

A MORPHOLOGICAL ANALYSIS OF BINDING OF A HYDROPHOBIC PROBE TO CELLS

JACOB DYCKMAN and JOEL K. WELTMAN. From the Miriam Hospital, Departments of Pathology and Medicine, Division of Biomedical Sciences, Brown University, Providence, Rhode Island 02912

Arylamino-naphthalenesulfonates are used as hydrophobic probes because of the enhanced fluorescence quantum yield of these compounds in media of low dielectric constant (1-3). One such probe, 8-anilino-1-naphthalenesulfonate (ANS), forms noncovalent fluorescent complexes with human leukocytes (4) and thus can be used to detect hydrophobic components on and within these cells. We report here a morphological analysis of the binding of ANS to human leukocytes and plasma cells performed by fluorescence microscopy.

MATERIALS AND METHODS

Leukocytes were obtained from the buffy coat of heparinized human venous blood of three normal subjects by sedimentation at 500 *g* for 15 min at 20°C. Plasma cells were obtained from a tumor that was excised from a patient with multiple myeloma. Cells were washed three times by centrifugation in 0.05 M

phosphate—0.15 M NaCl, pH 7.2 (PBS) and were finally suspended in 2.5×10^{-8} M ANS (K & K Laboratories Inc., Plainview, N.Y.) in PBS. Fluorescence microscopy was performed with a Zeiss microscope equipped with a cardioid dark-field condenser, a 200 w high pressure Hg light source and a 100 × oil immersion objective (NA 0.8). A BG-12 filter was placed in front of the light source and a Zeiss 53 filter was interposed between the microscope ocular and objective. Photomicrographs were obtained with a Polaroid camera and Type 146-L film. Total magnification before photographic enlargement was × 1000.

RESULTS

The typical pattern of fluorescence enhancement of ANS in small lymphocytes is shown in Fig. 1 A. The nucleus is round, nonfluorescent, and surrounded by a rim of highly fluorescent cytoplasm.

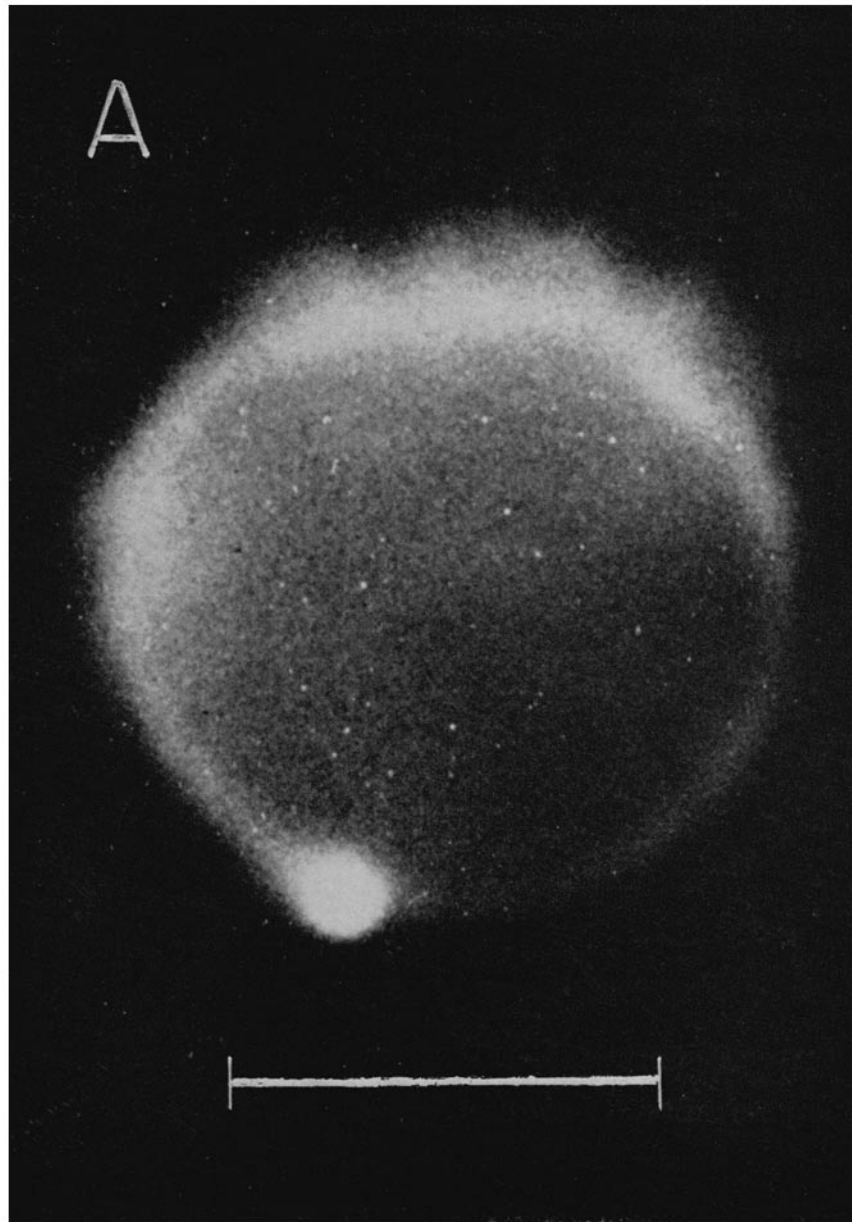


FIGURE 1 *A-D* Fluorescence photomicrographs of human cells. (*A*) lymphocytes in medium with 8-anilino-1-naphthalenesulfonate (ANS); (*B*) lymphocytes in medium without ANS; (*C*) polymorphonuclear leukocytes in medium with ANS; (*D*) plasma cells in medium with ANS. The scale indicates 5 μ .

Fig. 1 *B* is a photomicrograph of a lymphocyte taken under conditions identical to those used in Fig. 1 *A*, except that ANS was omitted from the medium. As shown in Fig. 1 *B*, background noise due to light associated with processes other than ANS fluorescence is negligible. A photomicrograph of polymorphonuclear leukocytes suspended in

medium that contains ANS is shown in Fig. 1 *C*. The nuclei of these leukocytes appear as non-fluorescent multilobed bodies within a highly fluorescent cytoplasm. The characteristic pattern of ANS fluorescence in plasma cells (Fig. 1 *D*) is also a dark nucleus within a brightly fluorescent cytoplasm. In the plasma cells, however, in addition

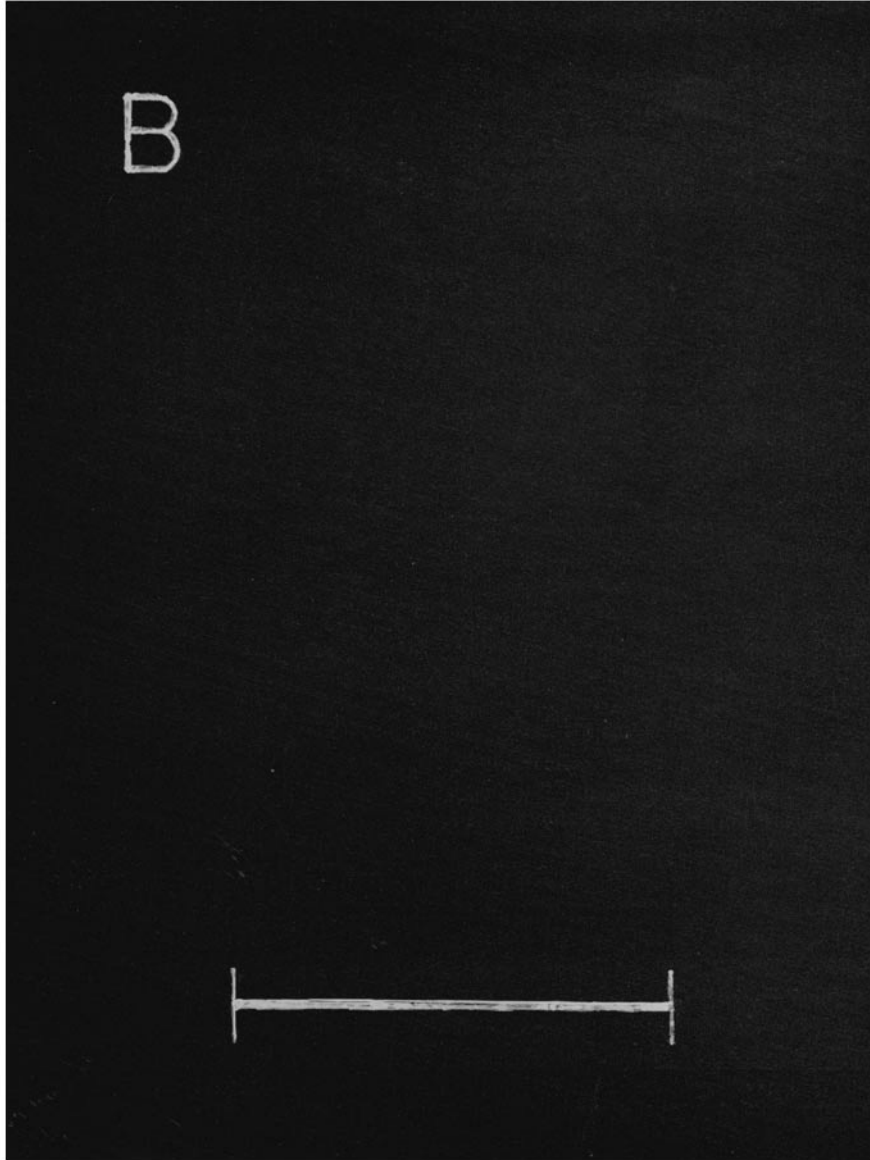


FIGURE 1 *B* See legend under Fig. 1 *A*.

to the fluorescent cytoplasm, a region of nucleolar fluorescence is also visible.

100 cells of each of the above cell types, i.e., lymphocytes, polymorphonuclear leukocytes, and plasma cells, were examined and all demonstrated the respective morphologic patterns of ANS fluorescence enhancement shown in Fig. 1. Furthermore, background in the absence of ANS was determined for polymorphonuclear leukocytes and plasma cells and was identical to that shown in

Fig. 1 *B*. The highly fluorescent localized deposits visible within the cytoplasm of the lymphocytes and granulocytes in the photomicrographs tend to develop in cell preparations under continued exposure to the Hg light source. With time, similar deposits also develop in regions of preparations devoid of cells. The localized deposits are thus photoinduced artifacts associated with exposure to the high intensity light source used for fluorescence photomicrography. These artifacts do not form

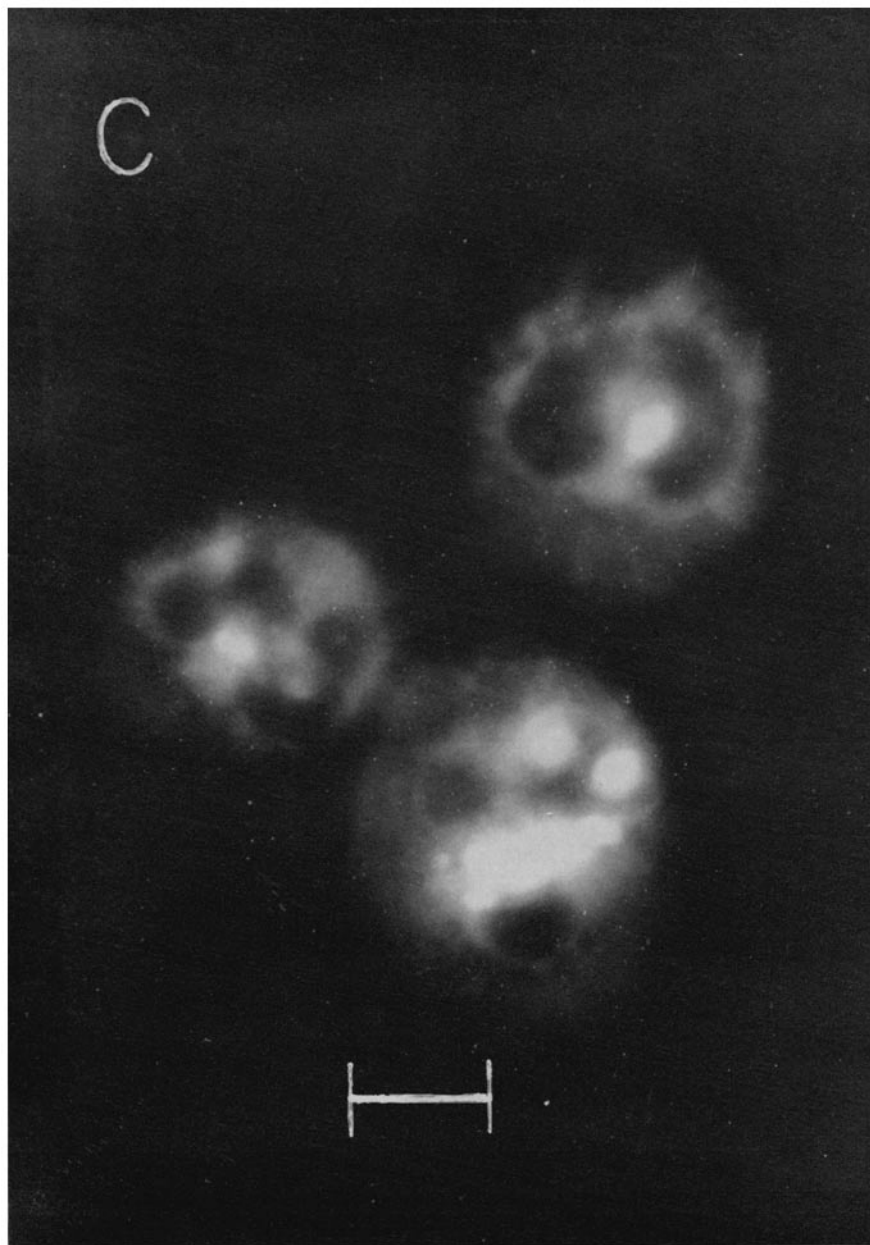


FIGURE 1 *C* See legend under Fig. 1 *A*.

under illumination with a tungsten lamp and visual observation of fluorescence. The dark oval shadow at the margin of the nucleus of the plasma cell in Fig. 1 *D* is unidentified.

DISCUSSION

The hydrophobic probe 8-anilino-1-naphthalene-sulfonate (ANS) fluoresces with a low quantum

yield in organic solvents with low dielectric constant or when noncovalently complexed to hydrophobic sites on macromolecules (1-3). Hydrophobic probes have yielded valuable insights into the relationships between structure and function in a variety of biological systems. For example, fluorescence enhancement of ANS has been used to study the time course of structural changes as-

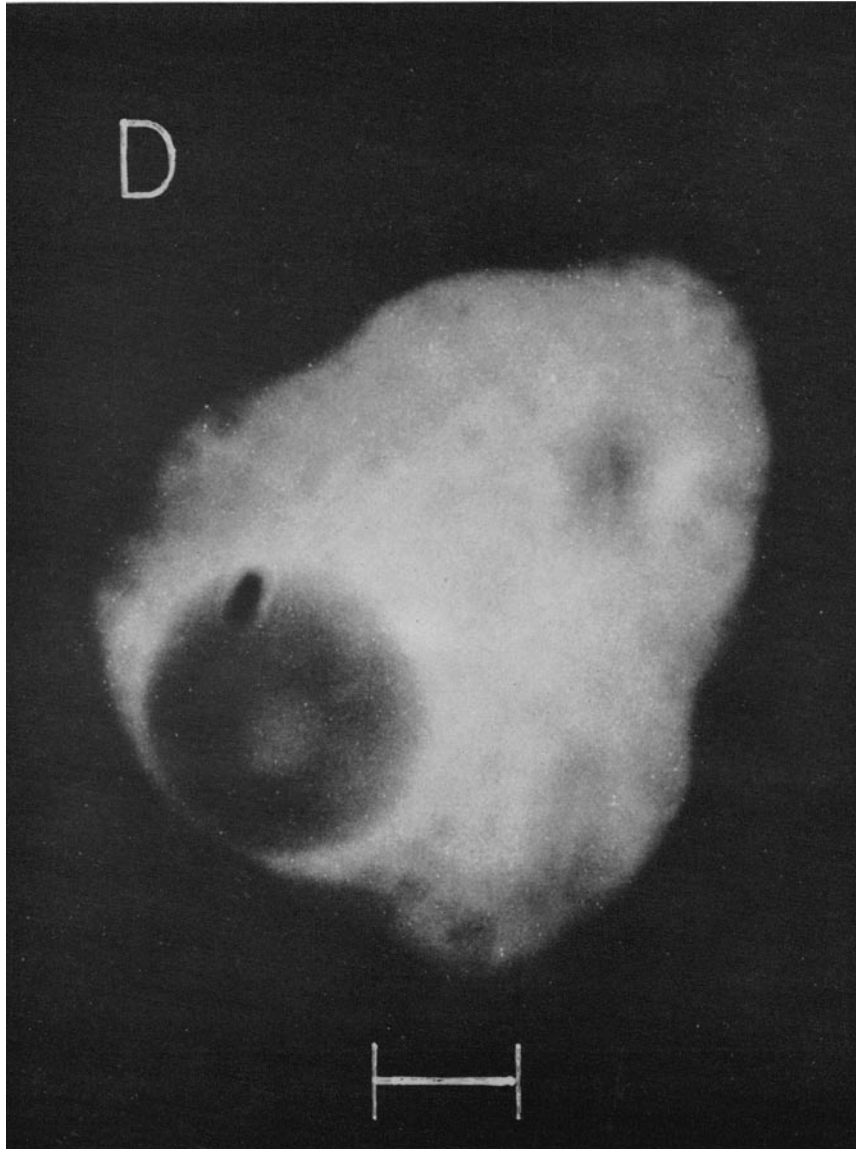


FIGURE 1 D See legend under Fig. 1 A.

sociated with electron transport and energy conservation in mitochondria (5). Tesaki et al. detected changes in the fluorescence intensity of ANS, which were synchronous with the action potential of spider crab axon (6). They concluded that ANS fluorescence yield varied with conformational changes associated with the conduction process in the axon membrane. Newton used a hydrophobic probe to study changes in *Pseudomonas* membrane permeability induced by poly-

myxin (7). Aronson et al. demonstrated that the fluorescence of ANS is sensitive to the conformational state of myosin (8).

Leukocytes enhance the fluorescence of ANS without a decrease in cell viability (4). Thus, fluorescence enhancement of ANS may be used to study relationships between structure and function of cellular components of leukocytes. An important step in such a study is the determination of intracellular localization of those components that en-

hance the fluorescence of ANS. We report here such a determination performed by fluorescence microscopy.

The fluorescence photomicrographs shown in Figs. 1 *A* and *C* respectively, indicate that hydrophobic components that bind ANS are located principally in the cytoplasm of lymphocytes and granulocytes. In view of the fact that the mean fluorescence intensity of ANS in granulocytes has been shown to be indistinguishable from that in lymphocytes (4), the photomicrographs in Figs. 1 *A* and *C* lead to the conclusion that either the concentration of hydrophobic components is greater in lymphocytes than in granulocytes, or that the dielectric constant per component is lower in lymphocytes than in granulocytes. It should be possible to distinguish between these two alternatives by direct chemical assay of purified cellular components that enhance the fluorescence of ANS or by microspectrofluorometry (9); i.e., higher concentration of hydrophobic components would be associated with greater ANS fluorescence in the absence of a spectral shift; in contrast, greater hydrophobicity (lower dielectric constant) of the components would be associated with a shift of the wavelength of maximal emission toward the blue (2, 3). Experiments concerning the further characterization of cellular components that enhance the fluorescence of ANS are in progress. These cellular components may include proteins and lipids of membranes (10, 11) and other organelles.

Changes in ANS fluorescence induced by altered cell functions may give insight into physicochemical bases of those functions. An example of a change in ANS fluorescence enhancement with altered cell function may be seen by comparing Fig. 1 *A* with Fig. 1 *D*. The lymphocyte is known to possess a small nucleolus (12), but this, however, does not significantly enhance the fluorescence of ANS (Fig. 1 *A*). In contrast, the nucleolus of the plasma cell shown in Fig. 1 *D* enhances ANS fluorescence. Since plasma cells are derived from lymphocytes by a transformation involving cell multiplication and differentiation (13), nucleolar fluorescence of ANS may be related to this transformation and activation of the nucleolar organizer genes. The plasma cells studied were obtained from

a patient with multiple myeloma and were engaged in the synthesis of large quantities of immunoglobulin. Experiments are in progress to determine whether nucleolar fluorescence enhancement of ANS is associated with activation of the nucleolar organizer or with processes specifically correlated with neoplasia.

SUMMARY

Hydrophobic components that enhance the fluorescence of 8-anilino-1-naphthensulfonate (ANS) were demonstrated in the cytoplasm of human lymphocytes, polymorphonuclear leukocytes, and plasma cells. Such hydrophobic components were not found in the nuclei of these cells but were demonstrated in nucleoli of the plasma cells.

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