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Epithelial regeneration in the bovine mammary gland: the closure of lesions produced by *Escherichia coli*

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[Plates 1–5]

The repair of lesions in the bilayered non-secretory epithelium of the bovine mammary gland was studied in cases of experimental coliform mastitis by means of scanning and transmission electron microscopy. Lesions were produced in the lactiferous and teat sinuses of six quarters of three cows by infusing small numbers (250–700 colony-forming units) of a virulent strain of *Escherichia coli* (B117). Glands were examined at intervals up to 30 h after infection. The earliest sign of lesion closure was observed in two quarters that had been infected for 15 h and 18 h respectively. The mounds of polymorphs that had accumulated over the lesions had dispersed to reveal a fibrin clot. Marginal cells of the basal epithelial layer became very flat, produced broad lamellipodia and started to migrate across the denuded basement membrane. These were followed by a succession of submarginal cells which usually moved as a sheet but were also capable of migrating independently. Some basal cells ingested small particles of extracellular material as they migrated under the fibrin clot.

Marginal cells of the superficial epithelial layer behaved in two, quite different ways. In some areas they were flattened, produced prominent lamellipodia and migrated relative to the basal cells. Their submarginal cells showed no sign of active movement and were probably pulled along by the marginal cells. Most marginal cells, however, were inactive. In these cases, the submarginal cells were very active and produced long basal processes that underlapped the adjacent marginal cells. Cell migration proceeded while bacteria were still present in the vicinity of the lesion. Cells derived from the basal layer eventually formed a continuous monolayer covering the lesion but the re-formation of a complete superficial layer was not followed in this study.

By comparing the progress of the disease in glands infected for different periods of time it was deduced that complete closure of lesions may take less than 5 h. The rapidity of closure and hence the restoration of the blood–milk barrier was attributed in part to the retention of an intact basement membrane during lesion formation. No increase in the incidence of mitotic figures was detected in the epithelia during the period of recovery studied.

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Introduction

Bovine mastitis produced by *Escherichia coli* is an acute disease of dairy cows that adversely affects milk quality and yield, causes the animal to become ill and in extreme cases may lead to death. It is characterized by a systemic toxaemia, by an intense local inflammatory response and by widespread lesions of the epithelium lining the lactiferous and teat sinuses (Frost *et al.* 1980, 1981). Recent studies have shown that at least two toxins are involved in the pathogenesis of the disease (Brooker *et al.* 1981) and that focal lesions are formed when both cell layers of the sinus epithelia are damaged and shed (Frost *et al.* 1980, 1981). The exposed basement membrane is usually left undamaged, but it is soon covered by polymorphs which migrate to the lumen of the gland in large numbers from neighbouring blood vessels. In cases where this polymorph response is rapid, the bacteria are soon eliminated and normal function of the gland is quickly restored (Hill *et al.* 1978). However, complete recovery of the udder and the return of normal milk composition can only occur when the areas denuded of epithelium have been repaired and the 'blood–milk barrier' (Linzell & Peaker 1971) has been re-established.

Although nothing is known of cellular repair in the mammary gland, several other mammalian organs, the skin in particular, have been used to study epithelial regeneration and wound closure *in situ* (McMinn & Pritchard 1972). These studies have shown that the repair of small lesions (< 100 μm diameter) takes place without any evidence of increased cellular proliferation either in the immediate vicinity of the damaged area or remote from it (Matoltsy & Sinesi, 1957; Bullough & Lawrence 1960). Even with wounds of macroscopic size, the absence of increased mitosis in the earliest stages of healing has come to be regarded as one of the characteristic features of regenerating epithelium. Instead, the integrity of the epithelium is rapidly restored by the migration of cells from the margins of the lesion or wound (Winter 1964; Krawczyk 1971; Kuwabara *et al.* 1976).

The cellular events involved in the repair of non-secretory mammary epithelium were investigated by means of scanning and transmission electron microscopy by following the closure of lesions produced by *E. coli*. In previous investigations of wound healing, the usual practice has been to damage the epithelium mechanically or by the use of corrosive agents. However, our intention was not only to study epithelial repair *per se* but also to record some of the events that lead to recovery of the gland from the effects of the toxins produced by *E. coli* during a very common disease of dairy cattle.

Materials and methods

Animals

Normal, healthy Friesian cattle in different stages of lactation were used: M1031, 172 days *post partum*; M1039, 267 days *post partum*; K477, 105 days *post partum*. The milk from all mammary quarters used was monitored for 7 days before experimental infection; the milk from the glands used was consistently free of pathogenic bacteria and had a somatic cell count below 150000 ml^{-1}.

Preparation of bacteria

The organism used was a serum-resistant *Escherichia coli*, strain B117 serotype 08 K85 K99, that was known to produce mastitis in lactating cows (Hill *et al.* 1978). The bacteria were maintained on nutrient agar (Oxoid) supplemented with 5 % (by volume) bovine erythrocytes. The bacteria for inoculation were grown as described previously (Frost *et al.* 1980) and the number of viable bacteria inoculated was determined by a surface plate count (Miles *et al.* 1938). The same counting method was used to determine the number of bacteria in milk samples taken from the gland before slaughter.

Somatic cell counting of milk

The milk was fixed with Somafix (Coulter Electronics) and the cells were electronically counted by means of a Coulter counter (Industrial D model, Coulter Electronics).

Experimental design

In a previous study it was shown that many epithelial lesions appear in the sinuses about 14 h after infection with small numbers (50–200 colony-forming units, c.f.u.) of *E. coli* (Frost *et al.* 1981). Thus, to study the closure of such lesions, the quarters of three cows were infected with small numbers of *E. coli* and, when at least 14 h had elapsed, samples were taken from the sinuses at intervals for microscopy.

Particulars of the quarters inoculated at various time intervals before autopsy are shown in table 1. The bacteria were suspended in 1 ml of pyrogen-free saline and were infused through the teat canal by means of a syringe fitted with an 18-G cannula. At the appropriate time, milk samples were taken from infected quarters just before slaughter, to determine the number of somatic cells and viable bacteria. The udder was quickly removed, suspended on a metal frame and the infected quarters infused with 3 % (by volume) glutaraldehyde (Taab Labs) in 0.1 M cacodylate buffer (pH 7.2) by means of a 60 ml syringe fitted with an 18-G cannula. Infusion was continued until the lactiferous and teat sinuses were slightly distended; the volume used was between 400 and 500 ml. After 1 h, the quarters were dissected and tissue removed for light and electron microscopy.

Scanning electron microscopy (s.e.m.)

Pieces of tissue approximately 10 mm^2 were carefully removed from larger pieces of excised lactiferous and teat sinus epithelium. Specimens were prepared by the methods described previously (Frost *et al.* 1980) and examined in an I.S.I. Super IIIA scanning electron microscope.

Transmission electron microscopy (t.e.m.)

Lesions that had been examined by s.e.m. were located by means of a binocular microscope and selected for further study. They were then excised, soaked for 2 h in propylene oxide and embedded in Araldite. Thin sections were cut by means of a Reichert Om U2 ultramicrotome and stained with lead citrate and uranyl

TABLE 1. DETAILS OF THE QUARTERS EXPERIMENTALLY INFECTED WITH *ESCHERICHIA COLI* AND THE RESULTS OF EXAMINATION OF THE MILK, EXPRESSED BEFORE INOCULATION AND AT AUTOPSY

cow	quarter	inoculum (c.f.u.)	duration of infection before autopsy / h	lesions: +, present, −, absent	10^{-3} t.c.c. before inoculation	10^{-3} t.c.c. at autopsy	c.f.u. per millilitre of milk at autopsy
M1031	r.h.	700	18	+	49	1000	10^6
	l.f.	700	30	−	73	3500	0
M1039	r.h.	450	15	+	48	1000	$> 10^6$
	l.f.	450	20	−	37	9000	1.4×10^4
K477	r.f.	250	20	−	133	200	5.7×10^6
	l.h.	250	24	+	141	1300	2.0×10^3

Abbreviations: c.f.u., number of colony-forming units; l., left; r., right; h., hind; f., fore; t.c.c., total somatic cell count per millilitre of milk.

acetate before examination in a Hitachi HU-11E electron microscope. This approach not only obviated the laborious task of searching for lesions by sectioning but also allowed the the results obtained by t.e.m. and s.e.m. to be compared for any given area of epithelium.

Light microscopy (l.m.)

Tissue samples were taken from the lactiferous and teat sinuses and fixed in 10 % (by volume) neutral buffered formalin. They were embedded in paraffin wax, sectioned (5 μm) and stained with haematoxylin and eosin.

RESULTS

Normal sinus epithelium

The lactiferous and teat sinuses of the bovine mammary gland are lined by an epithelium consisting of two layers (figure 1, plate 1). In the superficial (luminal) layer, cells are joined to their neighbours by a full junctional complex (Farquhar & Palade 1965) and by interdigitations along their lateral margins. They are generally cuboidal in shape but may also appear columnar or squamous depending on the degree of distension of the sinus when fixed for microscopy. They are attached to the underlying cells by desmosomes and produce narrow processes which extend to the basement membrane. When examined by s.e.m. their surface is studded with microvilli and their cell outline is polygonal (figures 2, 3, plate 1).

The cells of the basal layer cover most of the basement membrane and are joined to it by hemidesmosomes. Although they have sometimes been referred to as myoepithelial cells (Linzell & Peaker 1971), their contractile function has never been demonstrated. The term 'myoepithelial' has probably been applied to these cells because their cytoplasm contains some filament bundles and because they correspond in position to cells found in the walls of ducts and alveoli for which there is very good experimental evidence of contraction (Richardson 1949; Linzell 1955).

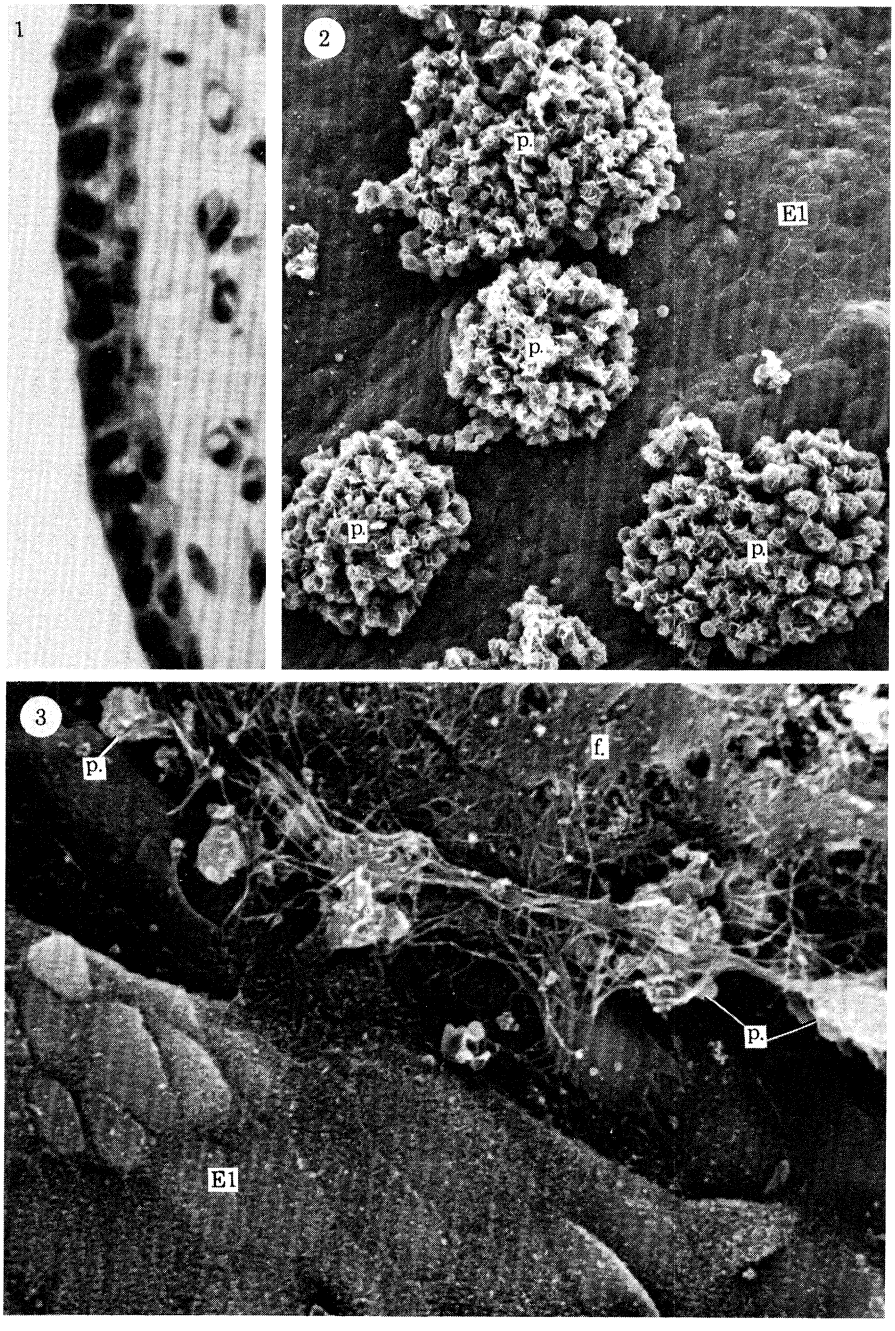
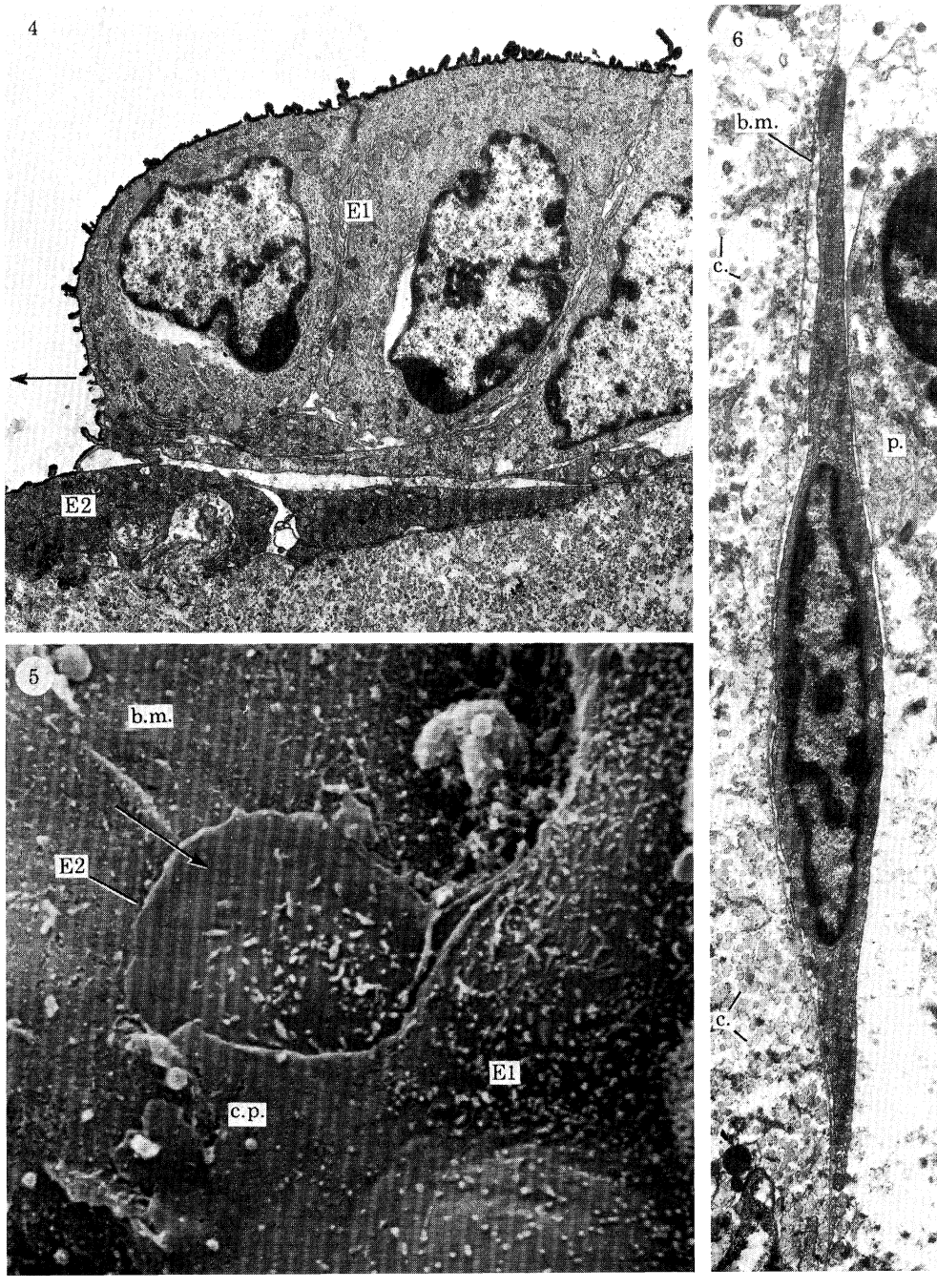


FIGURE 1. Section of normal lactiferous sinus showing the bilayered arrangement of cells. (Haematoxylin and eosin. Magn. ×960.)

FIGURE 2. Lactiferous sinus studded with mounds of polymorphs (p.) which overlie epithelial lesions. This is the appearance of the epithelium before lesion closure begins. E1, normal epithelium. (S.e.m. Magn. ×650.)

FIGURE 3. The edge of a lesion in the lactiferous sinus of cow M1031 18 h after infection with *E. coli*. Only a few polymorphs (p.) remain enmeshed in a fibrin clot (f.). The patchwork appearance of normal epithelium (E1) is visible at the bottom of the micrograph. (S.e.m. Magn. ×1600.)

(*Facing p.* 84)



FIGURES 4–6. For description see opposite.

Epithelial lesions

The events leading to the formation of epithelial lesions by *E. coli* have been described in detail by Frost *et al.* (1980, 1981). Lesions were formed when areas of the sinus (up to 0.3 mm diameter) were denuded of both epithelial cell layers. At the margins of these lesions, the superficial cell layer was often detached from the basal cells and milk proteins were able to penetrate into the intercellular spaces. The epithelium was oedematous with large fluid-filled spaces between the two cell layers. Polymorphs migrated from the margins of the lesions and through the exposed basement membrane and accumulated to form characteristic mounds which totally obscured the underlying epithelial damage (figure 2, plate 1). Lesions with this appearance occurred about 14 h after infection, but showed no evidence of epithelial repair when examined by transmission electron microscopy.

Lesion closure: epithelial cell migration

The lactiferous and teat sinuses from quarters that had been infected for 15–18 h (table 1) were found to contain not only small lesions and isolated damaged cells identical to those seen in earlier stages of the disease (Frost *et al.* 1980, 1981) but also areas of damaged epithelium in which repair had already started. In these lesions, the mounds of polymorphs were no longer present and the denuded basement membrane was covered to a variable extent by a network of fibrin in which cellular debris and some polymorphs were enmeshed (figure 3, plate 1). Most of the polymorphs had probably passed directly into the milk-filled lumen of the sinus since there was no sign of their lateral migration across the epithelium when samples were examined by s.e.m. Although the epithelium still appeared oedematous in places, the superficial marginal cells were no longer separated from the cells beneath. They had reorientated themselves so that their lateral surface, which had been exposed, was now in contact with the cell membrane of the cells beneath, i.e. the epithelium had 'resealed' itself at the margins of the lesion (figure 4, plate 2).

In the description that follows, some cells in both epithelial layers are described as submarginal. This term refers to cells that are situated further away from the margin of the lesions than are the marginal cells.

The first sign that cellular repair had begun was the production of broad lamellipodia with smooth, convex edges by marginal cells of the basal epithelial

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DESCRIPTION OF PLATE 2

FIGURE 4. The edge of a lesion in the lactiferous sinus after 15 h, showing the reorientation of the marginal cells of the superficial layer (E1). A basal cell (E2) has started to migrate into the lesion (arrow). In this and some succeeding t.e.m. micrographs, the black layer of material covering the cells is the thin deposit of gold that was applied for s.e.m. (T.e.m. Magn. ×8000.)

FIGURE 5. The edge of a lesion from the same gland as in figure 4, showing the lamellipodium (arrowed) produced by a basal cell (E2) at the beginning of cell migration over the basement membrane (b.m.). A cell in the superficial layer (E1) appears flattened and is producing a cell process (c.p.) which partially overlaps that from the basal cell. (S.e.m. Magn. ×4700.)

FIGURE 6. A flattened basal cell moving independently over the basement membrane (b.m.) of a lesion in the teat sinus of cow M1031 (18 h). Abbreviations: c., collagen fibres of connective tissue; p., polymorph. (T.e.m. Magn. ×13800.)

layer (figure 5, plate 2). This heralded the start of a cell migration across the basement membrane that would eventually restore the integrity of the epithelium. The basal cells appeared very flattened as they emerged from the margin of the lesion and moved across the basement membrane. Although they often moved as a continuous sheet with each cell maintaining contact with its neighbour on all sides, gaps between cells were common and single cells often moved in isolation across the basement membrane (figure 6, plate 2). Submarginal basal cells were also very flat (figure 4, plate 2; figures 7, 8, plate 3), but this appearance became progressively less obvious with distance travelled away from the lesion. It appeared that, as the basal cells migrated, the cells behind them flattened to occupy the space vacated and that this process spread outwards from the lesion as migration continued.

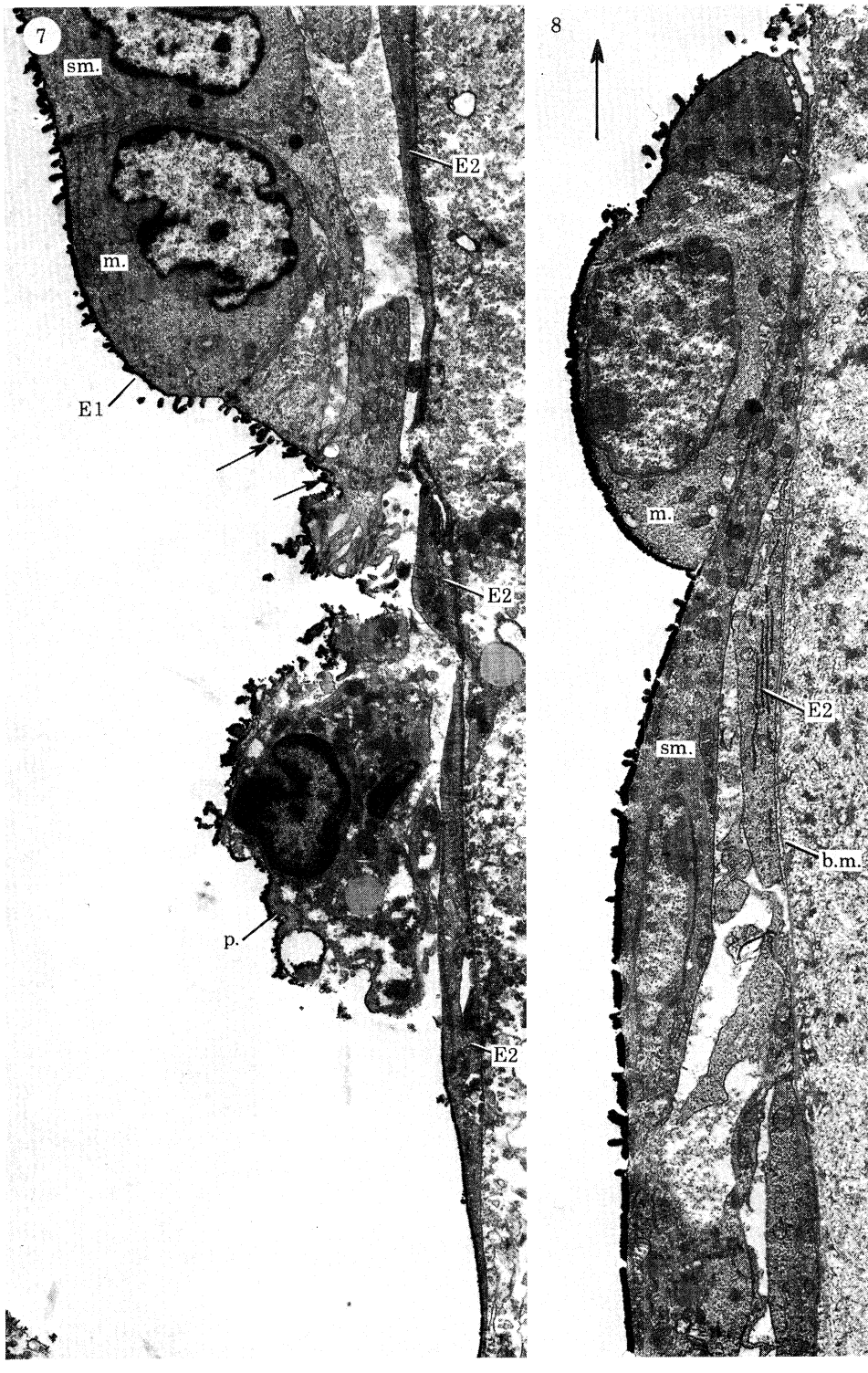
Throughout lesion closure, this migration of basal cells was always in advance of the superficial cells (figure 7, plate 3). Cells passed underneath large objects such as polymorphs (figure 7, plate 3) and the fibrin clot but small pieces of debris were often ingested as the cells moved over them (figure 9, plate 4). Thus, serial sections examined by t.e.m. showed that the cytoplasm of some migrating cells contained closed vacuoles filled with material that was identical in appearance to debris seen on the basement membrane and there were many profiles of cell membrane invaginations that were interpreted as phagocytosis in progress.

The behaviour of marginal cells in the superficial layer of the epithelium was varied. In many cases, the cells became very flattened and produced lamellipodia that were able to displace the fibrin network as they moved forward (figure 10, plate 4). Indirect evidence of movement by these cells was obtained from sections such as that in figure 9, plate 4. These showed that cells of the superficial epithelial layer were loosely attached to basal cells that must have already migrated from the margin of the lesion because some of them had ingested, and were still ingesting, extracellular material on the basement membrane. More frequently, however, the superficial layer of marginal cells remained inactive and retained their normal appearance with no sign of lamellipod formation (figures 7, 8, plate 3). In these cases, a number of the submarginal cells nearby produced basal processes that underlapped the marginal cell and reached the edge of the lesion. Here, the cell processes formed a junctional complex with the marginal cell or with adjacent basal processes and developed microvilli on their exposed surface (figure 7, plate 3). The net result was that many submarginal cells had two quite separate areas of their cell membrane forming part of the exposed surface of the epithelium. In some places, because of a gap in the basal cell layer, there was no basal cell at the

Description of plate 3

Figure 7. Lactiferous sinus, 18 h. Edge of a lesion showing the migration of flattened basal cells (E2) in advance of the superficial layer (E1). In layer E1, the marginal cell (m.) appears inactive but it is underlapped by processes from submarginal cells (sm.) which bear microvilli at the edge of the lesion (arrows). Other abbreviation: p., polymorph. (T.e.m. Magn. ×8000.)

Figure 8. Lactiferous sinus, 18 h. A cell process from a submarginal cell (sm.) of the superficial layer underlaps an inactive marginal cell (m.) and reaches the basement membrane. Arrow indicates the direction of the lesion. (T.e.m. Magn. ×8000.)



FIGURES 7 AND 8. For description see opposite.

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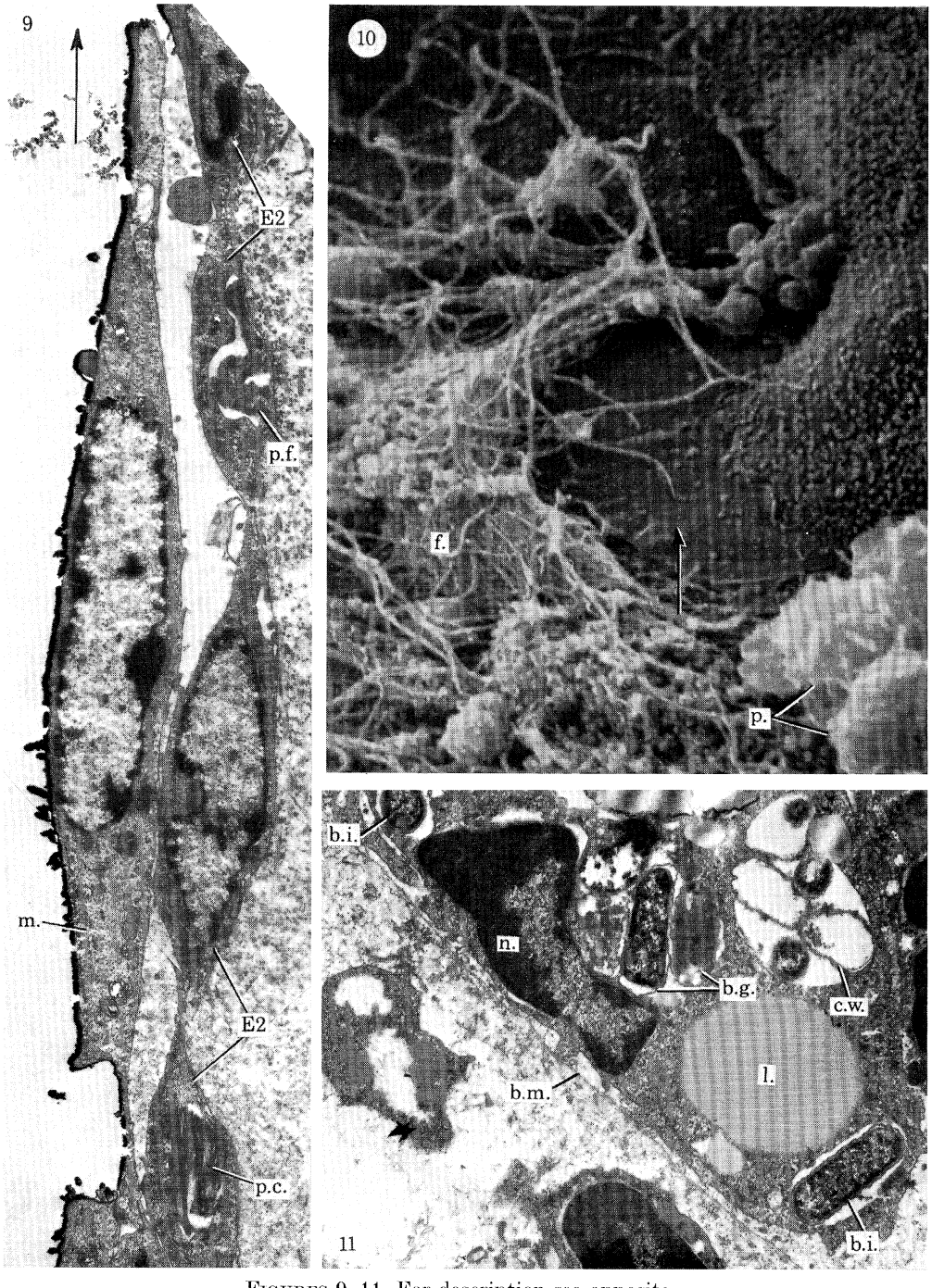


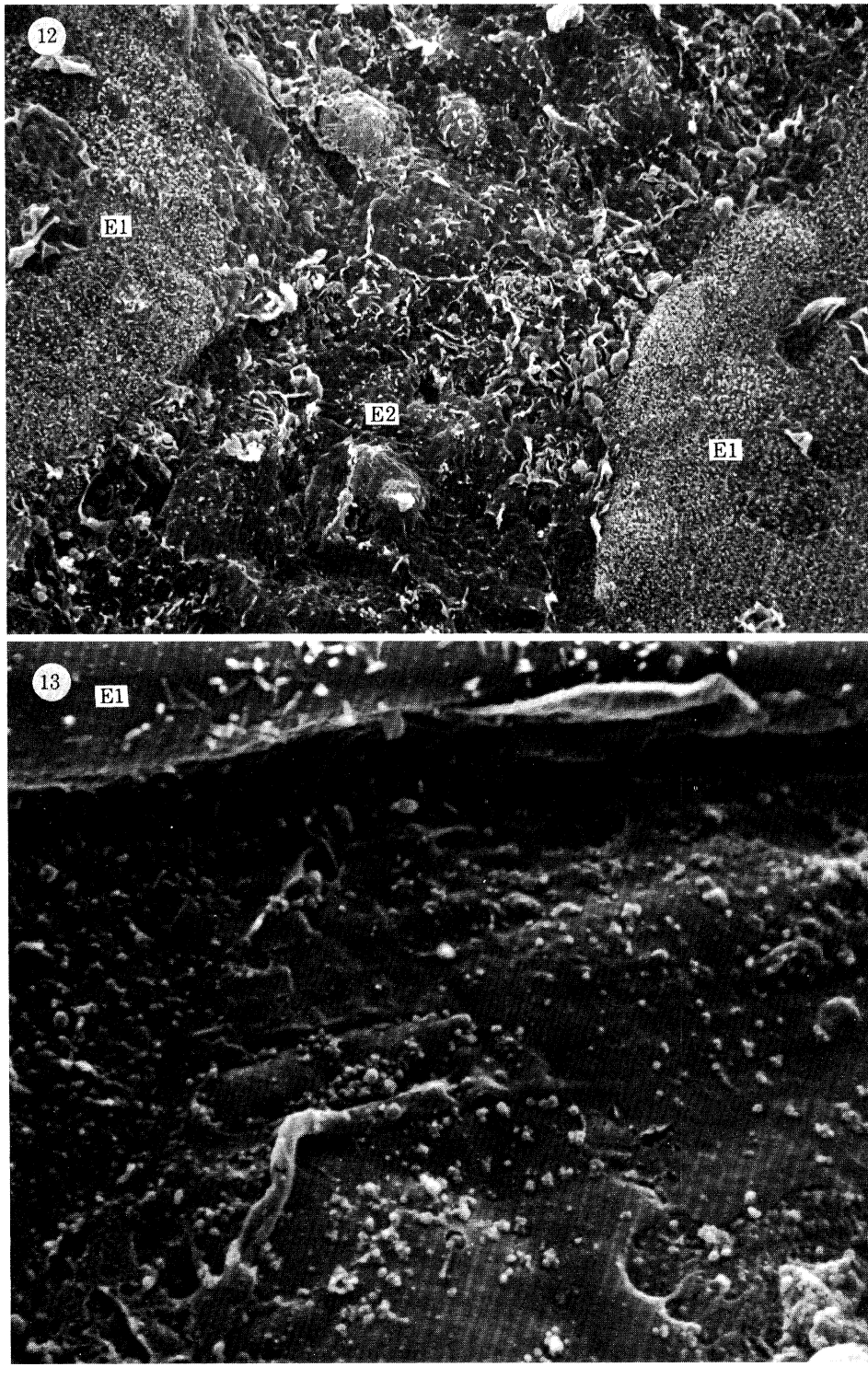
FIGURES 9–11. For description see opposite.

Description of plate 4

Figure 9. Teat sinus, 15 h. In the continuous layer of basal cells (E2) there is evidence of the ingestion of extracellular material in completed (p.c.) and forming (p.f.) phagocytic vacuoles. The leading edge of a migrating marginal cell (m.) in the superficial layer is separated from the cells beneath. Arrow indicates direction of movement. (T.e.m. Magn. ×8000.)

Figure 10. Teat sinus, 15 h. A marginal cell of the superficial layer is producing a broad lamellipodium (arrowed) that is displacing the fibrin clot (f.); p., polymorph. (T.e.m. Magn. ×4100.)

Figure 11. Lactiferous sinus, 18 h. Section through a polymorph on the denuded basement membrane of a lesion in which closure has already started. Phagocytic vacuoles contain intact (b.i.) and partially digested (b.g.) bacteria. In some cases, only the bacterial cell walls (c.w.) remain. Other abbreviations: n., nucleus; b.m., basement membrane; l., fat droplet. (T.e.m. Magn. ×13000.)



FIGURES 12 AND 13. For description see opposite.

lesion margin. The processes from submarginal cells then became very extended, reached the basement membrane and produced lamellipodia (figure 8, plate 3). It was apparent, therefore, that the lateral contacts of submarginal cells with their neighbours did not prevent locomotory activity at their basal surface. However, there was no evidence to suggest that submarginal cells were able to separate from their neighbours in order to continue their migration under a marginal cell.

It is important to note that all of the events described above took place while considerable numbers of *E. coli* were still present in the lumen of the gland. This was apparent not only from the plate counts of milk expressed at slaughter (table 1) but also from profiles of polymorphs on the exposed basement membrane that showed intact and partially digested *E. coli* in phagocytic vacuoles (figure 11, plate 4).

The left hind quarter of cow K477 (table 1) contained lesions in which closure was at a more advanced stage. In these, cells derived from the basal epithelial layer formed a monolayer covering the entire surface of what had been exposed basement membrane (figures 12, 13, plate 5). This could be clearly seen by s.e.m. because, although some polymorphs were still present, very little cell debris and fibrin remained over the lesion to obscure observation. The basal cells were still very flat so that the position of the nucleus was often marked by a bulge on the cell surface. There was little overlapping of cells at their margins (figure 13, plate 5).

In these lesions, the continuity of the epithelium had been re-established but wide gaps persisted in the superficial layer of cells. Later stages in lesion closure which might have shown the reformation of a double-layered epithelium could not be studied satisfactorily because of the difficulty of locating and identifying lesions that had reached an advanced stage of repair. This was a consequence of producing lesions by a pathogen, which did not allow the precise position and distribution of the damaged areas to be predicted.

Sinus epithelia that had been collected up to 30 h after infection were examined by light microscopy but showed no increase in the incidence of mitotic figures either at the margins of lesions or in adjacent areas of normal epithelium.

Rate of lesion closure

It has been seen from the results presented above that the right hind quarter of cow M1031 contained lesions in the earliest stages of closure 18 h after infection. The left fore quarter of the same animal had been infected with *E. coli* for 12 h longer but there was no sign of lesions. If it is assumed that the course of the disease and lesion closure proceeded at the same rate in both quarters, it appeared that complete lesion closure could take less than 12 h. The results obtained from cow

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Description of plate 5

Figure 12. Cow K477 lactiferous sinus, 24 h. This lesion, running from the top to bottom of the micrograph, has been closed by a monolayer of basal cells (E2). Except for some marginal cells, the superficial layer of the epithelium (E1) appears normal. (S.e.m. Magn. ×2400.)

Figure 13. The same lesion as that in figure 12, showing that the basal cells are flattened, possess few microvilli and do not appreciably overlap their neighbours. A marginal cell (E1) of the superficial layer is also visible. (S.e.m. Magn. ×7300.)

M1039 suggested that lesion closure could be even more rapid. The left fore quarter contained no lesions even though bacteria were still present in the gland. The duration of the infection in the right hind quarter was only 5 h less, yet there were many lesions in which cell migration was just starting.

Discussion

Pattern of cell movement

Although cell migration is responsible for wound closure in a variety of epithelia, several different patterns of cell movement have been recognized. It has been suggested, for example, that, during the closure of mucosal epithelium and epidermis, cells slide over one another and attach to the substrate in succession in a manner that is reminiscent of a 'caterpillar track' (Winter 1964; Krawczyk 1971; Sciubba 1977). Alternatively, entire sheets of cells can move either actively 'like a herd of animals' (Weiss 1961; Odland & Ross 1968; Martinez 1972) or passively as a result of exertions by the marginal (Vaughan & Trinkaus 1966; DiPasquale 1975; Pfister 1975) or basal (Radice 1980) cells only.

In mammary epithelium, the presence of gaps and of isolated cells in the advancing basal layer suggests that the movement here is active and that the mechanism of tissue closure is similar to that envisaged by Weiss (1961). However, the superficial cells behave in a more complex manner. In some areas the marginal cells produce lamellipodia and migrate actively relative to the basal layer. Their neighbouring submarginal cells show no morphological evidence of an independent active movement and are probably pulled along by virtue of their firm attachment to the marginal cells. The behaviour of cells in the superficial epithelial layer is made complicated by the fact that most marginal cells remain inactive. This inactivity cannot be satisfactorily explained by a phenomenon akin to the contact inhibition observed in fibroblasts (Abercrombie & Ambrose 1958; Trinkaus *et al.* 1971) and epithelial cells (Middleton 1972; DiPasquale 1975) *in vitro* because the adjacent submarginal cells make obvious attempts to move forward by producing elongate basal processes and, when the basement membrane is exposed, lamellipodia. One possibility is that marginal cell immobilization is a minimal effect of the bacterial toxin(s) that produces cell damage and shedding during lesion formation. The contribution of submarginal cell activity in these areas to the movement of the superficial layer is difficult to assess but there is evidence from avian epithelia *in vitro* that submarginal cells may participate actively in the spreading of the cell sheet by producing underlapping basal processes similar to those found in the present study (DiPasquale 1975).

The combination of active and passive cell movements during lesion closure demonstrates that more than one pattern of cell movement can operate within a bilayered epithelium. It is similar to the situation found by Radice (1980) in amphibian epidermis, where basal cells migrate actively in a loosely connected sheet while the superficial layer moves passively *en masse*.

Cell migration

Although there is no evidence to suggest that basal cells migrating under the fibrin clot are capable of the fibrinolysis proposed for epidermal cells (Clark & Clark 1953), some extracellular material is ingested. Phagocytosis of fibrin, cellular debris (Odland & Ross 1968); Krawczyk 1971) and electron-dense tracers (Platt 1963; Gibbins 1968) during migration appears to be a common feature of cells that, in the intact epithelium, are not normally active in this respect. Krawczyk (1971) has suggested that, by consuming energy needed for migration, phagocytosis may slow down cell movement. This may be one rate-limiting factor in the repair of mammary epithelium but the presence of an intact basement membrane over which cells can immediately start their migration is probably of overriding importance. This is suggested by the rapidity of lesion closure in other tissues such as the cornea and epidermis when the basement membrane is left undamaged (Pang *et al.* 1978). The same point has been made by Krawczyk (1971), who showed that healing was much more rapid in intact epidermal blisters than in opened blisters where the basement membrane degenerates. It can be argued, therefore, that the ability of the lesion-forming toxin(s) produced by *E. coli* to cause cell damage without seriously affecting the basement membrane is important in explaining the rapidity of lesion closure and hence the recovery of the gland.

The experimental design of the present study does not permit an accurate determination of the time taken for lesion closure. Only by comparing the progress of the disease in glands infected for different periods of time is it possible to deduce that complete closure may take less than 5 h. Even if one accepts that this is an estimate, the rate of closure in the mammary gland compares favourably with that observed in other mammalian epithelia, where a long delay may precede cell migration. Rabbit corneal epithelium, for example, begins closing 1 h after injury (Friedenwald & Buschke 1944; Kuwabara *et al.* 1976) but palatal epithelium (Anderson & Fejerskov 1974) and epidermis (Odland & Ross 1968; Croft & Tarin 1970; Krawczyk 1971) may show a 12–24 h delay.

Mitosis

As in other epithelia, the primary event during the closure of lesions in the lactiferous and teat sinuses is cell migration. In other organs this is followed, after some delay, by increased mitotic activity (Bullough & Lawrence 1960; McMinn & Pritchard 1972), but no such increase was found during the limited recovery period considered in the present study. Further study of this aspect of epithelial repair in the mammary gland is needed, but since the lesions produced by *E. coli* are usually small (< 100 μm diameter) it is possible that the normal structure of the epithelium can be restored by reorganization of the existing cells.

Epithelium–E. coli interaction

Since no sign of cell migration was noted in lesions that were still covered by mounds of polymorphs, the dispersal of these phagocytic cells into the milk phase may be considered to mark the beginning of epithelial repair. The coexistence of healing lesions and of new cell damage in the same infected gland is consistent with

the idea mooted by Frost *et al.* (1980, 1981) that there are likely to be changes in the local concentration of the diffusible, lesion-forming toxin(s) as the disease progresses. Lesion closure can probably take place in the presence of this toxin because cell migration occurs while bacteria are still present in the vicinity. This is suggested by the occurrence of polymorphs containing newly ingested bacteria on the denuded basement membrane of the closing lesions. It would appear that either the toxin is present in a concentration below a critical threshold level needed to cause cell damage or the migrating cells are not susceptible to it.

The cellular reaction of cow K477 to *E. coli* was noticeably slower than that seen in the other two animals. Twenty hours after infection the number of bacteria in milk withdrawn from the gland was very high but lesions were not detected and the somatic cell count was relatively low. Although it is known that factors such as the stage of lactation affect the rate at which polymorphs are mobilized in the mammary gland when challenged with *E. coli* (Hill 1981), no previous study has adduced evidence to suggest that there is a corresponding variation in the susceptibility of the epithelium to *E. coli* toxins.

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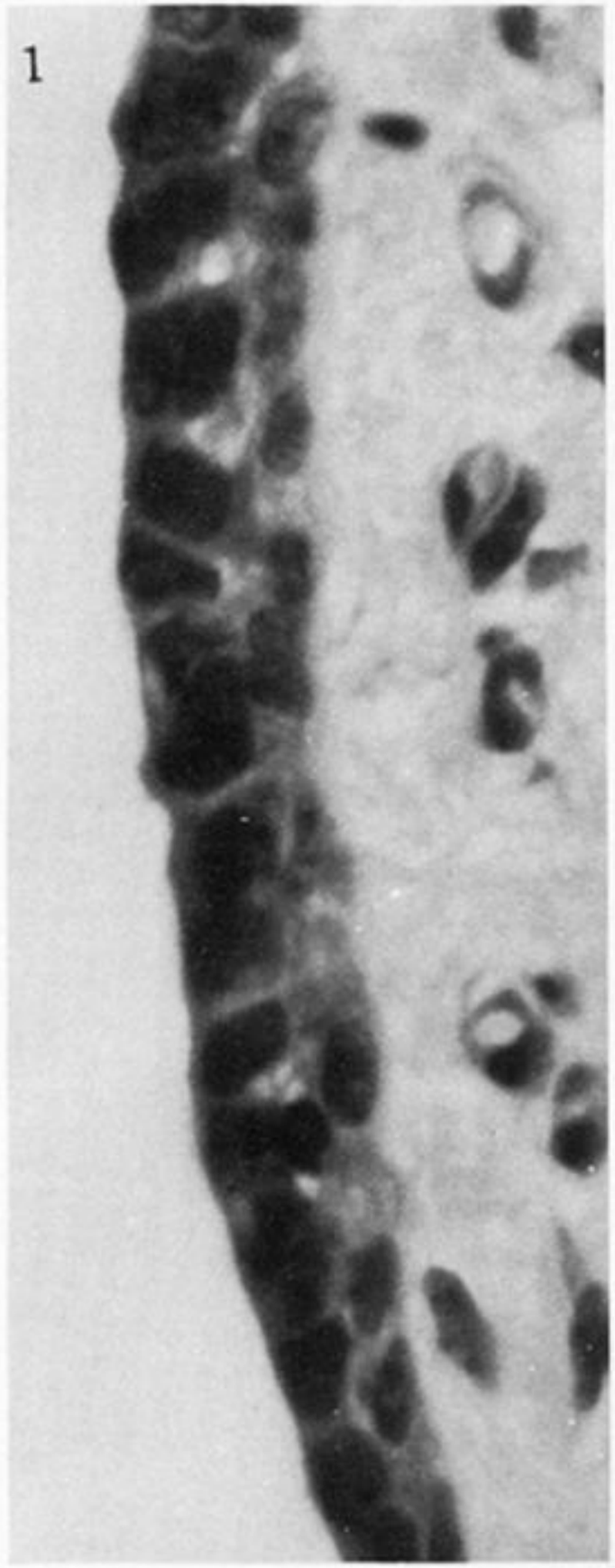

FIGURE 1. Section of normal lactiferous sinus showing the bilayered arrangement of cells. (Haematoxylin and eosin. Magn. ×960.)

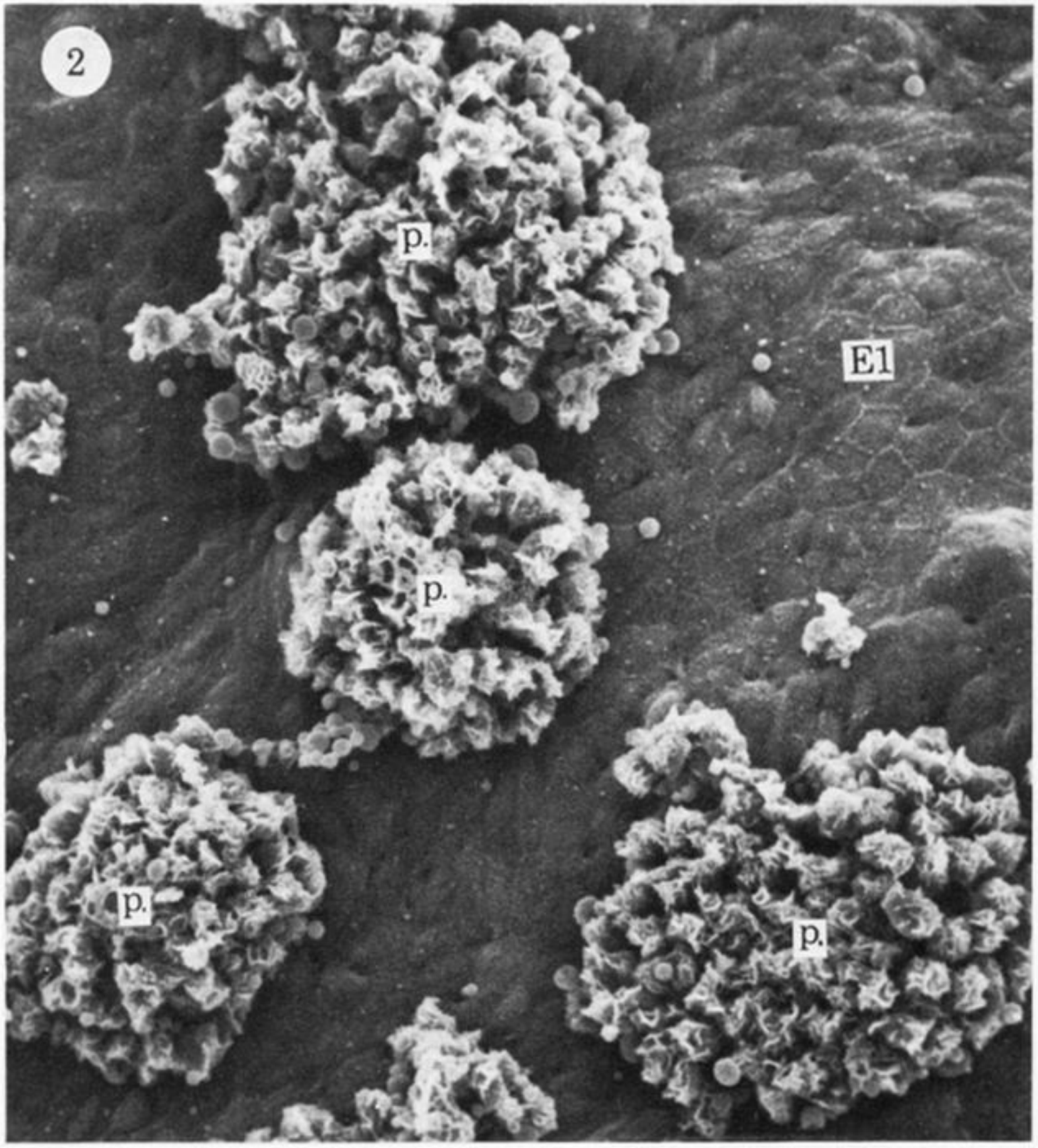

FIGURE 2. Lactiferous sinus studded with mounds of polymorphs (p.) which overlie epithelial lesions. This is the appearance of the epithelium before lesion closure begins. E1, normal epithelium. (S.e.m. Magn. ×650.)

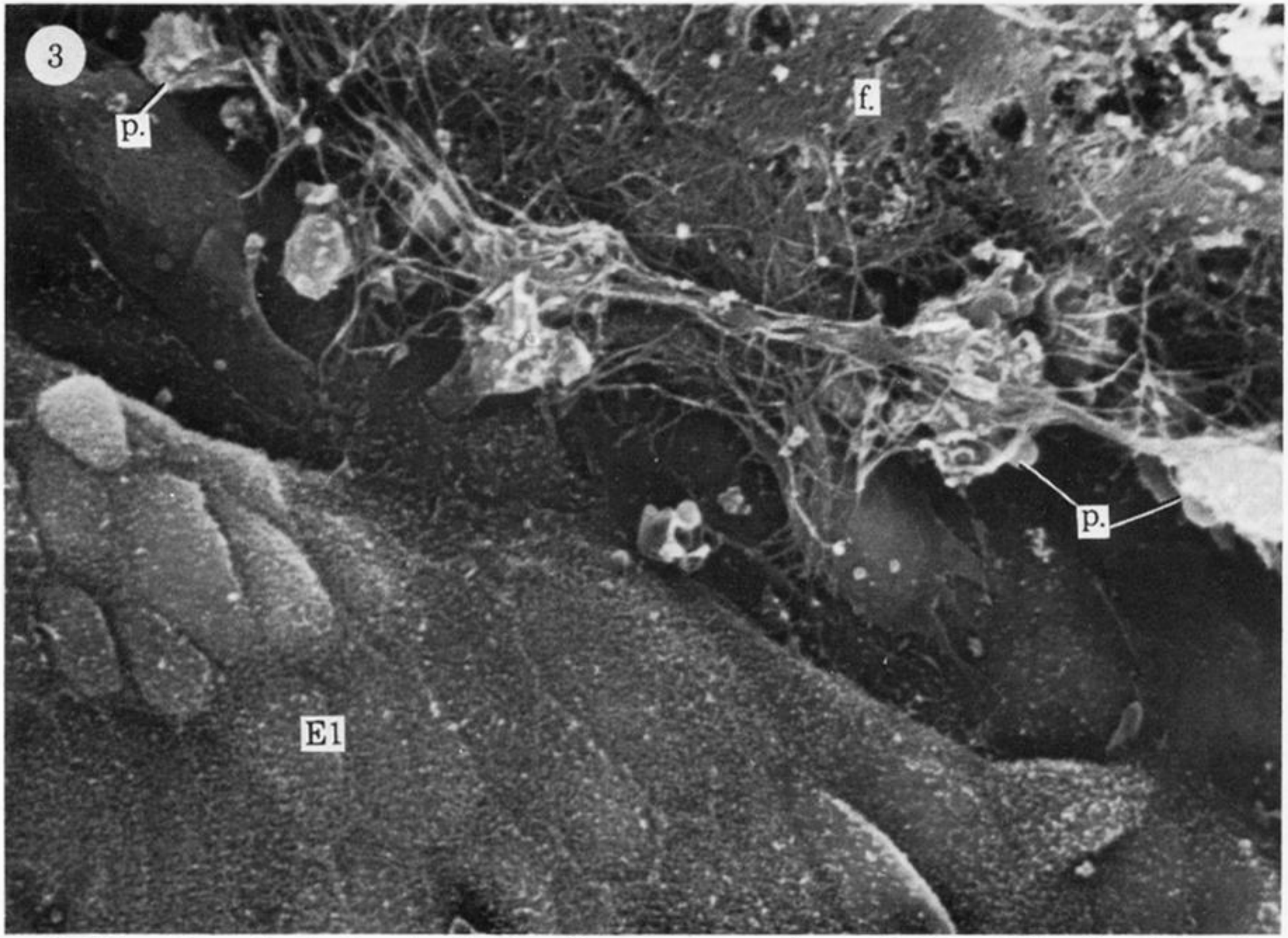

FIGURE 3. The edge of a lesion in the lactiferous sinus of cow M1031 18 h after infection with *E. coli*. Only a few polymorphs (p.) remain enmeshed in a fibrin clot (f.). The patchwork appearance of normal epithelium (E1) is visible at the bottom of the micrograph. (S.e.m. Magn. ×1600.)

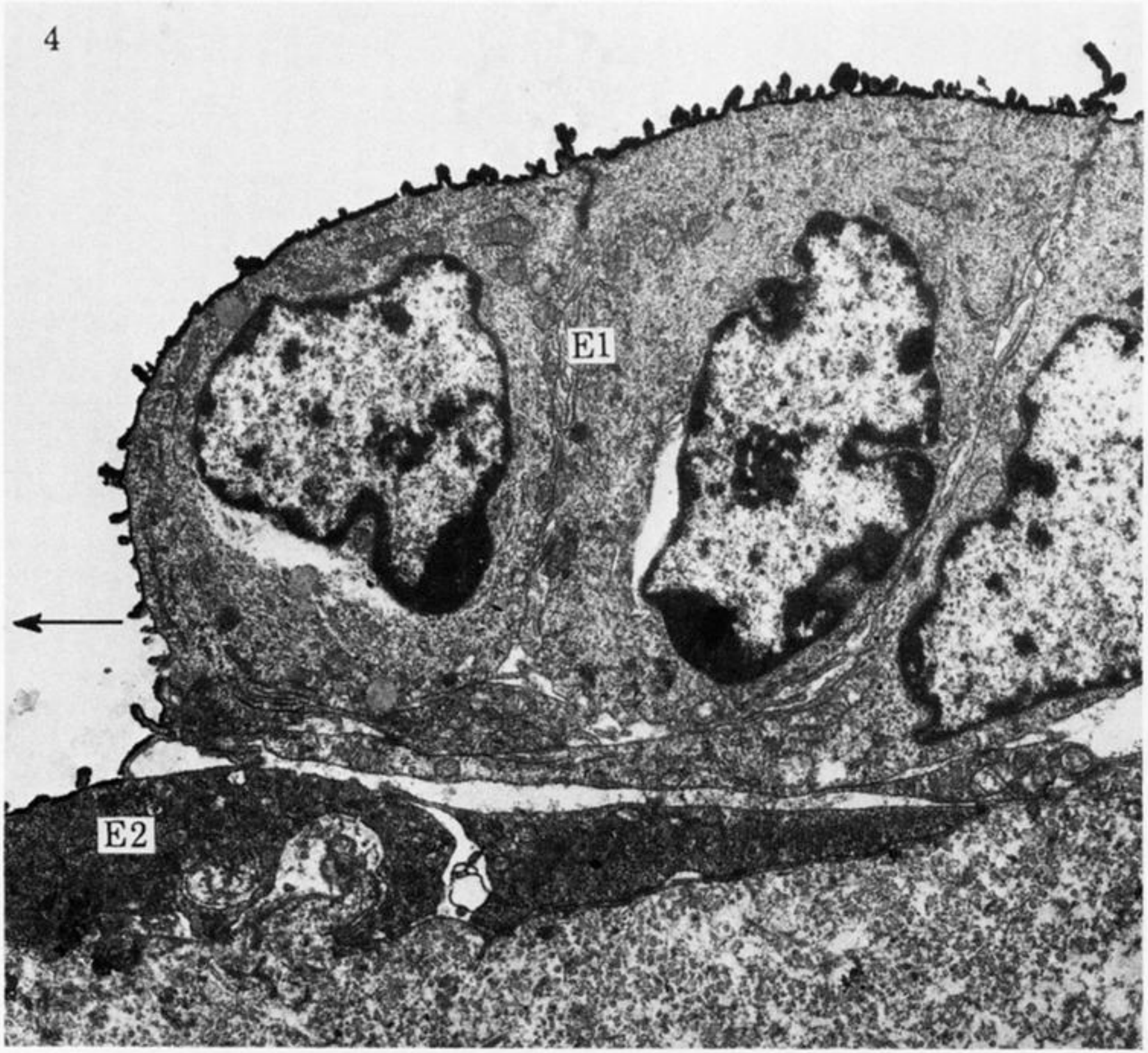

FIGURE 4. The edge of a lesion in the lactiferous sinus after 15 h, showing the reorientation of the marginal cells of the superficial layer (E1). A basal cell (E2) has started to migrate into the lesion (arrow). In this and some succeeding t.e.m. micrographs, the black layer of material covering the cells is the thin deposit of gold that was applied for s.e.m. (T.e.m. Magn. ×8000.)

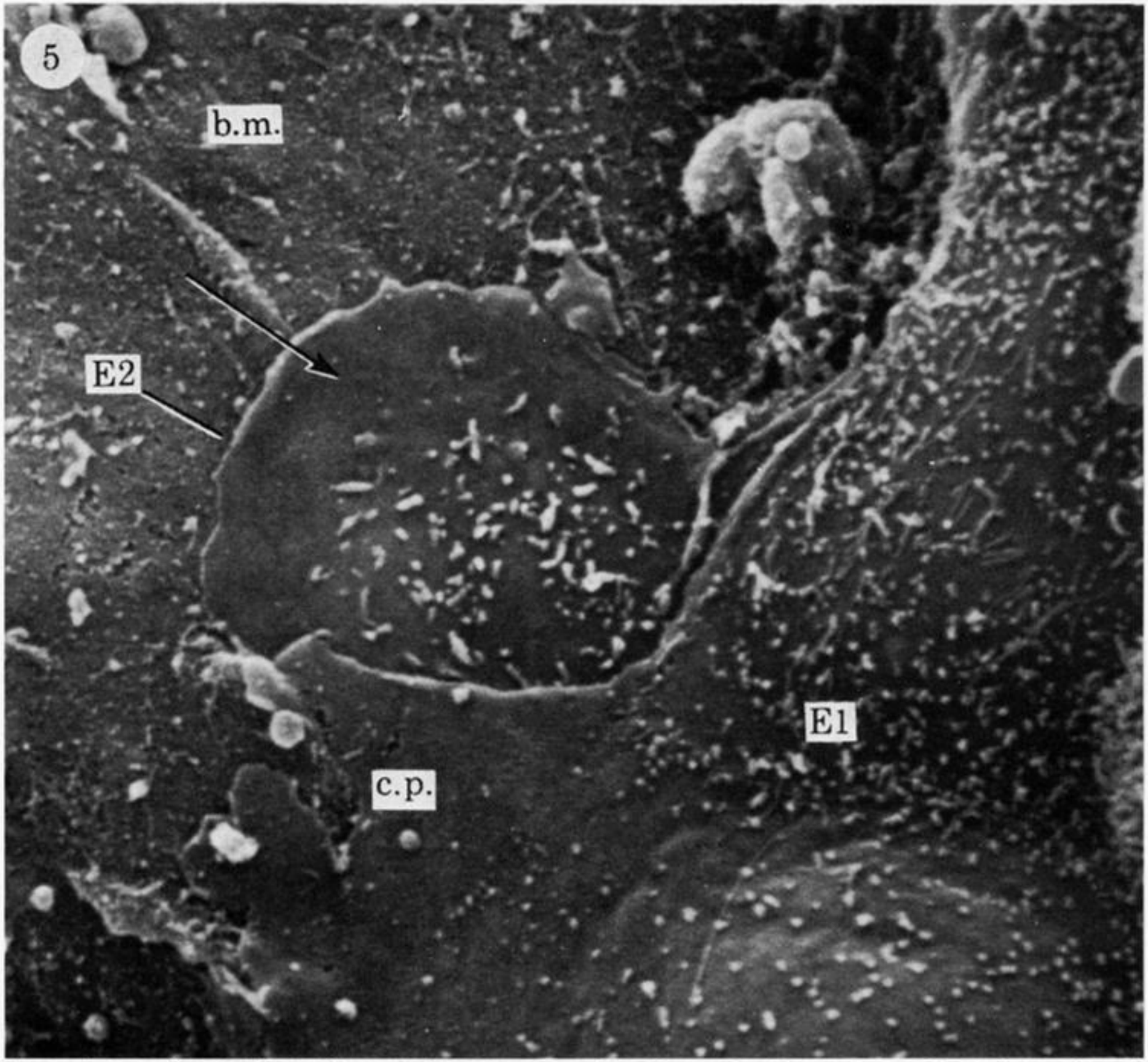

FIGURE 5. The edge of a lesion from the same gland as in figure 4, showing the lamellipodium (arrowed) produced by a basal cell (E2) at the beginning of cell migration over the basement membrane (b.m.). A cell in the superficial layer (E1) appears flattened and is producing a cell process (c.p.) which partially overlaps that from the basal cell. (S.e.m. Magn. ×4700.)

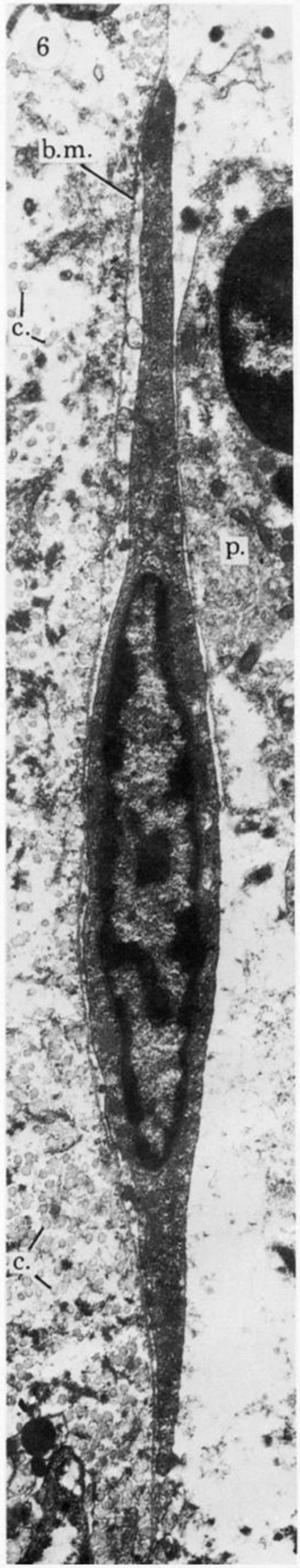

FIGURE 6. A flattened basal cell moving independently over the basement membrane (b.m.) of a lesion in the teat sinus of cow M1031 (18 h). Abbreviations: c., collagen fibres of connective tissue; p., polymorph. (T.e.m. Magn. ×13800.)

FIGURE 7. Lactiferous sinus, 18 h. Edge of a lesion showing the migration of flattened basal cells (E2) in advance of the superficial layer (E1). In layer E1, the marginal cell (m.) appears inactive but it is underlapped by processes from submarginal cells (sm.) which bear microvilli at the edge of the lesion (arrows). Other abbreviation: p., polymorph. (T.e.m. Magn. ×8000.)

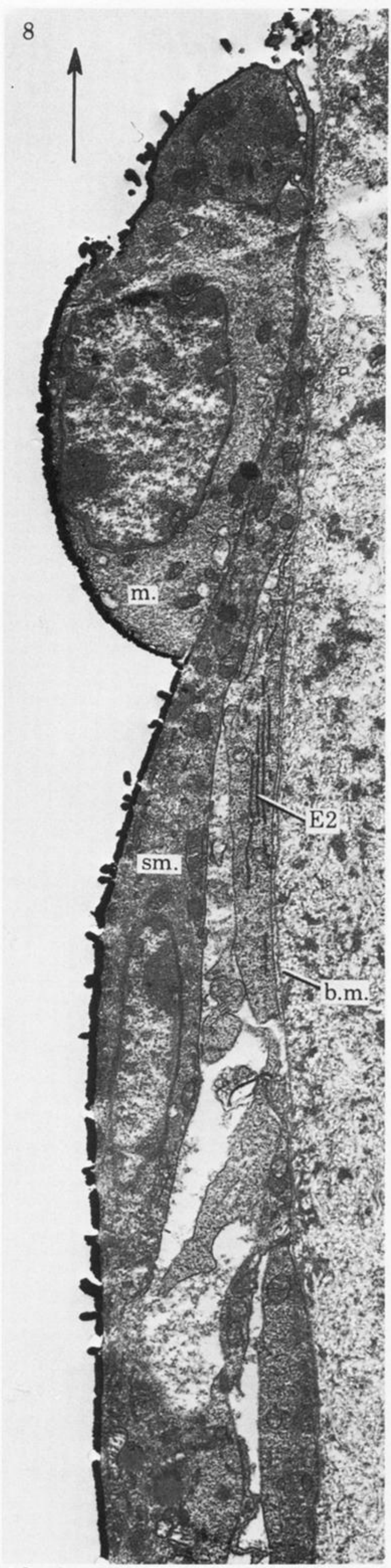

FIGURE 8. Lactiferous sinus, 18 h. A cell process from a submarginal cell (sm.) of the superficial layer underlaps an inactive marginal cell (m.) and reaches the basement membrane. Arrow indicates the direction of the lesion. (T.e.m. Magn. ×8000.)

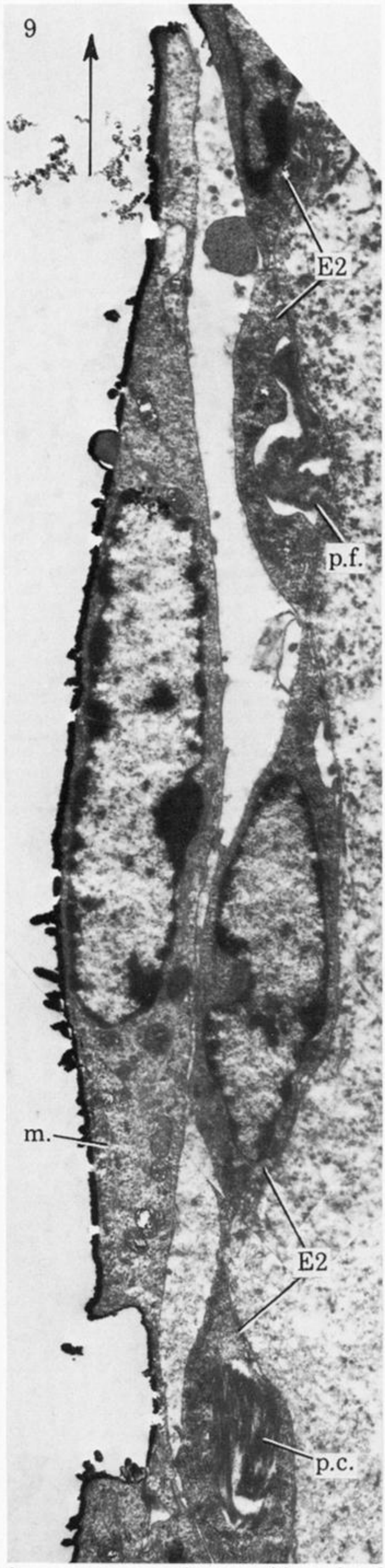

FIGURE 9. Teat sinus, 15 h. In the continuous layer of basal cells (E2) there is evidence of the ingestion of extracellular material in completed (p.c.) and forming (p.f.) phagocytic vacuoles. The leading edge of a migrating marginal cell (m.) in the superficial layer is separated from the cells beneath. Arrow indicates direction of movement. (T.e.m. Magn. ×8000.)

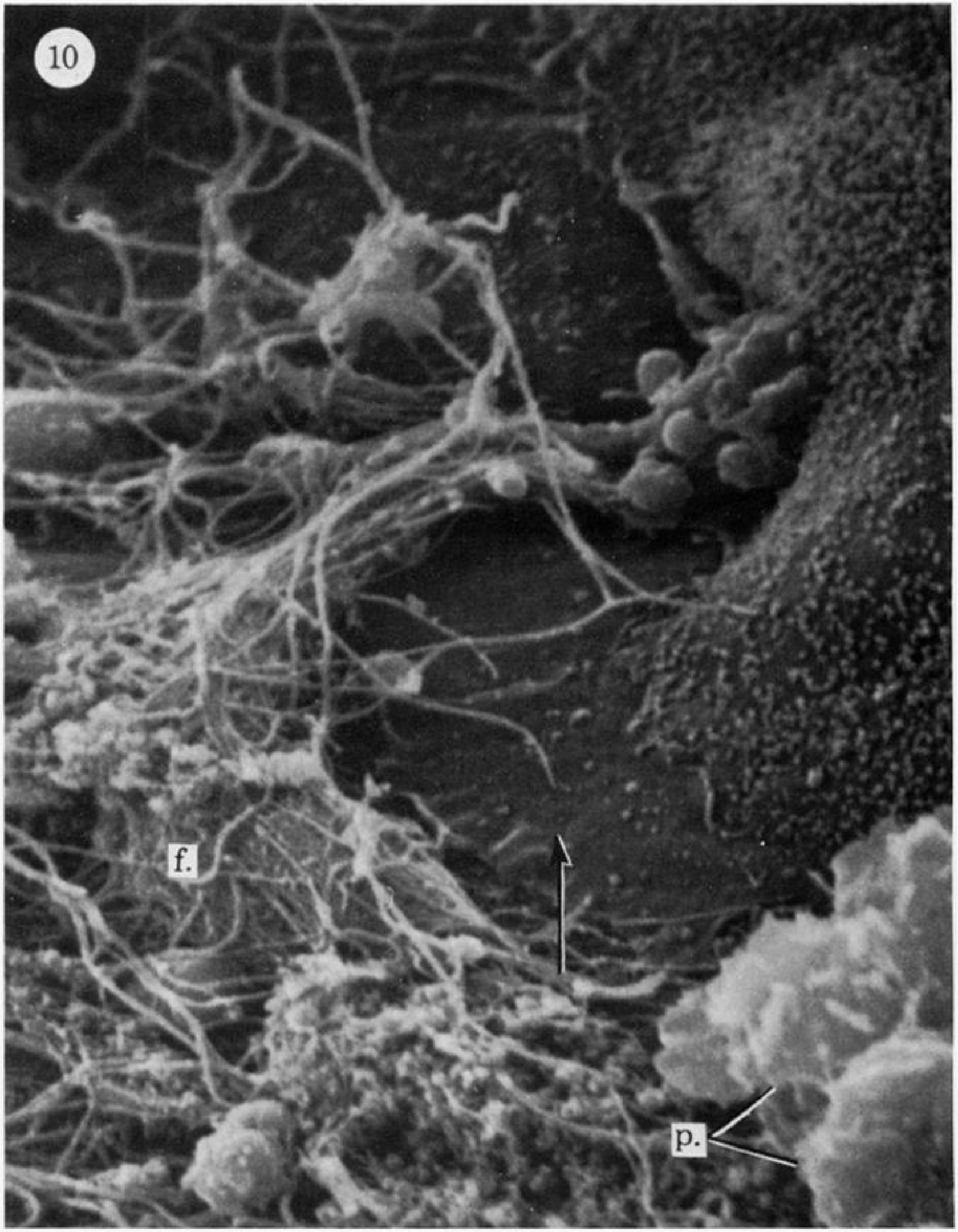

FIGURE 10. Teat sinus, 15 h. A marginal cell of the superficial layer is producing a broad lamellipodium (arrowed) that is displacing the fibrin clot (f.); p., polymorph. (T.e.m. Magn. ×4100.)

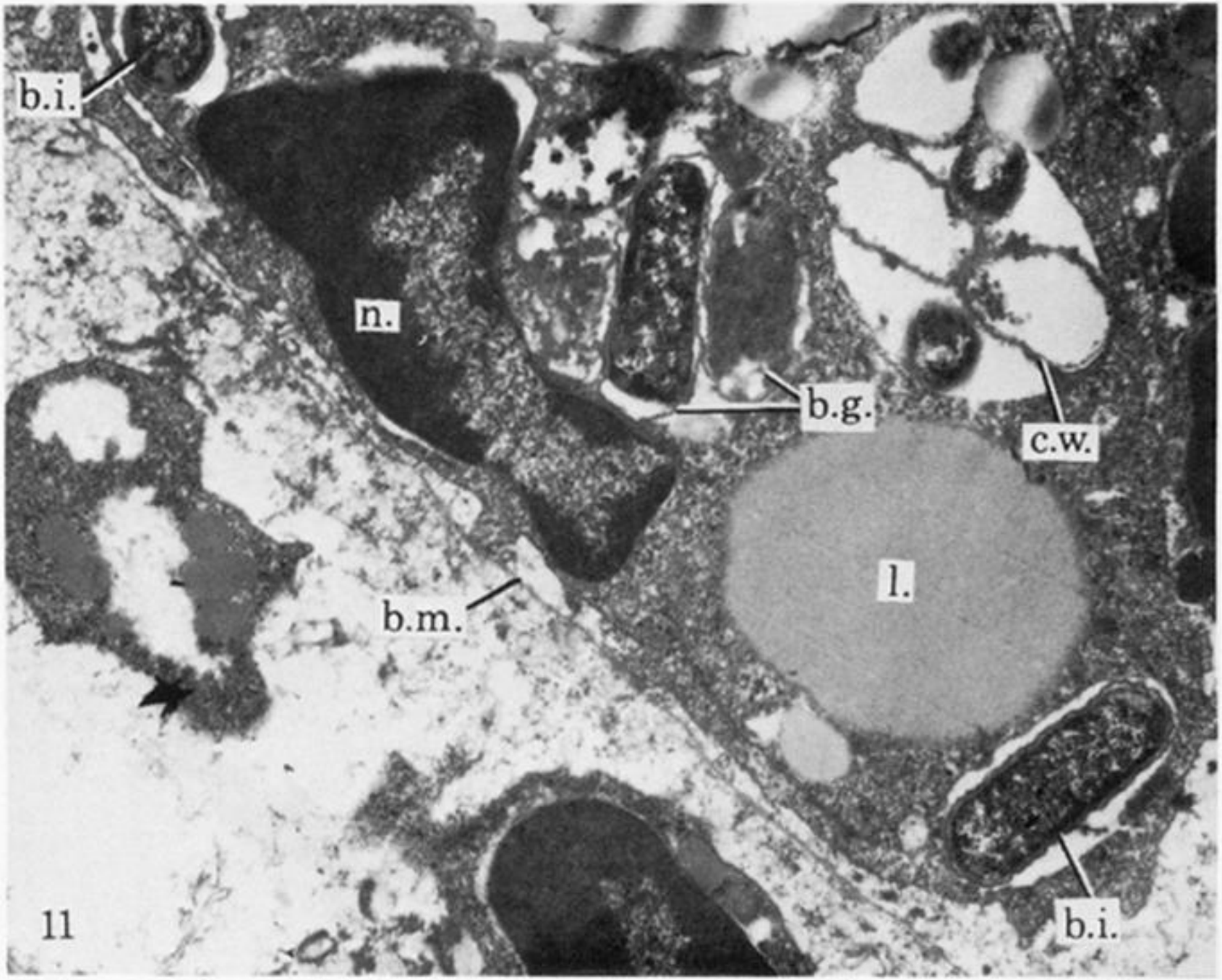

FIGURE 11. Lactiferous sinus, 18 h. Section through a polymorph on the denuded basement membrane of a lesion in which closure has already started. Phagocytic vacuoles contain intact (b.i.) and partially digested (b.g.) bacteria. In some cases, only the bacterial cell walls (c.w.) remain. Other abbreviations: n., nucleus; b.m., basement membrane; l., fat droplet. (T.e.m. Magn. ×13000.)

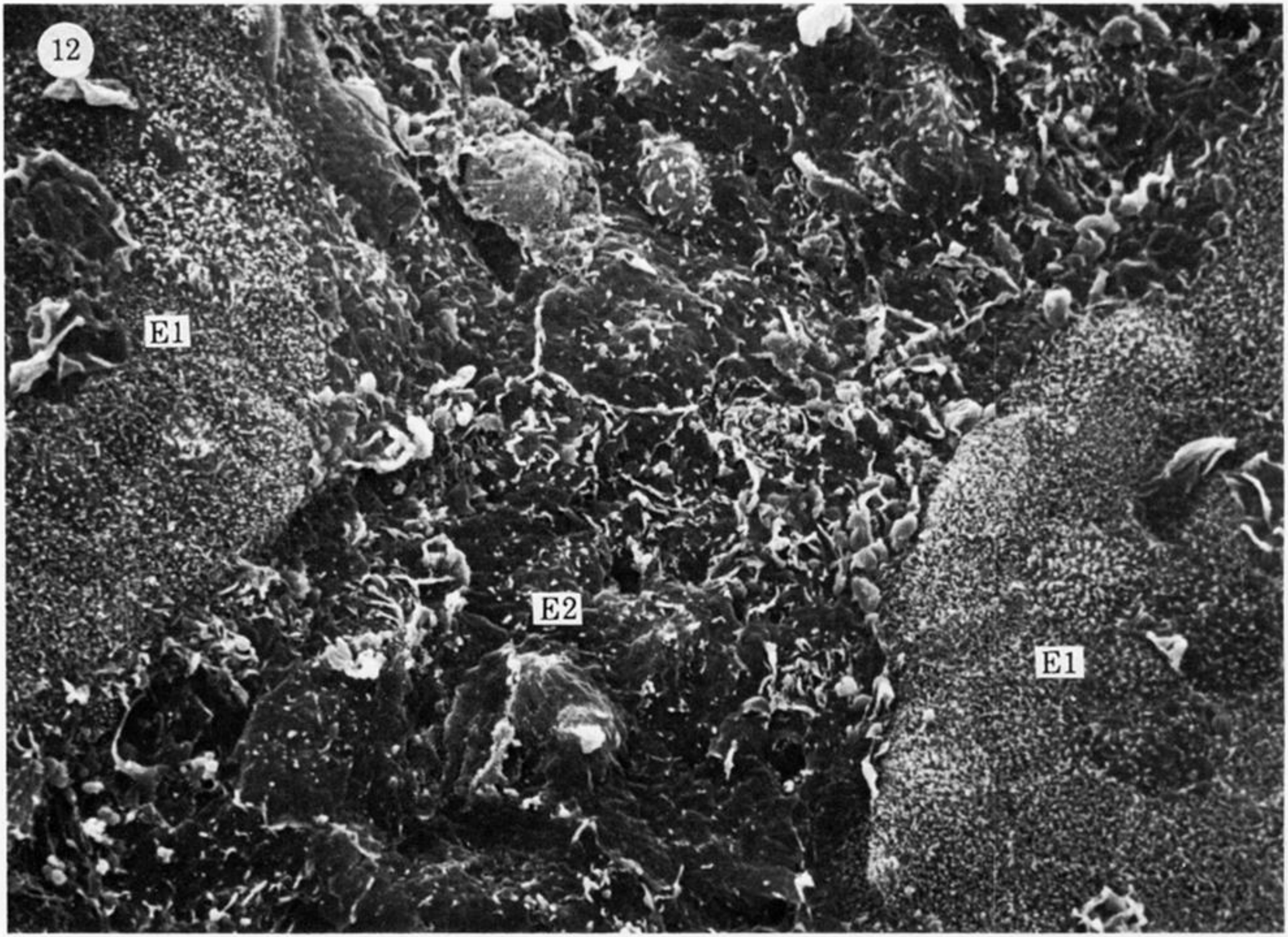

FIGURE 12. Cow K477 lactiferous sinus, 24 h. This lesion, running from the top to bottom of the micrograph, has been closed by a monolayer of basal cells (E2). Except for some marginal cells, the superficial layer of the epithelium (E1) appears normal. (S.e.m. Magn. ×2400.)

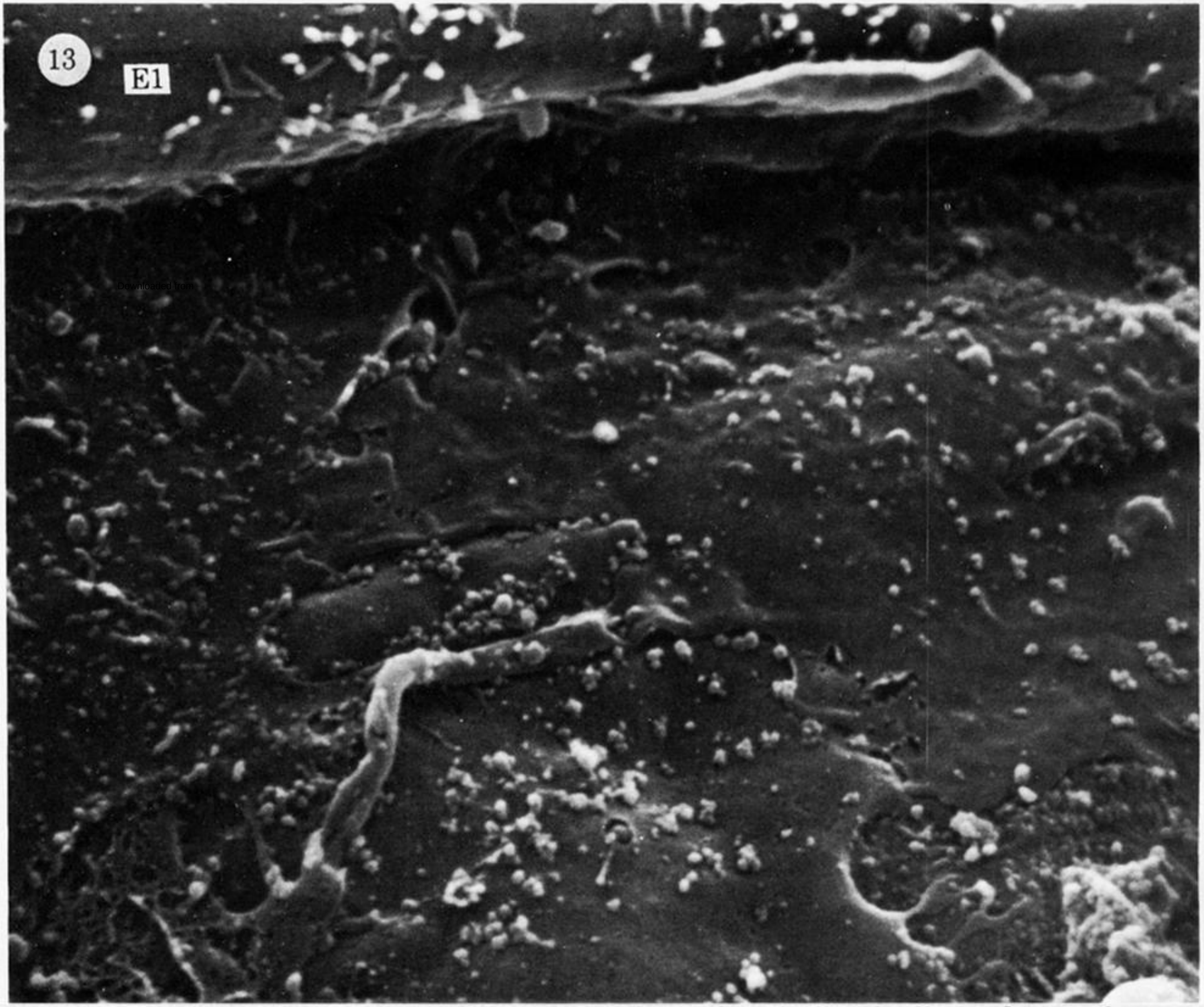

FIGURE 13. The same lesion as that in figure 12, showing that the basal cells are flattened, possess few microvilli and do not appreciably overlap their neighbours. A marginal cell (E1) of the superficial layer is also visible. (S.e.m. Magn. ×7300.)