living eye. Increased adsorption of hyaluronic acids may perhaps act as the trigger mechanism for subsequent adsorption of proteins on IOL, and in turn, this may induce an inflammatory reaction in the eye. Chronic inflammation in this case is an indication of incompatibility of the material with the surrounding media. Model investigations *in vitro* of this kind can provide speedy and reliable information on the adsorption properties of new materials for endoprostheses in ophthalmic surgery.

LITERATURE CITED

1. V. A. Agafonov, in: Morphological Aspects of Ophthalmology [in Russian], Moscow (1983), pp. 72-74.
2. V. A. Agafonov and S. I. Anisimov, Byull. Eksp. Biol. Med., No. 12, 761 (1984).
3. A. Pirie and R. Van Heyningen, Biochemistry of the Eye, Springfield, Ill., 1956.
4. S. Manabu (editor), Polymers of Medical importance [Russian translation], Moscow (1981).
5. S. N. Fedorov, Implantation of an Artifical Lens [in Russian], Moscow (1977).
6. S. N. Fedorov et al., in: Physiology and Pathology of Mechanisms of Adaptation of the Organ of Vision [in Russian], Vol. 4, Vladivostok (1983), pp. 132-134.
7. R. B. Packard et at., Br. J. Ophthal., 65, 585 (1981).
8. H. Ridley, Trans. Ophthal. Soc. UK, 71, 617 (1951).
9. Zhou Kai-yi, Chin. Med. J., 106, 175 (1983).

A METHOD OF PREPARATION OF LEUKOCYTES FOR ELECTRON MICROSCOPY

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The usual method of precessing and embedding leukocytes for electron-microscopic investigation has certain disadvantages. These disadvantages are characteristic of any material composed of a cell suspension. In order to concentrate the cells in a volume small enough for ultramicrotomy, the suspension is usually centrifuged and all subsequent manipulations are carried out with the solid residue formed in the centrifuge tube. Under these conditions the cell pellet is in contact over the greater part of its surface with the glass of the tube, and only a small part of the surface remains exposed for penetration of the dehydrating and embedding media. A result of this state of affairs is poor dehydration and embedding. In turn, this leads to morphological disturbances in the material and makes the preparation of semithin and ultrathin sections much more difficult. In addition, if the cells composing the suspension are nonhomogeneous, centrifugation before fixation will disturb their uniform distribution in the suspension and will create zones consisting mainly or entirely of cells of only one type. Subsequent fixation consolidates this zonality of the residue (Fig. 1). Usually several pieces are cut out of the fixed and embedded residue. Under these conditions, because of the uneven distribution of the cells in the residue, the object of concern to the investigator may be absent not only in the section, but even in the whole fragment. Epoxide resins with low viscosity,suggested by Spurr [1], and BEEM capsules, which are in short supply, are necessary. However, even theuse of these resins and capsules does not dispense with the need for additional centrifugation of the material when introduced into the capsules. Only if this is done can the necessary density of distribution of the cells be achieved. Centrifugation of capsules is a tedious operation, and if it is not done exactly some resin may fall into the centrifuge vessels, polymerize in them, and make them unfit for further work. Specimens from each centrifuge tube must be extracted with a separate instrument to avoid accidental transfer of the crumbling material from one sample into another.

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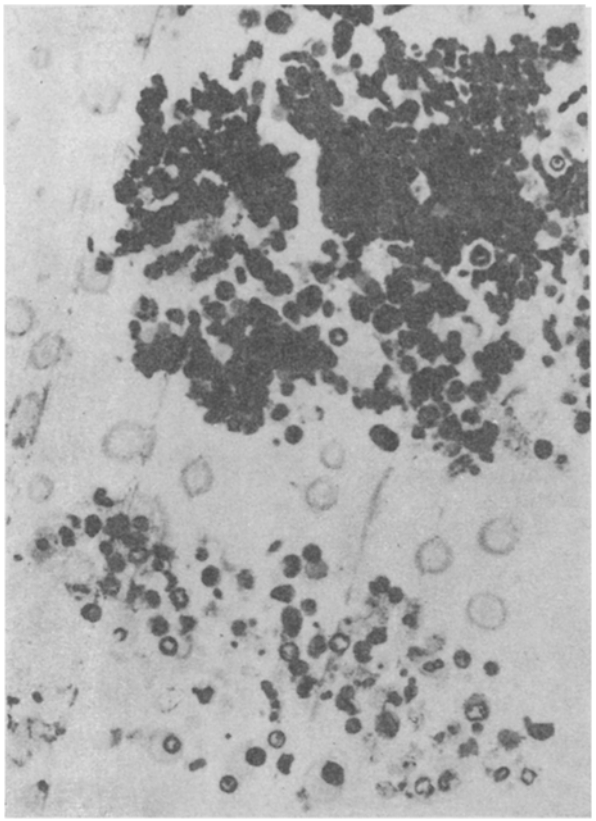

Fig. 1

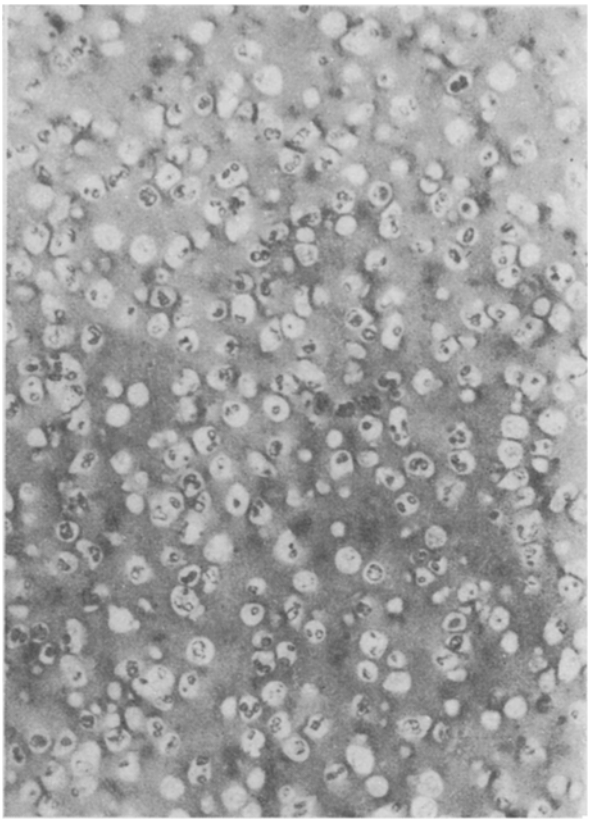

Fig. 2

Fig. 1. Suspension of leukocyte-enriched fraction of blood and bacteria prepared for electron microscopy by the usual method. Grouping of different cells in separate zones can be seen in the semithin section. Stained with toluidine blue. 270 ×.

Fig. 2. Semithin section of leukocyte-enriched blood fraction after double embedding: in gelatin and epoxide resin. Uniform distribution of cells in the block. Stained with toluidine blue. 270 ×.

To eliminate the disadvantages mentioned, the writers have developed a method which is distinguished by the following features: 1) each cell is bathed on all sides with fixing solution, for fixation takes place in suspension and not in the residue; 2) intermediate embedding of the fixed cells in gelatin is introduced; 3) the uniform distribution of cells of different types, characteristic of the suspension, is preserved throught the volume of the gelatin block; 4) dehydration and penetration by epoxide resin take place, not in the residue fixed to the bottom of the centrifuge tube, but in the fragment cut out of the gelatin block and totally immersed in the corresponding liquid. Treatment begins just as in the usual method with preparation of a residue of leukocytes in the centrifuge tube. Usually an attempt is made to keep this residue in the form of a solid pellet. For this purpose the fixing solution is layered carefully above the residue. If, come what may, this pellet is separated from the walls of the tube, sedimentation in the centrifuge is repeated. In our suggested method the residue of leukocytes is at once carefully resuspended in a 1% solution of glutaraldehyde in cacodylate buffer, pH 7.4, to obtain a uniform suspension of cells. The suspension is placed in a refrigerator at a temperature of 2-4°C. To fix the cells it is sufficient to keep the suspension in the glutaraldehyde solution for 2 h, but the cells can be left in it overnight. If fixation lasted 2 h, for the subsequent manipulations the cells must be sedimented in the centrifuge at a speed of 1000 rpm for 5 min. The cells left in the refrigerator overnight will sediment without the use of a centrifuge. After formation of the cell residue, the glutaraldehyde solution must be poured off and the residue rinsed with cacodylate buffer. If during addition of the buffer the residue floats to the top, it must again be sedimented by centrifugation at 1000 rpm for 5 min. The buffer solution is then completely poured off and the inner walls of the tube above the residue are wiped with filter paper. Next, 1 or 2 drops (depending on the volume of residue) of 10% gelatin solution,

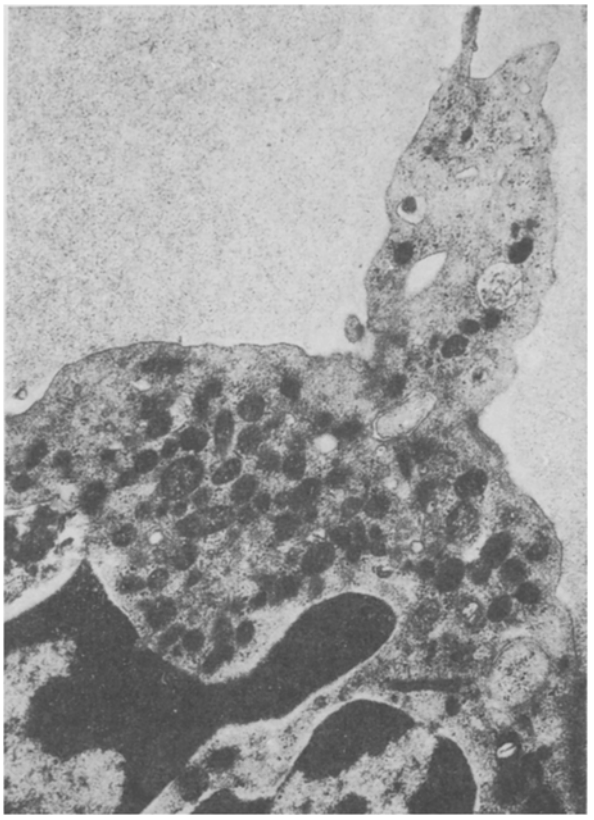

Fig. 3. Electron-microscopic section through part of a neutrophil with a pseudopodium, embedded in gelatin and epoxide resin. 22,000 ×.

heated to 40°C, are added to the residue. The residue is mixed with the gelatin solution by immersing the bottom of the tube periodically in a dish containing water heated to 40°C. The resulting cell suspension in gelatin is cooled in the refrigerator for 1 h. The gelatin block, solidified in the refrigerator, can be separated from the walls of the tube and removed from it by means of a cold thin, narrow spatula. Pieces measuring about 1 mm^3 are cut out of this block on ice and fixed with 1% glutaraldehyde solution. Subsequent manipulations with the fragments do not differ from the usual method of preparing fragments of solid tissues for electron microscopy.

Preliminary embedding in gelatin simplifies the method and enables ordinary epoxide resins and capsules of any kind to be used and, most important of all, it yields material which cuts well. The morphology of leukocytes prepared by the suggested method is illustrated in Figs. 2 and 3. Comparison of the structure of neutrophils prepared by the two methods differs appreciably. After fixation of the leukocytes, neutrophils in the residue are as a rule circular in shape without pseudopodia. After fixation in suspension the cells all have a similar shape with many pseudopodia, which is characteristic of living neutrophils observed under phase contrast.

The method described has been used to prepare not only leukocytes for electron microscopy, but also bacterial cultures, and it is evidently suitable for cell suspensions of all kinds.

LITERATURE CITED

1. A. Spurr, J. Ultrastruct. Res., 26, 31 (1969).