# Fluorescein-concanavalin A conjugates distinguish between normal and malignant human cells: a preliminary report

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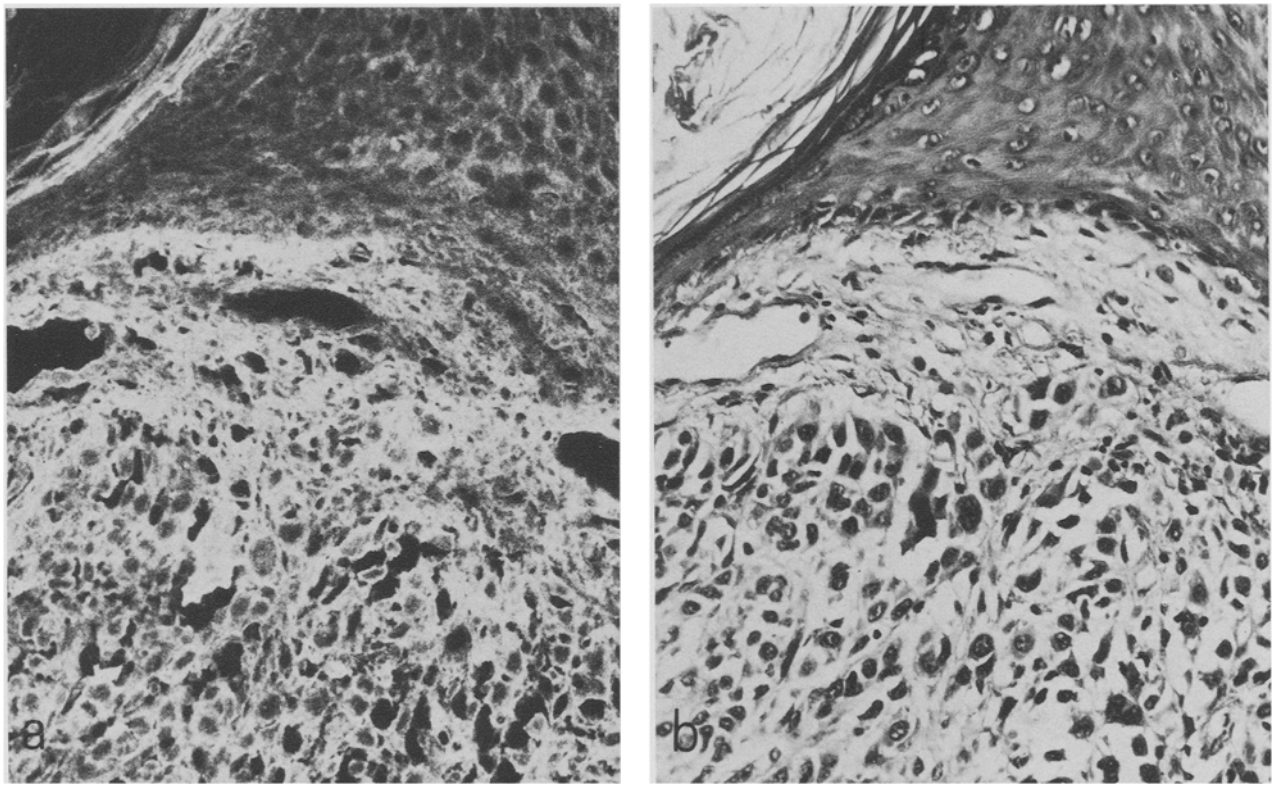
*Summary.* A method is described for using a fluorescein isothiocyanate concanavalin A conjugate to stain human cell membranes in formalin fixed paraffin embedded tissue. 57 neoplastic and normal tissue sites were examined. In 34 malignant tumours, bright green fluorescence was confined to the cell membranes while in 23 benign tumours and normal tissue sites, the membranes were unstained or showed a diminished level of fluorescence. The distinction between malignant and hyperplastic or normal cells was clear cut and definite.

Certain plant proteins (lectins) agglutinate cancer cells but not their nonneoplastic parent cells[1-3]. These lectins have been used extensively to identify differences in plasma membranes. They are thought to bind specifically to glycoprotein receptors located on the cell surface[4]. Recently, Bramwell and Harris[5] suggested that the lectin receptor on the plasma membrane of malignant cells is an abnormal glycoprotein in dimeric form, the normal cell glycoprotein receptor being monomeric. They believe the receptor to be part of the glucose transport system. We have investigated a variety of human tumours using a conjugate of concanavalin A (con A) with fluorescein isothiocyanate (FITC) as a histochemical stain. Here we report that in the cases examined FITC-con A conjugates seem to distinguish clearly and consistently neoplastic cells from their nonneoplastic parent cells.

The method was derived from conventional routine histological procedures. Formalin fixed paraffin embedded tissue sections were deparaffinized, washed and stained for 30 min with FITC-con A diluted in phosphate buffered saline (PBS) to a concentration of 0.015 mg/ml in a moist chamber. Then the slides were washed in 3 changes of PBS, mounted in saline glycerol and examined microscopically by Ploem illumination in UV light. Unstained sections prepared and mounted in the same way were used as controls for the identification of autofluorescence. In this system, the pattern of fluorescence is:

| | |
|---|---|
| FITC-con A | - Green |
| Autofluorescence | - Blue of yellow |

Green autofluorescence is rare. For comparison FITC-con A stained sections were photographed in UV light, then




Formalin fixed paraffin section prepared from the margin of a primary malignant melanoma of the skin and stained with FITC-con A conjugate: *a* Fluorescence photomicrograph showing fluorescing tissue on bottom half, non-fluorescing tissue above and some linear autofluorescence near top left corner; *b* same area as shown in (a) after washing the section in PBS and staining with haematoxylin and eosin, for comparison. The lower fluorescing area in (a) corresponds to tumour tissue and the fluorescence is confined to the surface of the tumour cells representing binding of FITC-con A conjugate to the cell membrane; the nucleus and cytoplasm do not bind conjugate. The non-fluorescing area above corresponds to normal stratified squamous epithelium which fails to bind conjugate. The linear autofluorescence is a blue-white colour and corresponds to keratin. × 200.

washed, stained with haematoxylin and eosin, and the same area rephotographed in white light.

In the tumours examined (table 1), bright green fluorescence was emitted only by malignant cells. Fluorescence was confined to the cell membrane. Cytoplasm and nucleus were not stained. On the other hand, normal cells were either unstained or showed a diminished level of fluorescence which was readily distinguishable from that emitted by malignant cells. Hyperplastic cells adjacent to malignant cells and normal cells failed to show a positive staining reaction. The observations recorded here apply to fully developed tumours and their metastases, and in all cases investigated, the distinction between malignant neoplastic and normal or hyperplastic cells was clear cut and definite. Examination of control sections for green autofluorescence is an essential requirement of this method. While we have found it to be rare, the possibility of it overlapping with the specific fluorescence of fluorescein should not be overlooked.

In the past many biochemical and morphological characteristics of tumour cells have been described. On further examination, none has proved to be absolutely distinctive and unique to the malignant cell. On the evidence currently available, it would appear that the increased capacity of malignant cells to bind certain lectins is the most likely characteristic to meet this ideal requirement. The adaptation of this finding to histological material has apparent applications in fundamental studies in cancer research and diagnosis. In difficult diagnostic cases when the specimen is small, the lesion arising in situ, or when the degree of morphological differention is marginal, this method may prove to be a potent tool in the recognition of the malignant cell regardless of its morphology.

Results obtained after staining various human tumours and normal tissues with FITC-con A conjugates

| Tissue | Condition | No. of cases | Rim fluorescence* |
|---|---|---|---|
| Epidermis | Squamous cells carcinoma | 5 | + + |
| | Basal cell carcinoma | 4 | + |
| | Irritated epithelium | 7 | − |
| | Malignant melanoma | 5 | + + |
| | Naevi | 7 | − |
| Breast | Lobulated hyperplasia | 2 | − |
| | Carcinoma | 3 | + + |
| | Metastases in lymph node | 2 | + + |
| | Adenosis | 2 | − |
| Lung | Bronchial mucosa | 3 | − |
| | Squamous cell carcinoma | 3 | + + |
| | Undifferentiated carcinoma | 2 | + + |
| Pancreas | Carcinoma | 2 | + + |
| | Malignant islet-cell tumour | 2 | + + |
| Liver | Hepatocarcinoma | 3 | + + |
| | Hepatoblastoma | 1 | + + |
| Muscle | Leiomyoma | 2 | − − |
| | Leiomyosarcoma | 2 | + + |

*The staining observed was a rim of green fluorescence on the surface of the tumour cells: (+ +), bright fluorescence; (−), absent or diminished fluorescence.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## Ultrastructural localization of glucose 6-phosphatase activity in the cells of the epididymis of the mouse


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*Summary.* Glucose 6-phosphatase activity is localized in the endoplasmic reticulum and nuclear envelope of all cell types composing mouse epididymis. It is higher in the principal cell than in other cell types in the terminal and caudal half of the middle segment.


Cytochemical localization of glucose 6-phosphatase (G6Pase) activity has been described for a variety of cell types in various organs. Allen[2] reported a detailed study of the mouse epididymis but the observations were carried out only with the light microscope and the pH of the incubation medium used was 5.5. This pH value is rather low in comparison with that (6.0–6.5) considered to be optimal for glucose 6-phosphate hydrolysis[3] and that (6.5) of the modified Wachstein-Meisel incubation media commonly used[4–6]. We have, therefore, investigated the ultrastructural localization of G6Pase activity in the cells of mouse epididymis, using a modified Wachstein-Meisel medium.

DD mice used were 3–6 months of age. Portions of the epididymis corresponding to the initial segment and 4 other parts (I, II, III and IV), from the middle and terminal segments[7], were dissected out as shown in figure 1. Thin slices of the portions, about 0.5 mm in thickness, were fixed in 2% glutaraldehyde buffered with 0.1 M cacodylate (pH 7.2) for 30–45 min at 4 °C and then washed in 0.1 M cacodylate (pH 7.2) containing 8% sucrose for 1 h at 4 °C. The slices were sectioned at 30 µm with a freezing microtome and incubated for 1 h at room temperature in a medium based on that described by Wachstein and Meisel[8], but containing 3 times as much substrate and at

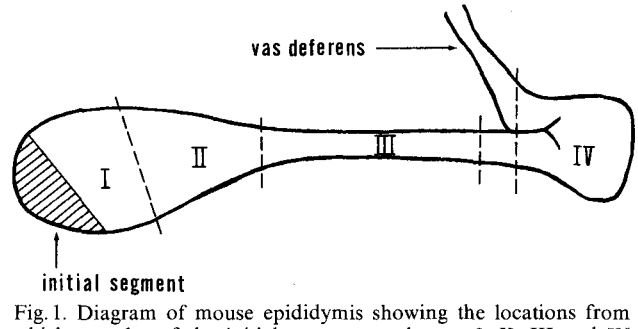


Fig. 1. Diagram of mouse epididymis showing the locations from which samples of the initial segment, and parts I, II, III and IV were obtained. Parts I, II, III and IV correspond to the middle and terminal segments.