their appearance within the hour. If treatment with the dye is prolonged, however, a second tier of dye-positive cells begins to become visible—optically in a deeper, anatomically in a more superficial stratum. These 'high-level' dye-positive cells are present in about the same numbers as the dye-positive cells of the basal layer, and although it is naturally impossible to examine them in any detail through the basal layer, it is usually possible to see that processes issue from them.

Cells of exactly the same type may be seen in pigmented guinea-pig's ear skin, though less clearly still, because of the opacity of the basal layer. It is best to use either albino ear skin or skin from the white ears of a recessively spotted animal; in neither are dye-positive cells to be seen in the basal layer, so that the dye-positive cells of the more superficial layers stand out more boldly.

None of these preparations is suitable for detailed histological analysis. A satisfactory vital preparation may, however, be made from white areas on the trunk skin of recessively spotted guinea-pigs (figures 13–15, plate 10). It has already been explained (above, p. 157) that 'split' preparations of guinea-pig's trunk skin have a crude hill-and-valley pattern, the hills representing the thicker parts of the epidermis in which alone, in pigmented skins, melanocytes are to be found (figure 5, plate 9). High-level dye-positive cells may be seen *through* the basal layer in the hill regions, but in the valleys they appear in abundance among and between the basal layer cells.

The cells are most clearly revealed by floating the split-skin preparations, ready-mounted on cover-slips, in a few ml. of a 0·01 % solution of brilliant cresyl blue in Ringer's solution containing 0·05 to 0·1 % $NaHCO_3$. The preparation passes through a phase of rather granular and purplish coloration before, after about 2 h, dye-positive cells begin to appear in the valley regions.

High-level dye-positive cells differ from the dye-positive cells (melanocytes) of the basal layer in the following ways. Their perikarya are smaller and at once more elongated and

more angular; their cytoplasm is more refringent and their nuclei are less well defined. They are, however, branched cells; their branches may be traced for two or three cells' distance between the Malpighian cells around them and may often be seen to undergo further subdivision (figures 14, 15, plate 10). Neighbouring cells are sometimes united by anastomoses of their branches.

Among tens of thousands of such cells that have been studied, not one has revealed chromosome figures or given any evidence of division. The somewhat angular, desiccated and refringent appearance of these cells, combined with the absence of division stages, give rise to the strong impression that they are moribund or dead. Their position in the epidermis among moribund Malpighian cells confirms this impression; their apparently anomalous occurrence between basal-layer cells in the valley regions of the epidermis of guinea-pig's trunk skin will be discussed later.

Cells of the same type can be seen in the same number in albino and pigmented as well as in 'recessive white' areas of trunk skin; nor are they much less easy to see in pigmented skins, for melanocytes are absent from the valley areas, in which the smaller and rather wiry dye-positive cells are most densely congregated. But, however deeply pigmented the skin may be, the 'high-level' dye-positive cells are wholly free from pigment, and all are dopa-negative.

The distribution of high-level dye-positive cells over the body surface may be summarized as follows. They occur only in those epidermal epithelia which, in fully pigmented animals, are pigmented; i.e. they occur only where melanocytes may also occur. We have been unable to find high-level dye-positive cells in the tongue of the guinea-pig, in the corneas of a variety of animals, or in the neck regions of hair follicles. In our experience, melanocytes are never found in these positions. On the other hand, high-level dye-positive cells are no less easily visible in albino skin and in 'recessive white' skin areas than in pigmented skin (indeed, for purely optical reasons, they are more easily visible). This distinction is important, for except at the 'points', which may darken on exposure to cold, the melanocytes of the skin of albino guinea-pigs are recognizable only as clear cells. Nor have we succeeded in devising a method which reveals wholly non-pigmentary melanocytes in the basal layers of the white skin areas of recessively spotted guinea-pigs; their occurrence in this position must for the present be regarded as an inference drawn from the occurrence in more superficial strata of the branched cells that are believed to be their degradation products.

It will be suggested later that the branched cells described in this section (cells of types *e*, *f* and *g*) are effete melanocytes which have discharged their pigment. They represent, however, only an intermediate stage of degeneration or degenerative specialization, and in still more superficial layers of preparations stained with brilliant cresyl blue it is possible to distinguish later stages of cellular involution. Cells of these later stages are better revealed by gold impregnation than by vital staining, and they will therefore be described in the next section.

Branched cells (type f*) revealed by impregnation with gold*

Branched cells revealed in the epidermis by the impregnation of skin with gold chloride were first described by Langerhans (1868), and Masson (1948*b*) has summarized the

lengthy history of the opinions that have been held about them. Langerhans himself was in no doubt that the cells he described occupied a superficial position in the epidermal strata; Masson, like many others, agreed with this opinion and, following Merkel, suggested that they are effete melanocytes passing through the epidermal strata in course of desquamation. In their earlier studies the present authors (Billingham 1948; Billingham & Medawar 1948) used Gairns's (1930) variant of gold chloride impregnation, a technique which, in spite of the very great clarity with which it reveals the fine dendritic processes (figure 16, plate 10), has the drawback that branched cells are seen to advantage only in squash preparations of the epidermis peeled away from the impregnated preparation. The use of such preparations, combined with the fact that after treatment with the strongly acid solutions required by this technique the epidermis does not usually cleave away at the dermo-epidermal interface, led to the erroneous conclusion that the cells of Langerhans occur in the basal layer. The present account corrects and supersedes our earlier interpretation.

Branched cells may be seen in the anatomically intact epidermis of split skin, whether pigmented or not, by treatment with Cohnheim's gold impregnation technique as follows.

The epidermis is split from the dermis and gummed upon coverslips. It is rinsed briefly in distilled water and immersed in a 0·5 % aqueous solution of yellow gold chloride until the whole preparation becomes pale yellow in colour. It is then washed for a few minutes in distilled water and allowed to undergo a slow 'natural' reduction by immersion in a vessel containing about 50 ml. of 2 % acetic acid which is left in a warm place and in the light. The preparation should be inspected daily. When the branched cells stand out in sufficient contrast to their background, the preparation should be cleared and mounted in absolute glycerol. Cohnheim's method produces neither maceration nor gross cellular distortion, but it is inferior to Gairns's method in its ability to reveal the finest dendritic processes.

The application of Cohnheim's technique to guinea-pig's ear skin epidermis, or human epidermis, makes it clear that the branched cells so revealed occupy a superficial position. They can be seen only *through* the basal layer. This is confirmed by orthodox vertical sections of skin slices, comprising both dermis and epidermis, after treatment by Gairns's method (figure 17, plate 10).

When Cohnheim's technique is applied to the epidermis of guinea-pig's body skin (figures 18, 19, plate 10), it becomes clear that the gold-impregnated cells and the 'dye-positive' cells described in the immediately preceding section are identical. In number, distribution and general form they are quite indistinguishable. What has been said of dye-positive cells therefore applies without qualification to Langerhans' cells. Langerhans' cells are invariably unpigmented; they are present in all epidermal epithelia, whether pigmented or not, in which melanocytes may also appear; they are absent from the tongue of the guinea-pig, the neck of hair follicles, and (Redslob 1922) the cornea.* Their dis-

* They are, however, present, together with melanocytes, in the pigmented conjunctival epithelium that forms a ring round the cornea, and Redslob expressed the view that the Langerhans cells were degeneration products of melanocytes. The corneal epithelium, it should be added, may become pigmented as a consequence of injury. If this pigmentation were due to the migration of melanocytes between corneal stroma and epithelium, it would be most interesting to determine whether Langerhans' cells made their appearance in the latter; but in fact it is due to the mass migration or 'slide' of the limbal conjunctiva as a whole over the corneal surface (Maumenee & Scholtz 1948).

tribution therefore corresponds exactly with that of the dye-positive cells of superficial strata.

At still more superficial epidermal strata than those figured in figures 18, 19, plate 10, the gold-impregnated cells are found to be smaller and to become rather more densely impregnated; processes are lost or are represented only by thin spines, and it is sometimes difficult to distinguish cytoplasm at all (figures 20, 21, plate 11). The occurrence of intermediate stages between fully branched and condensed, rounded-off stages in an anatomical sequence that must also be a chronological sequence makes it almost certain that the latter represent more advanced stages in the progressive involution of the former. (Exactly the same stages are revealed by staining with brilliant cresyl blue; see above.) Whatever doubts there may later prove to be about the derivation of Langerhans' cells from melanocytes, there can be little doubt that Langerhans' cells themselves are squamous, 'expendable' cells.

Clear cells (*type* g)

The methods that are appropriate to reveal clear cells in the basal layer of the epidermis will also reveal clear cells at more superficial levels. Masson (1948*a*, *b*) was the first to describe these cells adequately. 'Good' fixation is essential; Altmann's fixative (containing 1 % sodium chloride) has given satisfactory results (figures 22, 23, plate 11).

The clear cells of superficial layers occur in all epidermal epithelia in which melanocytes may also appear; we have not found them in the cornea or in the guinea-pig's tongue, but they occur in white human skin, and in white or albino guinea-pig's skin no less frequently than in pigmented skins. They are nucleated cells, and their nuclei are Feulgen-positive. 'High-level' clear cells are strikingly similar to the clear cells of the basal layer, and they occur in approximately the same numbers (figures 22 to 27, plate 11). In both, the cytoplasm is clear and not basiphilic, and is apt to collapse around the nucleus to leave an empty space. Just as clear cells of the basal layer are beyond doubt the perikarya of (weakly pigmented or unpigmented) melanocytes in the basal layer, so the 'high level' clear cells are almost certainly the perikarya of the branched high-level cells (types *e* and *f*) revealed by staining with brilliant cresyl blue or by impregnation with metallic gold. The cell bodies are often somewhat elongated and angular; even in ordinary vertical microtome sections it is usually possible to identify the stumps of the processes that issue from them; and in sections of formol-fixed skin impregnated on the slide by Holmes' silver method for axon staining (Holmes 1947) the processes, being more sharply defined, may be traced a cell's distance or more from the perikaryon (figures 24 to 27, plate 11). Vertical sections of skin previously impregnated by Gairns's technique (figure 17, plate 10) show that the perikarya of Langerhans' cells occupy the same epidermal strata as high-level clear cells.

High-level clear cells are unpigmented, no matter what the degree of pigmentation of the skin in which they reside. Chromosome figures and division stages have never been found.

At still more superficial levels (but beneath the granular layer) the clear cells revealed by Holmes's technique are visible as small obtusely angular or rounded formations which are rather more deeply impregnated with silver; the cytoplasm is reduced and processes are absent or represented by stumps. The correspondence of appearance and position makes it likely that these clear cells of the most superficial strata represent the advanced stages of cellular involution described in detail at the end of the preceding section.

Part 3. The melanocyte as a squamous cell

The evidence set out at length in part 2 of this paper may be summarized in the following terms. Branched cells of two topographically distinguishable classes reside in the epidermis: those of the basal layer, and those of more superficial layers. The former class comprises the melanocytes visible without special treatment in living skin; dopa-positive cells; cells rendered visible by supra-vital staining with certain quinone-imine dyes; and 'clear cells'. These are so many preparative variants or 'preparation images' of the same cell, the melanocyte or pigmentary dendritic cell; the 'clear cells' are merely the perikarya, seen in transverse sections, of melanocytes of such feeble pigmentary activity that their cytoplasm is not crowded with melanin granules. The branched cells of superficial levels are never pigmented, even in pigment-laden skin, and are never dopa-positive. They include cells rendered visible by supra-vital staining with quinone-imine dyes, cells impregnated with metallic gold after treatment by Gairns's or Cohnheim's techniques, and 'high-level' clear cells. It has been shown that these, too, are so many preparation images of the same cell.

We may now consider two possibilities. Either these two systems of branched cells are quite distinct, in the sense that they derive from different division lineages; or, alternatively, the high-level cells represent melanocytes which, having lost or discharged their pigment, are in course of passing through the epidermal strata to be flaked off at the skin surface. That such is the fate of the high-level branched cells themselves there can be little doubt (see above); the problem is whether the high-level cells are in their turn the products of the degenerative specialization of melanocytes. The evidence bearing upon this problem, one way or another, is presented step-by-step below.

Correspondence of structure and number

Pigmentation apart, the melanocyte and the high-level branched cell resemble each other in the following ways. They have much the same proportions. Both have compact and well-defined perikarya, from which there issue dichotomizing branches which weave between the Malpighian cells around them, occasionally to fuse with branches from neighbouring cells. Their spacing in the epidermis is such that they lie 'at arms' length' from one another, i.e. the territories defined by their branches are contiguous and do not overlap. Seen as clear cells in microtome sections, both have a clear non-basiphilic cytoplasm which, after imperfect fixation, which is not always easy to avoid, collapses around the nucleus in a distinctive way.

If high-level dendritic cells derive from melanocytes, there should be a close correspondence between their numbers—much as there is a correspondence between the number of Malpighian cells in the basal layer and in a single tier of the prickle-cell layer.

Estimates of the numbers of high-level branched cells are easiest to make in the 'valley' regions of the trunk epidermis of the guinea-pig (figure 5, plate 9), but for reasons that will be made clear in the next sections, such estimates are highly misleading. On the other hand, there is no reason why the melanocytes and high-level branched cells of ear skin should not stand in approximately one-to-one correspondence. Thin shavings of white ear skin were accordingly removed from three recessively-spotted guinea-pigs, 'split' (see

above, p. 153), subjected to Cohnheim's technique, and mounted in glycerol. Such preparations are too opaque for counting by projection microscopy, but they may be counted by the less convenient method of direct microscopy, a graticule being placed in the eyepiece. Twelve to eighteen areas of 0·025 mm^2 were so counted on each of the three specimens.

The three estimates of the number of high-level gold-impregnated cells in guinea-pig's ear skin were 893 ± 21, 1093 ± 19 and 1113 ± 25 cells/$mm^2 \pm$standard error of the mean. The mean number of melanocytes in pigmented ear skin (table 1) ranged from 515 ± 23 to 1751 ± 51 (overall mean 893 ± 85). The correspondence is close enough to justify the hypothesis of a one-to-one correspondence of number between the cells of the two types. High-level dendritic cells therefore occur in a number which is consistent with the belief that they derive from the melanocytes of the basal layer.

Position in the epidermis; 'vitality' of the cells

In human skin, and in those regions of guinea-pig's skin where the epidermis is thick (e.g. the ear), 'high-level' branched cells occupy exactly the position that would be expected if they were indeed the 'ghosts' of fully expended melanocytes. The strata they occupy are those in which cell divisions seldom, if ever, occur (cf. footnote p. 152), and no chromosome figures or division stages have been seen in any high-level branched cell. It is probable, then, that they are 'dead' in whatever sense the Malpighian cells of the more superficial layers of the epidermis are dead, and their somewhat angular, desiccated and refringent appearance confirms this supposition. Yet, although they are apparently incapable of division, their numbers are constantly maintained; and it will now be shown that their numbers are made good *pari passu* with the proliferation of a regenerating epithelium. The principle of the test is exactly the same as that which has been used to show the reproductive self-sufficiency of the epidermis as a whole (above, p. 153).

Thin shavings of skin were removed from the dorsa of the ears of an albino rabbit, and the epidermis disengaged by digestion with commercial trypsin solution (Billingham & Medawar 1951). The basal layer of the epidermis was scraped off and suspended in 0·6 % sodium citrate in 0·85 % sodium chloride; the coarse lumps were thereupon sucked in and out of a fine pipette until they were reduced to single cells. The entire epidermis and dermis, down to the layer of the panniculus carnosus, was removed over a square area of about 30 cm^2 from the skin of the chest; the suspension was then pipetted over the defect so formed. The area was dusted with sterile sulphadiazine powder, covered with tulle gras and then with plain and plaster-impregnated bandage (Billingham & Medawar 1951). By the 16th day after operation, the formerly raw area, now somewhat reduced by fibrous contracture, was found to be covered by a thick layer of opaque (because well cuticled) epidermal epithelium (cf. figure 1, plate 9). Such an epithelium may be removed simply by peeling it away, or by injecting air through an empty hypodermic syringe at the level of its plane of attachment to the bed. The treatment of squares of such epithelium with brilliant cresyl blue or by Cohnheim's method revealed an abundance of high-level branched cells, lying in a plane superficial to the basal layer.

This test makes it clear that the numbers of high-level branched cells are constantly made good within a proliferating epidermal epithelium—almost certainly from a source

within the epithelium itself, although this particular test does not wholly exclude the possibility of, say, the immigration of fibroblasts from the granulation tissue of the graft bed. But if the high-level branched cells are not themselves capable of division, they must be derivatives of cells which are. The rest of the evidence presented in this part of the paper makes it very probable that their precursors are indeed the melanocytes of the basal layer.

The apparently anomalous case of guinea-pig's trunk skin now requires special consideration. In the 'valley' regions of guinea-pig's trunk skin, it has been stated (p. 160), typical 'high-level' branched cells occur *between* cells of the basal layer (figures 14, 15, plate 10) as well as at more superficial levels; in the hill areas they occur only at more superficial levels. How is their position in the valley regions to be reconciled with the hypothesis that they are the 'ghosts' of melanocytes?

The valley areas of the trunk epidermis are those that lie in the acute angle of the emergent hairs; anatomically speaking, they can be regarded as part of, or as a continuation of, the trumpet-like flares of the openings of the follicles. The hill areas, in which the epidermis is about half as thick again, abut against the obtuse angle of the emergent hairs. Melanocytes occur only in the hill regions (figure 5, plate 9); yet the Malpighian cells of the valley regions contain a diffusely distributed complement of melanin granules. Earlier in their life the latter must therefore have been inoculated by the melanocytes of the hill regions, which suggests that there is a constant displacement of epidermal cells from the hills into the valleys. In a thick epidermis (the pattern of which is of steeply rising hills with deep canyons between them: figure 6, plate 9; figure 24, plate 11) the current of epithelial displacement must be predominantly outwards; in the relatively flattened epidermis of the trunk, with low hills and shallow valleys, the current of movement is both sideways and outwards. It should follow that the innermost layer of the epidermis of the valleys, though topographically a basal layer, consists of cells which correspond anatomically to those which occupy a more superficial stratum in a thicker epidermis. The distribution of melanin in the Malpighian cells of the valleys—diffuse rather than in the remains of a polar concentration at the distal end of the cell— supports this interpretation; but the most secure evidence is the complete or almost complete mitotic inactivity of the cells of the valley region themselves.

In each of three independent trials, a guinea-pig was injected subcutaneously with a 0·04 % aqueous solution of colchicine in a dosage of 1 mg/kg. Five hours later the guinea-pig was killed, a strip of trunk skin was excised, allowed to relax (as skin does upon removal), fixed in Bouin's fluid, embedded, and cut in a plane sagittal to the hairs. After staining with iron haematoxylin, a dot diagram indicating the position of mitoses in the epidermis in relation to the positions of emergence of the hairs was reconstructed from ten alternate sections in a continuous ribbon cut at 10 μ thickness. In the thicker region of the epidermis, abutting against the obtuse angle of the emergent hairs, mitoses were 10 to 15 times as frequent as in the thinner regions (corresponding, in whole mounts of 'split skin', to the valleys) which lie under the emerging hair shafts.

The almost complete mitotic indolence of the cells of the valley regions might have been guessed from the absence of melanocytes. It implies that the population of the valley regions is made good by the division of cells elsewhere—presumably in the hill regions, where the epidermis is thicker, melanocytes are present, and mitoses are abundant.

The occurrence of 'high-level' branched cells among the basal layer cells of the valley regions of trunk skin is therefore not anomalous. The cells of the valley region derive from the cells of mitotically active regions elsewhere, and represent chronologically later stages in whatever processes of involution they undergo. An exactly comparable situation obtains in the skin of the mouse's tail (Reynolds 1953).

Correspondence of distribution

It has been stated that 'high-level' branched cells, by whatsoever means they are revealed, occur only in regions of the skin where melanocytes may also occur, i.e. in regions which, in pigmented animals, are pigmented. Either melanocytes and high-level branched cells occur together, or neither occur; melanocytes are absent from the guinea-pig's tongue and from the cornea, and high-level branched cells, hypothetically the 'ghosts' of melanocytes, have been found in neither. Unless our technique is at fault or our survey too narrow, this accurate correspondence of distribution must be accepted as telling evidence in favour of the supposition that high level cells derive from effete melanocytes and represent a stage in their desquamation. There is, however, an exception to this generalization. In the skin of albino rats, melanocytes are visible only as clear cells (Taylor 1949). Of their presence in the albino guinea-pig there can be no doubt, for the albinism of the guinea-pig falls short of complete suppression of pigmentary activity, and the points of 'genetically coloured' albino guinea-pigs darken after prolonged exposure to the cold (Ginsberg 1944). Melanocytes are then easily visible in the living epidermis. In the white areas of recessively spotted rats (Taylor 1949) and guinea-pigs, however, the melanocytes of the basal layer are not identifiable with certainty even as clear cells; the occurrence of non-pigmentary melanocytes in these regions can therefore only be inferred from the occurrence of their presumed ghosts in more superficial layers of the epidermis.

Melanocytes in hair bulbs

The melanocytes of the hair bulbs and of the superficial epidermis are two normally independent but experimentally interchangeable systems (above, p. 154). If the melanocyte of the superficial epidermis is a squamous cell, that of the hair bulb should also be squamous. An experiment has already been mentioned in which a superficial epidermis has been caused to regenerate wholly by upward migration of the epidermal epithelium from the hair bulbs. (In clinical practice, the donor areas of Thiersch grafts do in fact heal in this way.) The melanocytes of the newly formed superficial epidermis can only have come from those originally resident in the hair bulbs, and treatment of the epidermis by the brilliant cresyl blue and by Cohnheim's method revealed a normal complement of branched cells at superficial levels (see also Pepper 1953). If our interpretation of the origin of these high-level branched cells is correct, this can only imply that the melanocytes of hair bulbs, like those of the superficial epidermis, are squamous cells. Presumably the effete melanocytes are incorporated into the substance of the hair shaft, for it would appear to be anatomically impossible for them to go elsewhere. The melanocytes of feather follicles, it may be added, are agreed to be 'expendable' cells (Rawles 1948; Willier 1948).

General conclusions; terminology

The gist of the foregoing argument is as follows. There are two systems of branched cells within the epidermis: the melanocytes of the basal layer and the 'high-level' branched cells of more superficial layers. The former are visible in the living epidermis without special treatment; among their preparation images are the dopa-positive cells of the epidermis, the quinone-imine dye positive cells, and the 'clear cells' of orthodox vertical sections that represent the perikarya of melanocytes. The latter include the branched cells that can be more or less specifically impregnated with gold chloride (Langerhans' cells), quinone-imine dye positive cells, and clear cells of superficial strata. These, too, are so many preparation images of a cell of one type.

The cells of the two systems are very similar in their general proportions, their manner and degree of branching, and their relationship to each other and to neighbouring Malpighian cells; indeed, they have been repeatedly confused by many students of the anatomy of skin, including the present authors. 'High-level' branched cells are of the 'expendable' or squamous type, and the products of their involution have been described and figured; yet they have never been seen to divide, although they are constantly manufactured anew and so maintained in numbers in a rapidly proliferating epidermis. It is therefore presumed that they themselves derive from generative cells elsewhere in the epidermis; more specifically, that they represent effete melanocytes which, having discharged or otherwise lost their pigment, participate in the general outward movement of epidermal cells to be eventually flaked off at the skin surface. This interpretation is supported by their close similarity of structure; by their appearance in an anatomically more superficial and therefore chronologically 'older' position in the epidermis; by the one-to-one correspondence of number between them and the melanocytes of the basal layer; and by their correspondence of distribution, which is such that either melanocytes and high-level branched cells occur together in the epidermis, or neither occur.

Against this interpretation may be set certain apparent anomalies which have been fully discussed in the body of the text; and it may also be argued that the evidence we have presented, like most histological evidence of cell transformations, is of a purely circumstantial character. Our interpretation cannot therefore be regarded as final.

In conclusion, something should be said of the choice of terminology. The synonymy of the branched cells of the epidermis is of bewildering complexity. 'Dendritic cell' (Becker 1927) is a non-committal and purely descriptive term that may be said to apply indifferently to them all, but it is to be hoped that the term 'melanocyte' (adopted here on the initiative of Drs Fitzpatrick, Lerner and Pinkus) will come to be the accepted name for the pigmentary dendritic cells of the basal layer and their various preparation images, of which the 'clear cell', appropriately so called, is merely one. There remains the difficulty of finding a suitable name for the dendritic cells of the more superficial epidermal layers. The term 'ghost cell' or 'melanocyte ghost', though in some ways apt, has the grave disadvantage of embodying within it a hypothesis concerning their mode of origin. Clumsy as it is, the term 'high-level branched cell' or 'superficial branched cell' has no such undertones, and should perhaps be kept in use until their mode of origin has been finally made clear.

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Plate 9

Figure 1. Illustrating the principle of the 'seeding' experiment described in the text. A suspension of epidermal cells of rabbit's ear skin was spread over an extensive area cut from the chest skin of the same rabbit; after 11 days the cells have united and grown to form a continuous sheet of thickly cuticled epithelium which covers almost the whole of the original defect. Note the ingrowth of epithelium from the margin of the lesion: this has also helped to provide epithelial cover. (Scale numbers show centimetres.)

Figure 2. A sagittal section through the epidermis of the trunk skin of a pigmented guinea-pig to show the abrupt disappearance of melanin-inoculated cells at the level of the neck of the hair follicle. The melanin has been intensified by treating the sections on the slide for 1 h with a 1:100000 solution of silver nitrate containing one drop of ammonia solution (sp.gr.0·88) per 100 ml. Stain: orange G. (Magn. × 78.)

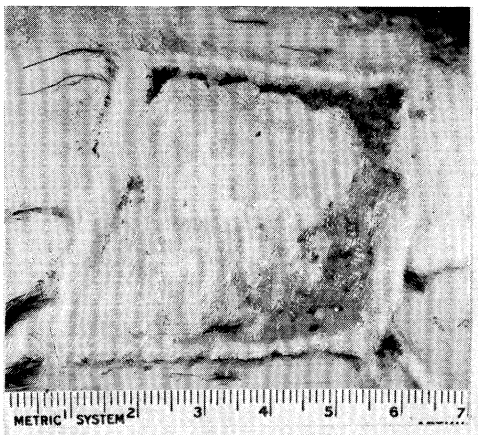
Figures 3, 4. The deliberate production of black hairs in white guinea-pig's skin by the action of melanocytes derived from the superficial epidermis. Figure 3 illustrates the pipetting of a suspension of epidermal cells from pigmented ear skin upon a lesion prepared in white skin by removing the superficial epidermis and exposing the bases of the hair follicles. Figure 4 shows the same area 35 days later: healing is complete; clumps of black hairs are mixed with the white, and superficial pigmentation extends over the entire area of the original skin defect. (Scale numbers show centimetres.)

Figure 5. Pigmented guinea-pig's trunk epidermis, seen from the inner (dermal) side; the preparation has been treated with the Dopa reagent but is otherwise unstained. Note the 'hill and valley' pattern referred to in the text: the melanocytes are wholly confined to the hill regions. Contrast figure 6. (Magn. × 20.)

Figure 6. Pigmented guinea-pig's dorsal ear-skin epidermis, seen from the inner (dermal) surface; the preparation has been treated with Dopa reagent. Contrast the pattern with that of trunk skin (figure 5), and note the prominence of the melanocytes. Such preparations are particularly suitable for conducting cell counts. (Magn. × 78.)

Figure 7. A field of the preparation illustrated by figure 6, in higher power: the melanocytes are uniformly and very densely impregnated with 'dopa melanin'; the Malpighian cells between which their branches weave, though inoculated with melanin granules, are dopa-negative. (Magn. × 335.)

Figure 8. A vertical section of human facial skin: Heidenhain's 'Azan' technique. Clear cells are prominent: their cytoplasm is not basophilic, and no 'prickles' unite them to neighbouring cells. (Magn. × 550.)



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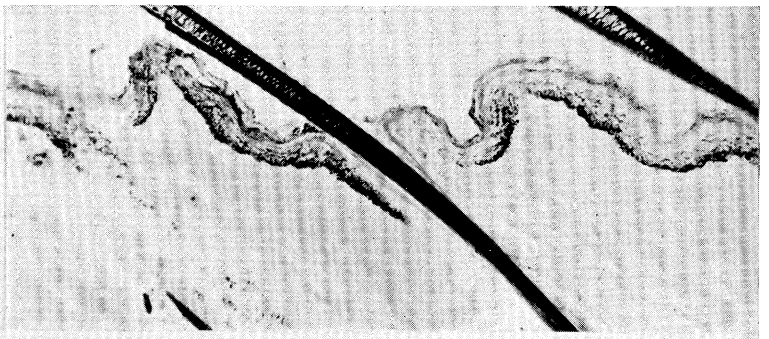

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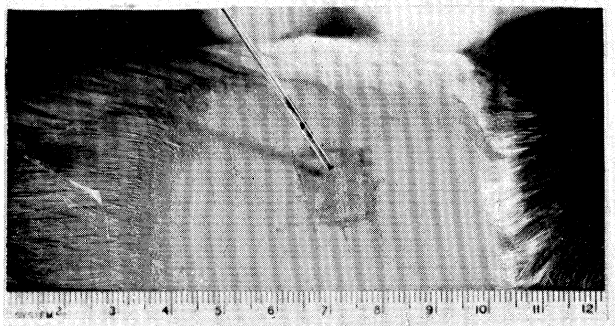

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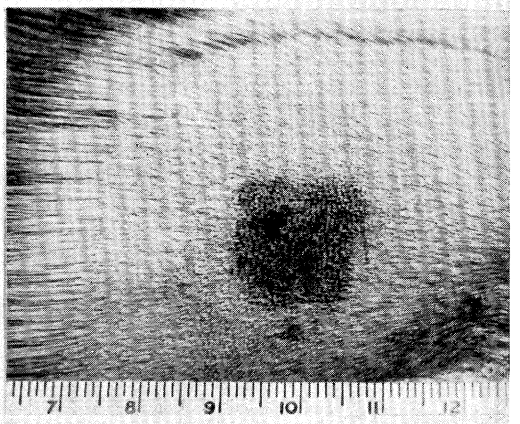

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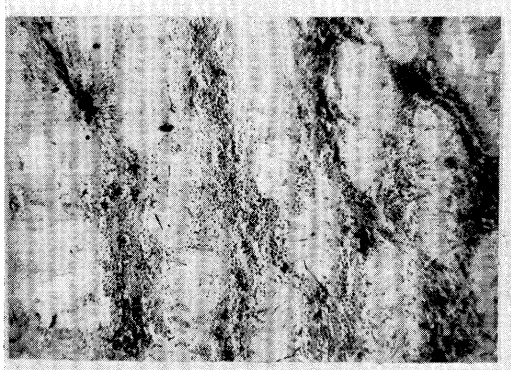

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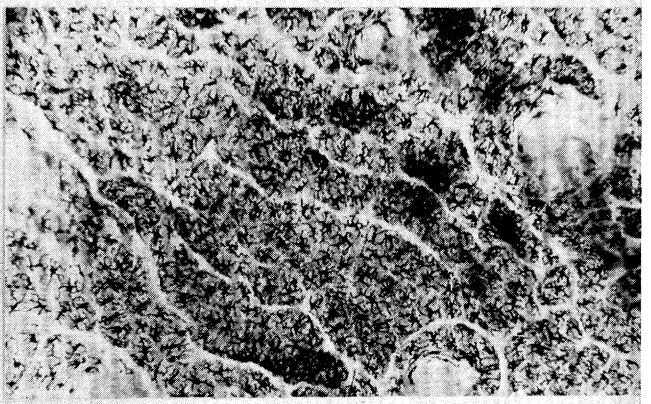

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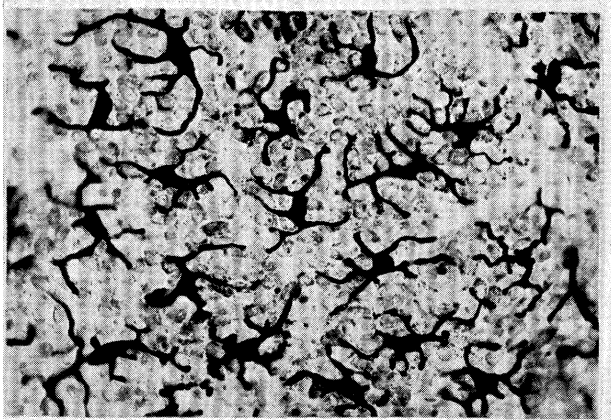

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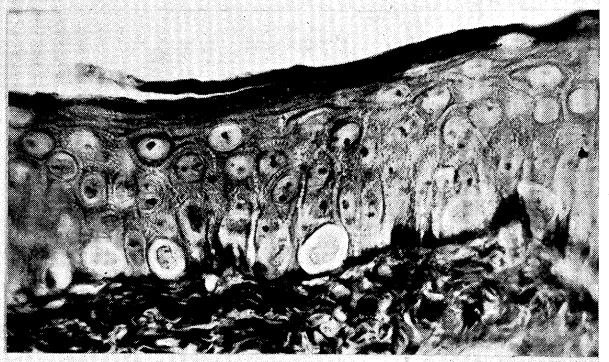

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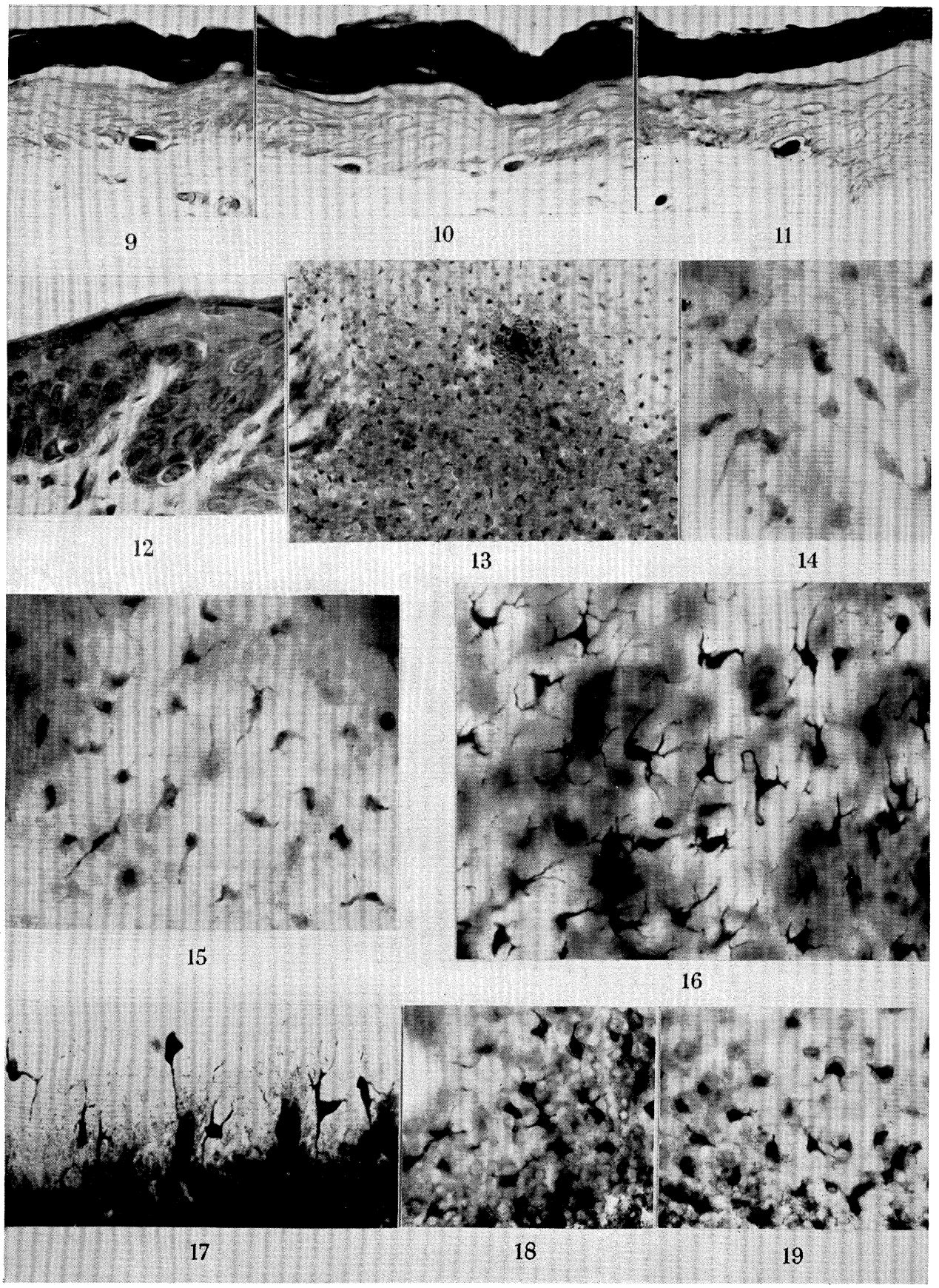
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Plate 10

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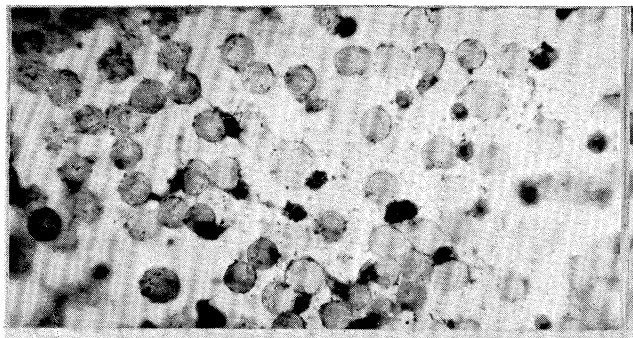

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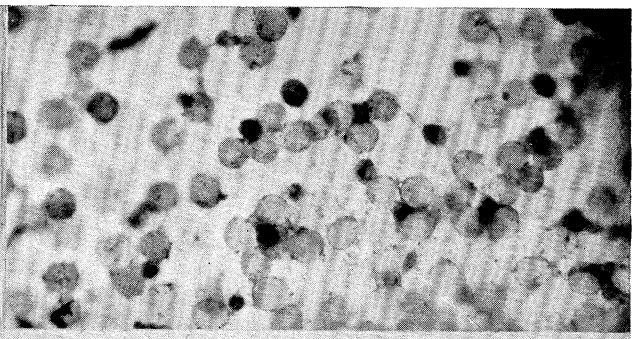

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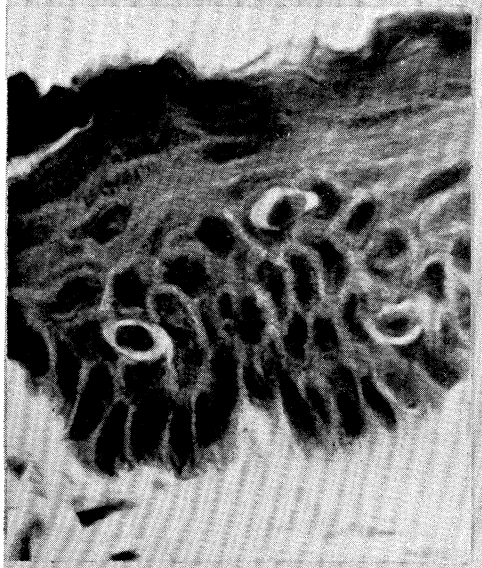

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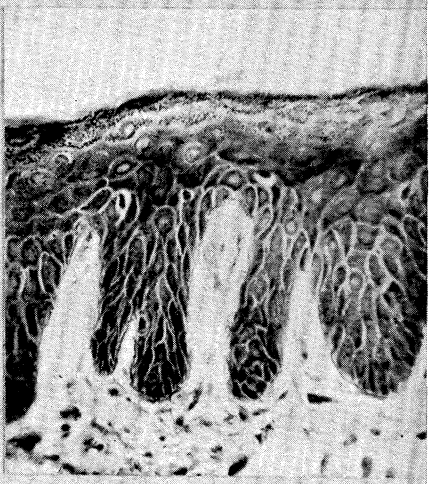

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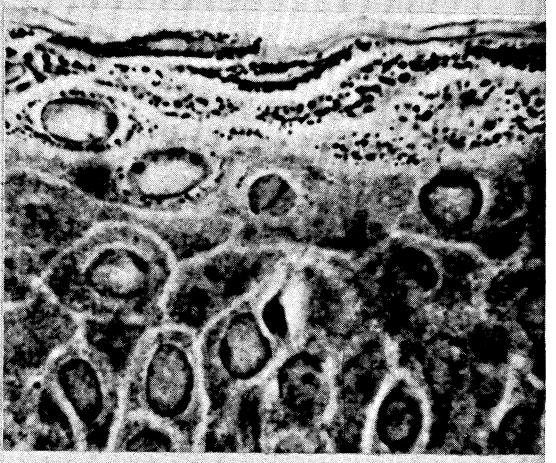

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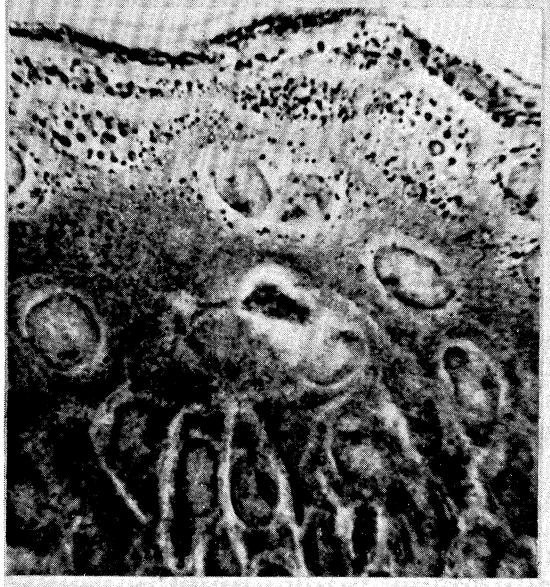

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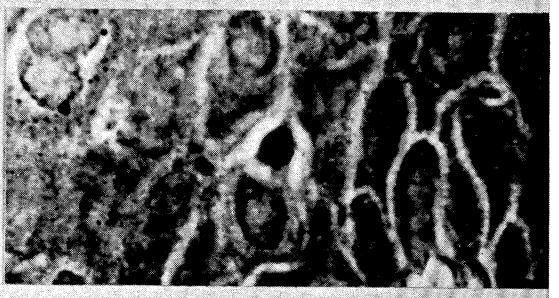

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Plate 11

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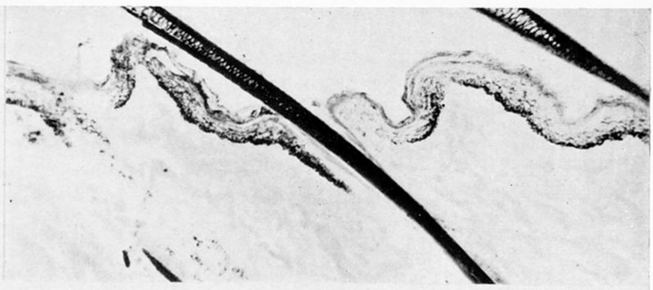

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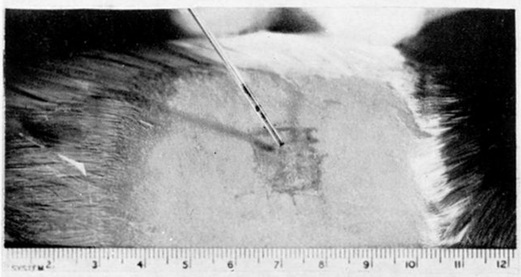

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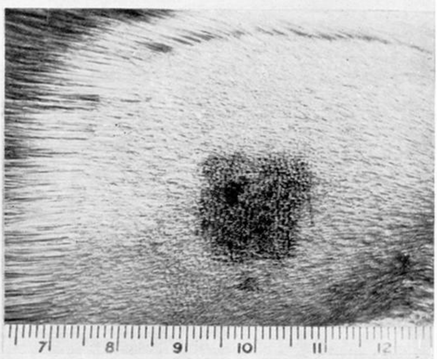

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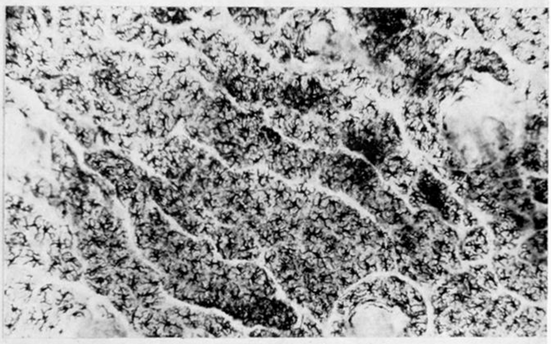

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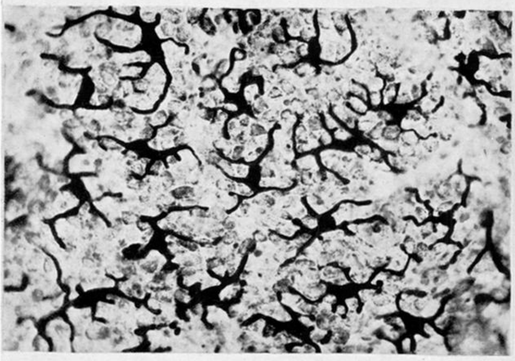

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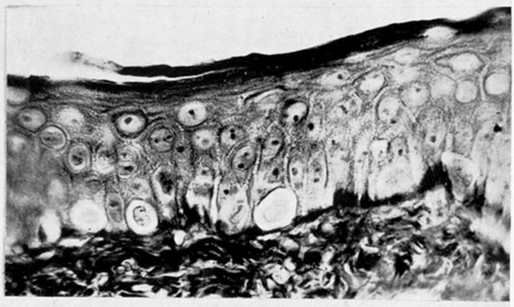

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Plate 9

Figure 1. Illustrating the principle of the 'seeding' experiment described in the text. A suspension of epidermal cells of rabbit's ear skin was spread over an extensive area cut from the chest skin of the same rabbit; after 11 days the cells have united and grown to form a continuous sheet of thickly cuticled epithelium which covers almost the whole of the original defect. Note the ingrowth of epithelium from the margin of the lesion: this has also helped to provide epithelial cover. (Scale numbers show centimetres.)

Figure 2. A sagittal section through the epidermis of the trunk skin of a pigmented guinea-pig to show the abrupt disappearance of melanin-inoculated cells at the level of the neck of the hair follicle. The melanin has been intensified by treating the sections on the slide for 1 h with a 1:100000 solution of silver nitrate containing one drop of ammonia solution (sp.gr.0·88) per 100 ml. Stain: orange G. (Magn. × 78.)

Figures 3, 4. The deliberate production of black hairs in white guinea-pig's skin by the action of melanocytes derived from the superficial epidermis. Figure 3 illustrates the pipetting of a suspension of epidermal cells from pigmented ear skin upon a lesion prepared in white skin by removing the superficial epidermis and exposing the bases of the hair follicles. Figure 4 shows the same area 35 days later: healing is complete; clumps of black hairs are mixed with the white, and superficial pigmentation extends over the entire area of the original skin defect. (Scale numbers show centimetres.)

Figure 5. Pigmented guinea-pig's trunk epidermis, seen from the inner (dermal) side; the preparation has been treated with the Dopa reagent but is otherwise unstained. Note the 'hill and valley' pattern referred to in the text: the melanocytes are wholly confined to the hill regions. Contrast figure 6. (Magn. × 20.)

Figure 6. Pigmented guinea-pig's dorsal ear-skin epidermis, seen from the inner (dermal) surface; the preparation has been treated with Dopa reagent. Contrast the pattern with that of trunk skin (figure 5), and note the prominence of the melanocytes. Such preparations are particularly suitable for conducting cell counts. (Magn. × 78.)

Figure 7. A field of the preparation illustrated by figure 6, in higher power: the melanocytes are uniformly and very densely impregnated with 'dopa melanin'; the Malpighian cells between which their branches weave, though inoculated with melanin granules, are dopa-negative. (Magn. × 335.)

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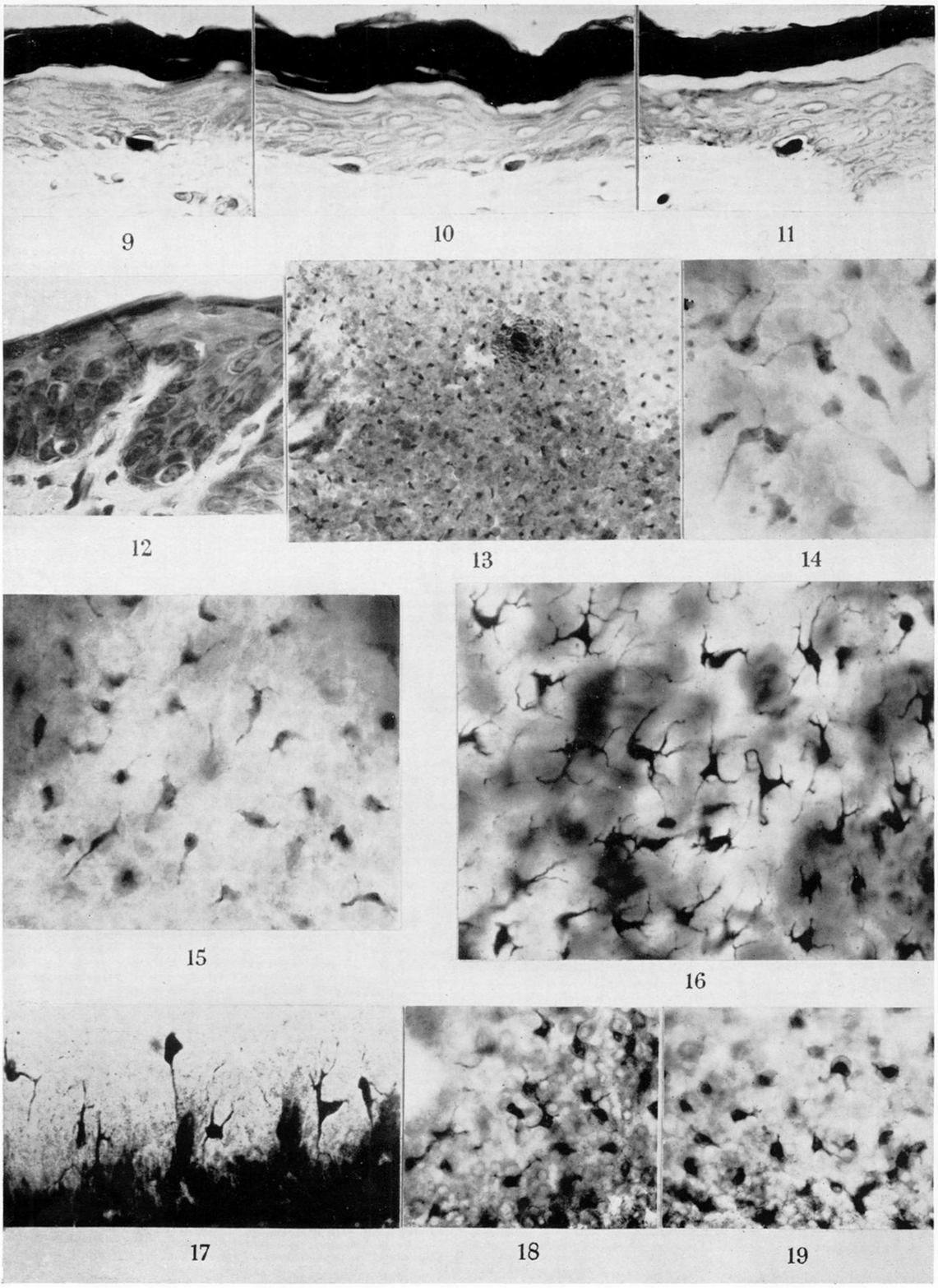

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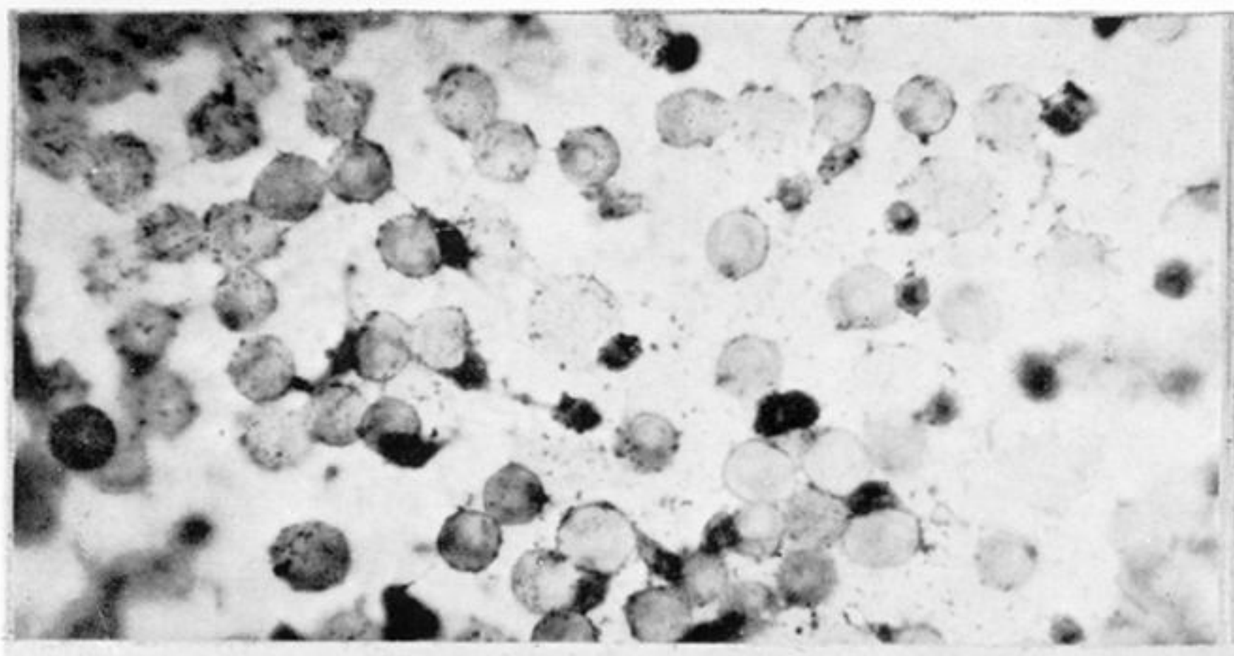

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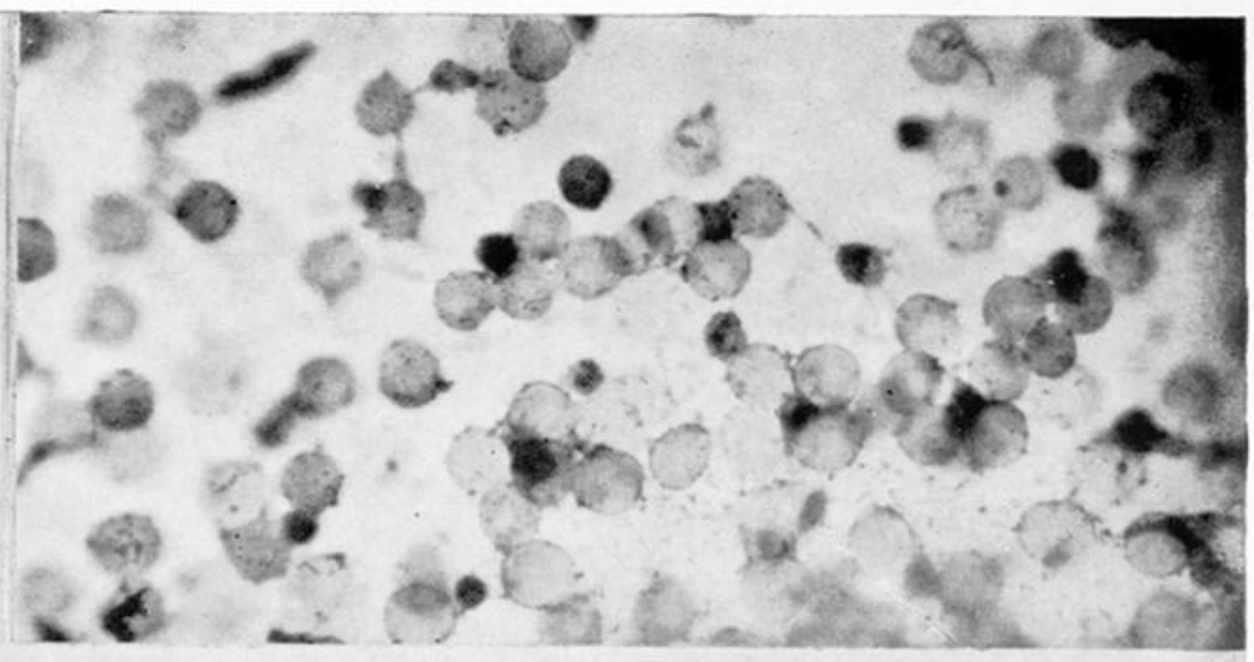

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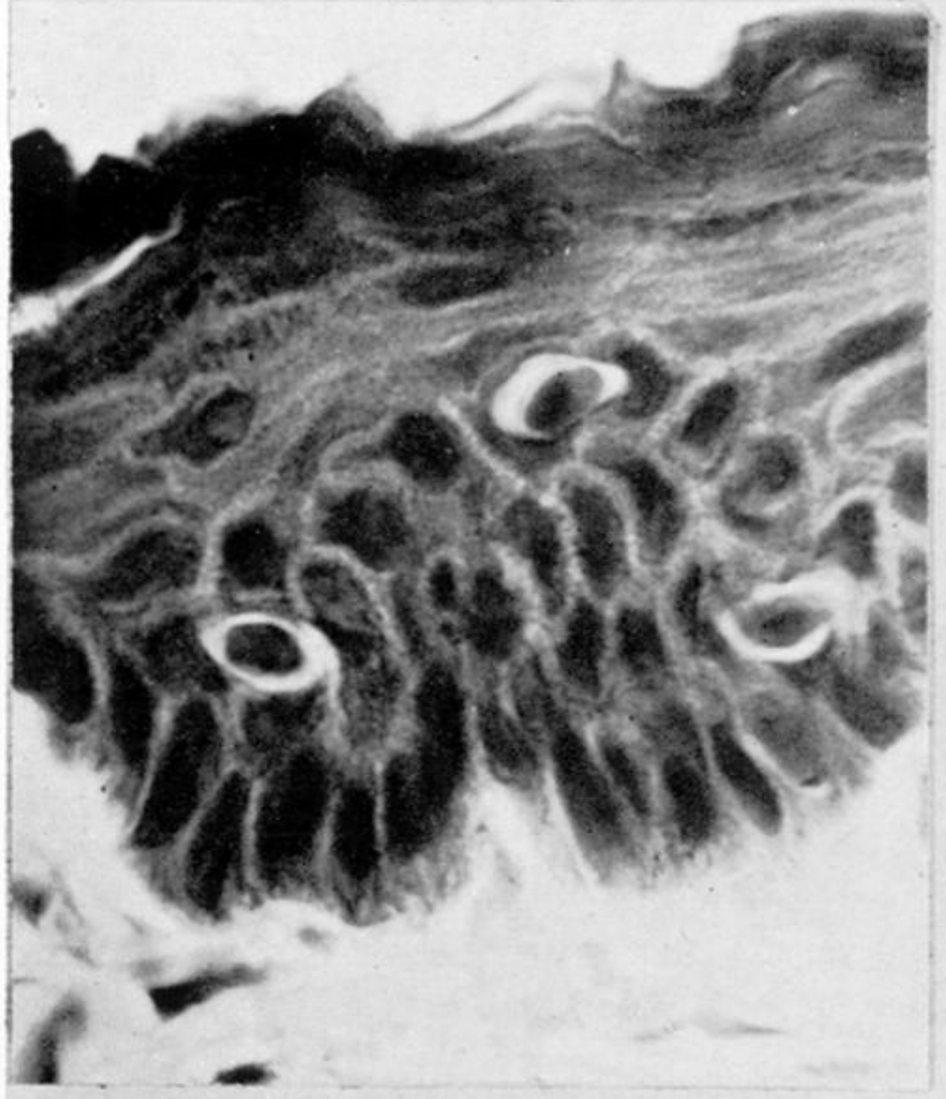

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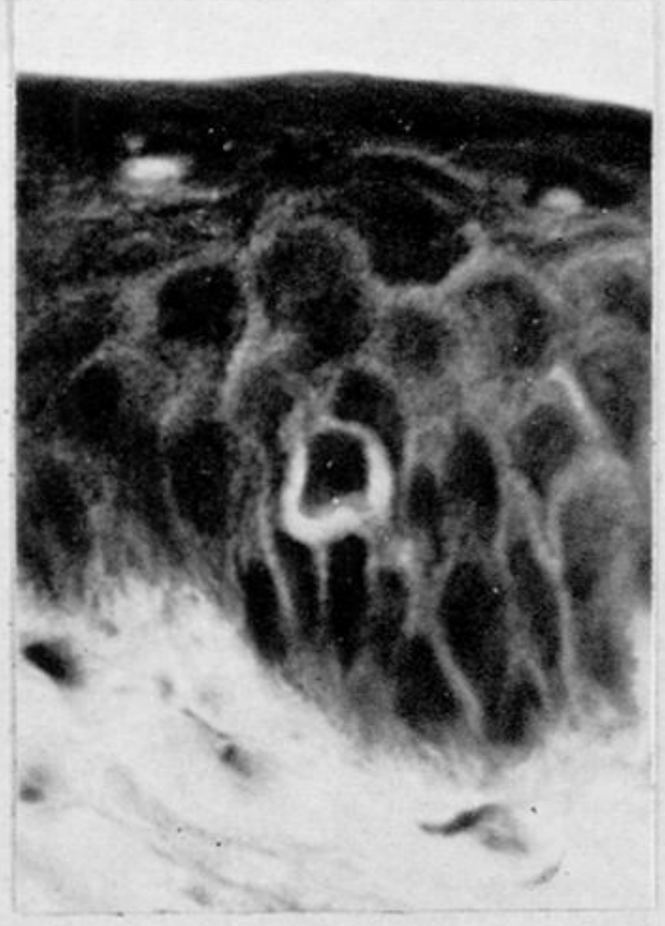

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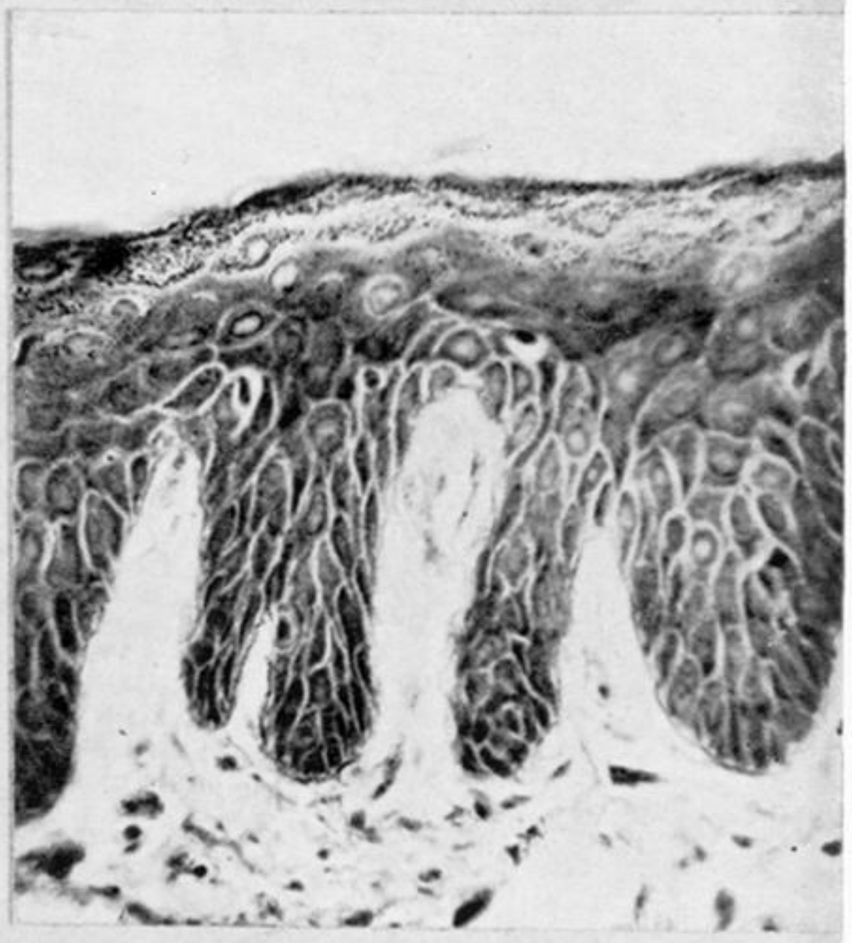

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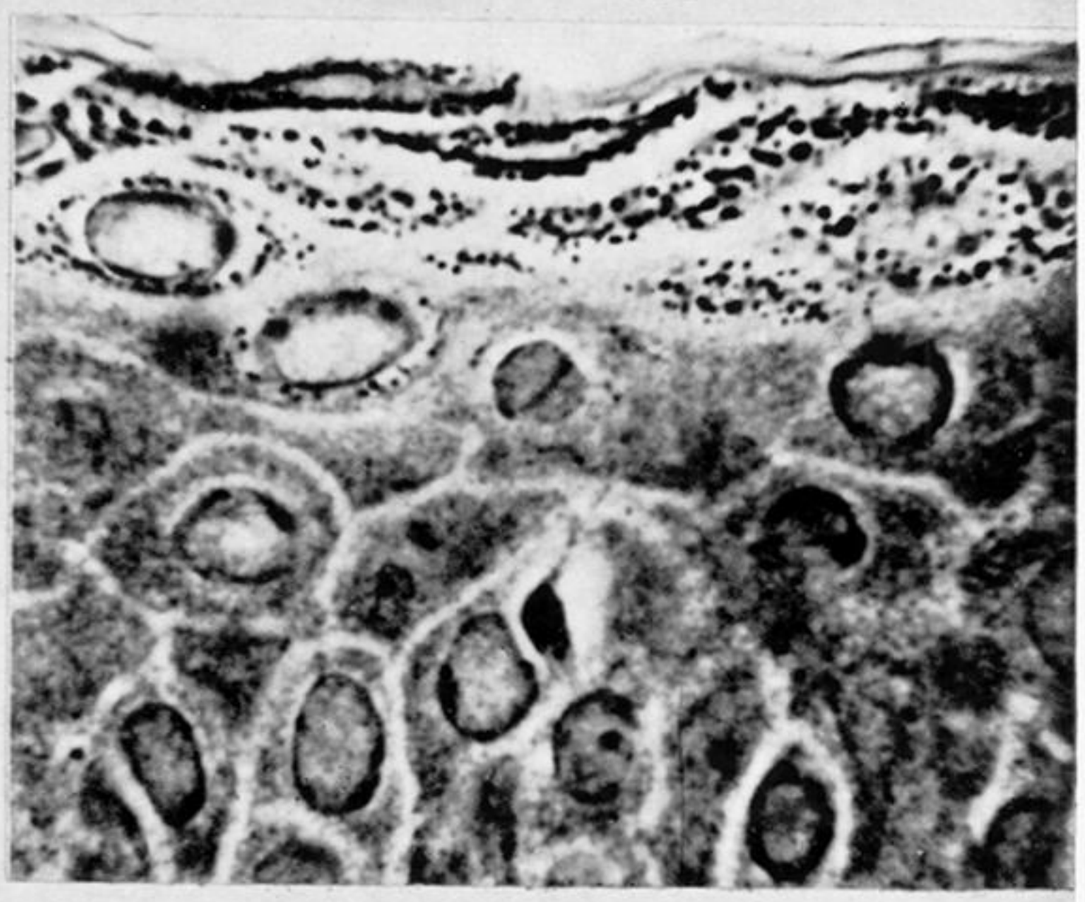

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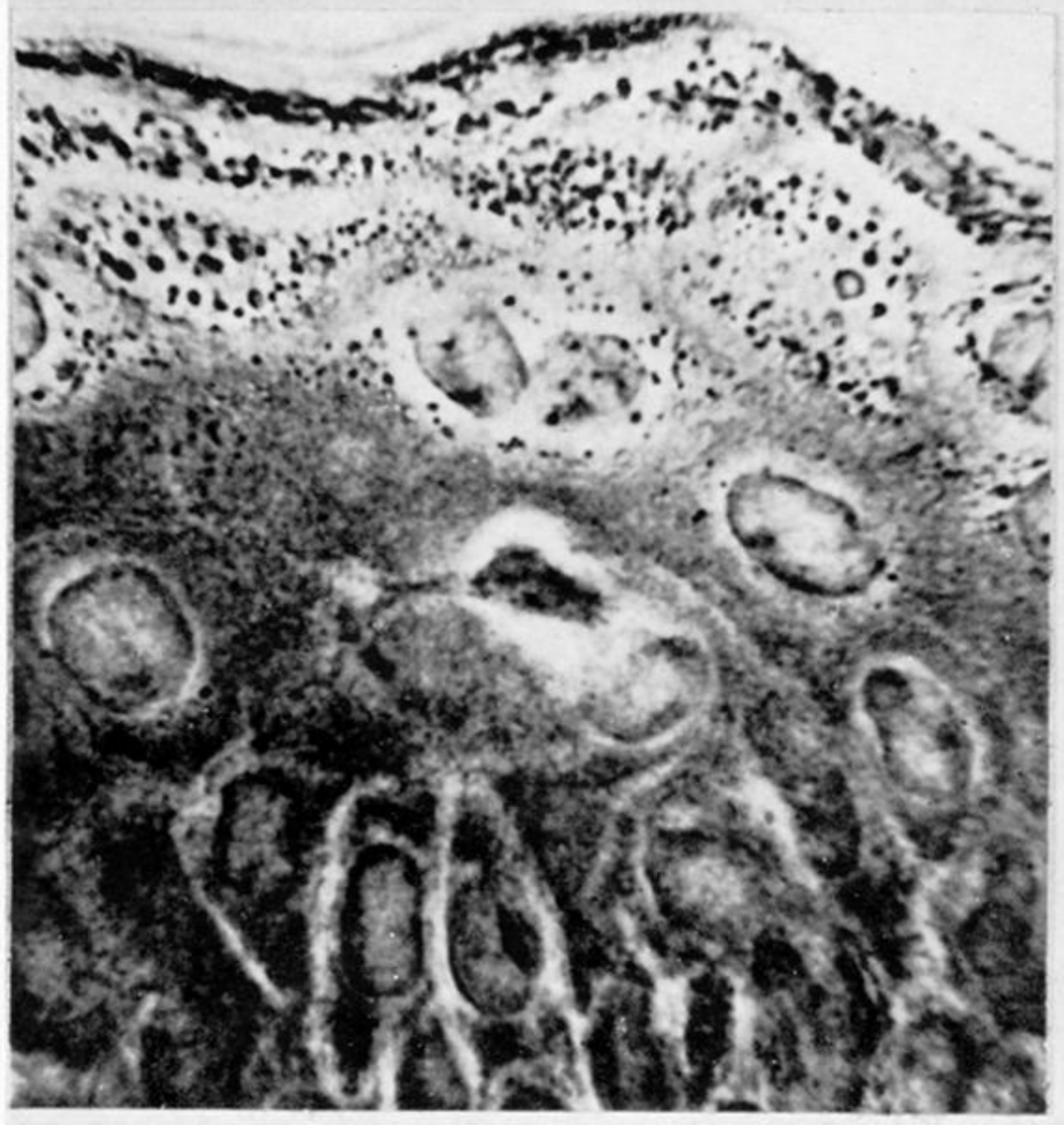

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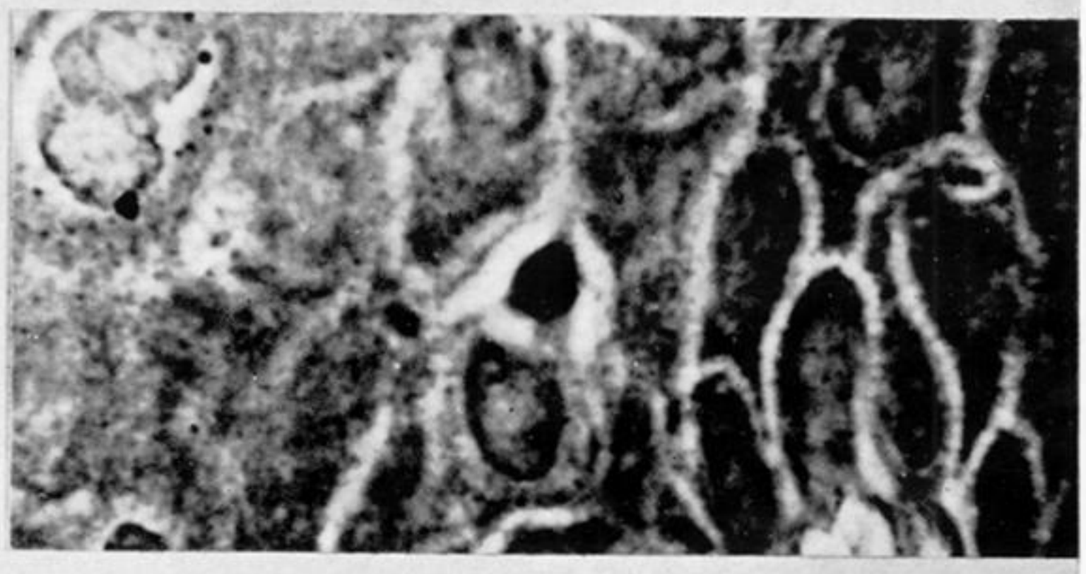

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