5-7 days, and when indicated for use together with solutions of drugs, with daily redressings, the state of deep burn wounds could be improved and the wounds prepared for skin autografting. With early covering of burn wounds with charcoal materials, the local use of strong antibacterial agents could be reduced. Several clinical cases could be cited.

Thus ACC and dressings based on it, in combination with necrolytic and antimicrobial agents, led to cleansing of burn wounds from purulent secretions, improved preoperative preparation of deep burn wounds, and accelerated the epithelization and healing of superficial burns.

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ORIGIN OF LIPOFUSCIN GRANULES IN HYBRIDOMA CELL CULTURE

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KEY WORDS: lipofuscin; electron microscopy; hybridoma cell culture.

Lipofuscin granules (LG) are lipoprotein-pigment inclusions which accumulate in the cells of various organs and tissues of man and animals during natural aging [15] or in pathology [7]. This phenomenon in pathology is particularly marked in genetically predisposed diseases of premature aging: the ceroid lipofuscinoses [5]. Investigations of LG accumulating with age in situ has not shed light on their genesis or functinoal role in the cells [5, 7, 10, 15]. Attempts have been made to create a model of their appearance in cells of young animals [3, 9], but this likewise has not yielded any basically new results. LG formation has recently been demonstrated in certain organotypic and cell cultures [12, 16]. However, ideas on the mechanisms of the intracellular genesis of LG still remain in dispute. Some workers consider that the precursor of LG is one of the intracellular organelles, for example mitochondria [16], whereas others associate their formation with a complex series of biochemical reactions taking place in several cell organelles [14]. Accumulation of LG in a culture of retrovirus-transformed hybridoma cells as found previously, and as a result of this it became possible to study LG on a relatively simple and convenient cell model.

We have studied the ultrastructure of LG in hybridoma cells. This paper gives details of their structure, and provides evidence in support of LG formation from the endoplasmic re-

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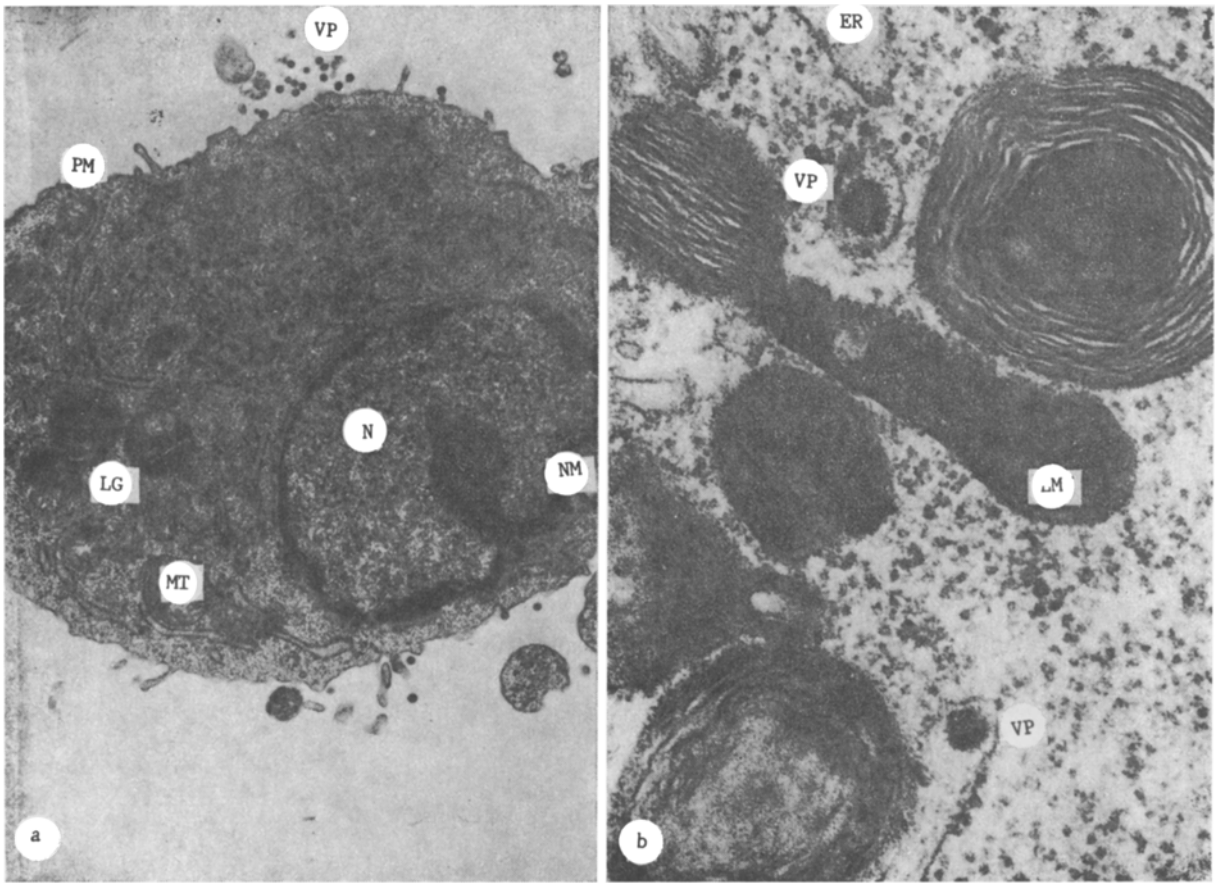

Fig. 1. Ultrastructure of hybridoma cell (a, b). Cluster of LG (light microscopy) in a hybridoma (b). Scale 0.5 μ. Magnification: a) 10,000, b) 80,000. Here and in Figs. 2 and 3: N) nucleus, NM) nuclear membrane, PM) plasma membrane, MT) mitochondria, VP) virus particle (fully formed retrovirus), ER) endoplasmic reticulum, LG) lipofuscin granules, LM) membrane surrounding lipofuscin granule.

ticulum (ER); biochemical and genetic investigations, the results of which support our ideas, also are analyzed.

EXPERIMENTAL METHOD

Hybrids of somatic lymphoid cells (hybridomas) were obtained by fusion of myeloma cells of mouse line Sp2//0-Ag14 with spleen cells of BaLb/c mice, immunized with bacteriophage λ and taken on the 4th day after the last injection. The hybridomas were cultured by the method described previously [1]. Cells at the end of the logarithmic and beginning of the stationary phases of growth were sedimented by centrifugation (1000g, 5 min) and fixed for 2 h in 2.5% glutaraldehyde, then postfixed for 2 h in 0.5% OsO_4 solution. Both fixatives were made up in 0.1 M Na-cacodylate buffer (pH 7.4). The hybridoma cells, dehydrated in ethanol-acetate, were embedded in Epon and Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in the JEM-100B electron microscope (JEOL, Japan), under an accelerating voltage of 80 kV.

EXPERIMENTAL RESULTS

The ultrastructure of a hybridoma cell is shown in Fig. 1a. Besides the known organelles, the cell cytoplasm contained clearly distinguishable electron-dense LG of different shapes and different internal contents, with clear ultrastructural individuality. They were found in hybridoma cells both as single LG and also as clusters (Fig. 1b), consisting of various numbers of separate LG. The diameter of LG varies from 0.3 to 0.8 μ, but larger groups also were seen. With age of the culture (at the end of the logarithmic and beginning of the stationary phases of growth) both the number of LG and their diameter in the hybridomas increased.

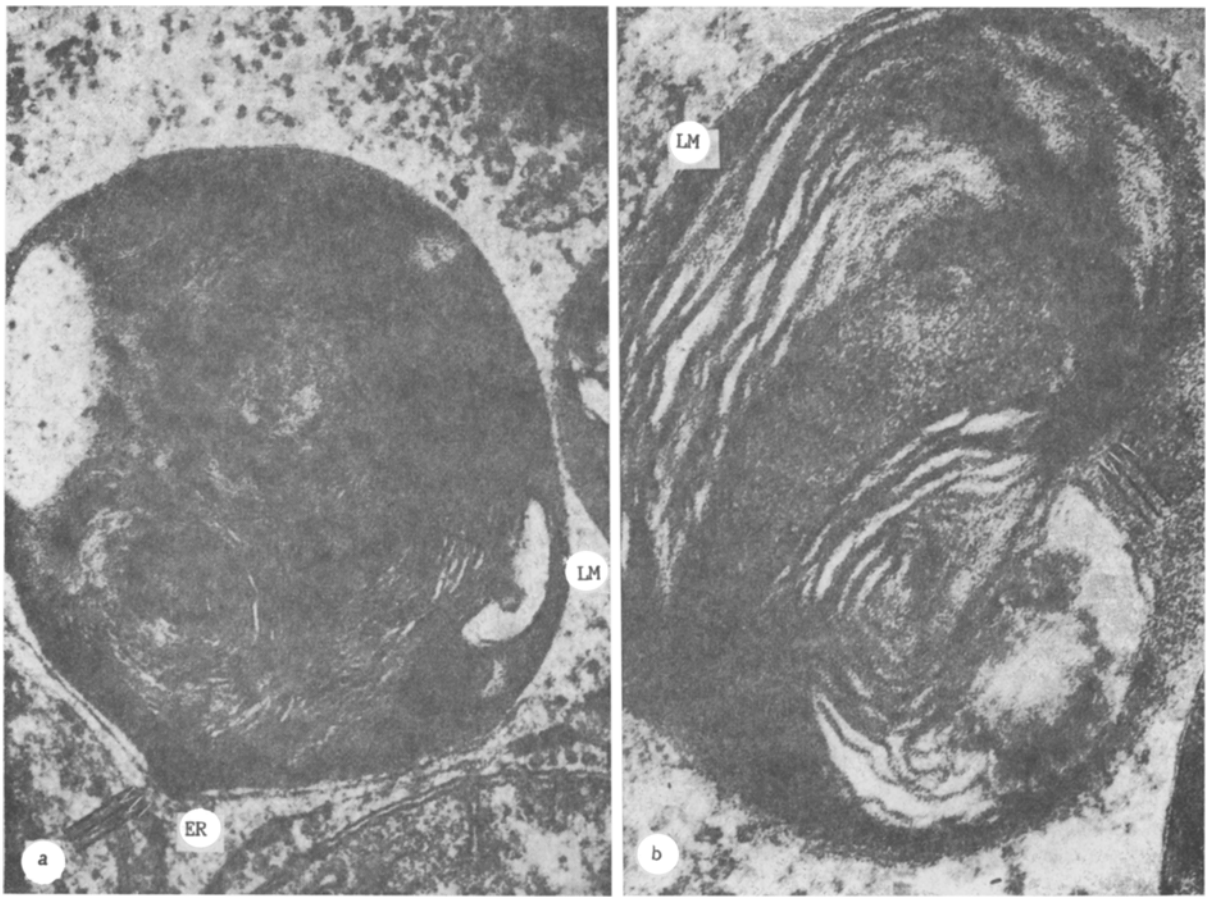

Fig. 2. Ultrastructure of single LG (a, b). Arrows indicate connection between ER and LG (a) and existence of multiple membranes in LG (b). Scale 0.5 μ. Magnification: a) 80,000, b) 120,000.

Each LG is surrounded by a bilayered membrane (Figs. 2 and 3), about 7 nm in thickness. The membrane of LG is a continuation of the membrane of the smooth ER. Communication between the internal contents of LG and a cistern of ER can be clearly distinguished in Fig. 2a. The main distinguishing feature of the ultrastructure of ER is the fact that three characteristic ultrastructural elements can be found in nearly all of them: electron-translucent amorphous vacuoles, an electron-dense fine-grain matrix, and lamellar-membranous (osmiophilic) formations. Relations between these elements vary, and it is this which determines the differing ultrastructural picture of individual LG. However, the following basic ultrastructural forms of LG can be distinguished morphologically: LG with more or less uniform electron density (preceroid and ceroid), LG with the above-mentioned three components clearly defined (the ordinary form of LG), and LG solidly packed with the lamellar-membranous component. This last type of LG has been called myelin-like structures or fingerprints [5, 7, 15]. The lamellar-membranous component in some LG may be folded compactly to form multiple-membranous structures with an interval of about 7 nm. In other LG the lamellar-membranous component has a tendency to separate into undulating layers. The impression is created of "peeling" of the internal matrix of LG. The undulating membrane-like separation of the layers of the internal matrix of LG is clearly distinguishable in Fig. 3a.

It will be noted that myeloma retroviruses were found in the internal space of LG (Fig. 3a). Since most retroviruses were gathered on the membranes of the cisterns of the hybridoma ER, their discovery in LG may be a further indication that LG are derivatives of ER. During luminescence microspectral investigations of LG the exciting ultraviolet radiation not only triggered photochemical reactions inside LG [2, 4], but also acted as a powerful agent after which some LG left the cell and emerged into the extracellular space. Although we were able to find LG near the cell membrane (Fig. 3b), induction of movement and actual movement of LG itself cannot yet be explained. This may be a topic for future research.

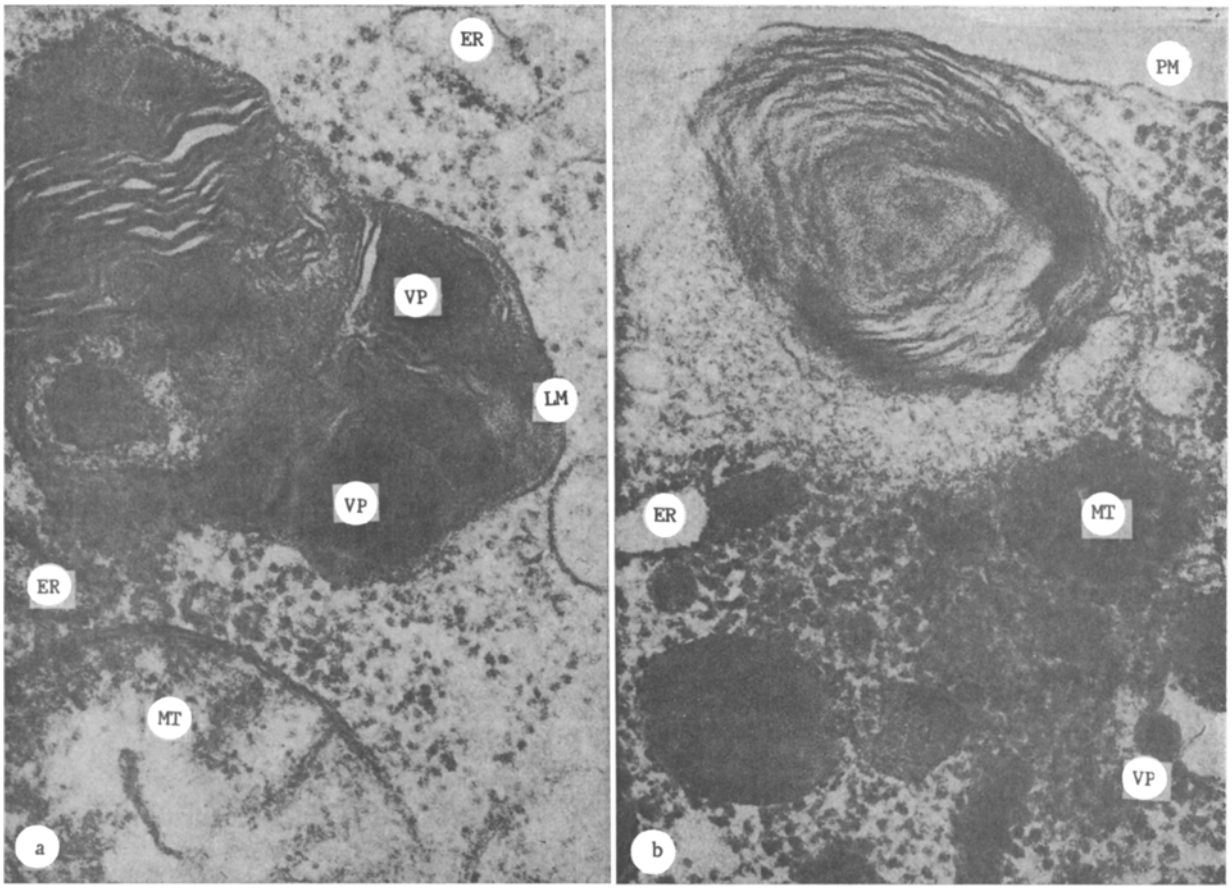

Fig. 3. Ultrastructure of a single LG with viruses (a) and LG near the cell membrane (b). Scale 0.5 μ. Magnification: 80,000.

Evidence for the formation of LG from ER also is given by data in the literature, including ultrastructural evidence recalling that given here: the formation of LG from an oblong form of smooth ER, resembling a cube [8]. However, the investigation cited has not received proper attention from research workers as a whole, for starting with the work of De Duve, most attention has been concentrated on lysosomes. The histochemical discovery of acid phosphatase, considered at that time to be an enzyme of purely lysosomal origin, connected functionally with autolysis of degrading elements in the cell, in LG served as the basis for creation of the hypothesis that lysosomes are involved in LG formation.

Interest in phosphatases as elements of the system determining the degree of phosphorylation of proteins and also, perhaps, of lipids, has increased at the present time in connection with many different aspects of regulation of cellular activity. It is interesting to note that dephosphorylation of phosphodolichol, a polyisoprenoid (retinoid) found in LG, acts as the rate-limiting factor in N-glycosylation of proteins [6] on the rough ER. In this case, after synthesis of the oligosaccharide bound with the polyisoprenoid, and its addition to the asparagine of the protein, phosphatase is essential for recycling of retinoids. Thus the early data on the presence of phosphatase activity in LG has acquired a new interpretation.

Data in the literature in support of a connection between processes of syntheiss of glycoproteins, glycolipids, and glycosaminoglycans of ER and LG formation in particular, show that LG accumulate in abundance when there is an abnormal increase of synthesis of heparan sulfate and gangliosides in ER, in the Sanfilippo Syndrome (mucopolysaccharidosis) [13]. On the other hand, neuronal ceroid lipofuscinosis [11] is accompanied by the appearance of defective forms of N- and O-bound oligosaccharides of glycoproteins in LG. Since these oligosaccharides are synthesized on membranes of ER, their discovery in LG is weighty evidence in support of our point of view.

Thus the direct and indirect evidence of the origin of LG from ER draws attention to the possibility of a link between LG formation and the presence of a disturbance in the gly-

cosylation of proteins and lipids, the result of which may be loss of functionally important cell surface receptors and, in turn, this may be a new and interesting aspect of the problem of aging as a whole.

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ELECTRON-AUTORADIOGRAPHIC STUDY OF HUMAN EPIDERMOCYTES IN CULTURE

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To treat patients with extensive burns, attempts have been made to graft layers of autologous epidermis, grown in the laboratory, to the affected region of skin. This advance in the treatment of burns has developed in recent years and is based on the most recent achievements of cell biology in the field of culture methods. At the present level of development of this science, it appears very promising. Its introduction into medical practice is entirely determined by the possibility of controlling proliferation and differentiation of cells in vitro. Several stimulators [6, 8-10, 12] and methods of cultures[4, 5, 7], capable of increasing the rate of multiplication of epidermocytes, have recently been suggested. Cytochemical and morphological criteria for assessing the level of differentiation of epidermocytes also have been described [3, 11]. An effetive method of studying cell proliferation and differentiation is electron-microscopic autoradiography, which combines an objective and precise

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