A NOTE ON THE SPECIFICITY OF THE CORNEAL EPITHELIUM

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It is a well-known fact that much of the finer detailed differentiation of the adult body is brought about by the direct adaptive responses of tissues to their environment. The architecture of a bone may be remodelled under a changed system of stresses; muscles enlarge upon constant use; skin thickens where it is irritated, darkens upon exposure to sunlight, and creases where it is repeatedly folded. With such evidence in mind it would be natural to suppose that the detailed histological differentiation that represents the final stage of embryonic development was also in large part governed by differences of environment and of habit of use. For example, the thick and heavily keratinized epidermis of the sole of the foot, like that of a corn or callosity, differs from the relatively quiescent skin of the body surface by its intense and sustained mitotic activity. This might very well be the consequence of the constant pressure and irritation to which the epithelium of the sole has to submit—the more obviously so, since callosities can be produced by such means at will. It has, nevertheless, been shown experimentally (Billingham & Medawar, 1948*a*) that the difference between sole and body epithelium, though primarily functional and not anatomical, is innate and inherited. A corn or callosity does not survive indefinitely the irritation that provoked its formation; but sole skin grafted from the foot of the guinea pig to its chest, where it is secure from irritation and protected by the overgrowth of hair from the skin around it, perseveres in its intense mitotic activity and continues to manufacture layer after layer of thick and now functionless cuticle throughout the life of the animal—in our experience, for more than two years after operation. The fact that there exist *inherited* differences between somatic cells of a type that can be and very often are mimicked by direct adaptive responses to the environment is of some importance in evolutionary speculation, since it offers special temptations to the Lamarckist. (The difference between 'inborn' and acquired flexure lines is in principle of this same sort: acquired flexure lines may disappear, but the plastic surgeon affirms that skin carrying 'inborn' flexure lines retains them wherever it may be transplanted.)

The epithelium of adult guinea-pig's tongue, claw, and ear skin (Billingham & Medawar, 1948*a*, and unpublished) have each likewise been shown to retain their exact specificity of histological type upon heterotopic transplantation. In this paper it will be shown that the same is true of the corneal epithelium, and that the maintenance of its peculiar properties does not depend upon its continuing to live in its physiologically unique environment. The epithelium of the cornea differs from that of body skin in its perfect transparency and in its mode of cell packing, which is peculiarly compact at all levels, no flaking cuticle being formed. Its mode of attachment to the stroma is not known, but it is known not to depend upon the presence of elastic fibres. Corneal epithelium is indeed peculiarly mobile upon its substratum, as

studies on corneal healing have repeatedly shown. So far as can be judged by mere microscopic observation, the corneal epithelium consists of cells of one lineage only, and the cells correspond with the 'ordinary Malpighian cells' of body epidermis. We have not been able to reveal an epidermal glial system of dendritic cells in the corneal epithelium (cf. also Redslob, 1922) such as is found within the basal layer of guinea-pig and human body skin epithelium (Billingham, 1948, 1949), though they are present in the conjunctival epithelium at the corneal limbus. (Peculiar branched wiry bodies may be identified immediately below the basal layer of corneal epithelium by their intense supravital staining with methylene blue (Pl. 1, figs. 5–7), but there is no evidence that these are dendritic cells or, indeed, that they are cells at all. They are not to be confused with the well-known branched connective tissue cells of the corneal stroma.) It is not known that the corneal epithelium contains any cells of the lineages which, in body skin, must be presumed responsible for formation of sweat and sebaceous glands and other epidermal appendages. Unfortunately, our knowledge of the cell lineages that comprise the epithelium of body skin is at present quite rudimentary, and no more can be said about them here.

The maintenance of the peculiar transparency and cell packing of the corneal epithelium might well depend in some degree on the peculiar physiological properties of its environment. Being moist, the corneal epithelium during waking hours is probably the coolest tissue in the body, and lacking a blood supply it must live at a much lower oxygen-tension than body epidermis. (Evidence will be presented later to suggest that the corneal epithelium may derive both oxygen and nutriment by diffusion through its outer surface as well as from the aqueous humour.) Nevertheless, it will be shown that the corneal epithelium retains its distinctive properties when grafted on to or below body skin, and, conversely, that body skin epidermis survives as such when transplanted to the cornea.

METHODS AND RESULTS

Although experiments with guinea-pigs are referred to, the majority of those which gave indicative results were done on rabbits. All the operations to be described were done aseptically under ether anaesthesia.

The grafting of corneal tissue to the skin (Pl. 1, figs. 1–3). After steadying the eyeball by grasping a fold of loose conjunctival tissue at a single point with fine dog-toothed forceps, a 4–8 mm.^{2} square or rectangular 'Thiersch' graft may be cut from the cornea by pushing the point of a very sharp cataract knife very superficially and in an all but tangential direction into the stroma somewhat to one side of the anterior pole of the cornea. (Since the majority of the bundles of connective tissue fibres of the stroma form thin, multi-layered lamellae disposed parallel to the surface, the cornea 'splits' fairly easily at any desired level.) The shallow blind pocket so formed may be enlarged by a gentle to-and-fro motion of the blade until tissue of the required area has been lifted from the surface. The three attached edges of the pocket are then cut through and the entire graft lifted off and placed inner side downwards in a Petri dish lined with filter-paper moistened with saline solution. The anterior chamber is not pierced by this method of cutting a graft and the success of the operation does, indeed, depend upon its remaining intact. The graft consists of a sheet of corneal epithelium underlain by a layer of stromal connective tissue of from four to six times

its thickness. In spite of its speed of healing, the graft donor area is still visible to oblique inspection after a week as a shallow depression on the corneal surface representing the defect of stromal tissue.

In order to allow of proliferation and epithelial outgrowth, such grafts have been transplanted to the centre of 9–16 cm.^{2} square or rectangular raw areas prepared from the skin of the right or left side of the rabbit's chest (Pl. 1, figs. 8, 9, 11), following the technique of dressing described in full detail by Medawar (1944). We have found it advisable to prepare the raw area 4 days before the graft is transplanted to it, in order to allow the blood supply of the graft bed to become enriched by capillary proliferation in the ordinary course of 'granulating'. Transplantation then involves no more than removing the dressings, placing the graft in a more or less central position in known relationship to the superficial thoracic vessels that overlie the panniculus carnosus, and then applying fresh dressings.

A study of six rabbits so operated upon shows that epithelial outgrowth from the corneal graft proceeds in the same manner and at the same rate as from Thiersch grafts of body or ear skin. Outgrowth may be slight or, because of the transparency of the epithelial sheet, imperceptible on first inspection at the 7th to 10th post-operative day, but the graft centre is easily recognizable as a raised, rounded and transparent button in the midst of the granulation tissue. It reaches its maximum between the 17th and 25th days post-operation, when the graft is surrounded, not always symmetrically, by an outgrowth of perfectly transparent corneal epithelium averaging from ten to thirty times the area of the original graft (Pl. 1, figs. 8, 9). The finest details of the dermal vasculature may be seen through the epithelial sheet as clearly as through a glass window (Pl. 1, fig. 9). In vertical section (Pl. 1, fig. 10) the corneal epithelium, not exceeding 0·008 mm. in thickness, showed the characteristic appearance of actively migrating epidermal epithelium, the long axes of the basal-layer cells tending to lie parallel to the plane of the integument. The cell packing remained perfectly compact, the outer surface was sharply defined and the superficial keratinized layers showed no hint of a tendency to flake. Corneal epithelium showed no tendency to invade the underlying newly formed fibrous tissue to form inwardly directed 'pegs'. The epithelial outgrowth of Thiersch grafts of body or ear skin under just the same conditions (Pl. 1, fig. 11) is completely opaque; the epithelium may reach or exceed 0·02 mm. in thickness and is bounded superficially by a multilayered flaky cuticle (Pl. 1, fig. 12).

The attachment of the corneal epithelium to the substratum is mechanically very weak: the lightest scraping will damage it and cause bleeding. With the contraction of the raw area combined with the ingrowth of body skin epithelium from its edges, the corneal epithelium rather rapidly disappears, and a month's persistence in each of the two rabbits allowed to survive so long is the most we have recorded. We believe that this disappearance is due to the invasive replacement of the one epithelium by the other. This interpretation is supported by the behaviour of corneal grafts of a slightly different type made upon four guinea-pigs. The corneal grafts were transplanted to the centre of small raw areas, hardly larger than the grafts themselves, cut from the centre of small pigmented 'marker' grafts earlier transplanted to white chest skin to help in the identification of the transparent corneal grafts. Primary healing, revealed by a first inspection after 10 days, was satisfactory

and the grafts were recognizable as small transparent corneal buttons; but the grafts ceased to be visible by the 20th day, by which time native epithelium had closed the raw area and undermined the grafts.

Corneal grafts transplanted subcutaneously. In each of two guinea-pigs a single corneal Thiersch graft was implanted below the skin of the dorsal midline of the head by means of a forwardly directed trochar insertion beginning at a small incision roughly midway between the bases of the ears. Both animals were killed after 4 months, and the two implanted grafts proved to be similar in every respect. Each had formed a hard, raised, palpable nodule about the size of a small pea in the subcutaneous fascia. The nodules were in fact cysts (Pl. 2, figs. 15–17) of a pearl-like transparency containing a clear watery fluid under considerable pressure. Sections show the cyst to be bounded externally by an epithelium showing the characteristic conformation of cells under persistent tension. So far as the polarity of the cyst can be made out, it appears to have formed 'externally', i.e. the original outer surface of the corneal epithelium is directed inwards. Accumulations of squame cells such as would be expected to accumulate within a cyst of body skin epidermis are wholly absent. The origin and nature of the cyst fluid is obscure, though it must be supposed to bear some special relationship to the physiological activity of the corneal epithelium.

The transplantation of body or ear skin epidermis to the cornea. Because of the special ease with which it may be cut and handled, and the greater thickness of its epithelial layer, we have found ear skin to be superior to general body skin for grafting to the cornea. Square Thiersch grafts of side $1\frac{1}{2}$–$2\frac{1}{2}$ mm. were cut by defining the outline of the graft with very light scalpel incisions on the dorsum of the ear and slicing off the area within the incisions as thinly as possible with a no. 11 Swann-Morton scalpel blade. Such Thiersch grafts are always invested by a layer of dermal collagen below the epidermis.

The insertion of such a graft into the cornea proved to be easy. The first stage was to prepare a flat, very shallow, pocket in a plane parallel to but just below the surface of the cornea, exactly as in the cutting of a corneal graft (Pl. 1, figs. 1–3). Into this pocket the skin graft, shaped to fit whenever it proved necessary, was simply slid, with the dermal side facing inwards (Pl. 1, fig. 4). Enclosed round three of its four margins and held down under light pressure from above by the outer flap of the corneal pocket, such grafts always healed securely, and we have no technical failures to report.

In two preliminary experiments in which pigmented guinea-pig's ear skin was transplanted in this manner to the eye, the corneal stroma became richly vascularized by vessels penetrating inwards from the limbus, so defeating the object of the experiment—to grow body skin in the physiological environment characteristic of the normal corneal epithelium. (In our experience, all but the most trivial surgical injuries to the guinea-pig's cornea are followed by vascular invasion and a mass migration of pigmented conjunctival epithelium over certain sectors of the cornea.) Autopsy and histological examination at 11 days post-operation revealed exuberant epithelial proliferation with copious exfoliation of cuticle, accompanied by a general traumatic inflammation of the graft bed (Pl. 2, fig. 22). 'Clear cells' (cf. Billingham, 1948, 1949), representing the cell bodies of dendritic cells, stand out boldly in section (Pl. 1, fig. 13).

Ear skin grafts transplanted to the eyes of each of five rabbits proved not to provoke vascular invasion of the cornea. The grafts were examined *in situ* and removed for histological examination after periods ranging from 3 to 6 weeks.

It was our theoretical expectation that the thin corneal flap superficial to the skin graft, being shut off by the impermeable, richly cuticularized skin graft from access to material diffusing from the aqueous humour, would wither and die, so exposing the healed skin graft on the surface of the eye. It is a matter of some physiological interest that this did not happen: the corneal flap persisted throughout, although bounded by a corneal epithelium of very much reduced thickness (Pl. 1, fig. 14; Pl. 2, figs. 19, 23). These results seem to us to suggest that the corneal epithelium may derive some of its nutriment and oxygen supply by diffusion through its outer surface—a conclusion reinforced by unpublished experiments by one of us (R.E.B.) in which the skin grafts were replaced by very much larger 'grafts' of tantalum foil.

In practice, it proved that the skin epithelium tended to make use of the pocket housing it to form a long, shallow, but open cyst (Pl. 1, fig. 14), the epidermis breaking through to the surface at the opening of the pocket (Pl. 2, figs. 22, 24). The formation of layers of flaking cuticle showed that the epidermis continued to keratinize in the usual way (Pl. 2, figs. 19, 23), but the epidermis layer thinned out considerably and showed the subdued mitotic activity characteristic of non-vascularized skin grafts (cf. Medawar, 1948). Hairs and sebaceous glands did not differentiate *de novo*.

The epidermal component of skin grafts does not persist indefinitely in the eye, but submits to invasive replacement by the native corneal epithelium—the exact converse of what happens to a corneal graft when transplanted to skin. Pl. 2, fig. 18 shows clearly the overgrowth of skin dermal collagen, easily recognizable by its staining properties and the size and mode of packing of its fibres, by corneal epithelium. It seems clear that under the physiological conditions prevailing in the cornea the native epithelium is at an advantage both in division rate and in migratory activity.

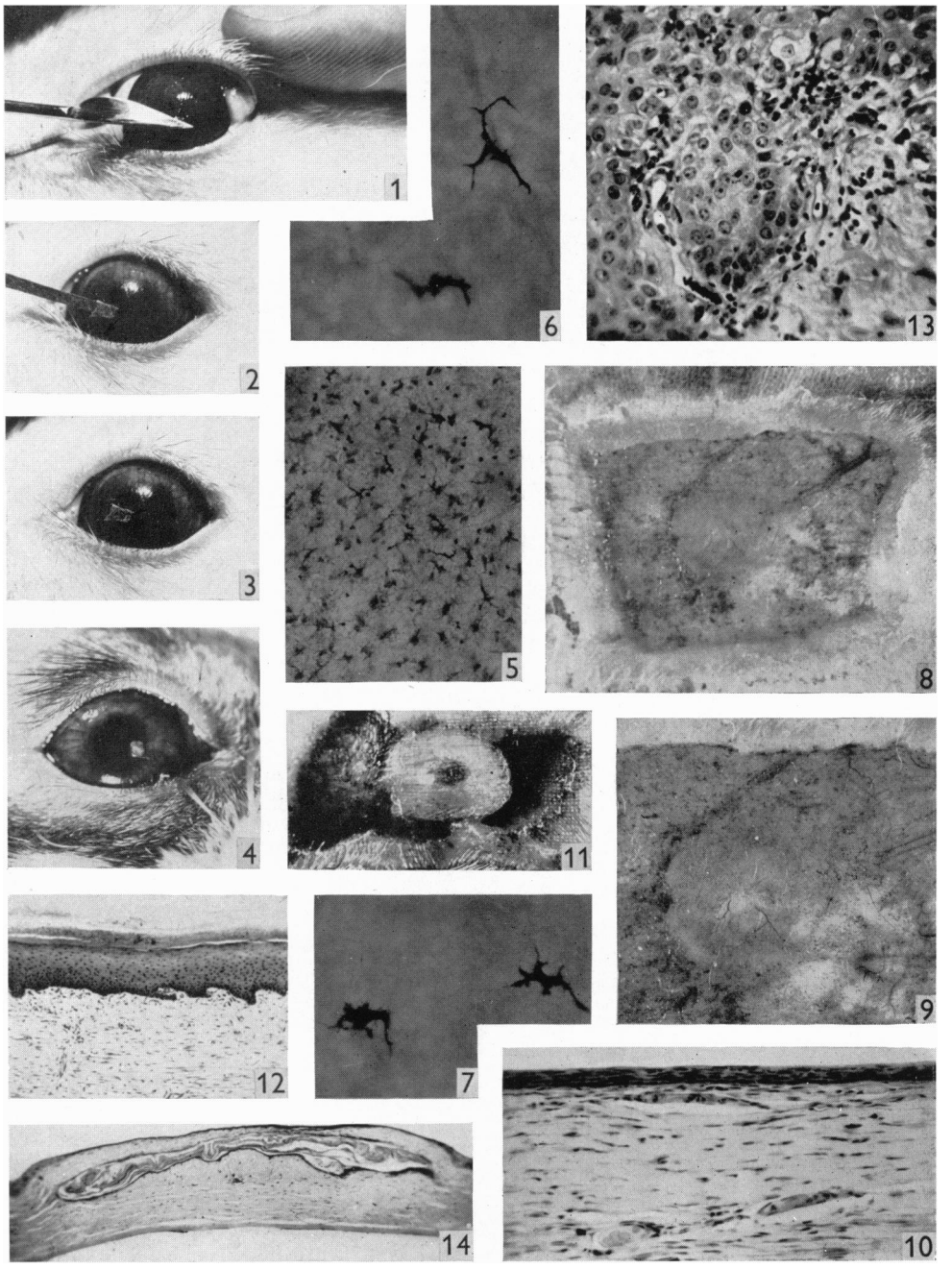
The experimental results recorded in this paper make it clear that the peculiar histological differences between adult corneal and body-skin epithelium are inherited or 'genetic', and are not to be attributed to the physiological differences between the environments in which they normally live. The subdivision of the genus 'epidermal epithelium' into its several genetic species—the epithelia of sole skin, body skin, cornea, tongue, claw, etc.—reinforces our belief that such a hierarchical classification is peculiarly appropriate to the cells of the body, a matter already discussed elsewhere (Billingham & Medawar, 1948*b*). We believe that the classification of the cell lineages of the body is an essential part of descriptive embryology and an indispensable preliminary to understanding the mechanism of embryonic differentiation.

SUMMARY

1. Operations are described by means of which corneal epithelium may be transplanted to body skin and body skin may be transplanted to the cornea.

2. Both epithelia retain their specificity of histological type in a physiologically and anatomically foreign environment.

3. Corneal epithelium is invasively replaced by skin epithelium when transplanted



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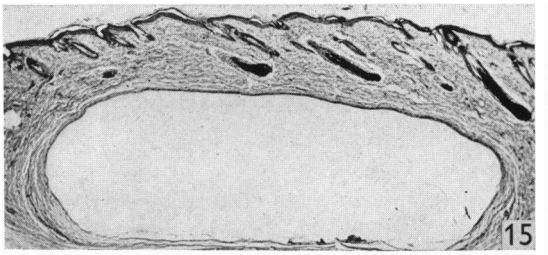

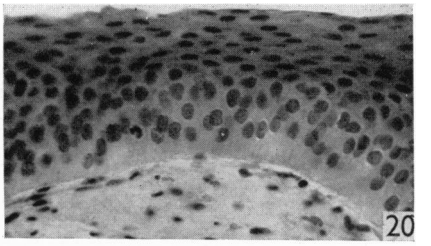

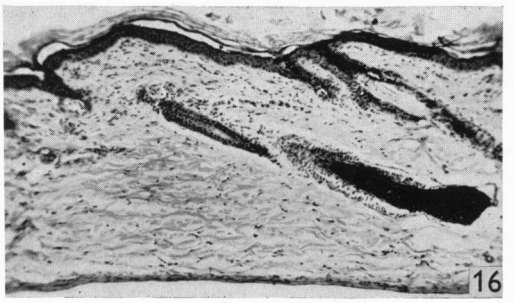

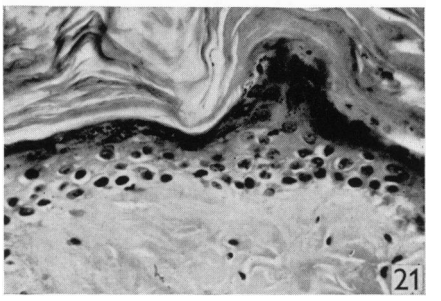

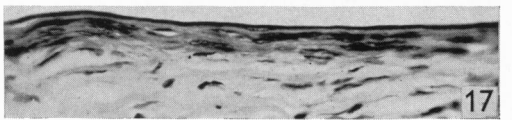

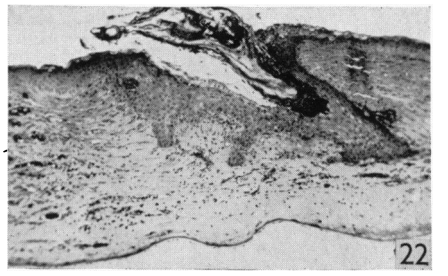

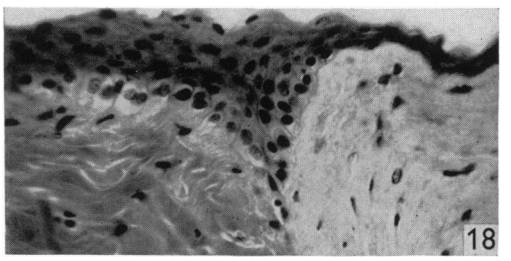

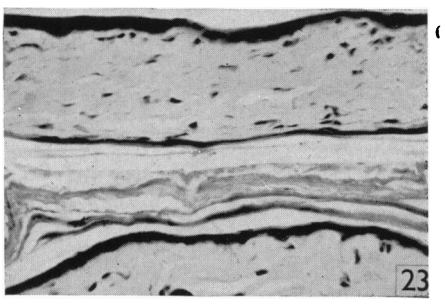

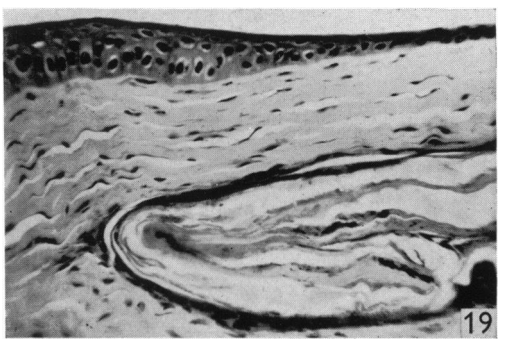

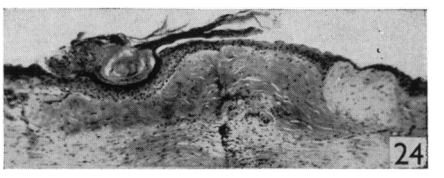

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to the skin, and skin epithelium is invasively replaced by corneal epithelium when transplanted to the cornea.

4. The subdivision of the genus epidermal epithelium into its several genetic species is discussed.

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REFERENCES

BILLINGHAM, R. E. (1948). Dendritic cells. *J. Anat., Lond.*, **82**, 93–109.

BILLINGHAM, R. E. (1949). Dendritic cells in pigmented human skin. *J. Anat., Lond.*, **83**, 109–115.

BILLINGHAM, R. E. & MEDAWAR, P. B. (1948*a*). Pigment spread and cell heredity in guinea-pigs' skin. *Heredity*, **2**, 29–47.

BILLINGHAM, R. E. & MEDAWAR, P. B. (1948*b*). 'Infective' transformations of cells. *Brit. J. Cancer*, **2**, 126–131.

MEDAWAR, P. B. (1944). The behaviour and fate of skin autografts and skin homografts in rabbits. *J. Anat., Lond.*, **78**, 176–199.

MEDAWAR, P. B. (1948). Immunity to homologous grafted skin. III. The fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. *Brit. J. exp. Path.* **29**, 58–69.

REDSLOB, E. (1922). Étude sur le pigment de l'épithelium conjunctival et cornéen. *Ann. Oculist., Paris*, **159**, 523–537.

EXPLANATION OF PLATES

PLATE 1

Figs. 1–3. Illustrating the technique of preparing pockets in the cornea, or alternatively for cutting corneal Thiersch grafts. For description, see text. In fig. 2 a paper strip has been inserted into the pocket to reveal its length and depth.

Fig. 4. A small rabbit's ear skin Thiersch graft 23 days after transplantation into the type of pocket illustrated by fig. 3. Note the absence of any trace of corneal opacity or vascularization.

Figs. 5–7. The 'peculiar branched wiry bodies' immediately below the corneal epithelium revealed by supravital staining with methylene blue. In fig. 5 ($\times 80$) they stand out at a different focal plane from the more diffusely stained stromal branched cells. In figs. 6 and 7 ($\times 334$) note the relationship of the bodies to the just perceptible outlines of the basal layer cells of the corneal epithelium. These bodies are not dendritic cells. The preparations were made from the cornea of the ox and were rendered permanent by the ammonium molybdate method.

Figs. 8 and 9. Showing the widespread outgrowth from rabbit corneal Thiersch grafts transplanted to raw areas cut from the skin of the chest. Fig. 8 ($\times 1$) shows the boundaries of the outgrowth; fig. 9 ($\times 1\frac{1}{2}$) shows the original graft as the small rather dense central patch. Notice that the pattern of the dermal vasculature can be made out in the finest detail through the perfectly transparent outgrowth of corneal epithelium.

Fig. 10. A transverse section through the outgrowth of corneal epithelium illustrated by figs. 8 and 9. Note the characteristic packing of the cells and the complete absence of a desquamating cuticle. Ehrlich's haematoxylin and eosin, $\times 210$.

Fig. 11. Contrast figs. 8 and 9. Widespread symmetrical outgrowth of opaque desquamating epidermal epithelium from a rabbit's ear skin Thiersch graft 20 days after transplantation to a raw area prepared from the skin of the chest. The central graft is darker and has grown a sparse pelt of hairs since transplantation, $\times 1\frac{1}{4}$.

Fig. 12. A transverse section through the outgrowth of ear skin epidermis illustrated by fig. 11: contrast fig. 10. The epithelium is hyperplastic and strongly exfoliating. Ehrlich's haematoxylin and eosin, $\times 50$.

Fig. 13. (See Pl. 2, fig. 22.) Guinea-pig ear skin 11 days after transplantation to the cornea, showing Malpighian cells and clear cells. The cornea has become vascularized, and shows inflammatory changes. Ehrlich's haematoxylin and eosin, $\times 216$.

Fig. 14. (See also Pl. 2, figs. 19 and 23.) A cyst of rabbit's ear skin epithelium 23 days after transplantation to a corneal pocket such as that illustrated by fig. 3. Note the thinness of the epithelium, the accumulation of cuticular debris within the cyst, and the persistence over the cyst of an intact layer of corneal epithelium of reduced thickness. The dermal collagen of the skin graft is still recognizable. Ehrlich's haematoxylin and eosin, × 38.

PLATE 2

Figs. 15–17. Cyst of guinea-pig's corneal epithelium 126 days after transplantation below the skin of the dorsal midline of the head. The cyst, originally fluid filled and containing no cellular debris, is bounded throughout by a thin, 'taut' layer of corneal epithelium, illustrated in higher power by figs. 16 and 17. Ehrlich's haematoxylin and eosin; fig. 15, × 15; fig. 16, × 51; fig. 17, × 210.

Fig. 18. A view in higher power than fig. 24 of the junction between corneal stroma and skin dermis in a rabbit's ear skin Thiersch graft 43 days after transplantation to the cornea. The skin dermal collagen to the left can be distinguished from the corneal stroma to the right by the deeper staining of its fibres, their greater stoutness, and their three-dimensional mode of packing. Both connective tissue elements are bounded above by *corneal* epithelium, which has invasively replaced the skin epithelium. Ehrlich's haematoxylin and eosin, × 210.

Fig. 19. Transverse section of the margin of the cyst illustrated by Pl. 1, fig. 14 (rabbit's ear skin 23 days after transplantation to a corneal pocket). Note that the corneal epithelium on the outer surface of the eye still survives in progressively diminishing thickness over the epithelial cyst, although virtually deprived of access to materials diffusing from the aqueous humour. See also fig. 23. Ehrlich's haematoxylin and eosin, × 210.

Figs. 20 and 21. Contrasting the appearance of hyperplastic corneal epithelium (fig. 20) with ear skin epithelium (fig. 21) 43 days after the transplantation of rabbit's ear skin to the cornea (see also fig. 24). The pyknotic appearance of the nuclei in fig. 21 is a characteristic of skin growing under conditions of reduced oxygen tension. Contrast the richness of exfoliating cuticle in fig. 21 with its absence in fig. 20. Ehrlich's haematoxylin and eosin, × 210.

Fig. 22. Cf. Pl. 1, fig. 13. General sectional view of a graft of guinea-pig's ear skin 11 days after transplantation to the cornea, which has since become richly vascularized. The section passes vertically through the edge of the corneal pocket into which the skin graft was originally tucked, and exfoliating epidermal epithelium has broken through to the surface of the cornea. Ehrlich's haematoxylin and eosin, × 44.

Fig. 23. A view in higher power of a vertical section through the middle of the cyst shown in Pl. 1, fig. 14, one formed by rabbit's ear skin epithelium 23 days after transplantation to a pocket in the cornea. The corneal epithelium that bounds the specimen above is still surviving, though of reduced thickness (cf. fig. 19). The skin epithelium bounding the cyst within the corneal stroma consists of only one or two cell layers. Ehrlich's haematoxylin and eosin, × 210.

Fig. 24. Cf. also figs. 18, 20, 21. A general sectional view of a rabbit's ear skin graft 43 days after transplantation to the cornea. Skin dermal collagen may be recognized by the criteria mentioned in the legend to fig. 18. The non-exfoliating corneal epithelium is invasively replacing the body skin epithelium from right to left: see fig. 18. Ehrlich's haematoxylin and eosin, × 38.