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Moulting in *Rana esculenta*: Development of Mitochondria-Rich Cells, Morphological Changes of the Epithelium and Sodium Transport

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Summary. The present study concerns moulting of the skin in *Rana esculenta* in vivo and in vitro. The evolution of mitochondria-rich cells (MRC) and changes in the epithelium during moulting were followed. The greater part of the MRC are lost during moulting, either because they remain attached to the old *stratum corneum* or because they are left in contact with the external medium and degenerate. The cells thus lost leave deep impressions in the new *stratum corneum* which disappear progressively. Before an MRC is shed, a cell of the *stratum intermedium* contacting it differentiates to form a new MRC to replace the old. Isolation of the skin triggers moulting in the excised pieces. This moulting does not cause changes in the short-circuit current or in the transepithelial resistance. Aldosterone (10^{-6} M) added in vitro to the serous side appeared to facilitate the detachment of the slough, however, no clear-cut moult-inducing effect of the hormone was seen.

Key words: Moulting – Epithelium – Sodium transport – *Rana esculenta.*

The multilayered epithelium of the adult amphibian skin is composed of two main cell types: typical epithelial cells and "mitochondria-rich cells" (MRC) also known as "flask cells". The latter can be recognised by their numerous mitochondria and by their pyriform shape, the apical pole being in contact with the external layer (*stratum corneum*) and the basal pole being level with the third or fourth cellular layer (*stratum intermedium*).

The earliest hypothesis on MRC function included the tenet that these cells played a role in the periodic moulting of the *stratum corneum* so characteristic of Amphibia (Schulze, 1867; Pfitzner, 1880; Dennert, 1924). Since 1971, numerous workers have considered a possible participation of MRC in transepithelial movement of various substances. Lodi (1971) proposed a role in osmoregulation, and Whitear (1972) suggested that the external barrier of the sodium transport

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compartment was at the level of the apical pole, while Voûte and coworkers considered the MRC as possible aldosterone receptors (Voûte et al., 1972; Voûte et al., 1975). Ehrenfeld et al. (1976) showed that these cells possessed a specialised organic-base excretory mechanism and suggested that they form a transepithelial excretory system, but do not play a significant role in sodium transport.

Little is known about the replacement of MRC during moulting. Two contradictory opinions have been proposed: according to Pfitzner (1880) the majority of MRC is shed with the old *stratum corneum*, while Dennert (1924) considered that most of these cells become covered by the new *stratum corneum* and are thus not renewed. The present study is concerned with the renewal and fate of the MRC during spontaneous moulting.

Nielsen (1969) in *Rana temporaria* and Hviid Larsen (1969) in *Bufo bufo* described a moult-promoting effect of aldosterone on isolated skin and the accompanying typical changes of the electrical parameters. As shown in the present investigation, isolation of skin triggers a moult in the absence of all hormonal intervention. It was therefore necessary to reexamine the interrelation between moulting, aldosterone and changes in electrical parameters in isolated skin.

Materials and Methods

Frogs (*Rana esculenta*), averaging 100 g in weight, were kept in running tap water at a constant temperature (15° ± 1° C) before use. They were studied within one week of arrival in the laboratory.

The histological study of spontaneous moulting was made on skins of frogs in which it could be seen that the slough was on the point of being shed: the frogs were double-pithed and pieces of ventral skin cut off. Sloughs were stained with a 0.5 % filtered solution of methylene blue and mounted flat between slide and coverslip. Skins were fixed in Carnoy's solution and embedded in paraffin. Sections cut at 8 µm thickness were stained with Ramón y Cajal's trichrome or with Schiff's reagent after oxidation by periodic acid (see Gabe, 1968). These techniques were also used for studying the morphological changes occurring in skins after isolation, one piece of skin being fixed immediately after removal, while another was mounted in an Ussing chamber betwen two Ringer solutions and kept alive for at least 6 h before fixation. The difference in electric potential (PD) between epithelial and serosal bathing solutions was measured with calomel electrodes and agar KNO_3 bridges. The skin was short-circuited by means of an apparatus which automatically annulled the transepithelial potential difference. The counter potential was introduced by Ag-AgCl electrodes in containers filled with a saturated KCl solution. Contact with the solution bathing the skin was by agar bridges saturated with KNO_3. The sign of the PD is given relative to the external face. To check any effect of the mounting in Ussing's chamber on changes in the epithelium, in certain experiments additional pieces of skin were kept alive in aerated Ringer solution beakers. The Ringer composition was as follows (in mM): NaCl, 111; KCl, 2; $NaHCO_3$, 2.4; $CaCl_2$, 1; glucose, 11.

The number of MRC was determined from sections fixed in Champy-Maillet's liquid (Gabe, 1968) which is a selective stain for these cells (Lodi, 1971).

In experiments designed to study the effect of aldosterone on moulting, 100 µg of the hormone (Sigma Chemical Co.) were dissolved in 50 µl of acetone and diluted 66 times with Ringer. This solution was added to the chamber on the serous side of the skin to give a final concentration of 10^{-6} M. The same solution but without hormone was added to controls: no effect on the bioelectric parameters was found.

The study of the development of new MRC was made on moulting skins of frogs injected with 0.5 ml of a filtered solution of methylene blue in Ringer (10^{-3} M) into the cutaneous artery. 5 to 20 min after injection pieces of abdominal skin were removed and treated with Erlich's technique: 30 min in contact with air followed by 1 h in 10 % ammonium molybdate solution. Sections (15 µm) were made with a freezing microtome, submerged in ammonium molybdate solution, and then mounted in glycerol for microscopic examination.

Results

I. Evolution of MRC and Superficial Epithelial Layers During Moulting

When a slough is shed naturally during moulting a considerable number of MRC remain attached to the shed *stratum corneum* at their apical poles. At some sites the slough consists of one layer of cells (*stratum corneum*) and at other sites of two cell layers (corneal+granular layers, see Fig. 1). This was seen to be the case in numerous flat-mounted sloughs examined at all seasons. Skin sections of animals moulting naturally confirm that the sloughs may consist of one (str. corneum) or two (str. corneum and granulosum) layers of cells. In Fig. 5 the str. corneum alone has become detached at the top but, after a sharp transition, the central region of the photograph shows a two-layered slough with four imprisoned MRC.

In histological sections of moulting skins when the str. corneum is only partly detached, MRC may be seen in three types of situation: (1) Some remain attached to the old str. corneum and are thus separated from the epithelium (Fig. 2) which shows pit-like concavities marking the sites of the detached MRC. The fate of these pits and their relationships with the neighbouring epithelial cells will be discussed below. (2) Some MRC become detached from the str. corneum as this structure is being sloughed off and remain in contact with the external medium (Fig. 3). (3) Some MRC are covered by the new str. corneum and thus separated from the exterior (Fig. 2). Only MRC in this last situation are seen in the typical intermoult skin. MRC in which the apical pole remains in contact with the outside medium are finally shed; in sections of skins well advanced in the moulting process these cells can be seen lodged in concavities in the new str. corneum, while the necks of the cells often extend freely into the external medium (Fig. 3). The concavities are bordered by several cells of the newly formed str. corneum, while their bases are thin and meniscus-shaped, convex toward the epithelial (Fig. 4). They shrink progressively. Degenerating MRC can also be seen as round bodies within the corneum. During these changes the new str. corneum becomes thinner and the impressions left by the original concavities become increasingly more shallow until only slight meniscus-shaped traces remain on the corneal surface. Concavities left by MRC that remained attached to the sloughed str. corneum follow the same series of changes as described above.

The greatest number of mitoses in the germinal layer occurred once the old str. corneum was detached and levelling of the old MRC concavities had begun (Fig. 6). It could suggest that cellular renewal awaits the completion of the moult.

II. Induction and Evolution of New MRC

It has recently been shown that the MRC in the skin of *Rana esculenta* concentrate and excrete methylene blue, whereas the other epithelial cells do not have this capacity, at least not in resting skins (Ehrenfeld et al., 1976). In skins of moulting frogs, however, cells of the *stratum intermedium* in contact with the MRC also concentrate the stain. These cells only appeared at one moment of the moulting cycle, i.e. when the old str. corneum had begun to become detached, but was still in

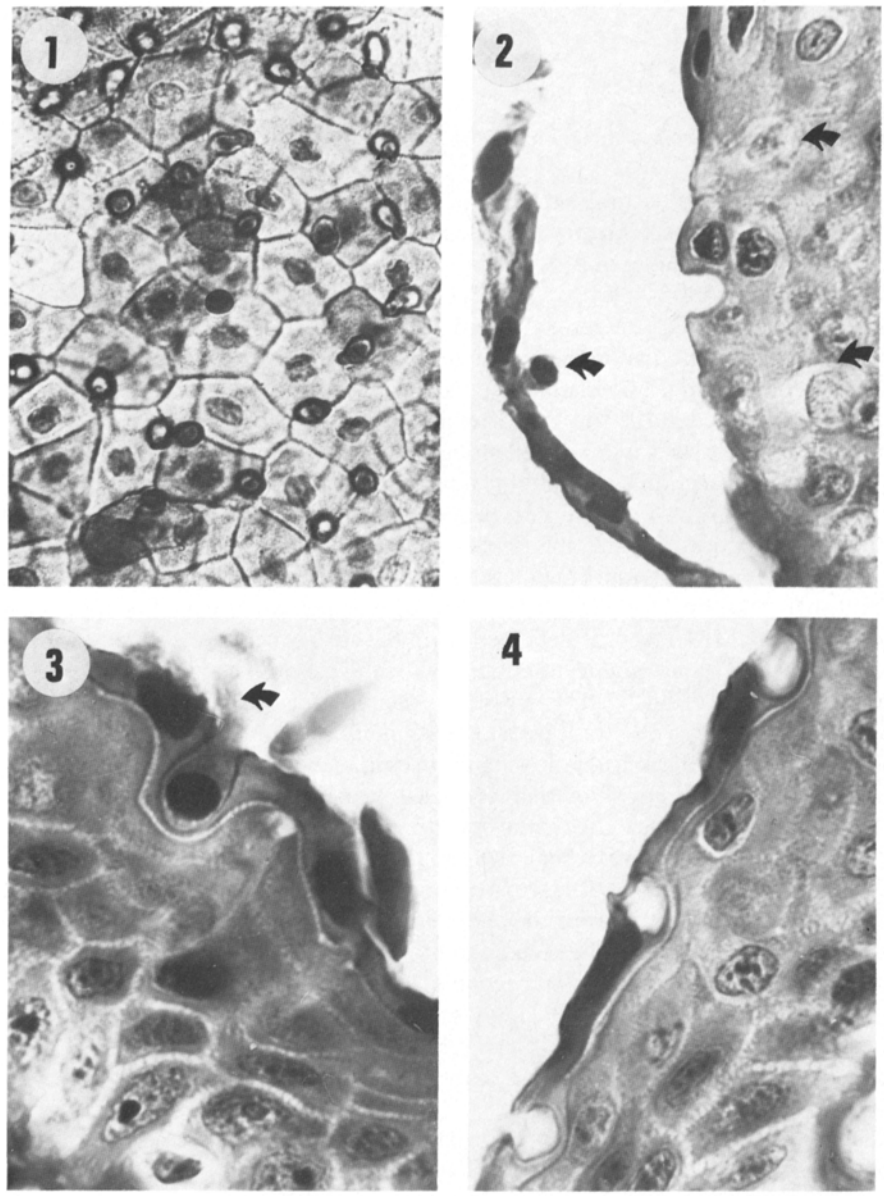


Fig. 1. View of the upper surface of a spontaneously-detached slough. Numerous pear-shaped MRC are located at the boundaries between the pavement cells. The cell outlines of a lower layer of pavement cells can be seen.

Fig. 2. Section of moulting skin. An MRC (*arrow*) remaining attached to the slough has left a deep depression in the new corneum. Below, a MRC which has not been renewed can be seen, and above, one in which the apical pole is in contact with the external medium. Trichrome of Ramón y Cajal. × 800

Fig. 3. An MRC (*arrow*) being expelled from the epithelium after shedding of the old str. corneum. The cell body lies in a depression of the new str. corneum, while its apical pole is in contact with the external medium. Trichrome of Ramón y Cajal. × 1100

Fig. 4. Three depressions in the recently-differentiated new str. corneum have been left by expelled MRC. This stage in the moulting processes follows that shown in Fig. 3. Trichome of Ramón y Cajal. × 1100

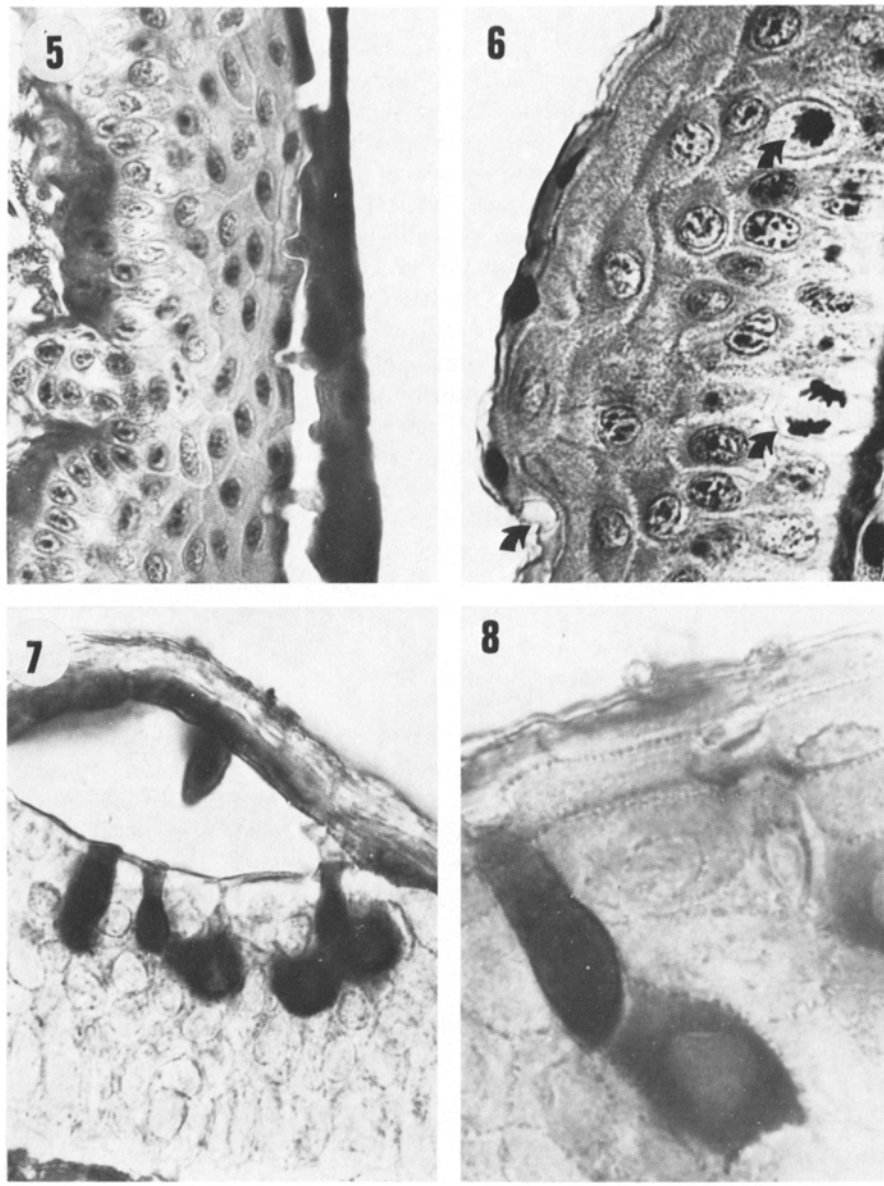


Fig. 5. A slough showing different depths of cleavage: at the top, it consists of a single cell layer, and below, the splitting occurs more deeply. The basal parts of MRC being shed with the slough can be seen in the sub-corneal space, and the depressions they leave are visible in the new str. corneum. Trichome of Ramón y Cajal. × 310

Fig. 6. Section of skin with a depression indicating a shed MRC (*arrow*). Sloughing has therefore recently occurred. The Malpighian layer shows two cells in different stages of the mitotic cycle (*arrows*). Trichrome of Ramón y Cajal. × 880

Fig. 7. Section of skin of frog injected with methylene blue solution into the cutaneous artery, showing the formation of new MRC during moulting. Two types of cells can be seen to have taken up the stain: the MRC in contact with the external medium and about to be shed, and certain cells of the stratum spinosum contiguous with the MRC. × 520

Fig. 8. Section of skin of frog injected with methylene blue solution, showing new MRC formation during moulting. For explanation, see text. × 1200

contact with the lower layer. Figs. 7 and 8 illustrate these observations. In Fig. 7 a detached MRC can be seen adhering to the separated corneum, while others, destined to be eliminated, remain in the skin but with their apical poles directly in contact with the external medium. At the bases of these cells and contacting them, isolated cells of the *stratum intermedium* have taken up the methylene blue.

Fig. 8 shows this at a higher magnification: between the stained cells of the *stratum intermedium* and other cells of the same layer, desmosomes are clearly visible, but not between stained cell and the MRC. To the right, a further stage of this association can be seen: a shrunken MRC has not concentrated the stain, and between it and the stained cell of the *stratum intermedium* the separation is clear. We consider that this represents the situation immediately preceding elimination of the old MRC. Subsequently, the old MRC retract toward the new str. corneum, thus leaving a passage for the new MRC cells forming at their bases (Fig. 9). These cells then evolve from the polygonal shape characteristic of cells of the *stratum intermedium* (Figs. 7–9) to the elongated shape characteristic of MRC (Fig. 10). At one stage during the movement of these cells toward the surface, they appear very elongated, with a long neck protruding between other epithelial cells (Fig. 11).

III. Moult-Promoting Effect by Isolation of the Skin

A histological study was made of 195 pieces of skin, 86 being fixed immediately after death of the animal and 109 after at least six hours, during which the isolated skins were kept alive in Ringer solution. Table 1 summarises the results. In the control group 34% of the skins were moulting and 66% resting, whereas in the "in vitro" skins these percentages were reversed: 67% moulting and 33% resting. This difference is highly significant statistically. Isolation of the skin must thus trigger

Fig. 9. Section of skin fixed immediately after detachment of slough. An MRC (*arrow*) has contracted leaving a space between its basal pole and the new MRC, which has concentrated the methylene blue injected in vivo. ×1200

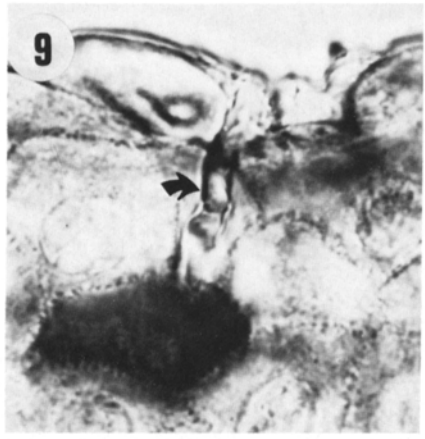
Fig. 10. Section of skin at the stage following shown in Fig. 9. The old MRC has been expelled leaving a depression (*arrow*). The new MRC, which has taken up the methylene blue, has now acquired the typical shape and lies in the axis of the depression. ×1200

Fig. 11. Section of skin showing last stage of MRC formation. The cell body has concentrated the methylene blue, and a very long neck (*arrows*) lies between the cells of the stratum intermedium. To the left a depression indicates an expelled MRC. ×1200

Fig. 12. Section of skin kept in vitro for 21.5 h. Three successively moulted corneal layers can be seen. Trichrome of Ramón y Cajal. ×400

Fig. 13. Section of skin kept in vitro for 21 h showing a slough consisting of several cell layers, i.e. a completely detached str. corneum, one partially detached and three deeper layers. The surface of the new str. corneum shows no sign of artificial separation. Champy-Maillet. Congo Red. ×400

Fig. 14. Section of skin moulting as a result of isolation. Two sloughed layers and in the centre a MRC with apical region in the slough can be seen (*arrows*). Periodic Acid-Schiff. ×1000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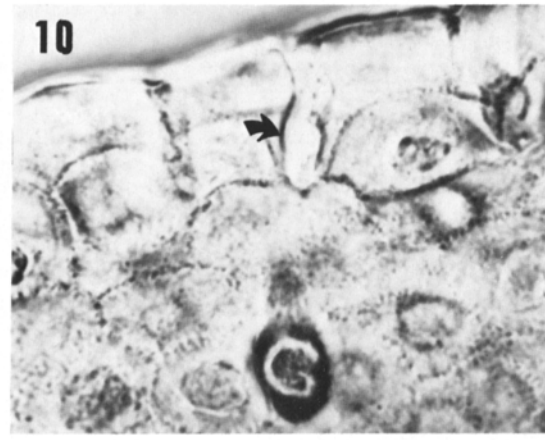
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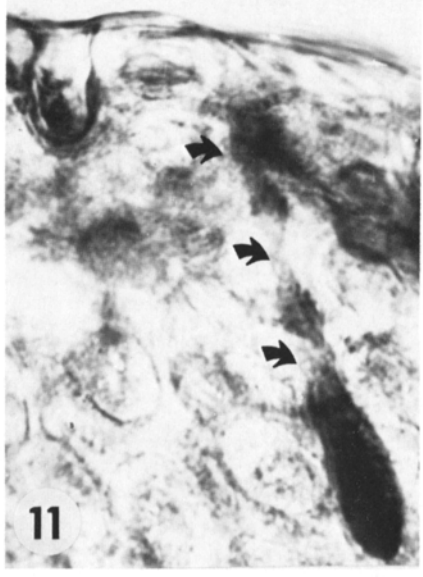
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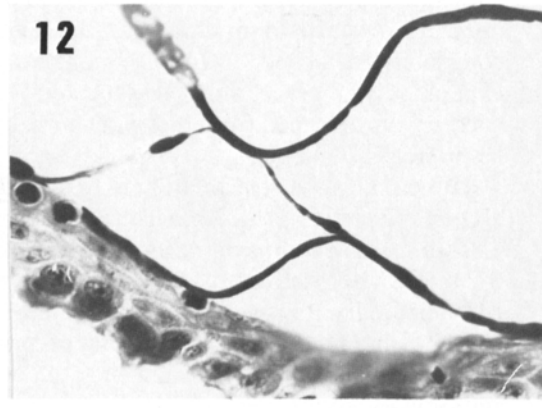
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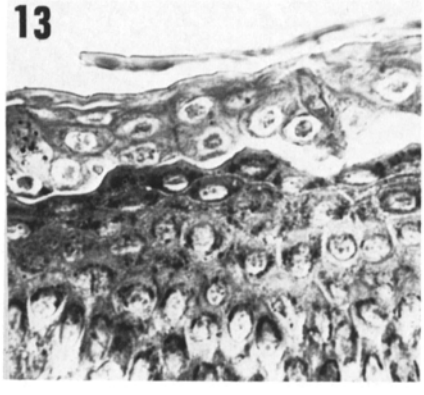
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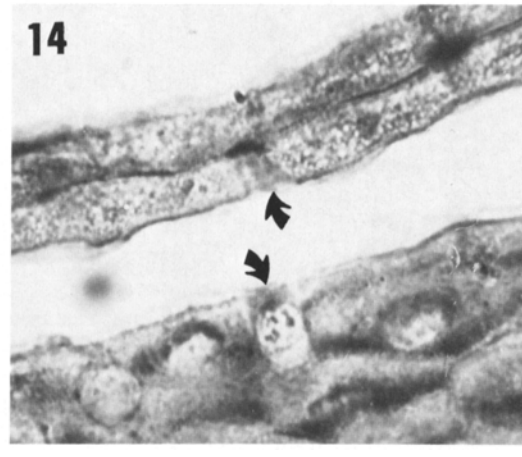
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Table 1. Number of skins in moult or intermoult in a group of skins fixed immediately after removal (controls), and in a group fixed after six hours in vitro in aerated Ringer solution

Group	Moult	Intermoult
Controls ($n=86$)	29 (34%)	57 (66%)
After 6 h in vitro ($n=109$)	73 (67%)	36 (33%)

$\chi^2 = 21.3$, $P < 0.00003$

the moulting process. Treatment of the isolated skins (i.e., mounting in a Ussing chamber, or leaving in a beaker) did not change these results.

The moult induced by isolation of the skin begins by splitting of the str. corneum, as in a natural moult (Fig. 12), but very frequently the new str. corneum immediately starts to moult again, sometimes even before the old str. corneum is completely detached. Multiple moults were especially seen in the longer experiments. Fig. 12 shows a skin kept "in vitro" for 21.5 h: Three corneae, successively detached from the epithelium, can be seen, i.e. a moult occurred every seven hours.

When skins are kept alive for 16 to 21 h, the slough may consist of several (up to six) cell layers, all of which become detached at the moult; nevertheless, the new *stratum corneum* appears normal. This condition is shown in Fig. 13.

Many MRC are destroyed in "in vitro" moults because the apical parts of the cells remain fixed between the cells forming the slough (Fig. 14). Thus, the number of MRC diminishes, as the following experiment shows: Pieces of skin from nine frogs were fixed at the beginning and at the end of a 20 h experiment; in those fixed immediately after removal there were 9.0 ± 0.8 MRC per 200 µm, while in those fixed 20 h later there were only 4.0 ± 0.3 MRC. The difference between these two groups is highly significant (5.0 ± 0.7; $P < 0.001$).

IV. "In vitro" Moulting and Evolution of the Short-Circuit Current

The possibility that the isolation-provoked moult affects transepithelial sodium transport was also studied. Isolated skins were mounted in Ussing chambers and their sodium transport measured by the short-circuit technique of Ussing and Zerahn (1951). At the time of mounting, small pieces of skin were taken for histological control; only results of specimens shown not to be in a moulting state were considered. Six hours later the mounted skins were also fixed for histological examination. In Fig. 15 the changes of short-circuit current of 18 skins which remained in intermoult throughout the whole period (six hours) are compared with those of 10 skins just completing the moulting process during this period, i.e. with cornea completely detached. No critical differences in short-circuit current associated with moulting appear to exists. Skins presenting a partial separation of the slough were not considered, but the evolution of the short-circuit current in these skins was the same as that of skins included in Fig. 15.

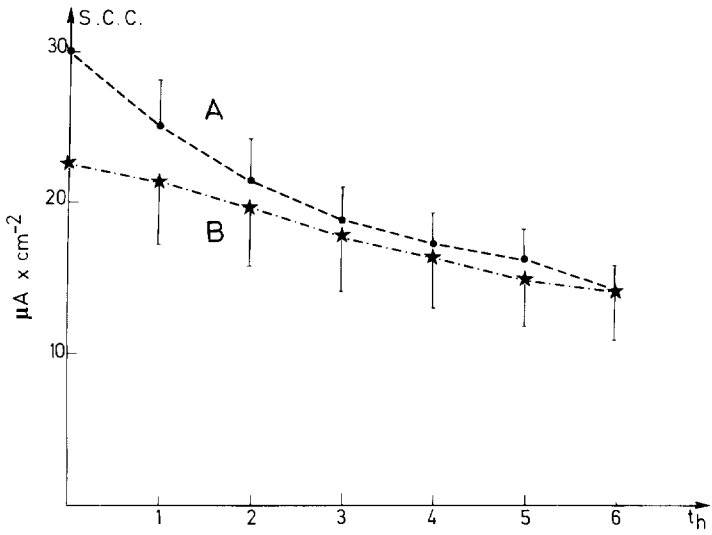

Fig. 15. Comparison between changes of short-circuit current (mean ± SEM) of 18 skins not moulting after 6 h in vitro (*A*) and 10 skins in which the str. corneum became completely detached during the same period (*B*)

V. Moulting and Aldosterone

Aldosterone has been reported to initiate moulting in "in vitro" skins (Hviid Larsen, 1969; Nielsen, 1969). Since many "in vitro" skins moult spontaneously, skins treated with 10^{-6} M aldosterone were compared with control untreated skins, the two being mounted in parallel and fixed after the aldosterone had begun to increase the short-circuit current. Table 2 summarises the results obtained. The number of experimental skins was too small to permit a direct statistical analysis of the significance of the difference in the two groups; however, applying the χ^2 test with Yates' correction (Bancroft, 1957) for the low theoretical frequencies recorded in Table 2 indicated that the number of skin specimens showing a moult in the aldosterone-treated skins was not significantly different from that of control skins.

Table 2. Comparison of number of moults in a group of in vitro-skins treated with 10^{-6}M aldosterone and a control group. The aldosterone was added to the internal chamber and the skins fixed after the hormone produced an increase of the short-circuit current, a control skin being fixed at the same time

Group	Moult			Intermoult
	Completely detached	Partly detached	Total	
Controls ($n=14$)	4	6	10 (71%)	4 (29%)
Aldosterone ($n=14$)	10	3	13 (93%)	1 (7%)

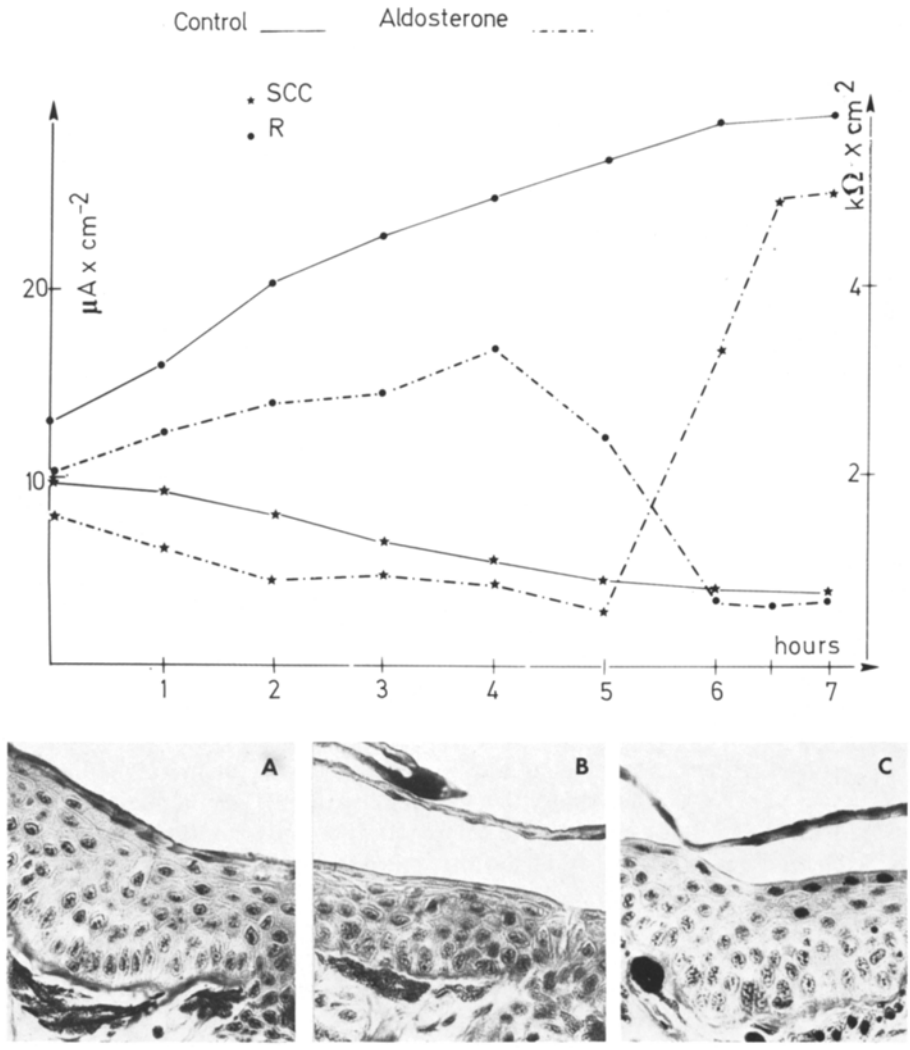

Fig. 16. Evolution of short-circuit current and resistance in two samples of skin from the same animal, one treated with 10^{-6} M aldosterone at time 0 and the other serving as control. A third sample **A** fixed immediately after removal showed that the skins were in a intermoult period. After 7 h in vitro both hormone-treated **B** and control **C** had moulted, their cornea being completely detached. Trichrome of Ramón y Cajal. A, B and C ×220

In any case, the incidence of moulting in the control skins was similar to that of the skins recorded in Table 1. Thus, even if aldosterone causes moulting it could only do so in 30% of the skins in which isolation alone would not have had this effect. Table 2 also shows that the proportion of sloughs completely detached is higher in the aldosterone-treated group than in the controls.

Changes in current and resistance induced by the hormone are independent of moulting. A typical experiment with two pieces of skin from the same frog mounted

in parallel is shown in Fig. 16 (1 and 2). Although the str. corneum had become detached in both, in the control the resistance increased and the current decreased regularly throughout the experiment, whereas in the other skin specimen aldosterone treatment caused a sudden reduction of the resistance after 3.5 hours and a current increase after 4.5 hours.

Discussion

a) Spontaneous Moulting and MRC

The histological study of a large number of skins moulting naturally showed that the moult is not homogeneous over the whole surface of the body, different relationships between slough and MRC being present side by side.

The slough consists not only of corneal cells but also retains many MRC, as already recorded by Pfitzner (1880) and Spanhof (1959), and more rarely and irregularly, cells from the granular layer. The fate of the MRC remaining behind depends on whether they are in contact with the external medium or not. In the former case they degenerate and are finally shed leaving pits which are subsequently leveled. In the latter case, the MRC persist, being recovered by the new corneum, a process recorded by several workers (Dennert, 1924; Alderman, 1933; Whitear, 1975). Sometimes one or the other of the above relationships is so predominant that certain workers have recorded it as the essential situation, whereas in fact all the possible relationships form part of the general phenomenon of sloughing.

The first function attributed to MRC was the purely mechanical anchoring of the str. corneum to the lower epithelium, the separation of these cells or of their necks from the adjacent cells enabling the detachment of the slough (Pfitzner, 1880; Fahrenholz, 1927; Spanhof, 1959). This function can no longer be considered: separation of the corneum starts when the desmosomes between the old str. corneum and the replacement layer are broken (Whitear, 1975; Budtz and Larsen, 1975).

Certain workers have postulated that the MRC secrete a substance at the moult which facilitates the separation of the corneum (Schulze, 1867; Schultz, 1889; Muhse, 1909; Dennert, 1924; Porto, 1936). Our observations do not confirm this role: if the MRC secreted a substance from their apical poles they could not also remain attached by this region to the old *stratum corneum* and shed with the slough as so frequently occurs. In fact, there is no evidence to assign any role in moulting to the MRC. That they show modifications is due to the fact that, along with the other cells of the superficial layers, they are involved in the moulting process. Recent workers attribute functions more in keeping with their structure (Voûte et al., 1972; Voûte et al., 1975; Ehrenfeld et al., 1976). An excellent review of this subject was given by Whitear (1975).

b) MRC Renewal

Whitear (1975) suggested that MRC are originally differentiated from epidermal cells, probably after cell division in the basal layers: MRC have occasionally been

observed among the cells of the germinal layer (Budtz and Larsen, 1975; Whitear, 1975). Whitear (op. cit.) considered that very few MRC were renewed at each moult, an opinion shared by Budtz and Larsen (1975). Our histological study, however, showed many MRC being lost during each moult. In view of this, the exact location of these cells in relation to the corneal cells is of significance. A MRC almost always occurs beneath the junction of two corneal cells (Muhse, 1909, Fig. 1; Alderman, 1933; Whitear, 1975; Ehrenfeld et al., 1976). When the majority of MRC remains in situ and becomes covered by the new str. corneum (Alderman, 1933) their final position is understandable (Whitear, 1975); however, when many MRC are shed with the slough it is difficult to explain why the new MRC, leaving the basal layer, should always be located finally under the junction of two corneal cells. The present study has shown that a new MRC is formed from a cell of the *stratum intermedium* in close contact with an MRC destined to be renewed, the departure of which always occurs between two cells of the renewed corneum. The new MRC follows the old one in its path toward the exterior, occupying finally the site vacated by the eliminated MRC. This process explains the final location of MRC under the junction of corneal cells. Occasional differentiation of MRC from cells of the germinal layer is not excluded.

c) Moulting Induced by Isolation of the Skin

The histological study of pieces of skin fixed some hours after isolation showed a highly significant increase in the proportion of moults, often repetitive, compared with controls. Isolation must, therefore, trigger the moulting process and the question of the endocrine control of moulting and its effects on transepithelial ionic flux are relevant in this connection.

According to Jørgensen and Larsen (1964), the normal moult falls into two main main phases. During phase I the *stratum corneum* separates and is transformed into a slough, while phase II comprises the subsequent processes of adoption of the moulting position, secretion of mucus and shedding and consumption of the slough. In vivo studies in urodeles and anurans have shown that the hypophysis is necessary for the normal processes of Phase II and for moulting periodicity, but not for phase I. Hypophysectomised animals thus accumulate several layers of undetached sloughs (Giusti and Houssay, 1924; Scharrer, 1934; Aubrun, 1935; Adams and Gray, 1936; Osborn, 1936; De Groot et al., 1948; Jørgensen and Larsen, 1961, 1964). It seems unlikely that the hypophysis-independent processes (i.e., phase I) are controlled by aldosterone, since its secretion is largely dependent on ACTH in amphibians (Johnston et al., 1967; Dupont et al., 1976). Furthermore, it would be difficult to explain how in hypophysectomised animals the remaining aldosterone level could be controlled cyclically to produce the periodically-occurring phase I. Even though moulting may be controlled by the endocrine system, the capacity for the cyclic formation of new cornified layers and separation of the old is presumably inherent to the skin (Jørgensen and Larsen, 1960, 1961). This would explain the continued cornification and separation of old cornified layers in the absence of hormones in the isolated skin. Why isolation of the skin should precipitate repeated moults is not clear. Is it due to the liberation of

activating substances in the wounded tissues or does isolation remove an inhibition maintained by humoral factors?

In anurans, shedding (phase II) is controlled by the hypophysial-interrenal axis (Jørgensen and Larsen, 1961, 1964; Stefano and Donoso, 1964). Subsequently, numerous workers have shown that aldosterone added to the isolated skin produces a moult (Hviid Larsen, 1969, 1970, 1971a, 1971b; Nielsen, 1969, 1972, 1973; Nielsen and Tomlinson, 1970; Voûte et al., 1969). For these investigators "moult" meant phases I and II. We have discussed above that cornification and separation of the old corneum are not under hormonal control in vivo, only detachment of the slough is endocrine controlled. In the studies cited above, with the exception of that of Voûte et al. (1969), proof of moulting was obtained by artificially peeling off the corneum, an insensitive method in which separation sometimes remains undetected. Jørgensen and Larsen (1964) have in fact described histologically-detected complete separations of the str. corneum from regions of skin in which it was impossible to peel off the old str. corneum. Studies performed to show the induction of moulting by aldosterone in vitro in reality only show that the steroid facilitates the detachment of the old str. corneum, since cornification and separation could well have remained undetected by the techniques employed. This being so, there is no difference between in vivo and in vitro skins as regards phases under endocrine control. The work described here agress with this conclusion: there was no clear effect of aldosterone on in vitro moulting, but the steroid did seem to facilitate corneal detachment.

A stimulating effect of aldosterone on sodium transport across the isolated frog skin was described by Maetz et al. (1958) and by Crabbé (1964). More recently, this stimulation was studied as a function of time. In isolated skin of *Rana temporaria,* aldosterone produces first an inhibition of the short-circuit current and an increase in the resistance, followed after some hours by an increase of the current and a decrease in resistance (Nielsen, 1969; Voûte et al., 1969; Eigler, 1970; Smith, 1975). Similar effects, except in the length of the inhibition period, have been found in isolated skins of *Bufo bufo* (Hviid Larsen, 1970; Smith, 1975) and *Bufo marinus* (Crabbé et al., 1971; Porter, 1971). The various changes in the short-circuit current caused by aldosterone have been explained in terms of the moult induced by the hormone. The inhibition period, that we very rarely saw, would correspond to slough formation (Nielsen, 1969, 1972, 1973) and the stimulation period to the shedding (Nielsen op. cit; Hviid Larsen, 1970 1971a, b, 1972). Hviid Larsen (1971b) and Nielsen (1973, p. 225) also recorded that spontaneous moults gave rise to the same bioelectrical changes as occurred in aldosterone-produced moults. The present work, comprising both a study of short-circuit current and resistance changes and histological examination of numerous skins not treated with aldosterone, has shown that, at least under our experimental conditions, the different phases of the moult are not accompanied by current or resistance changes. When an aldosterone-treated skin and its untreated control from the same intermoult frog skin show complete detachment of the corneum at the end of an experiment, it is only in the treated skin that critical current and resistance changes occur. The moult in itself therefore does not alter the conductivity or transport capacity of the skin. It is, however, possible that the different phases of the current and resistance changes induced by aldosterone are the result of varying effects of the

hormone at different stages of the moulting process. Thus, when there is not moult, the steroid-induced stimulation starts more rapidly and the current increase is regular (Lang et al., 1975). It is not excluded that under certain experimental conditions moulting may result in a sudden increase in sodium transport, but in any case the fact that under our conditions there was no change in the bioelectric parameters implies that this is not an essential accompaniment to moulting. The problem of the evolution of the tight junctions during moulting is relevant in this connection: To explain the absence of critical changes in resistance it is necessary to postulate the re-establishment of these junctions before the beginning of the moulting process. According to Lindemann and Voûte (1977) *zonulae occludentes* sometimes develop before the moult in the layer below the *stratum granulosum* destined to become the new str. corneum. Katz (1978) has recently shown that in *Bufo viridis* the conductivity of the external barrier for potassium increases during the moult; with our techniques it is not possible to state whether a similar change occurs in the skin of *Rana esculenta* during moulting.

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