$$\ln N_{st} \sim K\Delta\Phi \quad \text{or} \quad \ln \frac{N_{st}(\Delta\Phi)}{N_{st(o)}} = K\Delta\Phi + \text{const},$$

where $N_{st}(\Delta\Phi)$ denotes the level to which the population tends after absorbing energy $\Delta\Phi$, and $N_{st(o)}$ indicates the number of the population in the absence of irradiation.

The experimental data on dependence of the number of the population on dose at long times after irradiation show that these functions are valid and can constitute the basis for more detailed models of the dynamics of the mitochondrial population. The suggested model for a functionally active class of mitochondria is characterized by a high degree of agreement with the experimental results, and for that reason similar assumptions can be used with advantage for the numerical assessment of data in morphological and functional studies of cell organelles and, in particular, during determination of the effect of either pathological or of other damaging factors on the mitochondrial population.

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LIPOFUSCIN DETECTION IN HYBRIDOMA CELL CULTURES

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It was shown a long time ago that lipid-rich cytoplasmic inclusions, known as lipofuscin granules (LG), accumulate in many postmitotic cells of various mammalian organs and tissues with age, and that they are indeed a characteristic sign of aging [5, 7, 8, 12]. The function of these granules remains a topic for debate [3-5, 7, 8, 12]. Elucidation of the mechanisms of formation and accumulation of LG and their functional role in the cells remains an important task not only of modern gerontology but also of cell biology.

Cell cultures from various tissues and organs provide convenient model systems for research in gerontology [6, 7, 9, 10, 13-15]. Various organotypical [6, 13, 14] and cell cultures [6, 9, 10, 15] have been used for this purpose.

The aim of this investigation was to show that hybridomas constitute a very convenient model with which to study LG formation and accumulation, for they are natural producers of various monoclonal antibodies and they occupy an important place in medico-biological research and modern biotechnology.

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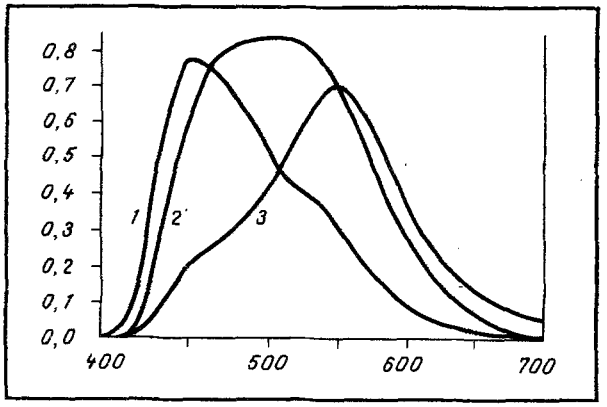

Fig. 1. Intrinsic luminescence spectra of LG clusters in hybridoma cells. Abscissa, wavelength (in nm); ordinate, intensity of luminescence (in relative units).

EXPERIMENTAL METHOD

Hybrids of somatic lymphoid cells (hybridomas) were obtained by fusing mouse myeloma cells of strain Sp2/0-Ag14 with spleen cells of BALB/c mice immunized with bacteriophage λ and used on the 4th day after the last injection [2]. The clones were tested for the presence of specific antibodies to phage λ 2-4 weeks after fusion by determining the inactivating ability of the culture medium. Subsequent cloning was carried out by the low density seeding method (not more than one cell per flask), using mouse peritoneal macrophages as the feeder. Cells of the myeloma line and hybridoma cells were cultured in Eagle's medium in Dulbecco's modification ("Gibco," Scotland) with the addition of 10% embryonic calf serum ("Gibco," "Serva," West Germany) or 5% hog serum and antibiotics: penicillin and streptomycin 100 U/ml each, gentamicin 80 U/ml.

Soviet luminescence microscopes (ML-3 and LYUMAM-IZ, Leningrad) were used for microscopic investigation of the hybridomas. For spectral analysis of LG a microspectrofluorometer for morphological research [1] was used, with excitation wavelength of 365 nm, separated by a UFS-6-3 filter. The diameter of the microspectrofluorometric probe was 7 μ.

The ultrastructural analysis was carried out on a JEM-100B electron microscope ("JEOL," Japan) under an accelerating voltage of 80 kV. Cells were sedimented by centrifugation (1000g, 5-10 min) and fixed in 2.5% glutaraldehyde solution for 34 h, followed by postfixation for 4 h in 0.5% OsO_4 solution. Both fixatives were prepared in 0.1 M Na-cacodylate buffer (pH 7.2-7.4). After dehydration in a series of alcohols of increasing concentration and in 100% acetone, the cell residue was mounted in Epon-Araldite. Ultrathin sections were stained in uranyl acetate and lead citrate in the usual way [11].

EXPERIMENTAL RESULTS

Hybridoma cells in culture medium were applied in a drop to a coverslip. A few minutes until they had settled the coverslip was inverted and placed on a slide. Hybridomas are clearly visible in such a preparation in transmitted light. Their diameter varied mainly between 15 and 30 μ, but occasionally even larger cells could be observed.

On dark field microscopy we observed granules clearly visible against the background of the cytoplasm because of the difference in their refractive indices. LG are known [3, 4, 8, 12] to possess intrinsic luminescence when excited in the near ultraviolet (UV). Accordingly, a luminescence microscope also was used to observe the LG because it could show that not all particles visible in the dark field are LG. In this way the LG detected could be seen to be localized in the cytoplasm both separately and in the form of clusters. Although during aging of the culture the number of LG in the cells increased, nevertheless the study of LG is most important at the end of the logarithmic and beginning of the stationary phases, for at that time the content of exogenous and cellular metabolites can still fully meet the normal requirements of cell metabolism, and the number of LG is sufficient to allow their identification by microspectral analysis.

The luminescence microspectral characteristics of LG are shown in Fig. 1. According to these data the spectra of intrinsic luminescence of LG consist of two bands at 440-460 and 540-560 nm; a tendency toward luminescence in the red region also is characteristic of al LG. That is why visually LG usually appear to be yellow (Fig. 1, curve 2), with a weak luminescence

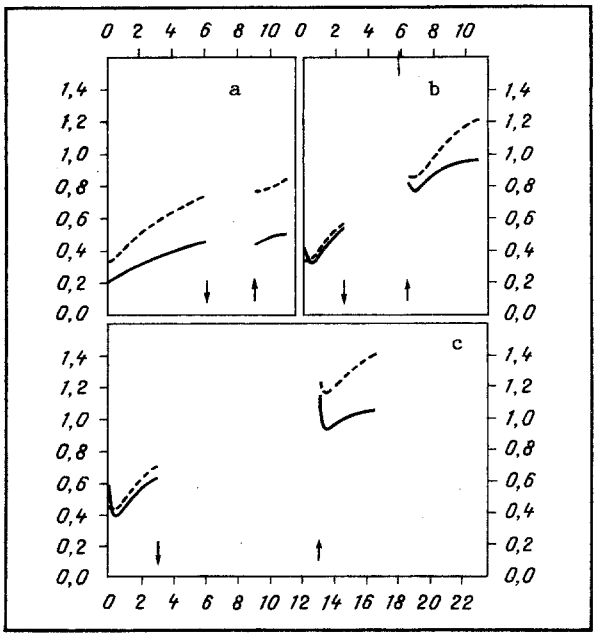

Fig. 2. Changes in intensity of luminescence of LG on excitation with UV (365 nm) at wavelengths of 460 nm (continuous line) and 540 nm (broken line). a-c) Kinetics of intensity of luminescence of LG from three different clusters. Arrows indicate end (downward) and beginning (upward) of exposure to UV. Ordinate, intensity of luminescence (in relative units). Abscissa, time (in min).

spectrum. Besides these granules, some whitish (Fig. 1, curve 1) and yellowish-brown (Fig. 1, curve 3) granules may also be observed. LG in various human and animals tissues in situ possess similar spectral characteristics [3, 4, 8].

It was shown previously that the intensity of the 540-560 nm band in the spectrum of intrinsic luminescence of LG in situ in various human tissues [3] increases in response to exposure to exciting UV (365 nm), and this was later confirmed in experiments on isolated LG. The same phenomenon also is characteristic of luminescent granules in hybridomas (Fig. 2a-c), further evidence of the lipofuscin nature of these granules. The kinetics of the increase in intensity of luminescence at 540 nm has an initial small drop of intensity irrespective of which band (440-460 nm or 540-560 nm) predominates at the zero time point. On stopping exposure to exciting UV (Fig. 2a-c, downward arrows) the process of increase of intensity of luminescence continued. On repeated application of UV (Fig. 2a-c, upward arrows) the shape of the initial kinetic curves was repeated, but at a higher level of intensity. This may mean that the exciting light at the zero time point acts as the trigger stimulus for certain photochemical conversions in LG.

We found a tendency for some LG to move toward the cell membrane after irradiation, and even to be released into the culture medium where, after a certain time, they lost their luminescence.

Ultrastructural analysis (Fig. 3a, b) revealed round formations surrounded by a bilayer membrane in the hybridomas. The ultrastructural characteristics of these inclusions are typical for LG [5-7, 9, 10, 12-15]. They vary in diameter from 0.5 to 1.5 μ, although sometimes larger LG can be found. An important ultrastructural characteristic of LG is their high osmiophilia, evidence mainly of their high lipid content. These lipids are present mainly as multilamellar structures (Fig. 3). It is a noteworthy fact that multilamellar structure is evidently spontaneous in character and is linked with the compartmentalization of the internal space of LG. Besides multilamellar structures, definite contribution to the compartmentalization of the internal space of LG is made by bilayer structures, which may evidently be connected with the presence of various proteins in LG. This, in turn, confirms the possible role of LG in cell metabolites [5, 7, 8, 12].

Incidentally, the morphology of LG varies considerably. For that reason, in many cases it is impossible to distinguish between LG and lysosomal formations. This also is attributable to the fact that in some investigations LG have been regarded as secondary lysosomes [5, 7, 12]. Comparison of our spectral and electron-microscopic data provides direct evidence of the existence of LG in the hybridomas, irrespective of their genesis.

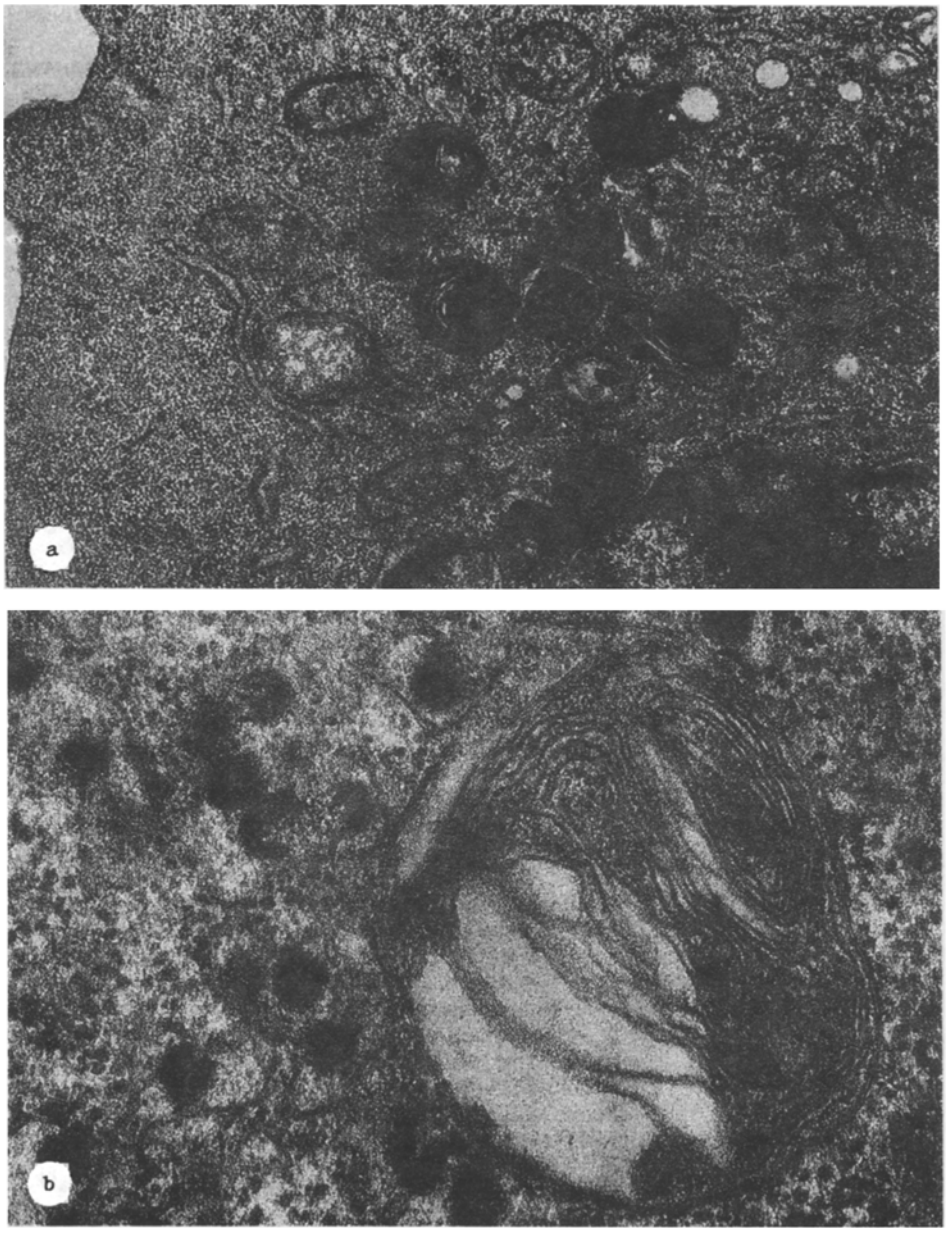

Fig. 3. Ultrastructure of LG in hybridoma cells. Formation of clusters of LG (a), morphology of individual LG (b). Scale 0.5 μ. Magnification: a) 20,000×, b) 100,000×. c) Type C retroviruses at different stages of morphogenesis.

Another characteristic feature of hybridomas is that they produce retroviruses (Fig. 3). However, the problem of the relationship between retrovirus production by hybridomas and LG formation in these same cells is not yet clear. Meanwhile, the accumulation of LG which we found not only in the postmitotic cells in situ [3, 4], but also in immortalized hybridoma cells, may indicate that LG formation is not so much a product of aging of cells as a product of changes in their metabolic activity.

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EFFECT OF DEGREE OF HEMOGLOBIN POLYMERIZATION ON CUMULATION AND ELIMINATION

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KEY WORDS: hemoglobin, pyridoxylated hemoglobin, artificial oxygen carrier.

Chemically modified hemoglobin (Hb) and, in particular, pyridoxylated polyhemoglobin (PPHb) is regarded by many investigators as a potential artificial oxygen carrier (AOC) [2, 3, 8, 9]. In experiments to study blood replacement by solutions of such compounds, their ability to maintain life of animals for a long time was demonstrated [3, 10].

Studies of various modified hemoglobins have shown that they can circulate in experimental animals for periods ranging from 2-4 h [5, 11] to 48-72 h [8]. The life span of Hb derivatives in the bloodstream is estimated relative to their half-elimination time. As has been shown, this characteristic is affected by the injected dose of Hb [5] and the degree of its chemical modification [1].

The half-elimination time is determined by measuring the change in concentration of the injected substances in the plasma during its circulation disregarding its excretion with the urine and its possible accumulation in the organs.

The aim of this investigation was the simultaneous estimation of the quantity of PPHb circulating in the plasma, excreted with the urine, and accumulating in the organs during the first few hours after injection. Various times of PPHb, differing in their degree of modification, were studied after intravenous injection into animals in a dose of 1 g/kg body weight.

EXPERIMENTAL METHOD

Modification of Hb with pyridoxyl-5-phosphate and glutaraldehyde was carried out by the method described previously [1]. The Hb concentration in the solutions was measured on a "Co-Oximeter 1L-282" instrument. The content of polymeric components in the samples was determined by high-pressure liquid chromatography on a TSK-250 column ("Bio-Rad," USA) in 0.1 M phosphate buffer, pH 6.5, with detection at 405 nm. The biological experiments were conducted on 42 male Wistar rats

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