3) Cells which can be reproducibly obtained and maintained with good viability.

4) Cells which can be divided into subclasses according to biological function, subclasses which might be related to cell volume or cell fluorescence. For these reasons, we chose to work on thoracic duct populations of lymphocytes, which are:

1) Divided into at least 3 size subclasses.

2) Naturally obtained in single cell suspension and do not form clumps of 2 cells or greater.

3) Can be maintained in a viable state with appropriate media for as long as 24 hours.

4) Have at least two significant biological criteria for size-subclass separation:

a) DNA synthesis: Large and medium thoracic duct lymphocytes are in a continuous cycle of cell division. At least 80% of their cell cycle is devoted to DNA synthesis, and therefore short-term incubation of thoracic duct lymphocyte population with radioactive DNA precursors will selectively label large and medium cells. Small lymphocytes only rarely divide.

b) Small lymphocytes are "immunologically competent cells", whereas populations of large and medium lymphocytes are not. We have developed rapid and easy methods for testing immunological competence.

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c) Both of the above assays detect these biological functions in viable cells only. Therefore, since we have a system (described previously) in which only viable cells fluoresce, we can test the fluorescent cell separator's action also.

The following are a few of the medically and biologically significant experiments we hope to perform:

1) Test purified cell populations for cell-cell interactions in the induction, development, and execution of the immune response (in conjunction with Professor L. Herzenberg and Dr. I. Weissman).
   a) Isolation of antigen-processing cells by phagocytosis of fluorogenic substrate.
   b) Isolation of immunologically competent cells by size.
   c) Isolation of antibody-forming cells by size following adherence of large antigenic particles.
2) Isolation of cells in mitosis by size criteria in order to establish cell lines in vitro which are synchronously cycling. These will be useful to determine the actual cellular and molecular events which determine the differential sensitivity of cells to radiation and certain drugs as a function of their place in the cell cycle (in conjunction with Prof. George Hahn, Department of Radiology).
3) Detection and isolation of cancer cells in the blood stream (metastases) in order to determine the type of cancer therapy most appropriate for the patient (in conjunction with Prof. H. S. Kaplan, Executive Head of Radiology and Radiotherapy Prof. R. Kallman, Radiology Department).

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4) Isolation and testing of cell types in Hodgkins Disease, (a cancer of the lymph node system) in order to determine:

   a) The malignant cell, its biochemistry and radiosensitivity

   b) The cell type (in these patients) responsible for widespread immunological deficiency, and how this deficiency is maintained in conjunction with Professor H. S. Kaplan.

5) Isolation and testing of the cell type in the bone marrow theoretically designated as the "stem" cell, which is responsible for redevelopment of normal blood cell types following irradiation In conjunction with Dr. Weissman.

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