The Effect of Neutrophil Migration on Epithelial Permeability

Linda C. Milks, Gregory P. Conyers, and Eva B. Cramer

Department of Anatomy and Cell Biology, State University of New York, Health Science Center at Brooklyn, Brooklyn, New York 11203. Dr. Milks' current address is Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, California 92037.

Abstract. To reach an inflammatory lesion, neutrophils must frequently traverse the epithelium of an infected organ. Whether the actual migration of neutrophils alters the epithelial permeability is unknown. Through the use of an in vitro model system it was possible to directly determine the effect of neutrophil emigration on the transepithelial electrical resistance of the monolayer. Human neutrophils (5 \times 10^6 cells/ml) were placed in the upper compartment of a combined chemotaxis/resistance chamber and stimulated for 40 min by a gradient of 10^{-7} M n-formyl-methionyl-leucyl-phenylalanine to traverse a confluent monolayer of canine kidney epithelial cells grown on micropore filters. Neither the chemoattractant alone (10^{-8}-10^{-9} M) nor the accumulation of an average of eight neutrophils per millimeter of epithelium lowered the transepithelial electrical resistance. However, under certain conditions the migration of neutrophils temporarily increased the permeability of the monolayer.

The resistance fell \sim 48\% within 5 min if the migratory cells were stimulated to reverse their migration across the same monolayer. As re-migration continued, the resistance returned to its initial levels within 60 min. Doubling the initial neutrophil concentration to 10 \times 10^6 cells/ml resulted in the accumulation of an average of 66 neutrophils per millimeter of epithelium and an average fall in resistance of 46\% (r = 0.98; P < 0.001) in 40 min. If the resistance had fallen <45\%, removal of the neutrophils remaining in the upper compartment resulted in a return of the transepithelial electrical resistance to its initial level within 65 min. However, when the fall was >45\%, the resistance only recovered to 23.5\% of its initial levels within the same time frame. Thus, these results suggest that the integrity of an epithelium can, under certain conditions, be affected by the emigration of neutrophils, but that this effect is either completely or partially reversible within 65 min.

The accumulation of leukocytes is an important part of the inflammatory response. To reach the site of inflammation, leukocytes must be able to traverse the endothelium lining blood vessels and the epithelium lining an infected organ (i.e., transitional epithelium during a bladder infection or tubular epithelium in pyelonephritis; 9, 24, 40, 41). While many in vitro studies have examined the effect of leukocyte migration on the permeability of the vascular endothelium (1, 18-22, 26-28, 34, 39, 42, 44, 46), relatively few studies (8, 36, 43, 45) have determined the effect of inflammatory mediators or neutrophil migration on the permeability of organ epithelia.

The paracellular or intercellular permeability of the epithelium is regulated by the zonulae occludentes (tight or occluding junctions; 5, 11). Ultrastructurally, these junctions appear as regions of fusion between the outer leaflets of the plasma membrane of adjacent epithelial cells and are thought to form an occluding belt around the circumference of each cell (3, 10). The tightness of these junctions can be assessed by transepithelial electrical resistance measurements (II, 37).

When leukocytes traverse epithelia, they must migrate either through the cells or between the occluding junctions. From previous studies in our laboratory (6, 7, 36) as well as work by others (8, 24), neutrophils have been shown to migrate between epithelial cells. The effect of this process on the transepithelial electrical resistance of the epithelium is unknown. To determine the effect of leukocyte migration on epithelial permeability we have used an in vitro model of neutrophil transepithelial migration (6). With this system a confluent polarized monolayer of Madin-Darby canine kidney (MDCK) epithelial cells are grown on micropore filters. Transepithelial electrical resistance measurements were monitored continuously as human neutrophils traversed the epithelium in response to the chemoattractant, n-formyl-methionyl-leucyl-phenylalanine (fMLP). The effect of the chemoattractant, neutrophil concentration, and the repeated migration of the same cells across the same epithelium on the permeability of an epithelial monolayer was assessed.

A preliminary report of portions of this work has already been published (35).

1. Abbreviations used in this paper: CMEM, MEM with 10% vol/vol fetal calf serum; fMLP, n-formyl-methionyl-leucyl-phenylalanine; Gey's, Gey's balanced salt solution containing 0.5% wt/vol bovine serum albumin; MDCK, Madin-Darby canine kidney epithelial cells.
Materials and Methods

Epithelial Cell Culture

MDCK epithelial cells were maintained in culture by serial passage in Eagle's medium with Earle's salts (MEM), penicillin (100 U/ml), streptomycin (100 μg/ml), and 10% vol/vol fetal calf serum (CMEM), as described previously (6). Neutrophil migration began after 5 min of incubation. As a test of the confluence of the epithelial monolayer, the transepithelial electrical resistance of the monolayer was measured at the 40 ± 2 min timepoint using Student's t test.

Preparation for Electron Microscopy

Micropore filters containing both neutrophils and epithelial cells from both experimental and control groups were fixed for 1 h in 2.5% vol/vol glutaraldehyde in 0.1 M phosphate buffer (pH 7.3), washed in buffer, and then processed for transmission and/or scanning electron microscopy. For transmission electron microscopy, the filters were postfixed in 1% vol/vol OsO4 in 0.1 M phosphate buffer (pH 7.3), washed in 0.85% saline, dehydrated in ethanol, and embedded in Epon 812. Thin sections, stained with uranyl acetate and lead citrate, were examined with the JEOL 100C electron microscope. For scanning electron microscopy, the filters were impregnated with OsO4, using the thio-carbohydrazide/osmium tetroxide technique (30), dehydrated in ethanol, critical-point dried (Samdri 790; Tousimis Research Corp., Rockville, MD), and examined with the JEOL 100C in the scanning mode.
Results

Effect of Media and Chemoattractant on the Permeability of Epithelial Monolayers

Under control conditions (media on both sides of the monolayer, no neutrophils or chemoattractant present) the transepithelial electrical resistance of epithelial monolayers remained relatively stable for 40–45 min. As seen in Fig. 1, neither the changing of solutions nor the addition of the chemoattractant (10^{-7} M fMLP) to either the lower or both compartments altered the epithelial resistance. In fact, fMLP at all concentrations (10^{-5}–10^{-9} M) tested had no significant effect on the transepithelial electrical resistance of the monolayer (data not shown). Most studies were performed in Gey's with 0.5% BSA. However, similar results were observed when experiments were performed in media with (CMEM) or without (MEM) 10% fetal bovine serum. All cells were viable as determined by trypan blue exclusion even after continuous transepithelial electrical resistance measurements lasting 180 min or after a 40-min exposure to a high concentration (10^{-5} M) of the chemoattractant.

Continuous Resistance Measurements during Neutrophil Migration

Continuous transepithelial electrical resistance studies were performed during neutrophil migration. Neutrophils suspended at a concentration of 5 x 10^6 cells/ml were placed above the apical epithelial surface. They were stimulated to traverse the epithelium grown on 0.45-μm pore micropore filters by 10^{-7} M fMLP, placed in the lower compartment. As can be seen in Fig. 2, the resistance remained stable throughout the ~40-min incubation period. At the end of the incubation the filters were fixed, embedded, and the number of neutrophils that traversed each monolayer per millimeter of epithelium was determined. As previously observed (36), the number of neutrophils that traversed the monolayer was dependent on the initial transepithelial electrical resistance of the monolayer. Consequently, more neutrophils traversed epithelia with lower starting resistances. However, even when as many as 18 neutrophils per millimeter of epithelium had traversed the monolayer, the resistance remained stable (Fig. 2).

Effect of Neutrophil Concentration on Epithelial Permeability

To determine whether the concentration of neutrophils stimulated to traverse the epithelium had any effect on the permeability of the monolayer, neutrophils were isolated from the same donor, suspended at concentrations of 5 and 10 x 10^6 neutrophils/ml, and stimulated to migrate across epithelial monolayers with comparable transepithelial electrical resistances (Fig. 3). While the resistance of the monolayer remained stable when a concentration of 5 x 10^6 neutrophils/ml was used, a concentration of 10 x 10^6 neutrophils/ml caused the transepithelial electrical resistance to fall during the 40-min incubation period. To assess the variability in this response and the statistical difference between groups, the transepithelial electrical resistance measurements from six different experiments were compared (Table I). The mean starting resistance of the epithelial monolayers used for either the 5 or 10 x 10^6 neutrophils/ml experiments was 185.7 ± 22.7 (range, 266.3–121.6) and 197.2 ± 32.6 (range, 305.3–117.2), respectively. There was no statistical difference between their starting resistance (P > 0.40). As seen in Table 1, a suspension of 5 x 10^6 neutrophils/ml stimulated to traverse the epithelium in response to 10^{-7} M fMLP caused no significant difference (P > 0.475) between the mean starting resistance and mean ending resistance after a 40-min incubation. In contrast, a concentration of 10 x 10^6 neutrophils/ml, under chemotactic conditions, caused an average fall in resistance of 45.7% (range, 31–65%) to 109 ohms-cm² (P < 0.05) by 40 min. When cross-sections of these experimental monolayers were examined by light microscopy (Fig. 4, a and b), the epithelium appeared intact.
Neutrophils that had traversed the epithelium were caught beneath the monolayer at the surface of the small pore size filter (0.45 μm). Occasionally, areas of the epithelium exposed to 5 × 10⁶ neutrophils/ml (Fig. 4 a) formed domes over large clusters of neutrophils that had emigrated beneath the monolayer. In contrast, all epithelia exposed to 10 × 10⁶ neutrophils/ml (Fig. 4 b) had areas of dome formation over large accumulations of neutrophils.

The number of neutrophils that migrated across the filter and the number of invasion sites where neutrophils had traversed the monolayer were quantitated for both experimental groups (Table I). When the neutrophils were suspended at a concentration of 5 × 10⁶ neutrophils/ml, a mean of 8.4 ± 2.5 neutrophils/mm epithelium traversed the epithelia. This was equivalent to the mean total emigration per monolayer of ~54,000 neutrophils. At concentrations of 10 × 10⁶ neutrophils/ml, there was an eightfold increase in the average number of neutrophils/mm (66.0 ± 19.4 neutrophils/mm epithelium, P < 0.01). This resulted in an average total accumulation of 425,500 (P < 0.01) neutrophils that had traversed per monolayer. It is of interest that only 2.2% of the 5 × 10⁶ neutrophils/ml and 8.5% of the 10 × 10⁶ neutrophils/ml traversed the monolayer in 40 min. In addition, at the higher concentration of neutrophils (10 × 10⁶ cells/ml) there was a fivefold increase in the number of invasion sites per millimeter of epithelium (0.6 ± 0.2 vs. 3.5 ± 0.8; P < 0.005) and a doubling in the number of neutrophils that traversed each invasion site (6.5 ± 1.1 vs. 3.5 ± 0.3; P < 0.0125).

**Variation in Pore Size of the Filter**

While the use of 0.45-μm pore filters enables one to quantify both the number of emigrated neutrophils and the number of neutrophil invasion sites, it does result in a lifting of the epithelium over the cells that accumulate at the surface.

---

**Table I. Effect of Neutrophil Concentration on Epithelial Permeability**

|                  | 5 × 10⁶ PMNs/ml* | 10 × 10⁶ PMNs/ml |
|------------------|------------------|------------------|
| Starting resistance† | 185.7 ± 22.7 †   | 197.2 ± 32.6     |
| Ending resistance  | 188.9 ± 20.8     | 109.3 ± 23.6 †   |
| Percent change in resistance | +2.6 ± 2.8       | -45.7 ± 5.7 †    |
| No. of PMN invasion sites/mm epithelium | 2.3 ± 0.6        | 10.6 ± 2.4 **    |
| No. of PMNs traversed/mm epithelium   | 8.4 ± 2.5        | 66.0 ± 19.4 †    |
| No. of PMNs traversed/PMN invasion site | 3.5 ± 0.3        | 6.5 ± 1.1 †     |
| No. of PMNs traversed/monolayer       | 53,965 ± 16,191  | 425,506 ± 124,713‡ |
| Percent of PMNs traversed/monolayer   | 2.2 ± 0.6        | 8.5 ± 2.5 †     |

* 0.5 ml of a suspension of neutrophils (PMNs) were placed above epithelial monolayers (n = 6) and stimulated to traverse the epithelium in response to 10⁻⁷ M fMLP for 40 ± 2 min.
† In ohms · cm².
§ Mean ± SEM.
Comparison between 5 and 10 × 10⁶ PMNs/ml:
† P < 0.025
‡ P < 0.0005
** P < 0.005
†† P < 0.01
§§ P < 0.0125
Figure 4. Light micrographs of neutrophil migration across epithelial monolayers in response to a gradient of $10^{-7}$ M fMLP for 45 min. (a) Neutrophils ($5 \times 10^6$ cells/ml) have migrated across the epithelium and are trapped beneath the monolayer at the surface of the 0.45-µm pore filter. (b) When the neutrophil concentration was doubled ($10 \times 10^6$ cells/ml) greater numbers of neutrophils traversed the epithelium and were caught at the surface of the 0.45-µm pore filter. The epithelium formed domes over these cells. (c) A larger pore size filter (3.0 µm) permitted neutrophils ($10 \times 10^6$ cells/ml) to continue their migration through the filter. This reduced the number of neutrophils that accumulated beneath the monolayer and the lifting of the epithelium. (a–c, toluidine blue; Bar, 31.1 µm)

of the filter. To be sure the fall in resistance was not an artifact of the system, the design of the experiment was modified. Monolayers were grown on 3.0-µm pore filters. This pore size enables neutrophils that have traversed the epithelium to continue their migration through the filter. When $10 \times 10^6$ neutrophils/ml were stimulated to migrate across these monolayers for 40 min, the epithelium remained intact and adherent to the filter surface (Fig. 4 c) but the transepithelial electrical resistance of the monolayers still fell. The reason for the fall in resistance was not morphologically apparent even at the ultrastructural level. The transepithelial electrical resistance measurements from five experiments in which $10 \times 10^6$ neutrophils/ml were stimulated to traverse the epithelium grown on 3.0-µm filters were normalized. The results were averaged and compared with the results obtained from five monolayers grown on 0.45-µm pore filters under similar conditions (Fig. 5). There was no significant difference ($P > 0.1$) between the mean starting resistance of these two groups. The transepithelial electrical resistance of the monolayers grown on either filter fell similarly to an equivalent degree (41% on 3.0 µm vs. 45% on 0.45 µm; $P > 0.3$) after $10 \times 10^6$ neutrophils/ml were stimulated to traverse the epithelium for 40 min.

Recovery of Resistance

The process of directed migration of $10 \times 10^6$ neutrophils/ml across monolayers grown on 3.0-µm pore filters for 40 min caused a fall in resistance that ranged between 27.7 and 56.2%. At this time the remaining neutrophils in the apical compartment and the chemoattractant in the lower compartment were removed. The monolayers were washed carefully in buffer and then incubated further in Gey's. Monolayers whose resistances had dropped <45% were able to totally recover their resistance between 30 and 60 min (Fig. 6). Those monolayers in which the resistance decreased $\geq$45% recovered to within 23.5% ± 1.1% (range, 21.2–26.1) of their original resistance in 30–60 min. The failure of these monolayers to completely recover did not appear to be due to the death of any of the epithelial cells. This was assessed by exclusion of trypan blue. Total recovery of the transepithelial electrical resistance of monolayers with falls $\geq$45% may take longer than we measured.

Effect of Random Migration and Chemokinesis

The previous studies indicate that when $10 \times 10^6$ neutrophils/ml are stimulated to traverse the monolayer under conditions of chemotaxis, the transepithelial electrical resistance of the monolayer decreases with time. However, it is not clear whether the mere presence of a similar concentration of cells above the epithelium would have a similar effect. To examine this, monolayers were exposed to 5 or $10 \times 10^6$ neutrophils/ml under conditions of either random migration (Gey's in both compartments) or chemokinesis ($10^{-7}$ M fMLP in both compartments; data not shown). Regardless of cell concentration or condition, less than one neutrophil per millimeter of epithelium traversed the monolayer. In addition, there was no significant decrease in resistance ($\sim 4\%$). Thus, the physical presence of neutrophils at the apical epithelial surface in the presence or absence of fMLP did not appear to influence the transepithelial electrical resistance. This is consistent with the results reported by Sugahara et al. (43).

Reversal of Neutrophil Migration

Neutrophils at a concentration of $5 \times 10^6$ cells/ml were able to migrate across the monolayer once without affecting the epithelial permeability. It was not clear whether the return migration of the same neutrophils across the same monolayer, and possibly the same junctions, would affect the permeability of the epithelium. Therefore, the transepithelial electrical resistance of an epithelium grown on a 0.45-µm pore filter was continuously monitored while $5 \times 10^6$ neutrophils/ml first traversed the epithelium from the apical to basal direction (Fig. 7). After 30 min the neutrophils that had
not migrated were removed and the chemotactic gradient was reversed. This stimulated the emigrated neutrophils to migrate (now in a basal to apical direction) back across the same area they had just traversed. This reversal resulted in a ~33–58% fall in resistance within 5 min. In general, the higher the starting resistance of the epithelium, the less the fall in resistance of the monolayer 5 min after reversal. After this sudden fall, the resistance of the monolayer gradually returned to its initial levels by ~55 min (Fig. 7). This decrease in resistance was not observed under control conditions when the chemotactic gradient across the monolayers was reversed in the absence of neutrophils.

To understand the relationship between the electrical measurements and the migratory process, filters were fixed at various times during the reversal process and examined by either light microscopy or scanning electron microscopy. Before reversing the gradient, neutrophils could be observed beneath the monolayer at many locations, either in small clusters, in pairs, or singly. Within 5 min of reversing the chemotactic gradient, neutrophils could be observed beginning to migrate back across the monolayer (Fig. 8a). Scanning electron microscopic examination of these cells revealed pseudopods emerging from the intercellular crevices between epithelial cells (Fig. 9a). As remigration continued (15–30 min) those regions where many neutrophils had originally accumulated beneath the monolayer now contained groups of emerging neutrophils. When these cells reached the apical epithelial surface they formed clusters and adhered by fine tendrils to one another and to the newly traversing neutrophils (Figs. 8b and 9b). When the last neutrophil in

---

**Figure 5.** The effect of neutrophil migration (10 × 10⁶ neutrophils/ml) across epithelial monolayers grown on 0.45- and 3.0-μm pore size filters. Neutrophils were stimulated to migrate across epithelial monolayers in response to 10⁻⁷ M fMLP for 40 min at 37°C. The normalized measurements with similar time points (±2 min) from each experimental condition were averaged. The resistance decreased at a similar rate regardless of whether the emigrated neutrophils accumulated beneath the monolayer at the surface of the 0.45-μm pore filter (10, X, n = 5) or if they were able to migrate into the 3.0-μm (10, open box, n = 5) pore size filter. By 20 min the resistance had decreased significantly (P < 0.05) from the Gey's control (G, solid diamond, n = 8).

**Figure 6.** Recovery of the transepithelial electrical resistance after the migration of neutrophils at a concentration of 10 × 10⁶ neutrophils/ml. The transepithelial electrical resistance of the epithelial monolayer was continuously monitored. After 35 min neutrophils were placed in the apical compartment (10) and were stimulated by 10⁻⁷ M fMLP to traverse the epithelium grown on 30-μm pore size filters for 22 min. The resistance of the monolayer decreased 35.2%. The cells that had not migrated and the chemoattractant were then removed. The monolayers were washed and allowed to incubate in Gey's (G). The resistance returned to its initial values within 31 min.
of neutrophils (5 × 10^6 cells/ml) did not increase the transepithelial electrical resistance of the epithelial monolayer that these invasion sites are also impermeable to macro-molecules. Since a basic function of epithelia is to form a wall between two different environments, the ability of neutrophils to traverse an epithelium without disturbing this barrier is very important.

Relatively few of the added neutrophils actually migrate across the renal epithelium. Keller et al. (25) and Harvath and Leonard (17) have shown that in chemotactic studies only 15–40% of the available neutrophils respond to a gradient of fMLP and migrate into plain micropore filters. The renal epithelium appears to restrict the number of emigrating neutrophils even further (<10%). This may be due to the tightness of the epithelial occluding junctions and the need to have a population of neutrophils that are not only able to migrate but are able to open tight junctions.

In experiments in which the chemotactic gradient was reversed, the emigration of the lower concentration of neutrophils (5 × 10^6 cells/ml) was associated with a fall in the transepithelial electrical resistance. In this situation, the emigrated neutrophils were stimulated to migrate back across the same, possibly weakened, junctions; the direction of migration; or a combination of these. Further studies are necessary to resolve this issue.

Figure 7. Continuous transepithelial electrical resistance studies during the migration and remigration of neutrophils at a concentration of 5 × 10^6 cells/ml. The transepithelial electrical resistance of the epithelial monolayer was continuously monitored at 37°C. After 65 min neutrophils were added to the apical compartment (5) and stimulated to migrate across the monolayer for 30 min. The resistance of the monolayer remained stable during this period. The chemotactic gradient was then reversed (R). This caused a rapid drop (51.6%) in resistance within 5 min. Over the next 55 min the resistance returned to its initial starting level.

Discussion

Through the use of an in vitro model it has been possible to eliminate serum and connective tissue factors and to examine the effect of the chemoattractant and the migratory leukocytes on the permeability of a renal epithelium. The synthetic peptide fMLP, which is equivalent to the major chemoattractant produced by bacteria (31), was used to stimulate neutrophil emigration across the epithelium. fMLP has been shown in vivo to affect the permeability of the intestinal epithelium (29). However, in the present study, neither the concentration of the chemoattractant nor its location above or below the monolayer elicited a change in the transepithelial electrical resistance of the renal epithelium. This confirms previous reports that fMLP has no effect on the permeability of blood vessels (23, 44) and endothelial (16) or MDCK monolayers (8).

The apical to basal migration of the lower concentration of neutrophils (5 × 10^6 cells/ml) did not increase the permeability of the epithelium, as the transepithelial electrical resistance of the monolayer remained stable throughout a 40-min migration period. This was true regardless of either the starting resistance of the monolayer (226–122 ohms-cm^2) or when an average of eight neutrophils per millimeter of epithelium traversed the monolayer. The number of invasion sites per millimeter of epithelium varied with the starting resistance and ranged from one to five sites per millimeter of epithelium with an average of two and one-third sites per millimeter of epithelium. From a previous study (36), we know that these invasion sites are also impermeable to macromolecules. Since a basic function of epithelia is to form a
no affect on epithelial permeability. This suggests that it is the process of migration of many neutrophils across the monolayer rather than just their presence above the monolayer that causes the fall in resistance.

The decrease in resistance caused by the migration of $10 \times 10^6$ neutrophils/ml was totally reversible within $\sim 1$ h if the transepithelial electrical resistance was initially lowered <45%. A decrease in resistance of >45% delayed the recovery period such that monolayers only recovered an average of 23% of their initial resistance by 60 min. A similar variability in recovery of resistance occurred in MDCK monolayers after removal of extracellular calcium (4, 14, 32). In these experiments the recovery period appeared to be dependent upon the length of time (>20 min) the junctions were kept open rather than the percent fall in resistance. In our studies the time period for neutrophil migration across the monolayer was always 40 min, although the length of time each junction was kept open probably varied from one invasion site to another. Falls in resistance >45% may reflect monolayers in which a number of invasion sites were kept open for longer lengths of time, thereby requiring longer time to repair.

Both Griepp et al. (13) and Gonzalez-Mariscal et al. (12) found that in established MDCK monolayers de novo protein synthesis was not required for the resealing of junctions after calcium chelation. In our studies, the rapid recovery of monolayers whose resistance fell <45% also suggests that protein synthesis is not required for junctional repair after neutrophil emigration. Whether protein synthesis is required after falls in resistance of >45% remains to be determined.

Recently, Evans and co-workers examined the migration of
The emigration of peritoneal exudate cells decreased the rat peritoneal exudate cells across a low and high resistance strain of kidney MDCK epithelial cells (8). These cells (62% macrophages, 34% neutrophils, and 4% lymphocytes), which were collected in response to 1% oyster glycocepin, appeared to have a more disruptive effect on the integrity of the kidney epithelium than human peripheral blood neutrophils. The emigration of peritoneal exudate cells decreased the transepithelial electrical resistance of the kidney epithelium at least 30% even with low migratory cell concentrations (4.1 × 10^5). The repair of these monolayers required at least 20 h to re-establish the initial transepithelial electrical resistance. The cause for the different reaction to the migration of human peripheral blood neutrophils and rat peritoneal exudate cells may be due to the presence of macrophages and lymphocytes in the exudate, species differences, or the amount of cell activation.

Through the use of an in vitro model system, it is possible to determine the factors that affect the permeability of an epithelium during an inflammatory response. By eliminating connective tissue and serum factors, the direct effect of neutrophil migration on the permeability of an epithelium was assessed. The results of this study indicate that neutrophil migration can occur with or without an increase in epithelial permeability. The fall in transepithelial electrical resistance that sometimes accompanies neutrophil emigration does not appear to result from destruction of the epithelium, but rather from a temporary loosening of the occluding junctions.

We would like to thank Edmund Folkes and Alex Fulop for their technical assistance, Jack Illari and Louis Dienes for their photographic work, Dr. Tom Easton for his advice and help, and Andrew Valenti and Antonio Sabatini. 1978. Polarized monolayers formed by epithelial cells on a permeable support.

Through the use of an in vitro model system, it is possible to determine the factors that affect the permeability of an epithelium during an inflammatory response. By eliminating connective tissue and serum factors, the direct effect of neutrophil migration on the permeability of an epithelium was assessed. The results of this study indicate that neutrophil migration can occur with or without an increase in epithelial permeability. The fall in transepithelial electrical resistance that sometimes accompanies neutrophil emigration does not appear to result from destruction of the epithelium, but rather from a temporary loosening of the occluding junctions.

We would like to thank Edmund Folkes and Alex Fulop for their technical assistance, Jack Illari and Louis Dienes for their photographic work, Dr. Tom Easton for his advice and help, and Andrew Valenti and Antonio Sabatini. 1978. Polarized monolayers formed by epithelial cells on a permeable support.

References
1. Bjork, J., P. Heiqvist, and K. Arefors. 1982. Increase in vascular permeability induced by leukotriene B4 and the role of polymorphonuclear leukocytes. Inflammation. 6:189–200.
2. Boyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood: isolation of mononuclear cells by one centrifugation, and granulocytes by combining centrifugation and sedimentation at 1G. Scand J. Clin. Lab. Invest. 21 (Suppl.):77–89.
3. Bandigaard, M. 1984. The three-dimensional organization of tight junctions in a capillary endothelium revealed by serial-sectional electron microscopy. J. Ultrastruct. Res. 88:1–17.
4. Cerejido, M., E. S. Robbins, W. J. Dolan, C. A. Rotunno, and D. D. Sabatini. 1978. Polarized monolayers formed by epithelial cells on a permeable and translucent support. J. Cell Biol. 77:853–880.
5. Cerejido, M., E. Stefani, and A. Martinez-Palomo. 1980. Occluding junctions in a cultured transporting epithelium: structural and functional heterogeneity. J. Membr. Biol. 53:19–32.
6. Cramer, E., D. E. Crumley, and G. E. Paule. 1986. Junctional complexes in various epithelia. J. Cell Biol. 102:1868–1877.
7. Evans, C. W., J. E. Taylor, J. D. Walker, and N. L. Simmons. 1983. Transglutamylase of rat peritoneal exudate cells. Br. J. Exp. Pathol. 64:644–654.
8. Fall, M., J. L. Johansson, and A. Vahlne. 1985. A clinicopathological and virological study of interstitial cystitis. J. Urol. 133:771–773.
9. Farquhar, M. G., and G. E. Palade. 1963. Junctional complexes in various epithelia. J. Cell Biol. 17:375–412.
10. Gumbiner, B., and K. Simons. 1986. A functional assay for proteins in lipid membranes. Proc. Natl. Acad. Sci. USA. 83:4151–4155.
11. Harper, J. M., B. Schwartz, M. A. Reidey, S. Schwartz, H. D. Ochs, and L. A. Harker. 1985. Activated neutrophils disrupt endothelial monolayer integrity by an oxygen-independent mechanism. Lab. Invest. 53:140–150.
12. Harvath, L., and E. J. Leonard. 1982. Two neutrophil populations in human blood with different chemotactic activities: separation and chemotactic inhibition. Infect. Immun. 36:443–449.
13. Hefflin, A. C. Jr., and K. L. Brigham. 1981. Prevention by granzyme depletion of increased vascular permeability of sheep lung following endotoxemia. J. Clin. Invest. 68:1253–1260.
14. Howes, E. L., Jr., K. L. Wong, K. T. Hartilia, R. O. Webster, and J. T. Rosenbaum. 1985. Complement and polymorphonuclear leukocytes do not determine the vascular permeability induced by intracellular LPS. Am. J. Pathol. 118:35–42.
15. Hurley, J. V. 1963. An electron microscopic study of leukocytic emigration and vascular permeability in rat skin. Aust. J. Exp. Biol. Med. Sci. 41:171–188.
16. Hurley, J. V. 1964a. Substances promoting leukocyte emigration. Ann. NY Acad. Sci. 116:918–935.
17. Hurley, J. V. 1964b. Acute inflammation: the effect of concurrent leukocytic emigration and increased permeability on particles retention by the vascular wall. Br. J. Exp. Pathol. 45:627–633.
18. Issekutz, A. C. 1981. Vascular responses during acute neutrophilic inflammation. Their relationship to in vivo neutrophil emigration. Lab. Invest. 45:435–441.
19. Ivanyi, B., J. Ormos, and L. Lantos. 1983. Tubulointerstitial inflammation, cast formation, and renal parenchymal disease in experimental pyelonephritis. Am. J. Pathol. 113:300–308.
20. Kettler, H. U., J. H. Wiessler, and B. Damara. 1981. Diverging effects of chemotactic peptide and synthetic Fmet-leu-phe on leukocyte locomotion and adhesion. Immunology. 42:379–383.
21. Koh, M., L. Parente, and D. Willoughby. 1978. Immunological and non-immunological pleural inflammation. The effects of anti-inflammatory and anti-rheumatic drugs. Eur. J. Rheumatol. Inflammation. 1:204–211.
22. Kopanick, M. M., and H. Z. Movat. 1983. Kinetics of acute inflammation induced by Escherichia coli in rabbits. II. The effect of hyperimmunization, complement depletion, and depletion of leukocytes. Am. J. Pathol. 110:13–29.
23. Logan, G., and D. Wilhelm. 1963. Ultra-violet injury as an experimental model of the inflammatory reactions. Nature (Lond.). 198:968–969.
24. Magnusson, K. E., C. Dahlgren, and A. Sjolander. 1985. Effect of N-formylmetionyl-leucyl-phenylalanine (FMLP) on gut permeability. Inflammation. 9:365–373.
25. Malick, L., and R. Wilson. 1975. Evaluation of a modified technique for SEM examination of vertebrate specimens without evaporated metal layers. In Scanning Electron Microscopy/1975. Part 1. O. Johari and I. Corvin, editors. IIT Research Institute, Chicago. 259–266.
26. Marasco, W. A., S. H. Pian, H. Kutzsch, H. J. Showell, D. E. Feltner, F. Rairn, E. L. Becker, and P. A. Ward. 1984. Purification and identification of formyl-methionyl-leucyl-phenylalanine as the major peptide neutrophil chemotactic factor produced by Escherichia coli. J. Biol. Chem. 259:5430–5439.
27. Martinez-Palomo, A., L. Meza, G. Beaty, and M. Cerejido. 1980. Experimental modulation of occluding junctions in a cultured transporting epithelium. Cell Biol. 87:736–745.
28. McCell, E., J. Povey, and D. C. Dumonde. 1981. The culture of vascular endothelial cells to confluence on microporous membranes. Thromb. Res. 24:417–431.
29. Michel, L., and L. Dubertret. 1985. A simple method for studying chemotaxis, vascular permeability and histological modifications by mediators of inflammation in vivo in man. Br. J. Dermatol. 113(Suppl. 28):61–66.
30. Milks, L., and C. Danner. 1984. Transglutamylase of rat peritoneal exudate cells. Proc. Natl. Acad. Sci. USA. 77:4069–4073.
31. Misfeldt, D., S. Hamamoto, and D. Piteka. 1976. Transglutamylase transport in cell culture. Proc. Natl. Acad. Sci. USA. 73:1212–1216.
32. Phillips, H. 1975. Dye exclusion tests for cell viability. In Tissue Culture. J. Kruse and H. Patterson, Jr., editors. Academic Press, Inc., New York. 406–410.
33. Pedegon, A., A. Moore, A. Al Duaij, and D. Willohabit. 1985. Studies into the association between leukocyte accumulation and oedema formation. Agents Actions. 17:209–213.
40. Sivan, Y., B. Griffel, O. Medalia, and M. Aronson. 1982. Comparative histology of the mouse bladder following initial infection and re-infection with Escherichia coli. J. Pathol. 138:353-364.
41. Smith, A. F. 1984. A light and electron microscopic study of urothelium from patients with chronic cystitis. Eur. Urol. 10:272-275.
42. Staub, N. C., E. L. Schultz, K. Koike, and K. H. Albertine. 1985. Effect of neutrophil migration induced by leukotriene B4 on protein permeability in sheep lung. Fed. Proc. 44:30-35.
43. Sugahara, K., G. R. Cott, P. E. Parsons, R. J. Mason, R. A. Sandhaus, and P. M. Henson. 1986. Epithelial permeability produced by phagocytosing neutrophils in vitro. Am. Rev. Respir. Dis. 133:875-881.
44. Wedmore, C. V., and T. J. Williams. 1981. Control of vascular permeability by polymorphonuclear leukocytes in inflammation. Nature (Lond.). 289:646-650.
45. Welsh, M. J., D. M. Shasby, R. Husted, P. Karp, and P. Spory. 1985. Oxidants increase paracellular permeability in a cultured epithelial cell line. J. Clin. Invest. 76:1155-1168.
46. Willoughby, D., and W. Spector. 1968. Inflammation in agranulocyte rats. Nature (Lond.). 219:1258.