Response to "Comments on the Fluorescein Excitation and Emission Polarization Spectra in Living Cells"

Dear Sir:

With respect to buoyant density distributions and functional properties, the peripheral blood lymphocytes are a heterogeneous population of cells (1). The phenomenon of changes in the intracellular fluorescein fluorescence polarization of lymphocytes on phytohemagglutinin (PHA) stimulation (structuredness of cytoplasmic matrix [SCM]-test) is defined for a specific lymphocyte subpopulation, in which significant changes in polarization are observed at 510 nm on preferential excitation at 470 nm. The polarization changes observed depend on the experimental conditions used during the isolation of these lymphocytes (2–7). We have examined the leukocyte isolation procedure used by Price[1] and find their isolation conditions to be different from those used by us (2,3) and other laboratories which confirmed the SCM-test (4–9). Furthermore, the intracellular fluorescein emission polarization spectrum recorded on these leukocytes (Fig. 1) is very different from that obtained on the subpopulation of lymphocytes used in our experiments (see Fig. 3 in reference 10). It follows, that the results of Price and Miller were obtained on a different leukocyte population. We confirm that on these leukocyte preparations, isolated from the blood of healthy donors, there is a decrease of 12–21% in the intracellular fluorescein polarization on PHA stimulation when the fluorescein is excited at 488 nm, or 472.8 nm, ($\Delta\gamma$ = 10 nm) and the emission wavelength is 540 nm ($\Delta\gamma$ = 15 nm), i.e, under spectroscopic conditions similar to those used by Price and Miller. However, the qualitatively similar changes in the intracellular fluorescein fluorescence polarization observed on different leukocyte populations do not necessarily imply that the same biological mechanisms are monitored. For example, we have recently discovered that changes in the intracellular fluorescein polarization when monitored at 510 nm, on excitation at 470 nm, are mainly associated with the structural changes in the mitochondria on their transition from the orthodox to the condensed conformation and vice versa (11). As the same stimulus can elicit concomitant changes in the physical state of organization, i.e., structuredness, in several cytoplasmic domains, qualitatively similar or opposite polarization changes could be observed under spectroscopic conditions of preferential excitation of fluorescein molecules in different intracellular domains. For example, a decrease in polarization values is observed on PHA stimulation both in the subpopulation of SCM-responder lymphocytes and in leukocytes used by Price and Miller. However, on stimulation of bone marrow cells by low molecular weight colony stimulating extracts we observed under our

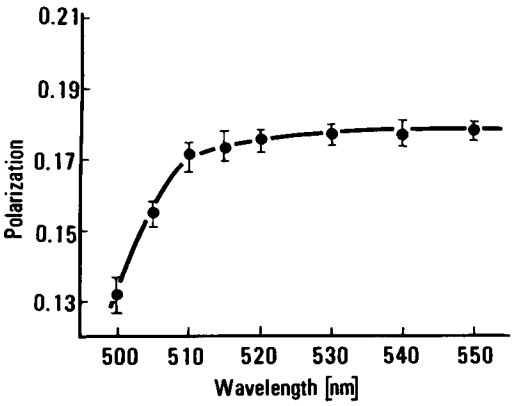

FIGURE 1 Fluorescein emission polarization spectra in leukocytes isolated according to the procedure of Price.[1] Excitation wavelength 470 nm ($\Delta\gamma$ = 20 nm). The error limits represent maximal deviations from the mean value of two independent experiments.

[1]Price, G. B. Personal communication.

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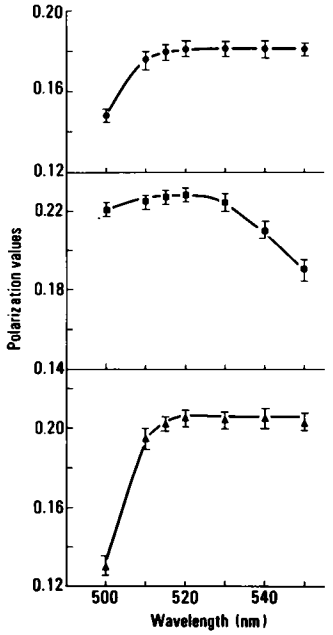

FIGURE 2 Fluorescein emission polarization spectra in synchronized He-La S3 cells in (*a*) mitosis (●), (*b*) G-1 phase (■), and (*c*) mid-S-phase (▲). Excitation wavelength 470 nm. The error limits represent maximal deviations from the mean value of two independent experiments.

spectroscopic conditions a decrease in polarization values, whereas an increase in polarization values was reported by Price and Miller (12). It therefore remains to be elucidated which particular cell structure is involved in changes of the intracellular fluorescein fluorescence polarization observed at 540 nm on excitation at 488 nm (or 472.8 nm) in the above cell system.

Lindmo and Steen (13), who used single cell measurements in a flow-cytofluorimeter and similar spectroscopic conditions as Price and Miller, did not observe any changes in polarization between G-1 and mid-S phase in synchronized, NHIK-3025 cells. Since also in our cell systems we did not observe any changes in polarization between G-1 and S-phase under spectroscopic conditions used by Lindmo and Steen it was obvious that the wavelength dependence should be pointed out as one of the possibilities for the disagreement (10). Furthermore, our recent results show that in synchronized He-La S3 cells (Flow Laboratories, Ltd., Irvine, Scotland), which were derived from human cervical cancer cells, i.e., of the same histological origin as the NHIK-3025 cancer cells used by Lindmo and Steen (13), the intracellular fluorescein emission polarization spectra during the cell cycle (Fig. 2) are very different from those observed in cell lines derived from normal cells (10). Therefore, the comparison of their results (13) with ours (10) was also in this respect not justified.

In conclusion, it follows from the above discourse that valid interpretations and comparisons of results between various laboratories are possible only if care is taken that identical biological systems and/or subpopulations of cells are studied under equivalent experimental and spectroscopic conditions. It is with respect to these points that Price and Miller appear not to have given due consideration to their comments on the validity of our interpretation of the fluorescence polarization spectra in living cells.

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L. CERCEK and B. CERCEK
Paterson Laboratories
Christie Hospital and Holt Radium Institute
Manchester, M20 9BX, England