propria, involving the participation of many lymphocytes and plasma cells, evidence of the development of inflammation with manifestations of local immune reactions in this part of the intestine.

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# ACCUMULATION OF LIPOFUSCIN GRANULES IN ACUTE MYOCARDIAL INFARCTION AS A MODEL OF CELL AGING


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**KEY WORDS:** lipofuscin; myocardial infarction; electron microscopy


The aging process in man and animals is characterized by the accumulation of specific structures in cardiomyocytes [1, 7, 8, 10, 12], known as lipofuscin granules (LG). They are generally considered to be a kind of marker of the kinetics of aging [11, 13, 15, 16]. However, natural aging lasts quite a long time, and investigators have therefore attempted to produce a model of aging which would narrow this time interval. For this purpose, various cell cultures, in particular, have been widely used [2-4, 6, 9, 14]. The main parameter used to monitor the aging process in this case is a change in the number of LG. The results we obtained could be realistically interpreted only recently, when the membrane hypothesis of aging has become more widely adopted [11, 13, 15, 16]. Establishment of the fact that a key role in the formation of LG is played by the endoplasmic reticulum [3, 10], and also data indicating that the drug centrophenoxine (meclofenoxate) reduces the number of LG in cells, through its action at the receptor level on the plasma membrane [2, 4, 9], has made possible the development of a membrane-receptor hypothesis of aging, which is based on regular cellular mechanisms.

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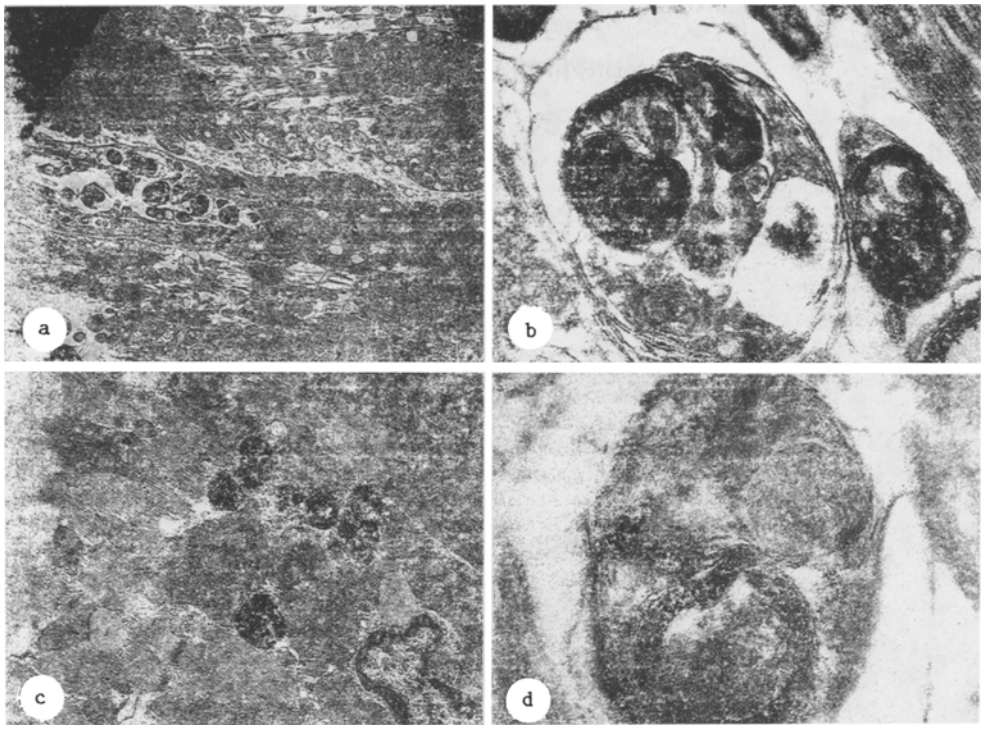


Fig. 1. Concentration (cluster at the light level) (a, b) and individual (c, d) LG in intact myocardium. Magnification: a) 10 $\mu$, b, c) 1 $\mu$, d) 0.5.

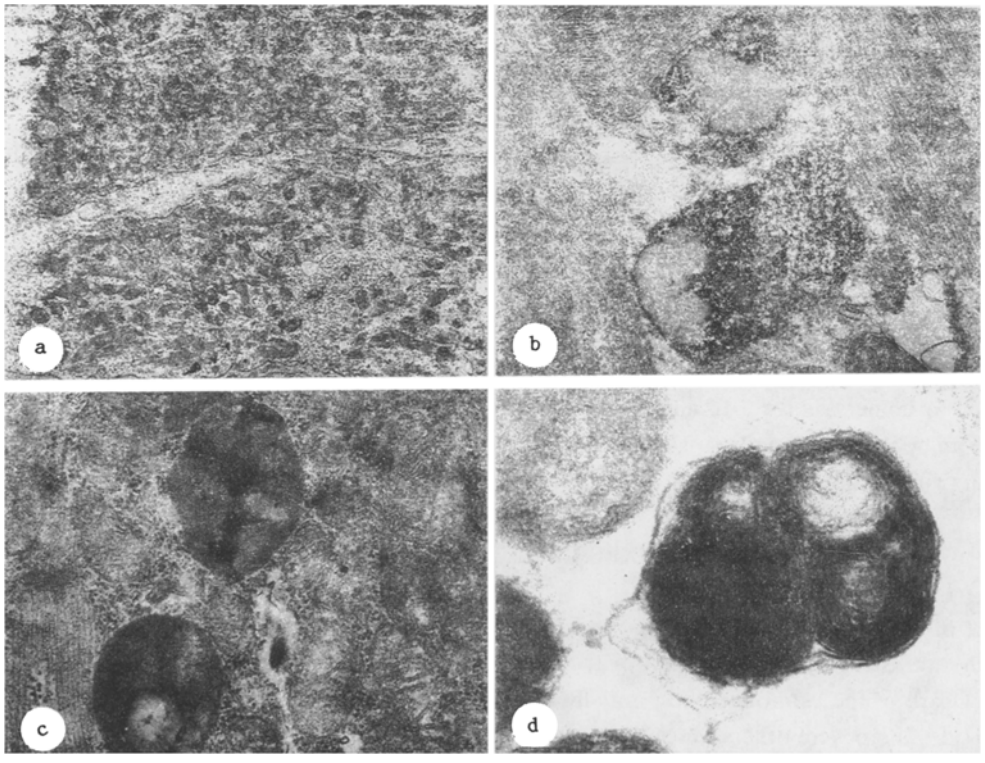


Fig. 2. Different ultrastructural types of LG in cardiomyocytes from infarct zone during 1st day of ischemia. Magnification: a) 4200; b, c) 35,000; d) 7000. Scale: a) 10 $\mu$; b, c) 1 $\mu$; d) 0.5 $\mu$.

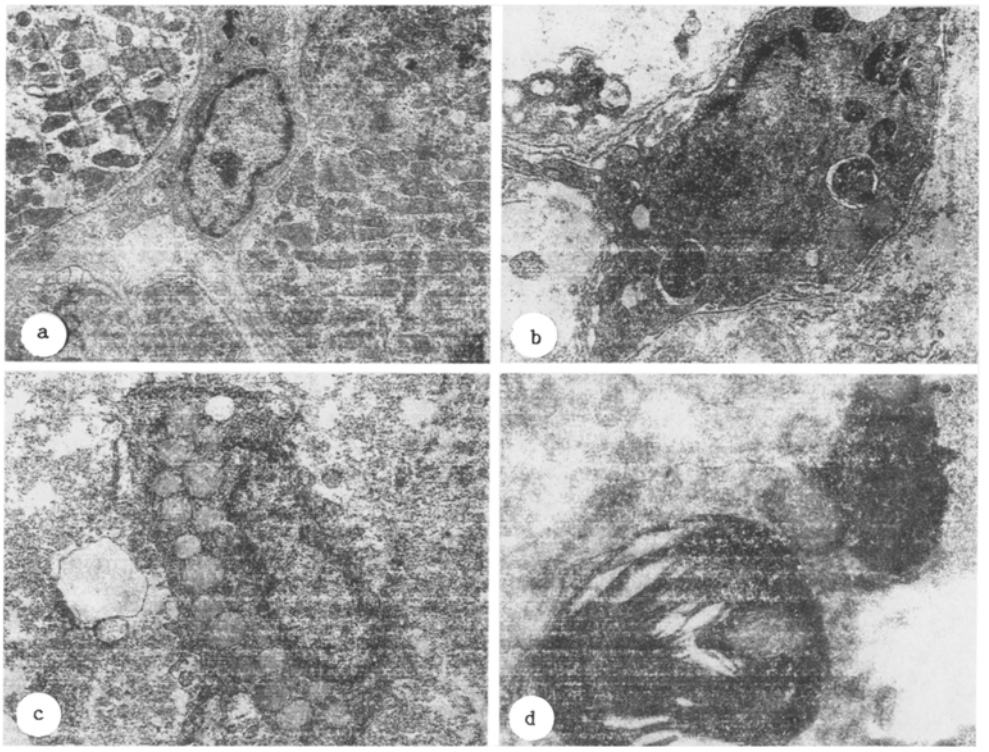


Fig. 3. Different ultrastructural types of LG in connective-tissue (fibroblast-like ) cells in zone of myocardial infarct. Magnification: a) 4200; b, c) 8400; d) 35,000. Scale: a) 10 $\mu$; b, c) 5 $\mu$; d) 1 $\mu$.

In the investigation described below, an artificially created acute myocardial infarct was used as a model of the natural aging process of myocytes of young rabbits, with monitoring of the kinetics of LG accumulation at the electron-microscopic level.

## EXPERIMENTAL METHOD

Experimental acute myocardial infarction was induced in young noninbred rabbits aged under 3 months and weighing about 2 kg, by applying Lavsan ligatures to the descending branch of the left circumflex coronary artery, under general thiopental anesthesia (35 mg/kg), after left-sided thoracotomy with resection of ribs IV-V, and with opening of the pericardium. Specimens of left ventricular myocardium from the central region of the infarct, the boundary zone, and the intact myocardium were fixed 1, 2, 3, 7, 14, and 30 days after ligation of the coronary artery. The material was fixed in 1% $OsO_4$ solution or in 2.5% glutaraldehyde solution, and then postfixed for 1 h in 0.5 $OsO_4$. The fixation time in each solution was at least 6 h. After dehydration in solutions of increasing concentration of ethanol and in 100% acetone the preparations were embedded in Epon-812. Ultrathin sections were stained by immersion for 5-10 min in a 70% solution of ethanol saturated with uranyl acetate, and for 15-20 min in lead citrate made up by Reynolds' method. The preparations were examined in the JEM-7 electron microscope (JEOL, Japan) under an accelerating voltage of 80 kV.

## EXPERIMENTAL RESULTS

Disturbance of the coronary circulation and the accompanying ischemia of a particular part of the myocardium quickly led to changes in the ultrastructural organization of the cells located in it. During the first 3 days a zone of necrosis formed below the ligature. Death of the cardiomyocytes, initially mosaic in character, was observed in this zone, i.e., side by side with damaged cardiomyocytes there were others which were normal. Death of the cells was preceded by swelling of the mitochondria with translucency of the matrix and fusion of the cristae, supercontraction and destruction of the myofibrlls, condensation of the nuclear heterochromatin, and swelling of the cells themselves. However, in several parts of the ischemic region, the degenerative changes took place at different rates. Whereas in the central part of the infarct the degree of ischemia was so great that the cardiomyocytes quickly died, nearer the periphery, degeneration of the cardiomyocytes took place more slowly. Degeneration of

cardiomyocytes in the myocardium must therefore be regarded as a two-parameter process, depending on the distance from the center of the lesion and the time after creation of the infarct. Naturally the degenerative changes progressed most rapidly in the center of the infarct zone.

Investigation of the intact areas of the myocardium in young rabbits showed that individual LG and concentrations of them were present in the cardiomyocytes, although very rarely (Fig. 1). Concentrations of LG could usually be found near the nucleus in the zone where the myocardium changes into connective-tissue tendons. These concentrations consisted of individual LG 0.5-0.8 $\mu$ in diameter, and differing in shape and in their internal contents. Connective tissue cells in the intact myocardium were characterized by total absence of LG.

In principle, the various LG in cardiomyocytes can be subdivided by their ultrastructure into three types: 1) granules surrounded by a membrane and having more or less uniform electron density, resembling lipid (lipoprotein) drops (LD); 2) granules with uneven electron density, incorporated into the membrane and consisting of one or more LD, arranged side by side with several clearly distinguishable membrane fragments of curvilinear profile; 3) granules containing densely packed membranous structures, sometimes separated into layers. Granules of the first type resemble those observed in the tissues in neuronal ceroid lipofuscinoses [11]. The second type, the one most widely distributed during aging in several pathological conditions, is considered to be the "classical" type of LG. Granules of the third type, the so-called "myelinlike structures," or "residual bodies," are known in the gerontologic literature also under the name of "fingerprints" [13]. This conventional morphological subdivision of LG describes the hypothetical sequence of their maturation [11, 13, 15, 16]. With age not only the number of LG in animals increases, but also the proportion of the Type 3 granules.

The number of LG of the first and second types in the intermyofibrillar space in the cytoplasm of the cardiomyocytes in the central part of the infarct zone increased during the first 3 days after ligation of the coronary artery. LG of Type 3 (membranous, Fig. 2) also were occasionally found.

Besides degeneration of the cardiomyocytes, swelling of the intercellular space and its filling with connective-tissue cells took place, i.e., contractile elements were replaced by supporting elements. Fibroblastlike cells and macrophages, migrating into the focus of infarction, gradually became enriched with LD and LG of Types 2 and 3 (Fig. 3). Maturation of connective tissue took place with the appearance of collagen fibrils. This process was almost completely finished by the end of the 2nd week.

The area of the zone of infarction increased during the 1st day after its creation. Depending on the character of the collateral circulation, the velocity, duration of increase, and final area of the zone differed. However, after the 2nd week, in all cases a tendency was observed for the area of the lesion to decrease. In this connection the visually determined boundary of the lesion was not constant in its location.

The ultrastructure of the boundary zone contained many LG of different types, in both cardiomyocytes and connective-tissue cells. When one month had elapsed after the operation and the connective-tissue scar was fully formed, all the above-mentioned types of LG could still be detected in those cardiomyocytes, and particularly clearly, connective-tissue cells which remained in the boundary region.

Experimental myocardial infarction is certainly a much more complex process than culture of a single type of cells, but its advantages for the study of aging are determined by the ability to pick out common elements in the behavior of cells with different functional roles during both degeneration and regeneration of the tissue. Regenerative processes are no less important than degenerative, for it is not yet clear whether the appearance of LG during aging is part of the regulatory programme of individual death of the cell or of its protection against internal stress. Results obtained in the present investigation showing the presence of LG in cardiomyocytes and fibroblastlike cells, both at the stage of development of the infarct and regeneration of the myocardium do not permit a choice to be made between these two possibilities. However, a common feature of both programmes is activation of protein and lipid synthesis in the endoplasmic reticulum.

Incidentally, the infarct model we have examined is an excellent object with which to study the ultrastructural organization of the contractile system. During degeneration of the myofibrillar apparatus intracellular membranous connections are better revealed. It can be clearly seen that the intracellular membranes of cardiomyocytes form a system of compartments perforating the sarcomeres in the region of the Z- and N-lines and of the H-zone, which is interconnected both with each other and with the mitochondria. This interconnected system of compartments in the intact cardiomyocytes evidently provides for transport of various ions and low-molecular-weight compounds that are involved in the regulation of muscular contraction. Synchronization of contraction within the cell may also be effected through this system.

Thus the fluorescent granules which accumulate in the zone of myocardial infarction in young rabbits [5], as regards their electron-microscopic characteristics, are LG which, most probably, are at different stages of their development [11, 13, 15, 16]. Accumulation of LG against a background of ischemia in cells which differ in their functional role and significance (cardiomyocytes and fibroblastlike cells), during both degeneration and regeneration, in the presence of intensive protein synthesis with phosphorylation of proteins and lipids simultaneously, suggests a common mechanism of genesis of this particular organelle, in which the principal role is played by the endoplasmic reticulum [3, 8, 10, 12]. From the standpoint of narrowing of the time interval of the processes of cellular aging, the zone of the myocardial infarct and the changes described above taking place in it, correspond to a unique type of culture of different cells in the tissues under natural conditions.

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