Successive Evaluation of Multiple Parameters in Individual Cells: The SEMPIC Photometric System

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Summary. An automated microphotometric system has been designed for the successive assessment of a maximum number of parameters from individual cells. The hardware and software elements are described as well as the various photometric functions that can be performed. The aim is to obtain a multiparameter analysis of morphology, cytochemistry, cell surface characteristics, and metabolic activity including cell cycle characteristics for any set of cells selected from a sample. The combination of DNA synthesis rate determination in a really quantitative approach with the analysis of features of nuclear chromatin texture opens up a new field for associating morphology with functional properties.

Examples of application are presented to demonstrate the applicability of the system in a heterogeneous cell population of a very limited sample size. For this purpose, human peripheral blood cells grown in diffusion chambers in preirradiated mice were investigated. It is shown that, depending on their functions, different cell types may have quite different nuclear-to-cell area ratios. Further, a crude morphological parameter, such as the degree of basophilia of Giemsa-stained cells, may be associated with the rate of DNA synthesis, thus bearing information on the replicative activity of a cell. Cell surface properties related to the leukemia-associated cALL antigen are investigated in a human-derived cell line and correlated with cell-cycle characteristics. It is concluded that different antigenic sites rather than cell-cycle dependent differences of antigen density account for the outcome of a bimodal distribution.

Introduction

Many questions in cellular biology are studied by attempting to correlate different parameters derived from the same cells. It is often desirable to confine
such measurements to distinct cell types mixed with others in a tissue. As far as the cells of interest can be separated according to their physical, chemical or immunological properties, this tends to be the procedure of choice for a selective investigation. In many instances, however, morphology is the only criterion available for distinction.

For such conditions, a photometric system has been developed allowing a multiparameter analysis of individual cells following data acquisition in consecutive steps. The hardware consists of a computer-controlled microphotometer equipped with a scanning stage and offering great optical versatility for performing different types of measurement. The software allows the relocation of cells once located on a slide, performance of various automated measurements, data processing including texture analysis, and the establishment of correlations between the different parameters of individual cells. The present report describes the hardware and software elements, the performance of measurements, and gives several examples of application.

**Material and Methods**

Mononuclear cells from normal human peripheral blood were separated on a Ficoll Isopaque gradient (Boyum, 1968) and subsequently grown in diffusion chambers implanted into preirradiated mice (Boyum et al., 1972) for either 3 or 7 days. Each chamber was filled with $5 \times 10^5$ cells in 0.1 ml of Hanks balanced solution. On the harvesting days the cell number per chamber ranged between $2 \times 10^3$ and $6 \times 10^3$. After shaking the chambers for 40 min in a 0.5% pronase solution in order to dissolve the fibrin clot, the chamber content was withdrawn, and the cells were subjected to the technique of quantitative $^{14}$C-autoradiography as described elsewhere (Dörmer, 1973a, 1973b; Dörmer and Thiel, 1976). Labeled and unlabeled cells were located on the slides at random, and their coordinates were stored by computer. At the same time, a morphological subdivision into small lymphocytes, lymphoid blast cells, and macrophages was performed and recorded along with the information “labeled” or “unlabeled”. The labeled cells were relocated in the next step for the measurement of their grain count and the evaluation of the thymidine incorporation rate. The silver grains were then removed using solutions of 7.5% potassium ferricyanide and 10% sodium thiosulfate, and the cells were re-stained with Giemsa stain as described elsewhere (Dörmer, 1972). Subsequently, all the cells were relocated and measured for total extinction and total area at 580 nm. At this wavelength it is predominantly the blue component of the Giemsa stain that determines the extinction values. Cytologically, this correlates with the degree of “basophilia” of a cell. It is of course not a quantitative cytochemical parameter but rather a crude morphological feature which, however, is of significance in qualitative cytology. After removal of the Giemsa stain in acidified ethanol the cells were Feulgen-stained, relocated and measured for total nuclear extinction and nuclear area at 546 nm.

In another set of experiments the NALM-1 cell line was investigated, which originally had been derived from a blastic crisis of chronic myeloid leukemia in a child (Minowada et al., 1977). This cell line is known to carry the cALL surface antigen predominantly found in acute lymphoblastic leukemias in man (Rodt et al., 1977). During the exponential phase of growth the cells were pulse-chased with $^3$H-thymidine, and subsequently labeled with FITC-conjugated rabbit anti-cALL antibody prepared and kindly provided by Dr. H. Rodt (Rodt et al., 1977). The cells were smeared onto slides and immediately thereafter measured for fluorescence intensity, while their coordinates were stored at the same time. Subsequently, they were fixed in methanol for 48 hrs and covered with AR. 10 stripping film. The previously measured cells were relocated on the Giemsa-stained autoradiographs and classified as labeled or unlabeled, which allowed the separation of cells synthesizing DNA from the rest.