The origin and continuous replacement of epidermal cells in the planarian *Polycelis tenuis* (Iijima)

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WITH TWO PLATES

RAY LANKESTER (1873) coined the term ‘Triploblastic’ and supposed that the gut, parenchyma and epidermis of Turbellaria corresponded to the germ layers of contemporary dogma. This idea is still current, though neither the origin, nor the maintenance of the epidermis of planarians has been investigated in detail. Most embryological studies have been restricted to early development, but Bardeen (1902) worked on embryos of a wide range of ages and claimed that their epidermal cells divide amitotically. Both Mattiesen (1904) and Fuliński (1916), however, denied that cell division occurs there, and since this has been confirmed for the epidermis of the adult (Skaer, 1961), the cells must be recruited from elsewhere. I suggested that the entire epidermis might be continuously renewed by migration of cells from the parenchyma to the periphery.

The idea that cells from the parenchyma might enter the epidermis has been put forward several times. Hallez (1887) described motile cells, equivalent to neoblasts, that enter the epidermis throughout development. Both Mattiesen (1904) and Fuliński (1916) mentioned (without further description) the presence of a second epithelial layer beneath the flattened blastomeres of the young embryo; they supposed that this layer was made up of replacement cells for the epidermis, although they found no evidence of replacement. Loman (1887) suggested that rhabdite-containing cells from the parenchyma might enter the epidermis of the land planarian *Bipalium*, but gave no evidence for this suggestion and did not even discuss it. Korotneff (1909) held that the epidermal cells of triclads were phagocytes of mesenchymal origin, and Sabussow (1911) claimed that free mesenchymal cells (recognizable as neoblasts) sometimes entered the epidermis of planarians from Lake Baikal. Epidermal replacement cells in Acoela from the Gulf of Finland were described by Luther (1912); he suggested that they arose in the parenchyma but he did not find them there. Dorey (1963),

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however, has produced unequivocal experimental evidence that a centrifugal migration of epidermal replacement cells from the parenchyma occurs in the acoel *Convoluta*. In the column of *Hydra*, cell replacement occurs through growth from a hypostomal growth zone (Brien & Reniers-Decoen, 1949); in triclads and acoels it is apparently achieved by the mass migration of differentiated cells.

The cells of the epidermis in adult triclads are unicellular glands containing a formed, cigar-shaped secretion product—the rhabdites. A demonstration that rhabdites first appear in the parenchyma of the embryo and only subsequently in the epidermis, would support the suggestion that the epidermal cells have performed a centrifugal migration. Rhabdites, in fact, act as intracellular markers (Skaer, 1961).

Electron microscopy has shown that the ducts of other types of unicellular glands in the parenchyma of triclads discharge their contents to the exterior through the cells of the epidermis (Török & Röhlich, 1959; Klima, 1961; Skaer, 1961). This raises the additional problem of how, despite continuous replacement of the epidermal cells, the ducts of the glands both penetrate the thick basement membrane and maintain a connexion with the exterior.

Sections of embryos have been searched for evidence of the origin in time of the system of cell replacement that was suggested for the adult epidermis, and for signs of relationships between unicellular glands and epidermal cells. This paper presents the results obtained by bright-field and phase-microscope studies of embryos and newly hatched animals.

**MATERIALS AND METHODS**

*Polycelis tenuis* (Iijima) was chosen for culture in the laboratory since, as mentioned by Reynoldson (1960) and as I have found, this species breeds very readily in the laboratory.

Before it is laid, an egg capsule can be detected as a bulge inside the parent. Immediately before the capsule is laid, the parent tends to adhere to the substratum by the margins of the body, while the rest of the body and the head are raised. This behaviour often enables the precise time of laying to be determined. When first laid, the capsules are a creamy-white colour, but after a few hours they darken to a dull brown (Nurse, 1950). The approximate age of a capsule can be gauged from its colour during this initial period. Capsules were kept at 19 ± 4°C, and the stage of development reached by the embryo was estimated from the age of the capsule since laying. This estimate can be relied on, as observations showed that, of fifteen fertile capsules allowed to complete their development, all, except two, hatched within 12 hr. of 21 days.

Owing to the extreme impermeability of the capsule wall, it was necessary to dissect off part of the wall before fixing the embryos. Not all the external yolk has been ingested by the embryos until 8½ days have past, and, until ingestion is complete, the embryos cannot be seen through the wall of the capsule. Further-
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more, before this time the contents of the cocoons appear to be under pressure, for no matter how carefully they are opened, yolk spouts out at the first incision, and it is difficult to open the capsule without damaging some of the embryos. Under a dissecting microscope the capsule was carefully pierced with a fine glass needle and the tear thus made was extended so the top of the capsule could be lifted off. Approximately 25 per cent. of the total number of capsules opened were infertile and contained only yolk cells. Reynoldson (1960) found that the proportion of infertile capsules laid in the field varied independently of the season from 0 to 34.5 per cent. In fertile capsules, however, from one to six, but usually three, white embryos are disposed like eggs in a nest. Nine-day and older embryos continue to develop in opened capsules; but no younger embryos survived for more than a few hours after the capsule had been opened. Almost all observations were made on embryos from freshly opened capsules, and much of this material was examined alive.

Eight-day embryos contain very little parenchyma and are spherical, but by 9 days they have become elongated, and show a median partition of parenchyma which divides the gut into right and left lobes posteriorly. More anteriorly the gut develops a scalloped edge. The posterior cleft widens, extends towards the head end, and in 10-day embryos the bud of the adult pharynx has developed in its most anterior region. Embryos of this age have started to flatten, the parenchyma has increased in bulk, and the gut has developed peripheral lobes. Twelve-day embryos have become flattened; the pharynx bud has moved still more anteriorly and is now situated near the centre of the animal.

For routine microscopic examination, embryos and young worms were fixed in 2 per cent. osmium tetroxide buffered at pH 7.4 with veronal-acetate. They were left in this solution for 1 to 2 hr. at 3°C., and were then stained, usually by the osmium-ethyl gallate procedure of Wigglesworth (1959). Other animals were fixed in freshly prepared 4 per cent. solutions of formaldehyde, buffered with veronal-acetate buffer at pH 7.4. Either araldite, or agar and ester-wax, was used for embedding, and sections were cut at 0.8 μ and 0.4 μ on a standard Cambridge rocking microtome. Sections of specimens that had not been treated with osmic-gallate were stained with methylene blue, or with 1 per cent. phloxine and methylene blue (Mullinger, 1964); some sections of animals that had been fixed in formalin and embedded in agar and ester-wax were stained by Heidenhain's Azan technique.

OBSERVATIONS

The embryological origin of the epidermis

The early embryo is bounded, as shown by all previous investigators, by blastomeres that become greatly flattened and form what will here be called—following Mattiesien (1904)—the 'primary epidermis'. In a remarkable early note, Kölliker (1846) described waves of contraction passing over these cells, and this observation has been confirmed. As the external yolk cells are ingested by the
embryo, the primary epidermis becomes progressively thinner, until, in 8-day embryos, it has been reduced to about 1 μ in thickness. It contains minute, round, refractile granules, stains a mid-tone of grey with osmic-gallate, and bears tufts of hair-like, birefringent protrusions which, as they do not move or contain basal bodies, may be large microvilli.

The cells beneath the epidermis of the 8-day embryo are closely packed and scarcely stained by osmic-gallate. At this stage, larger transparent cells that will emerge as 'secondary' epidermal cells distort the smooth contour of the primary epidermis into humps, and then pierce it. It seems likely that they pass between the original epidermal cells. The bulges they cause, and the large secondary epidermal cells themselves, can be seen on the surface of living 8-day embryos, for these cells flatten only slightly in the epidermis, and therefore appear as transparent 'pimples'. Their transparency is perhaps correlated with their characteristic paleness (as compared with the cytoplasm of the primary epidermis) when stained with osmic-gallate. Usually the cells are sparsely ciliated but they contain many mitochondria and a large nucleus with a prominent nucleolus (Plate 1, Fig. A). These nuclei are larger than those of any other type of epidermal cell found throughout development. Lipid droplets (in some cases droplets of triglycerides), identified by the black or blue colour of the reaction-product with osmic-gallate, are present in these and other cells of the embryo. One type of spherical inclusion that stains black with osmic-gallate, and is $0.4 \mu$ to $1 \mu$ in diameter, is almost always present and is situated immediately beneath the outer surface among the ciliary rootlets (Plate 1, Fig. A).

Secondary epidermal cells are plentiful in that hemisphere of the embryo that

### Explanation of Plates

All the figures are light micrographs at a final magnification of ×1760, and the scale represents 10 μ.

**Plate 1**

**Fig. A.** Osmic-gallate preparation. Median section of an 8-day embryo showing a large, ciliated, secondary epidermal cell that has entered the primary epidermis. The arrow indicates a granule, characteristic of these cells.

**Fig. B.** Osmic-gallate preparation. Median section of an 8-day embryo. A ciliated secondary epidermal cell is entering the epidermis; its nucleus and nucleolus are distorted by a muscle fibre.

**Fig. C.** Transverse section, stained with methylene blue, of the hind-end of a 9½-day embryo, showing the lack of adhesion between the epidermal cells and the underlying parenchyma, the irregular height of the epidermal cells and a large extracellular vacuole.

**Fig. D.** Transverse section, stained with methylene blue, of the hind-end of a 9½-day embryo, showing the variation in thickness of the epidermis. In the centre of the picture is a ciliated secondary epidermal cell, and on the right, a ciliated primary epidermal cell.

**Fig. E.** Osmic-gallate preparation. Transverse section of the ventral surface of a 9-day embryo. An epidermal replacement cell with darkly stained cytoplasm and including a vesicle lined with cilia is situated between two paler secondary epidermal cells.

**Fig. F.** Osmic-gallate preparation. Transverse section of the lateral margin of a 9-day embryo, showing an epidermal replacement cell in the parenchyma. The cell lies immediately beneath the primary epidermal cells; it has darkly stained cytoplasm with black granules and a vesicle lined by cilia.
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will form the future ventral surface; elsewhere, the epidermis is largely made up of primary cells. As more and more secondary cells enter the epidermis, the primary cells increase in height and diminish in width. At this stage of development, muscle fibres appear, and secondary cells entering the epidermis are deformed by them, for the circular muscles lie immediately beneath the epidermis and are closely packed (Plate 1, Fig. B). No basement membrane is present at this stage.

In 9-day embryos, secondary epidermal cells are numerous, but they are sparse in 10-day embryos, and totally absent from 11-day and older embryos. It seems unlikely that they are being sloughed off, even though the entire epidermis at the hind end of the animal adheres less closely than elsewhere to the underlying tissue and the individual cells are irregular in height (Plate 1, Figs. C & D). Sloughed cells would not disintegrate immediately but would probably persist as free cells within the capsule for a period. Free cells have never been found, however, in carefully opened 10-day capsules from which the embryos have been removed. It is also unlikely that free cells could be ingested by the embryo, as are the yolk cells, for in 9-day and 10-day embryos the embryonic pharynx is degenerating, and the future adult pharynx is probably not functional. One explanation of the disappearance of these secondary epidermal cells is suggested by observations to be described later (p. 134).

Certain cells in the ventral regions of 9-day embryos contain a vesicle, approximately 5 \( \mu \) in diameter, lined by cilia. So far only five such cells have been found; four were in the epidermis (Plate 1, Fig. E) and one was in the parenchyma (Plate 1, Fig. F). Those in the epidermis had no cilia except in the vesicle, although all the surrounding cells were typically ciliated, and the one in the parenchyma

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**PLATE 2**

Fig. G. Osmic-gallate preparation. Transverse section of the lateral margin of a 9-day embryo. In the parenchyma is a rhabdite-forming cell with darkly stained cytoplasm and one rhabdite, sectioned longitudinally.

Fig. H. Osmic-gallate preparation. Transverse section of the ventral surface of a 9-day embryo, showing a process from a parenchymal rhabdite-containing cell extending into a large, pale, secondary epidermal cell.

Fig. I. Adjacent section to that shown in Fig. H. The process is seen to ramify within the cytoplasm of the epidermal cell.

Fig. J. Osmic-gallate preparation. Longitudinal section of the dorsal surface of a 10-day embryo, showing epidermal rhabdites arranged with their long axis at an angle to the surface. This grouping would give a typical star-shaped pattern in surface view. A longitudinal muscle fibre runs beneath the epidermis, and there are two rhabdite-containing cells in the parenchyma.

Fig. K. Osmic-gallate preparation. Transverse section of the dorsal surface of an immature animal, showing a rhabdite-containing cell situated partly in the epidermis and partly in the basement membrane. The duct of a unicellular gland containing ovoid, darkly staining, acidophil granules is draped around the nucleus of the migrating cell like a string of beads. One of the epidermal cells is ciliated; this is uncommon on the dorsal epithelium. Notice the increase in height of the epidermis, compared with that in Fig. J, and the conspicuous orientation of rhabdites and other cellular components.
was situated immediately beneath a non-ciliated primary epidermal cell that was itself surrounded by ciliated cells. The cytoplasm of these vesicular cells, when stained with osmic–gallate, is dark grey and contains black granules (approximately 1 \( \mu \) in diameter) that are especially numerous around the vesicle. Near the vesicle of the cell found in the parenchyma was a minute, elongate, black granule too small for accurate identification but possibly a rhabdite.

With its dark, granular cytoplasm and internal ciliation, this cell-type so closely resembles parts of the flame-cell system that in a single section they are indistinguishable. The distinction, however, that the cilia of these cells line a vesicle rather than the lumen of a tube (as established by serial sections) is crucial, for preliminary studies on the differentiation of the flame-cell system suggest that, though it develops through the coalescence of intracellular vacuoles, its cilia are not formed until the system has a continuous lumen. The similarity between these cells, containing cilia within a vesicle, and the epidermal replacement cells of acoels described by Luther (1912) and by Dorey (1963) is so striking that it would seem reasonable to suggest that, for a few hours during embryonic development, cells containing a ciliated vesicle enter the epidermis. Subsequently the vesicle may be supposed to burst, so that its ciliated lining becomes part of the outer surface of the ventral epithelium. Mihálik (1935) has described a similar formation of vesicles lined with cilia in replacement cells of the tracheal and bronchial epithelium of several mammals.

The study of embryonic material has proved conclusively that rhabdites first develop in the parenchyma (Plate 2, Fig. G). The formative cells differentiate in the lateral margins of 9-day embryos which, at this stage, have become slightly flattened. The rhabdite-forming cells are small, and the bulk of their cytoplasm stains darkly with osmic–gallate. This dark staining may be correlated with the presence of copious endoplasmic reticulum, previously shown to be present in rhabdite-forming cells of the adult (Klima, 1961; Pedersen, 1961; Skaer, 1961). Cells of this type first appear in the epidermis of 9½-day embryos; they eventually make up the entire epidermis, which could be regarded, therefore, as a ‘tertiary’ epidermis.

Like the ventrally and laterally situated secondary epidermal cells, ventrally and laterally situated rhabdite-containing cells in the epidermis also bear numerous cilia. Nothing suggests, however, that rhabdite-forming cells develop cilia before they reach the epidermis, or that the cilia form within a vesicle rather than directly on the surface of the epidermis. Epidermal cells on the dorsal side of 9-day and older embryos are usually not ciliated.

Some sections suggest that rhabdite-containing cells penetrate, digest, and destroy the secondary epidermal cells (Plate 2, Figs. H & I). A long, dark, branching process of an invading parenchymal cell can occasionally be seen within a secondary epidermal cell, the cytoplasm of which, in the regional of this process, may be even paler than elsewhere. Whether or not this is a cause of their disappearance, no secondary cells remain in the 11-day embryo.
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Primary epidermal cells are still present in 11-day and somewhat older embryos. The secondary epidermal cells seem, indeed, to be largely supplementary rather than replacing cells. Rhabdite-containing cells, on the other hand, certainly replace the primaries, since no primary epidermal cells are to be found in 15-day embryos; at this stage, the epidermis consists entirely of rhabdite-containing cells.

A basement membrane is absent from 10-day and younger embryos, but a clear layer, approximately 1 μ thick, separates the epidermis from the underlying tissues in 11-day embryos. This layer becomes more and more strongly stainable with aniline blue until, in the hatchling, it has all the staining characteristics of the basement membrane.

In regions of the epidermis where only primary cells are present, rhabdite-containing cells migrate centrifugally to the surface, penetrate between the original cells and flatten. This process has been watched in a living 10-day embryo. At this stage, the rhabdites are longer than the cells of the epidermis are tall, and they come to be spread out like the spokes of a wheel in the flattened epidermal cell, forming a star-shaped pattern (Plate 2, Fig. J). In cells apparently passing into the epidermis the rhabdites form a bundle (the axis of which is approximately normal to the surface of the animal) extending from the epidermis into the parenchyma. With surface lighting a single cell in this position can be watched, and at the end of 3 hr. continuous observation, the rhabdites had splayed out basally, presumably because the lower part of the cell had reached the surface of the parenchyma, and the cell had started to flatten. At a slightly later stage, all the cells in this particular region were fully flattened, as was shown by the regular star pattern of their rhabdites.

Continuous intercalation of rhabdite-containing cells causes the epidermis to increase in height (the cells becoming more columnar as the number of cells per unit area increases) until sometime after hatching. This change in shape of the epidermal cells is correlated with the disappearance of the star pattern.

Relations between epidermal cells and gland cells

Gland cells appear to differentiate throughout the parenchyma, so that the probability of rhabdite-forming cells developing next to, or encountering, gland cells of another type is high. The cytoplasm of a rhabdite-forming cell frequently encloses the narrow duct of a gland cell. Some sections suggest that as the former cell migrates to the surface it drags the duct behind it like a train. Three rhabdite-containing cells have been found, apparently in process of migrating through the basement membrane, with the ducts of gland cells draped round their nuclei (Plate 2, Fig. K). When the epidermal cell and its contents become oriented in the epithelium, with rhabdites normal to the surface, the gland-cell duct may become similarly oriented and thus come to open to the exterior through the rhabdite-containing cell. The agent whereby the ducts of gland cells come to penetrate both basement membrane and epidermal cells, seems to be the actively migrating, rhabdite-containing cell itself.
DISCUSSION

The most striking feature of the development of the triclad epidermis is the parenchymal origin of all the types of epidermal cell with the exception of the peripheral blastomeres, the formation of which necessarily precedes the development of the parenchyma.

Since the secondary epidermal cells have so short a life, being present only in embryos between 8 and 10 days old, it is tempting to assume that their existence is associated with some precise but transitory function. Contemporary changes in the embryo, however, are so many and so drastic that it is not yet possible to suggest what this function might be. It seems unlikely that secondary epidermal cells change into rhabdite-forming cells, for the latter have smaller nuclei even though they are secretory. The nuclei of most cells enlarge when secretion commences (Kracht, 1954).

Although Metschnikoff (1883) found rhabdites in the parenchyma before they occur in the epidermis, Mattiesen (1904) and Prenant (1922) both claimed that rhabdites first develop in the epidermis. The embryo drawn by Mattiesen to show this, however, is at a relatively late stage of development and corresponds to a 10-11-day embryo in my material; it is to be expected that rhabdites would be present in the epidermis at this stage. Prenant did not publish a drawing of his specimen, but that of Metschnikoff is plainly younger than that of Mattiesen.

The fact that rhabdites first develop in the parenchyma is in accordance with the suggestion that rhabdite-containing cells undergo a centrifugal migration, but it is not, of course, part of the evidence for migration. The case for migration would not be undermined, however, even if rhabdites first appeared in the epidermis, for the epidermal cells (other than the blastomeres) are clearly reinforcements or replacements mobilized by centrifugal migration. Migration might precede the formation of rhabdites, and the two processes might then overlap only at a later stage. Studies are at present in progress to determine whether rhabdite-containing cells migrate only when they have a full complement of rhabdite material, or whether migration is independent of the presence of rhabdites but controlled by some other factor—for example, by the degree of wear and tear on the epidermis. The experiments of Dorey (1963) on the epidermis of the acœl Convoluta, show that the formation of epidermal replacement cells in the parenchyma can be provoked by damage to the epidermis.

Although replacement of the epidermis by centrifugal migration apparently occurs both in embryonic and adult triclads, and in adult acoels, it cannot be the sole method of replacement throughout the Turbellaria, for mitosis has been reported to occur frequently in certain cells of the epidermis of the catenulid rhabdocoeel Stenostomum (Keller, 1894; Stern, 1925; Pullen, 1957).

Rhabdite-containing cells are continuously lost from the epidermis, and it is necessary to consider what happens to their associated gland cells. It is possible that the life of an elongate gland cell is, in fact, shorter than that of an epidermal
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1. The primary epidermis of the embryo of *Polycelis tenuis* is formed by flattened blastomeres.
2. All subsequent types of epidermal cell apparently come from the parenchyma by centrifugal migration.
3. Secondary epidermal cells are large, transparent, and occur only in 8 to 10-day embryos.
4. For a few hours, cells with osmiophil cytoplasm and a vesicle lined by cilia enter the epidermis of 9-day embryos.
5. Rhabdites first develop in the parenchyma. This occurs in 9-day embryos and accords with the suggestion that centrifugal migration occurs in rhabdite-containing cells.
6. Rhabdite-containing cells invade the secondary epidermal cells, and in 15-day and older embryos they make up the entire epidermis.
7. Continuous intercalation of cells into the epidermis causes it to increase in thickness from 8-days onwards until several weeks after the planarian has hatched.
8. Replacement of epidermal cells seems to occur exclusively by centrifugal migration both in embryonic and adult triclads, and in acoels, but cell-division has been reported in the epidermis of a rhabdocoel.
9. When rhabdite-containing cells migrate they appear to drag the ducts of unicellular glands behind them.
10. The relationships between unicellular glands and epidermal cells are discussed in the light of centrifugal migration.
RÉSUMÉ

L’origine et le remplacement perpetuel des cellules épidermiques chez la planaire Polycelis tenuis (Iijima)

1. L’épiderme primaire de l’embryon du Polycelis tenuis est formé par des blastomères aplatis.
2. Toutes les cellules de types ultérieurs semblent provenir du parenchyme par le moyen de la migration centrifuge.
3. Les cellules épidermiques secondaires sont grandes, transparentes et se présentent uniquement dans les embryons âgés de 8 à 10 jours.
4. Les cellules, fournies de cytoplasme osmiophile et d’une vésicule munie de cils vibratiles, entrent dans l’épiderme des embryons de neuf jours. Ce phénomène se fait remarquer pendant quelques heures seulement.
5. C’est dans le parenchyme que les rhabdites se présentent pour la première fois. Ceci s’accorde avec la théorie que les cellules à rhabdites effectuent une migration centrifuge. Elles se manifestent dans les embryons à partir du 9ième jour.
7. L’intercalation continue de cellules fait épaissir l’épiderme à partir du 8ième jour, et cet épaississement dure jusqu’à plusieurs semaines après l’élosion de la planaire.
8. Le remplacement de cellules épidermiques paraît s’effectuer exclusivement par le moyen de la migration centrifuge tant dans les triclades embryonnaires que dans les adultes, et aussi dans les acœles, mais on a signalé la mitose dans l’épiderme d’un rhabdocoele.
9. Quand les cellules à rhabdites effectuent une migration, quelques-unes d’entre elles ont l’apparence de trainer des canaux de glandes unicellulaires.
10. Les rapports entre les glandes unicellulaires et les cellules épidermiques sont discutés du point de vue de la migration centrifuge.

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