that followed were, firstly, the appearance of a nuclear membrane around the newly consolidated 'nucleus' and, secondly, the outline of the microspheres assumed an amoeboid irregularity. While most of the cell-free microspheres gradually disintegrated, those which attained the above stage eventually transformed into miniature amoebae (Diameter 15–25 μm) (Figure d). These amoebae subsequently underwent encystment. They would perpetuate with an alternating cyst-trophic cycle through successive sub-cultures. They had no morphological similarity with the paramaecia.

The preliminary findings described above raised a number of searching questions rather than providing answers. Based on the limited data, the exact mechanism behind the phenomena observed defies analysis. Nevertheless, two essentially important issues have been raised.

First, the findings support the view that, at the lower grade of organization, the cells have innumerable genetic endowments and developmental possibilities than are expressed under the normal environmental set up[3]. These possibilities may be as paradoxical as has been described above and as versatile as well. Although the basis of argument may be slim, it is conceivable that the macronucleus of paramaecium is a repository of wider (but hidden) possibilities. The amino acid, by some hitherto obscure mechanism, possibly quite indirect, induced a pathway that blindly 'sliced out' a number of gene loci. These gene loci were exported out of the ciliate body along with the microspheres. Sometimes (not always), the microspheres carried self-sufficient genetic constellations which would transform into small amoebules following a novel pathway. All microspheres could not transform into amoebules, since all of them did not have the genetic capability to do so.

Secondly, the cell-free microspheres, which bore distinct marks of prokaryote-like organization, transformed into amoebules of eukaryotic make-up. This phenomenon provides a unique opportunity to trace the evolution of eukaryotic cells from prokaryotic predecessors. This is possibly a simulation of the phase, or, rather a succession of phases which represent the missing links of the greatest single evolutionary discontinuity to be found in the present day living world, namely, prokaryote to eukaryote evolution.

[3] E. N. WILLMER, in *Cytology and Evolution* (Academic Press, New York 1960), p. 399.

Unusual Membrane Fracture Faces in Polymorphonuclear Leukocyte Granules[1]

M. BAGGIOLINI, MILÈNE AMHERDT and L. ORCI

Research Institute Wander, a Sandoz Research Unit, Wander Ltd., P. O. Box 2747, CH-3001 Bern (Switzerland), and Institute of Histology and Embryology, University of Geneva Medical School, CH-1211 Genève (Switzerland),
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Summary. Freeze fracturing of azurophil and specific granules from rabbit polymorphonuclear leukocytes reveals unusually high numbers of intramembrane particles. Both granules have significantly higher particle densities on the B than on the A fracture face.

Membrane fusion is a selective process which is likely to be initiated by the mutual recognition of the merging membranes. The molecular basis of the recognition event is unknown. Its exploration would ideally require molecular interaction studies between membrane constituents whilst preserving their topographical arrangement. A promizing, if indirect, approach is the morphological freeze-fracture analysis of the membranes involved. So far, two systems have been studied with this technique, discharge of mucocysts[2] or trichocysts[3] in protozoa and secretion in the B-cell of the endocrine pancreas. In *Tetrahymena*[2] and *Paramecium*[3], granule fusion is preceded by the formation of a rosette of intramembrane particles in the plasma membrane. In the B-cell, however, no such re-arrangement of membrane structures can be seen at the presumptive sites of fusion, which appear as circular membrane areas almost totally devoid of intramembrane particles[4,5]. The apparent differences between these two cases seemed to justify the study of another system, the polymorphonuclear leucocyte (PMN), in which two distinct types of granules[6–11] fuse with the area of the plasma membrane which becomes internalized as a phagocytic vacuole[12]. This study has disclosed some very unusual features of the PMN granule membranes, which we wish to report briefly.

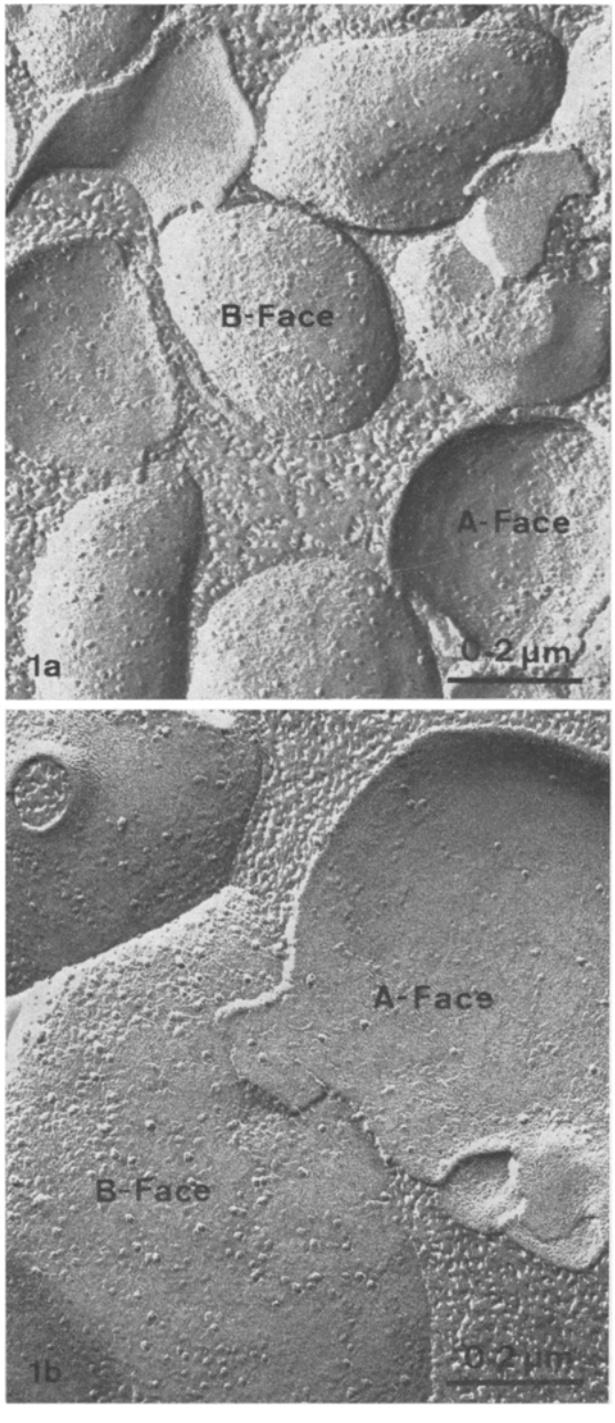
Numbers of intramembrane particles in the granule and plasma membranes of rabbit polymorphonuclear leukocytes

Type of membrane	Numbers of intramembrane particles per μm² of membrane surface (mean ± SEM)		Difference between A and B
	A Face	B Face	(p-value from t-test)
Azurophil granules	542 ± 32	813 ± 92	<0.01
Specific granules	630 ± 25	1002 ± 62	<0.001
Plasma membrane	989 ± 30	757 ± 38	<0.001

Methods. Rabbit PMNs were obtained from glycogen-induced peritoneal exudates[13] and were studied as such or fractionated by zonal differential sedimentation, as described previously[8]. All samples were prepared for freeze-fracturing after fixation in 1.5% glutaraldehyde buffered with sodium cacodylate 0.1 *M* at pH 7.4. Freeze-fracturing was performed according to procedures described previously[14]. From each preparation 3 replicas were made, and 20 randomly selected micrographs, together with a micrograph of a calibration lattice, were taken from each replica. On each micrograph, intramembrane particles in a total area of 1/8 to 1/4 μm² of flat surface were counted, using a counting lattice. In whole PMNs, granules were differentiated according to size. Diameter ranges of 350 to 700 nm and of 170 to 240 nm were taken for azurophil and specific granules, respectively. Intramembrane particles were counted on

complete profiles only. The membrane fracture faces are designated as A and B, respectively. They correspond to the P and E faces of the newly proposed nomenclature[15].

Results and discussion. The results are summarized in the Table. In azurophil and specific granules, freeze-fracturing revealed very high numbers of intramembrane particles on both membrane fracture faces. The average particle densities were slightly higher in the specific granule membrane than in the azurophil, but the difference was not found to be significant. In contrast to the findings in most membranes examined so far[16], we found in both PMN granules more intramembrane particles on the B than on the A fracture face. This unusual particle distribution was observed both in isolated granules and in granules which were examined in whole cells. This seems to rule out the possibility of a preparation artefact.



Freeze-fracture replicas of isolated specific (1a) and azurophil (1b) granules from rabbit PMN. In both granules, the numbers of intramembrane particles per unit area of fractured membrane is significantly higher on the B- than on the A-face. × 92,500.

In the PMN plasma membrane, we found similar densities of intramembrane particles as in the two types of granules. As expected, the intramembrane particles on the cytoplasmic (A) fracture face significantly outnumbered those on the B face, although the difference in particle densities between the two faces appears to be much less pronounced than in most other cells[16].

Our results indicate that the PMN differs in a number of features from other cells with comparable exocytotic functions. The membranes of the azurophil and specific granules of rabbit PMNs are much richer in intramembrane particles than those of secretory granules of the pancreatic B-cell[4,5], mastocyte granules, and zymogen granules of the exocrine pancreas[16]. Calculations show that the intramembrane particle densities in B-cell[4,5] and mastocyte granules are 2 to 4 times lower (A face), and up to 25 times lower (B face) than in PMN granules (Amherdt et al., unpublished observations). These data also indicate that the granules of mastocytes and of B-cells, in contrast to PMN granules, have a conventional intramembrane particle distribution, i.e. more particles on the A than on the B face. A further important difference between the PMN and secretory cells and mastocyte is that in the former the particle densities of granule and plasma membranes are similar.

As already mentioned, characteristic morphological membrane differentiations are seen in the B-cell of the pancreas at the presumptive sites of fusion[4,5]. Such structural changes could reflect the need for the fusing membranes to 'adapt' to each other before fusion since the intramembrane particle densities of granule and plasma membrane differ considerably. In the PMN, however, in which granule and plasma membranes have a similar freeze-fracture appearance, structural differentiation at the sites of fusion may not be required. A study of the fusion of PMN granules with phagocytic vacuoles should give further insight into this problem.

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