Macronuclear Response of *Paramaecium multimicronucleatum* to L-Lysine

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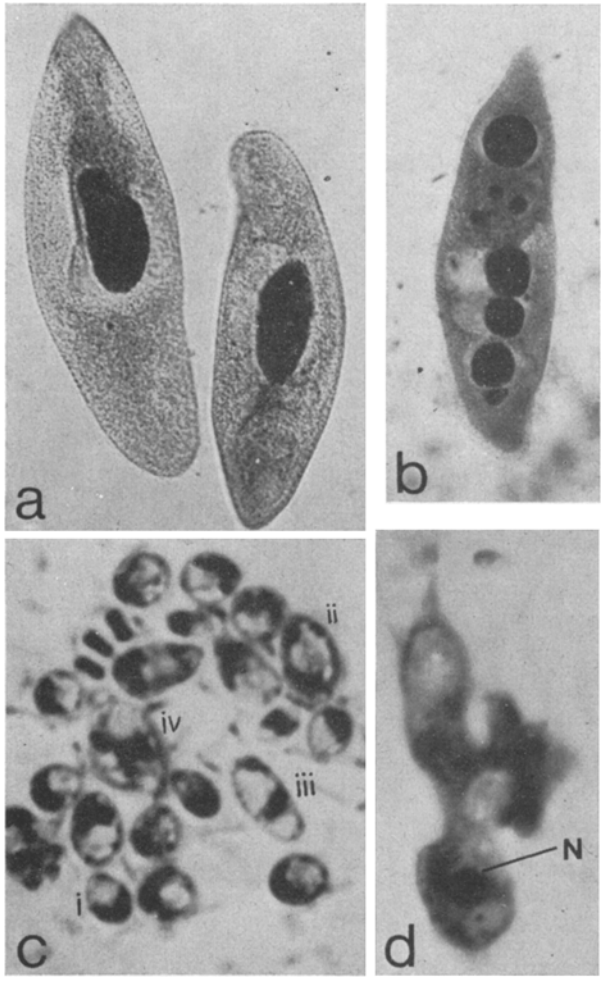
Summary. A strain of *Paramaecium multimicronucleatum* was exposed to a medium containing L-lysine; the concentrations of the amino acid were 0.1%, 0.5% and 1.0% for different sets of experiments. In these two latter concentrations, the macronucleus of the ciliate broke down into innumerable small fragments, the microspheres. The micronuclei remained inert. The microspheres left the body of paramaecium as cell-free, self-duplicating entities constituted of DNA and RNA and enveloped by a protein coat. They had no nuclear membrane and they resembled the prokaryotes. Grown in culture medium with 0.1% horse serum, the microspheres transformed into small amoebae having typical eukaryotic features. These amoebae maintained a typical cyst-trophic cycle during the successive sub-cultures; they had no similarity with the paramaecia.

The phenomenon of secretion of nuclear material from cells under experimental condition is well-known. For instance, excess DNA in UV-irradiated *Tetrahymena* was eliminated, during successive macronuclear divisions, as unusually large macronuclear extrusion bodies[1]. Unlike such a physical agency as described above, chemical intervention of one or a combination of a few amino acids added to the inorganic culture medium is known to cause similar extrusion of nuclear material from a species of soil amoeba, *Acanthamoeba* sp.[2]. But contrary to the happenings referred to earlier, in case of amoebae, the nucleus would break down into innumerable small fragments which in turn would leave the amoeba body as discrete self-duplicating entities. Grown in the culture medium, these particles would transform, beyond all recognition, into small amoeboid cells. A very similar phenomenon was observed when the same experiment was repeated on amoeboid cells germinated from fresh-water sponge gemmules (Mallik, unpublished).



a) Photomicrograph of the normal individuals of *Paramaecium multimicronucleatum*. × 300.

b) Ciliate after 48 h of treatment with 0.5% L-lysine. Note that the macronucleus has broken down into large and small lobes. × 300.

c) Cell-free microspheres at different phases of differentiation; i–iv represent progressive reorganization towards a centrally consolidated nucleus. Note the general prokaryote-like configuration of the particles. × 200.

d) Fully differentiated amoebule. N is the nucleus. × 450.

These findings, however, led to the present study with *Paramaecium multimicronucleatum* (Figure a) exposing the ciliate to the amino acid L-lysine. The primary reason behind the choice of the ciliate was that it has many distinct cytomorphological features which separate it instantly from any amoeboid protozoa. So, it was assumed, should the phenomena observed in the case of amoeboid cells be repeated in this case, the transformed organisms would definitely offer a better opportunity for comparison with the mother organism.

Materials and methods. A strain of *P. multimicronucleatum* constituted the material for this study. The culture was maintained in the laboratory at 21 °C and in the wheat infusion. For the purpose of experiment, varying concentrations of L-lysine, e.g., 0.1%, 0.5% and 1.0%, (w/v) in the culture medium were used. Standard methods were followed for enzymatic digestion studies.

Results and discussion. In 0.5% and 1.0% lysine medium, the macronucleus had broken down, within 36 to 48 h, into 4–6 smaller lobes, while each of these lobes further disintegrated into still smaller fragments (microspheres, having a diameter of 3–5 μm) (Figure b). These microspheres left the cell body of paramaecium as cell-free entities (Figure c). Notably, the micronuclei, which number 3 to 5 in this species, remained inert throughout this period. Outside the ciliate body, the microspheres divided at a fast rate; the overall manner of division simulated that of the yeasts. Feulgen preparations and enzymatic digestion studies with DNase and RNase suggested that these microspheres consisted of a DNA moiety that was non-specifically spread throughout the inner space of the microspheres and the rest was mostly RNA. The nuclear membrane was absent.

When these microspheres were cultured in the usual culture medium with a little horse-serum added (0.1%), they underwent radical structural transformations. Some of them (not all) gained in size and their nuclear apparatus gradually consolidated at the centre. Glimpses of such transforming stages can be seen from Figure c. Events

[1] D. C. Shepard, Expl Cell Res. *38*, 570 (1965).

[2] S. Mookerjee and S. Ghosh, Nucleus *9*, 139 (1966).

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]

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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