Since secretory activity of the calcitoninocytes is reduced when parasympathetic influences predominate, despite an increase in functional activity of the thyrocytes (due, it must be supposed, to an increase in thyrotrophin secretion by the adenohypophysis, which is intensified after partial thyroidectomy), it follows from the above account that, first, the functional state of the parafollicular cells depends on direct nervous impulses to a much greater degree than thyrocytes and, second, functional shifts in the thyrocytes and calcitoninocytes begin to follow the same direction only when adrenergic influences in the thyroid gland predominate over cholinergic; third, and last, the cause of this parallel is not the influence of thyrocytes on calcitoninocytes or of calcitoninocytes on thyrocytes, but sympathetic impulses reaching the thyroid gland and acting simultaneously on both thyrocytes and calcitoninocytes.

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CYTOPHOTOMETRIC ANALYSIS OF TRANSITION FROM PHASE G_0 TO PHASE G_1 OF THE CELL CYCLE

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Recently in connection with the appearance of modern precision scanning cytophotometers equipped with computers, new prospects have opened for the automatic analysis of the image of a cell and its individual structures. In particular, investigation of the geometric parameters of cell nuclei stained by Feulgen's method and of the distribution of optical densities has made it possible to determine quantitative changes in the ratio between condensed and diffuse chromatin and the geometry of the cell nucleus in cells of eukaryotes during their stimulation [8, 10], virus infection [7, 9], in the course of acid hydrolysis of DNA [4], and after exposure to ionizing radiation [5].

The object of this investigation was to study the possibility of separating cells in phases G_0 and G_1 of the cell cycle on the basis of cytophotometry of cell nuclei stained by Feulgen's method.

EXPERIMENTAL METHOD

Peripheral blood lymphocytes from a blood donor were isolated and cultured with PHA by the method described previously [2]. The cells were cultured for 24, 48, and 72 h. Films were fixed in a mixture of ethanol and acetone (1:1) for 30 min at room temperature. Hydrolysis in 5 M HCl was carried out at 37°C for 15 min, after which the preparations were stained in Schiff's reagent, made from basic fuchsine (from Reanal, Hungary) [3].

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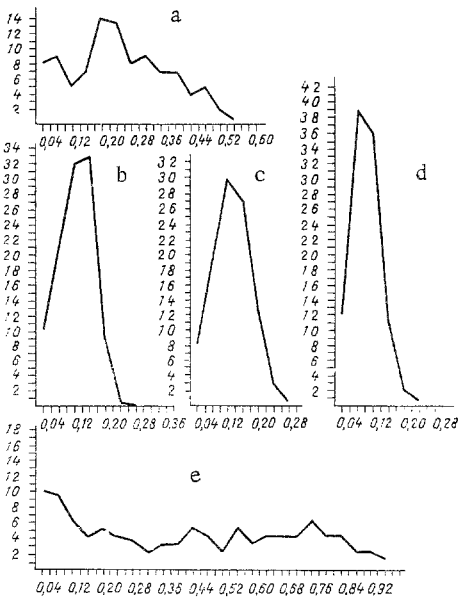

Fig. 1. Distribution of optical densities during scanning of nuclei of lymphocytes in various phases of cell cycle. a)G_0, b) G_1, c) G_2, d) S, and e) M. Abscissa, optical densities (in optical density units); ordinate, frequency of points with given optical density (in %).

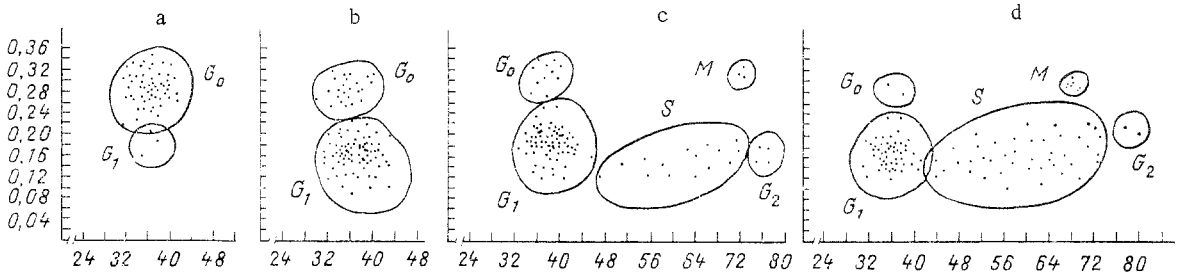

Fig. 2. Two-dimensional dot displays of IOD and MOD of lymphocytes during culture with PHA. a) Control, b) 24 h, c) 48 h, d) 72 h. Abscissa, IOD; ordinate, MOD.

Cell nuclei stained by Feulgen's method were studied on a "Univar" cytophotometer (from Reichert, Austria), connected to a Hewlett-Packard 9845A bench computer (USA), according to the "WONDER" Program. Scanning was carried out in the plane of the image by means of a scanning lens ("Reichert") with a step of 0.2 μ, and by means of a circular probe of the same diameter. A planapochromat × 100 immersion objective with 1.32 aperature was used and the magnification factor of the carrier optical system was × 2.5. Under these conditions the number of counting points during scanning of one nucleus was between 1500 and 4000. Optical densities below 0.04 were classed as background, those above 0.04 as object.

After completion of the scanning cycle a histogram of distribution of optical densities in the nucleus, the area of the nucleus, and its integral optical density (IOD) were obtained. The mean optical density (MOD), the ratio of IOD to the number of counting points, was calculated for each nucleus. A two-dimensional dot display was plotted for MOD and IOD. In each preparation 100 nuclei of cells in different phases of the cell cycle was studied.

EXPERIMENTAL RESULTS

During stimulation of PHA, peripheral blood lymphocytes, of which the overwhelming majority are in the resting G_0 phase, are known to commence the mitotic cycle and to pass successively through phases G_1, S, G_2, and M. The cells enter phase G_1 during the first few hours after exposure to PHA, and it lasts 20-24 h. During this time preparations are made for DNA synthesis and are accompanied, in particular, by an increase in the proportion of diffuse

chromatin, connected with an increase in DNA template activity, as an essential stage for synthesis of RNA and proteins during preparation of the cell for division [1]. The transition of the lymphocytes from G_0 into G_1 is largely asynchronous [6], and for that reason even after 72 h of culture, besides cells in phases G_1, S, G_2, and M, some lymphocytes are still in the G_0 phase.

Data on the distribution of optical densities for cells in different phases of the cell cycle, obtained from 48-h cultures, are given in Fig. 1. Clearly the predominance of low optical densities characteristic of diffuse chromatin corresponds to nuclei of cells in G_1, S, G_2; the regions of their distribution approximately coincide for cells in these phases. On the other hand, in cells in the G_0 and M phases, optical densities are mainly high, characteristic of condensed chromatin. This is particularly well expressed in mitotic cells (metaphases).*

The time course of passage of the lymphocytes through phases of the cell cycle during stimulation of PHA is clearly illustrated by the two-dimensional dot displays (Fig. 2). Before addition of PHA (Fig. 2a) nearly all the lymphocytes were in G_0, and this corresponds to their grouping in the zone of high MOD. After culture for 24 h with PHA (Fig. 2b) many cells have moved into the zone of low optical densities, to form the G_1 population. After 48 and 72 h (Fig. 2c, d) cells can be seen in all phases of the cell cycle, and some are still present in G_0.

Transition of cells from G_0 into G_1, characterized by a change in MOD, is a gradual process, as shown by the presence of an intermediate zone between the G_0 and G_1 groups on the two-dimensional distributions. Their separation is thus to some degree empirical. Mathematical analysis of these distributions requires complex algorithms [5]. Results satisfactory for practical purposes can be obtained, however, by simpler approaches. For example, considering the similarity between distributions of optical densities of nuclei in phases G_1 and S of the cell cycle (Fig. 1), the highest values of MOD of cells in the S phase can be taken as the boundary separating G_0 from G_1.

In view of the absence of reliable markers for the G_0 and G_1 phases of the cell cycle, the approach described in this paper may prove useful for assessing the kinetics of populations of normal and tumor cells.

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*The presence of low optical densities in metaphases can be attributed in particular to incomplete filling of the probe in the marginal regions of the chromosomes.