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13. ABSTRACT (Maximum During the past grant period we have developed a new assay to study the responses of large populations of cells to pulsating near infrared light signals. We propose to use them to determine the optimal light pulse patterns and to study the photo behavior of different cell types, states of transformation and phases of the cell cycle to artificially generated light signals. In addition we have developed the first infrared fluorescent microscope which we propose to develop further in order to test the hypothesis that the natural infrared light signals are emitted by the mitochondria of the cells. The concept of photo behavior of cells is entirely new and is potentially very important for biology and medicine. As more and more details of this phenomenon emerge we may become able to imitate the signals of specific cell types in order to be able to influence their behavior during development, wound healing, immune response and metastasis. In addition, the dependency of the photo behavior on pulse patterns rather than on total energy points to an elaborate data integration systems in cells that are, so far, unknown to biology and promise to revolutionize our concepts of cells.					
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Cellular localization of infrared
sources

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During the past grant period we have developed a new assay to study the responses of large populations of cells to pulsating near infrared light signals. We propose to use them to determine the optimal light pulse patterns and to study the photo behavior of different cell types, states of transformation and phases of the cell cycle to artificially generated light signals.

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Infrared, phototaxis, mammalian cells, tissue culture, motility, centrioles.

STATEMENT OF THE PROBLEM STUDIED.

Based on our earlier work of more than 18 years it appeared likely that mammalian tissue cells may be able to locate microscopic infrared light sources in their vicinity. It is important to investigate this possibility for the following reasons. The recognition of a cell's immediate and distant environment is likely to play a major role in the control of its motile behavior. Therefore, the investigation of phototaxis and photosensitivity in general of mammalian cells in the infrared may have far reaching consequences for all medical and biological processes that involve cell motility, e.g. the processes of malignant invasion and wound healing. Work preceding this grant had established that mammalian cells were able to 'see' pulsating near infrared light sources that emitted light around

800 nm wavelengths. It appeared that the centrosome, specifically the embedded pair of centrioles contain the cellular detection apparatus. Thus, 2 classes of questions arose.

- a. What are the natural emitters of pulsating 800 nm signals around cells?
- b. What is the mechanism of the cellular response to the pulsating light? Especially, what cellular structures mediate between the centrosome and the motile machinery of the cell in the cortex?

SUMMARY OF THE MOST IMPORTANT RESULTS.

Question a. What are the natural emitters of pulsating 800 nm signals around cells? (Albrecht-Buehler, G., *Autofluorescence of live purple bacteria in the near infrared*. (1997) *Experimental Cell Research* 236:43-50)

The most likely candidates for the natural emitters of near infrared pulses are the mitochondria of the cells. However, their signals must be very weak compared to the thermal background and, therefore, they can only be detected with appropriately cooled detectors and lock-on amplifiers. Alternatively, one can expect that the signal emitters will fluoresce if excited with their normal emissions. Therefore, an infrared fluorescence microscope would be required. Unfortunately, no such instrument is available. Therefore, we have developed a novel microscope to study the fluorescence of cells in the near infrared region ($\lambda = 750 \text{ nm} - 2500 \text{ nm}$). As one of its first application we report the autofluorescence of live purple bacteria, *Rhodospirillum rubrum*, and suggest that the autofluorescent component is bacteriochlorophyll. The rapid fading of the autofluorescence of fixed bacteria and of purified bacteriochlorophyll suggests that the live bacteria are able to regenerate their pigment with a time constant of approx. 20 s.

Question b. What is the mechanism of the cellular response to the pulsating light? Especially, what cellular structures mediate between the centrosome and the motile machinery of the cell in the cortex?

(Albrecht-Buehler, G., *Altered Drug Resistance of Microtubules in Cells Exposed to Infrared Light Pulses: Are Microtubules the "Nerves" of Cells? Cell Motility and the Cytoskeleton* (1998) (in press))

During the past grant period we developed the first quantitative assay of the response of an entire population of cultured mammalian cells to a pulsating near-infrared signal. The assay measures the change of resistance to nocodazole of reconstituted cytoplasmic asters of irradiated cells. Using this assay on CV1 cells, we obtained results suggesting that pulsating near-infrared signals reduced the stability of the radial microtubules around the centrosome. The results are consistent with the interpretation that the centrosome responded to the light by sending signals along its radial array of microtubules whose stability was then altered. In other words, the results point to the microtubules as the mediators between the cellular detection of the infrared light signals and the motile response of the cellular cortex. The results may be an example of a more general function of the centrosome to integrate exogenous signals and send response signals along microtubules to various sites inside the cell.

LIST OF ALL PUBLICATIONS DURING THE GRANT PERIOD.

- Albrecht-Buehler, G. Changes of cell behavior by near-infrared signals. *Cell Motility and the Cytoskeleton* 32:299-304 (1995).
- Albrecht-Buehler, G., Autofluorescence of live purple bacteria in the near infrared. (1997) *Experimental Cell Research* 236:43-50
- Albrecht-Buehler, G., Altered Drug Resistance of Microtubules in Cells Exposed to Infrared Light Pulses: Are Microtubules the "Nerves" of Cells? *Cell Motility and the Cytoskeleton* (1998) (in press)
- Albrecht-Buehler, G. The conceptual challenge of cellular gravi-sensing, In 'Frontiers of Biological Science in Space' Taiyo Ltd. Tokyo, Japan (ed. A. Sato) 54-65
- Albrecht-Buehler, G. Phagokinetic Track Assay of Cell locomotion in Tissue Culture, In 'Cells: A Laboratory Manual' Vol. 2, Cold Spring Harbor, NY (1997) 77.1-77.10

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REPORT OF INVENTIONS

No inventions.