time after injection of thymidine, i.e., anaphase and telophase 9 h after injection of the isotope (Fig. 3a). More exact evidence that precursors of Anichkov's cells pass through mitosis after doubling their DNA content is given by the results of cytophotometric determination of optical density of the nuclei, which show that most of Anichkov's cells are diploid (Fig. 3b).

The characteristic arrangement of chromatin in the form of a serrated band evidently appears about 20 h after the future Anichkov's cell has completed mitosis (the difference in time between the peaks of labeled mitoses and of labeled Anichkov's cells). Although the causes of this phenomenon and the further fate of the cells await explanation, it can be postulated that the origin of Anichkov's cells is linked with the end of proliferation rather than its beginning.

It must be recalled that not every mitosis in the heart is likely to lead to the formation of an Anichkov's cell. Nevertheless, the easily identifiable Anichkov's cell can serve as marker of mitosis completed 20 h before the material was taken, so that mitotic activity of heart cells at different times can be analyzed in the same preparation.

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ESTIMATION OF CELL AGGLUTINATION BY LASER NEPHELOMETRY

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Cell agglutination (CA) can be induced by plant lectins, which bind specifically with glycoproteins on the cell surface. The degree of CA is a characteristic of the state of the cell surface membrane. Changes in CA are observed during tumor development, morphogenesis and embryogenesis, and in various pathologic states [3, 6, 9, 10]. Quantitative estimation of CA is an urgent problem at the present time. Until now a laborious microscopic method has been used to estimate CA [2, 4]. Meanwhile the intensity of agglutination correlates directly with the sedimentation rate of cell agglutinates and translucency of the cell suspension. These parameters form the basis for development of quantitative instrumental methods of estimating CA [7, 8].

The aim of this investigation was to study whether the method of laser nephelometry (LN) can be used for quantitative estimation of agglutination of human tumor cells under the influence of lectins.

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EXPERIMENTAL METHOD

Cells of continuous lines of human osteosarcoma (Ca-4) [1] and adenocarcinoma of the lung (AKL) were used. Tumor cells were grown in medium 199 and in Eagle's medium with 10% embryonic calf serum, respectively. Both monolayer lines were obtained by E. A. Timofeevskaya (P. A. Gertsen Moscow Cancer Research Institute). Before testing, the cells, were incubated with EDTA solution for 5 min at 37°C, then washed twice with physiological saline, and then centrifuged, counted, and made up to the necessary concentration. Microscopic examination showed that the suspensions of Ca-4 and AKL cells thus obtained consisted of single cells and did not contain agglutinated cells. Tumor cells suspended in 1 ml of physiological saline were incubated in siliconized tubes at room temperture with different concentrations of phytohemagglutinin (PHA) — 0.05 or 0.2 ml (Difco, USA) or concanavalin A (Con A) — 25, 100, and 200 μg/ml (Sigma, USA), after which they were carefully suspended with a Pasteur pipette and the cell suspension was transferred to cuvettes. Scattering of light (SL) of the experimental and control (without lectins) cell samples was determined every minute on a laser nephelometer (Behringewerke, West Germany), volume of cuvette 1 ml, length of optical path 10 mm, wave length 632.8 nm). During quantitative estimation of CA the rate of decrease of SL in 1 min ($V\Delta SL$) and also the period of time (t_0) required for complete sedimentation of the cells (at $V\Delta SL = 0$) were considered. Parallel with this, in a "hanging drop" preparation, CA was estimated microscopically on a 4-point system (from 0 to +++) [4]. The results were subjected to statistical analysis, using Wilcoxon's T test.

EXPERIMENTAL RESULTS

Microscopic study revealed that after incubation of Ca-4 and AKL cells for 30 min with minimal concentrations of PHA (0.05 ml) and Con A (25 μg/ml) CA reached a maximum and thereafter did not increase. Considering this, kinetic determination of $V\Delta SL$ began after incubation of tumor cells with lectins for 30 min. The kinetic investigation showed that $V\Delta SL$ and t_0 depended on the concentrations of tumor cells and lectins. With a constant dose of lectins, an increase in the concentration of tumor cells (from $5 \cdot 10^5$ to $3 \cdot 10^6$) led to a considerable increase in $V\Delta SL$ and to a decrease in t_0 ($P < 0.01$; Fig. 1). With a constant concentration of Ca-4 cells maximal doses of PHA (0.2 ml) and of Con A (200 μg/ml) led to an increase in $V\Delta SL$ and to a decrease in t_0 compared with minimal doses of lectins ($P < 0.01$; Fig. 2). A similar dependence of $V\Delta SL$ and t_0 on cell and lectin concentrations also was found when AKL cells were used. The maximal differences in the values of $V\Delta SL$ between experimental and control samples, incidentally, was observed during the first 5 min of the investigation, and thereafter these differences diminished. When Ca-4 and AKL cells were used in a concentration of under $5 \cdot 10^5$/ml the number of cellular agglutinates decreased, and for that reason differences between the values of $V\Delta SL$ in the experiment and control became negligible. With an increase in concentration of tumor cells above $3 \cdot 10^6$/ml sedimentation of tumor cells was observed not only through agglutination, but also because of their high concentration, which led to a decrease in differences between the values of $V\Delta SL$ in the experimental and control samples. Parallel microscopic estimation of CA also showed a direct relationship between the increase in tumor cell and lectin concentration and the intensity of CA: With Ca-4 cells in a concentration of between $1 \cdot 10^6$ and $3 \cdot 10^6$/ml and with 0.2 ml of PHA the degree of Ca was assessed at +++, and with a concentration of $5 \cdot 10^5$ and 0.05 ml of PHA — as +. CA was always absent in the control samples.

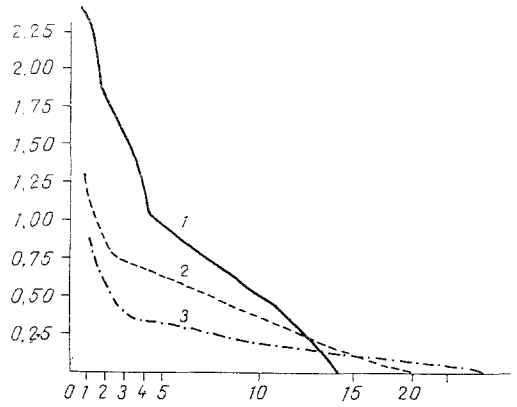

Fig. 1. Effect of various concentrations of Ca-4 cells with a constant dose of PHA (0.2 ml) on $V\Delta SL$ and t_0. 1) Concentration of Ca-4 cells $3 \cdot 10^6$/ml, 2) $1 \cdot 10^6$/ml, 3) $5 \cdot 10^5$/ml. Abscissa, time (in min); ordinate, $V\Delta SL$.

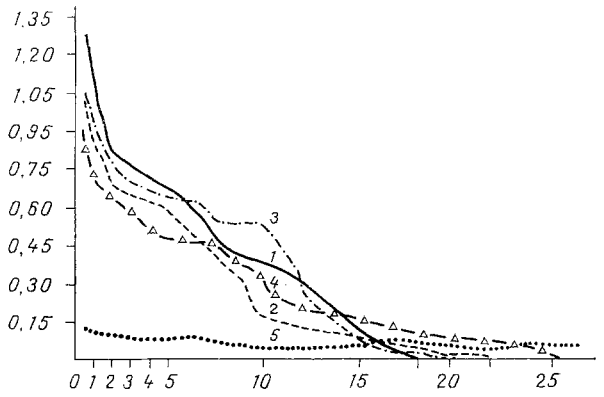

Fig. 2. Effect of different concentrations of PHA and Con A with constant concentration of Ca-4 cells ($1 \cdot 10^6$) on VΔSL and t_o. 1) PHA (0.2 ml), 2) PHA (0.05 ml), 3) Con A (200 µg/ml), 4) Con A (25 µg/ml), 5) control. Remainder of legend as to Fig. 1.

The results indicate that laser nephelometry can be used for quantitative estimation of CA. For CA it is recommended that laser nephelometry be used with the following established parameters: cell concentration $5 \cdot 10^5$ to $3 \cdot 10^6$/ml, determination of VΔSL in the first 5 min of the investigation, and a constant lectin concentration. Considering the widespread biological occurrence of the CA phenomenon, laser nephelometry for estimation of CA can be used in various fields of biology and medicine.

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