A study of the orignal specimens Nos. 2 and 3 by scanning electron microscopy showed that they differed in the type of distribution of adhesive. The predominant structure in specimen No. 3 was punctate, when most of the adhesive was distributed in the form of punctate zones where the fibers crossed. In specimen No. 2 the adhesive covered the fibers and was distributed in the form of segments in the lattices formed by the crossing fibers. After removal from the model of the wound surface, the structure of specimen No. 3 remained unchanged. However, fibrin clots accumulated in the meshes between the fibers. After removal of specimen No. 2 from the model, separation of the layer of adhesive, loosening of the surface of the fibers, and deposition of fibrin clots on them were observed (Fig. 2a-d). These particular features of the structure of dressing materials affect their atraumatic qualities.

Evaluation of the atraumatic properties of newly developed dressing materials is thus best carried out by the combined use of methods which supplement one another. The simplicity and reliability of the use of cytological study of squash preparations from wounds must be particularly noted.

LITERATURE CITED

1. T. T. Daurova, S. D. Andreev, V. Yu. Kassin, et al., Khirurgiya, No. 5, 100 (1980).
2. M. P. Pokrovskaya and M. S. Makarov, Cytology of the Wound Exudate as an Indicator of Wound Healing [in Russian], Moscow (1942).

USE OF COLLOIDAL LANTHANUM AS AN ELECTRON-MICROSCOPIC TRACER

V. G. Sharov

UDC 57.086.11.546.654

KEY WORDS: colloidal lanthanum; membrane permeability; calcium.

Colloidal particles of lanthanum and precipitates of its insoluble salts are electron-dense and so can be used for various purposes in electron-microscopy. Lanthanum nitrate is most frequently used as the original salt for preparing colloid. On titration of an aqueous solution of $La(NO_3)_3$ with NaOH solution, starting from pH 7.6 a colloidal solution of $La(OH)_3$ with a particle diameter of 2 nm is formed. Particles of colloid do not pass through membranes of normal cells but contrast their surfaces and the narrow slits between them clearly. This property was first utilized for studying the structure of intercellular junctions [8]. It was later found that penetration of colloidal lanthanum through the limiting membrane of the cell of contractile muscle can serve as an early morphological sign of injury to the striated muscle fiber following exposure to heat [3]. Similar observations with colloidal lanthanum were made in a study of cardiomyocytes located in the zone surrounding an experimental myocardial infarct [7]. Penetration of colloidal lanthanum into the sarcoplasm of cardiomyocytes in severe ischemia following experimental myocardial infarction is evidence of the development of early irreversible changes in these cells [5].

The use of colloidal lanthanum as transmembrane tracer has a number of distinguishing features. Different modifications of the classical method are used in different laboratories [8]. The modification described below has been developed and is used in the Department of Human Cardiovascular Pathology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR.

EXPERIMENTAL METHOD

A 3-4% solution of $La(NO_3)_3$ was made up in boiled deionized water and fresh 0.1 and 0.01 M solutions of NaOH were made, also in boiled deionized water. The $La(NO_3)_3$ solution

Department of Human Cardiovascular Pathology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR I. K. Shkhvatsabaya.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 12, pp. 757-759, December, 1981. Original article submitted April 10, 1981.

 0007-4888/81/9212-1748$07.50

was titrated against 0.1 M NaOH to pH 7.7, and the pH was adjusted to 7.80 by means of 0.01 M NaOH. During the titration the solution was continuously stirred by means of a magnetic mixer and the pH values were monitored with a pH meter. It is important to adjust the pH of the solution to values as close to 7.80 as possible but not to overtitrate. If the pH of the solution does not reach 7.80 this indicates that it contains lanthanum particles in the form of ions, and this may lead to misinterpretation of the results. At pH 7.81 the colloidal solution forms a precipitate. If the reagents are of sufficiently good quality, and if contact with phosphorus salts, CO, and CO_2 can be excluded, if the procedure is carried out at room temperature (a fall of temperature to 10°C or below, and also contact of the solutions with any phosphorus salt or with one of the oxides of carbon leads to rapid precipitation of the colloid), the colloidal solution is completely transparent and does not opalesce. Sometimes very small quantities of macroscopically visible thread-like impurities appear in the solution but they do not affect the quality of the results. Glutaraldehyde fixative is next prepared from 25% glutaraldehyde, 0.1 M cacodylate buffer (pH 7.4), and freshly prepared colloidal solution of $La(OH)_3$, so that it contains 1-1.5% of colloidal lanthanum and 3.5% of glutaraldehyde. Phosphate buffer must not be used for it causes rapid precipitation of the colloid. Before fixation the tissue is rinsed in 0.1 M cacodylate buffer to remove blood containing phosphates. Pieces of tissue are then fixed in freshly prepared fixative with constant stirring and at room temperature for 4-24 h. The pieces of tissue are rinsed in 0.15 M cacodylate buffer, prepared from 0.5 M cacodylate buffer (pH 7.4) by dilution with the colloidal lanthanum solution, and fixed in 1% osmic acid in 0.2 M cacodylate buffer containing colloidal lanthanum for 1 h at room temperature. The pieces are then again rinsed in 0.25 M cacodylate buffer containing colloidal lanthanum, after which they are dehydrated in 70% alcohol, prepared from 100% ethanol and colloidal lanthanum (5-10 min) and in 100% ethanol (four changes, 1-2 min in each). The colloidal lanthanum used for dilution must have pH 7.80. To fix the colloid which settles in the cells, cold 100% ethanol can be used. This is followed by two changes, 1 min each, in acetone or propylene oxide. The tissue is saturated with a mixture of acetone or propylene oxide with resin (1:1) for 5-10 min with constant stirring and is poured into capsules.

EXPERIMENTAL RESULTS

By the method described above colloidal lanthanum does not always stain the surface of the limiting membranes, but is well preserved inside the cells as an electron-dense deposit mainly around mitochondria [1, 2]. To study the membrane surfaces it is most convenient to use the classical method [8] or its modification [9]. As the result of a series of investigations in Buja's laboratory (Department of Pathology, Texas University, Dallas, USA) it has been shown that membrane defects detectable by the aid of colloidal lanthanum are formed during life and that fixation of the material does not affect their formation [4]. On the basis of these findings colloidal lanthanum can be recommended as the most informative and convenient transmembrane tracer. The diameter of the colloidal lanthanum particle is between half and one tenth of the size of particles of all other known tracers [6]. Since the method is not an intravital one, penetration of the colloid particles through the membrane by endocytosis is ruled out. For the same reason, it can be easily used on any material, including human.

The formation of early defects in membranes of cells with reversible changes that are still functioning in cardiomyocytes has been shown to be connected with the special features of calcium metabolism in them [1, 2]. In particular, fenestration of membranes of the internal organelles is formed only as a result of their direct contact with excesses of calcium, even in irreversibly changed cardiomyocytes. The colloidal lanthanum method can accordingly be recommended for the study of membrane defects, above all in cells in which calcium plays an important role in the regulation of energy metabolism and maintenance of the integrity of the membranes. These cells include cardiomyocytes, other contractile cells, and neurons. For the same reasons, it would evidently be incorrect to compare the degree of injury to cells of different types with the aid of colloidal lanthanum (for example, to study whether injury to endotheliocytes is primarily relative to cardiomyocytes), especially if the mechanism of formation of the defects of their membranes has not been studied beforehand. Interpretation of the results will be much more difficult, for the appearance of membrane defects in different cells, marked by differences in metabolism (including calcium), may be evidence of processes which do not lend themselves to comparison.

LITERATURE CITED

1. V. G. Sharov, R. B. Jennings, and H. K. Hotchkiss, Arkh. Patol., No. 10, 35 (1980).
2. V. G. Sharov, R. B. Jennings, H. K. Hotchkiss, et al., in: Energy Transport, Protein Synthesis and Hormonal Control of Heart Metabolism, Bethesda (1980), pp. 373-390.
3. H. D. Fahimi and R. S. Cottran, Am. J. Pathol., 62, 143 (1971).
4. H. K. Hagler, K. P. Burton, L. Sherwin, et al., in: Scanning Electron Microscopy, ed. O. Iohavi and R. P. Becker, Vol. 2, Chicago (1979), pp. 723-732.
5. H. K. Hawkins, V. G. Sharov, and R. B. Jennings, in: Ninth International Congress on Electron Microscopy, Vol. 2, Toronto (1978), pp. 306-307.
6. M. A. Hayat, Positive Staining for Electron Microscopy, New York (1975).
7. S. Hoffstein, D. E. Gennaro, A. C. Fox, et al., Am. J. Pathol., 79, 207 (1975).
8. J. P. Revel and M. J. Karnovsky, J. Cell Biol., 33, C7 (1967).
9. M. Shaklai and M. Tavassoli, J. Histochem. Cytochem., 25, 1013 (1977).

"MICROPIPET" METHOD OF RECORDING FAST INWARD IONIC CURRENTS OF SINGLE HEART MUSCLE CELLS

Yu. I. Zil'berter, E. N. Timin, Z. A. Bendukidze, and N. A. Burnashev

UDC 612.172.4.014.2.014.421.7

KEY WORDS: voltage clamp method; isolated heart cells; fast ionic currents; micropipet method.

Recently developed techniques for working with single heart cells provide an approach to the problem of creating an effective voltage clamp system for measuring fast ionic currents [2, 4, 6]. To obtain reliable voltage clamping and adequate resolutions between the capacitive current and the initial phase of the fast ionic current, it was decided to use the idea of the suction micropipet for recording the integral currents from a small area of the membrane of a single heart cell.

EXPERIMENTAL METHOD

Single heart cells were isolated by the method in [8]. The heart was removed quickly from rats weighing 200-300 g under ether anesthesia. The heart was then perfused through the aorta under a pressure of 90 cm water with a standard solution of the following composition: NaCl 140 mM, KCl 5.4 mM, $MgCl_2$ 1 mM, glucose 11 mM, Tris 10 mM, human serum albumin (from Reanal, Hungary) 1 mg/ml (pH 7.4). The temperature of the solution was 37°C. The solution was saturated with oxygen. Collagenase (type 1, from Sigma, USA) and $CaCl_2$ to final concentrations of 0.4 mg/ml and 25 μM respectively were added 4 min later to the perfusion fluid and perfusion continued for 15 min. The ventricles were then cut off and placed in 40 ml of a standard solution containing 0.2 mM $CaCl_2$. The tissue was then cut into pieces with scissors and the resulting cell suspension was filtered through nylon gauze. After sedimentation of the cells the supernatant was carefully poured out and replaced by 40 ml of a fresh standard solution containing 0.9 mM $CaCl_2$.

A cell chosen under the microscope (magnification 155) was transferred by means of a polyethylene pipet into the working chamber. The cell was then fixed in the pore of a V-shaped suction pipet [1] (diameter of pore 5-6 μ), in which a pressure of 50 cm water was created. Both the chamber and suction pipet contained standard solution with 0.9 mM $CaCl_2$. The experiments were carried out at room temperature (20-22°C).

A scheme of the experimental arrangements is shown in Fig. 1A. A command potential is applied to electrode E1. The signal on electrode E2 is held at the zero level by means of a

Scientific Techniques Department, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. Laboratory of Functional Biophysics, Research Institute for Biological Testing of Chemical Compounds, Kupavna, Moscow Region. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 12, pp. 759-761, December, 1981. Original article submitted April 6, 1981.

 0007-4888/81/9212-1750$07.50