# USE OF CALIBRATED MELAMINE-FORMALDEHYDE LATEX FOR LUMINESCENCE-MICROSCOPIC STUDY OF PHAGOCYTOSIS IN MACROPHAGE CULTURES

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KEY WORDS: phagocytosis; macrophages; latex.

In the study of phagocytosis particles of latex of a particular size are frequently used as an object resistant to intracellular digestion. Usually these are particles of calibrated polystyrene latex, manufactured by foreign firms [4]. However, in the USSR calibrated melamine-formaldehyde latex is more readily available, and so far as the writers know, it has not been used to study phagocytosis.

The aim of this investigation was to determine the ability of melamine-formaldehyde latex to be phagocytosed by macrophages, whether observations on latex particles phagocytosed by macrophages are possible by phase-contrast and luminescence microscopy, to discover their morphological differences from cell structures and absence of toxicity for macrophages, and finally, to study the possibility of using calibrated melamine-formaldehyde latex for luminescence-microscopic study of intracellular processes during phagocytosis.

## EXPERIMENTAL METHOD

A two-three-day culture of peritoneal macrophages from albino mice on coverslips, fluorochromed intravitally with acridine orange, was used. Culture and fluorochroming of the macrophages were carried out by the method adopted in the laboratory [1].

Calibrated melamine-formaldehyde latex (mean particle size 5-7 μm) was sterilized by boiling for 2-3 min, washed twice by centrifugation (5 min each time, 2000 rpm) in medium 199, and a suspension was prepared in the same medium. The number of particles was counted in a Goryaev chamber and the suspension was added to the fluorochromed culture of macrophages. The ratio of cells to latex particles in the experiments was 2:1, 1:1, and 1:5. For 1 h after incubation of the cells with latex at 37°C, unphagocytosed latex particles were washed off with medium 199. Cover slips with culture were taken from the tubes after 1, 3, 24, 48, and 72 h, chambers were made on slides for intravital observation, and these were examined in the luminescence microscope.

## EXPERIMENTAL RESULTS

With macrophages and latex in the ratio of 1:5 up to 95% of macrophages phagocytosed with latex particles; from 1 to 6 particles were found in the phagocytic cells and, in certain macrophages as many as 15. With cells and latex in the ratio of 2:1 and 1:1, the parameters of phagocytic activity were correspondingly lower. Because of their regular circular shape and size, the phagocytosed latex particles differed sharply from structural elements of the cell (Fig. 1).

During incubation for 1 h the phagocytosed latex particles, unlike the weakly luminescent particles found in the medium, acquired very bright green luminescence. With macrophages and latex particles present in the ratio of 1:5, because of the bright luminescence of the latex particles, luminescence of the organelles of the macrophages and, in particular, of the cytoplasmic granules (lysosomes) was indistinguishable.

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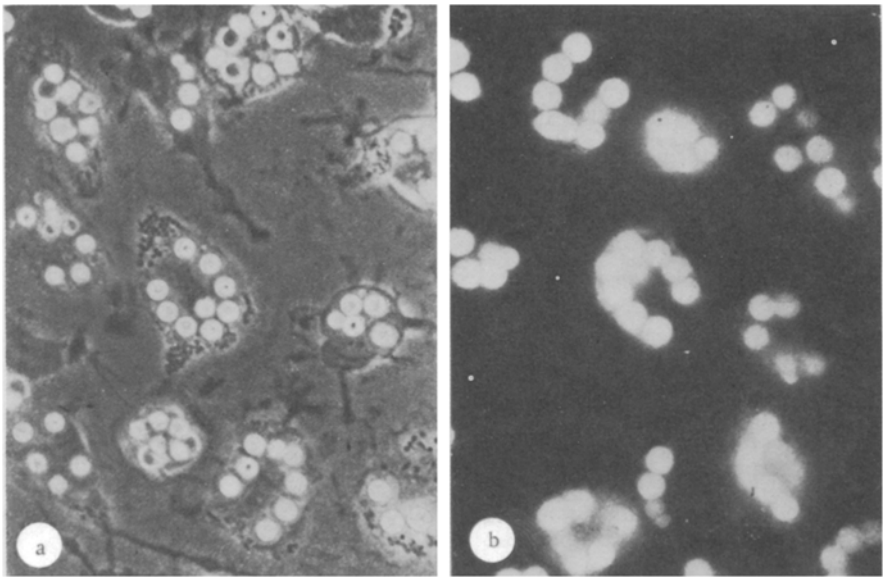


Fig. 1. Melamine-formaldehyde latex, phagocytosed by peritoneal macrophages in culture: a) phase-contrast microscopy, b) luminescence microscopy. 1600 ×.

If cells and latex particles were present in the ratio of 1:1 and 2:1, red cytoplasmic granules (lysosomes) were clearly distinguishable in the **macrophages.** Longer incubation (up to 24 h) of the macrophages with phagocytosed latex led to more intensive spreading of the macrophages compared with the control without latex. Meanwhile changes took place in the character of luminescence of the macrophagal lysosomal apparatus and of the phagocytosed latex similar to those observed previously when other objects of phagocytosis were used [2]. In the course of 24 h the intensity of the red luminescence of the cytoplasmic granules diminished sharply and, at the same time, a gradual change was observed in the color of luminescence of the latex particles from green to yellow and orange-red. Later, toward 72 h of incubation, the color of luminescence of the latex particles became mainly red. During incubation up to 72 h more rapid death of the macrophages in culture or appreciable pathological changes in the cells compared with the control were not observed.

The results are thus evidence that calibrated melamine-formaldehyde latex can be used for cytological (and, in particular, for the intravital luminescence microscopic) study of phagocytosis in a culture of macrophages, and that latex is free from toxicity toward macrophages. Luminescence-microscopic changes in phagocytosed latex particles are similar to those observed by the writers previously during phagocytosis of bacteria, and also of certain other objects, and they are further convincing proof that the change in color of luminescence of phagocytosed particles is not due to their death or intracellular digestion, but depends on transition of the luminescent complex, which is evidently a complex of RNA with acridine orange [3], from lysosomes into a phagosome, containing a latex particle.

The use of latex in conjunction with luminescence microscopy of phagocytic cells fluorochromed with acridine orange enables phagocytosed objects to be distinguished from extracellular objects depending on the character and color of their luminescence.

Consequently, melamine-formaldehyde latex is actively phagocytosed by macrophages in culture and has no marked toxic action on macrophages. Phagocytosed latex particles are clearly distinguishable from cell structures. Phagocytosed latex particles in macrophages fluorochromed intravitally with acridine orange change the character and color of luminescence in the same way as other phagocytosed objects, evidence that these changes are not connected with intracellular death and digestion of phagocytosed objects.

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# HIGH-RESOLUTION ELECTROPHORESIS IN A NEW POLYACRYLAMIDE GEL BLOCK

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Electrophoresis is widely used in modern biochemistry and molecular biology for fractionating and analyzing macromolecules. The undisputed merits of this method are its relative simplicity, sensitivity, high resolving power, and universality.

Polyacrylamide gel (PAG) is an exceptionally convenient supporting medium for the electrophoretic separation of proteins and nucleic acids. The chief advantages of PAG are its inertia, transparency, and the possibility of obtaining a gel of assigned pore size, so that high resolution can be achieved for macromolecules of different sizes. However, in homogeneous PAG it is impossible to obtain high resolution for a mixture of different macromolecules, and also of macromolecules with very close values of molecular weight. This problem has largely been successfully solved by Slater [5, 6], who used PAG with a concentration gradient for analysis of serum proteins, and in this way the resolving power of electrophoresis of proteins in this gel was increased to 1000 daltons (over a wide range of molecular weights).

Effective separation of macromolecules in a PAG gradient is based on the gradual slowing of movement of the particles to be separated on account of narrowing of the pores in the gel, as a result of which the molecules for analysis are effectively grouped into narrow zones, for molecules "in the lead" are retarded more strongly than the "laggards" [2]. The conditions of this kinematic focusing can be described formally as follows:

$$\frac{du}{dx} < 0, \tag{1}$$

where u(x) is the velocity of movement of the test **molecules.** The velocity of movement of the separated molecules during electrophoresis can be represented as follows:

$$u = \mu E, \tag{2}$$

where μ denotes mobility and E the intensity of the electric field. It follows from equation (2) that the velocity of movement of the particle during electrophoresis is proportional to its mobility (which is basically a combination of the properties of the supporting medium and the structure of the molecule itself) and the intensity of the electric field. It will be evident that condition (1) can be satisfied, i.e., the macromolecules for separation can be gradually retarded, by changing μ for E. During electrophoresis in a traditional PAG gradient,

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