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

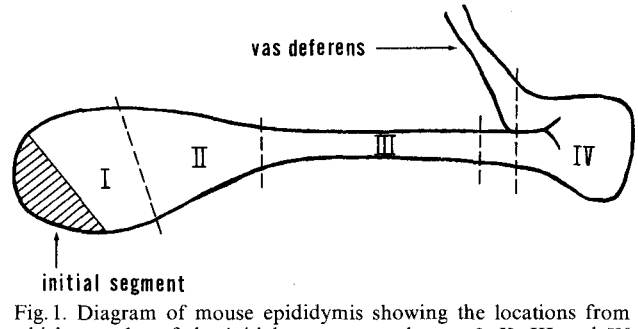
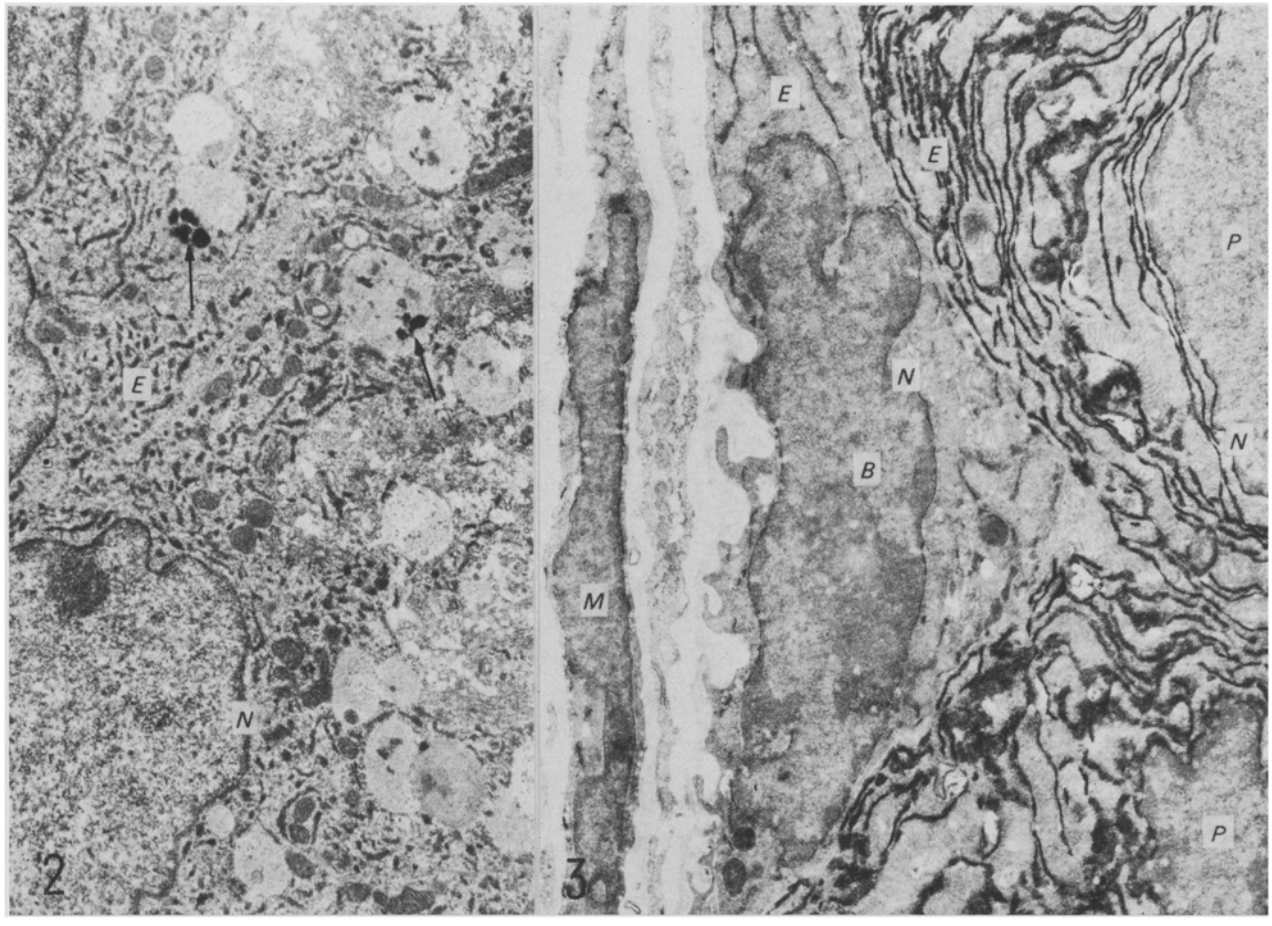


Fig. 1. Diagram of mouse epididymis showing the locations from which samples of the initial segment, and parts I, II, III and IV were obtained. Parts I, II, III and IV correspond to the middle and terminal segments.



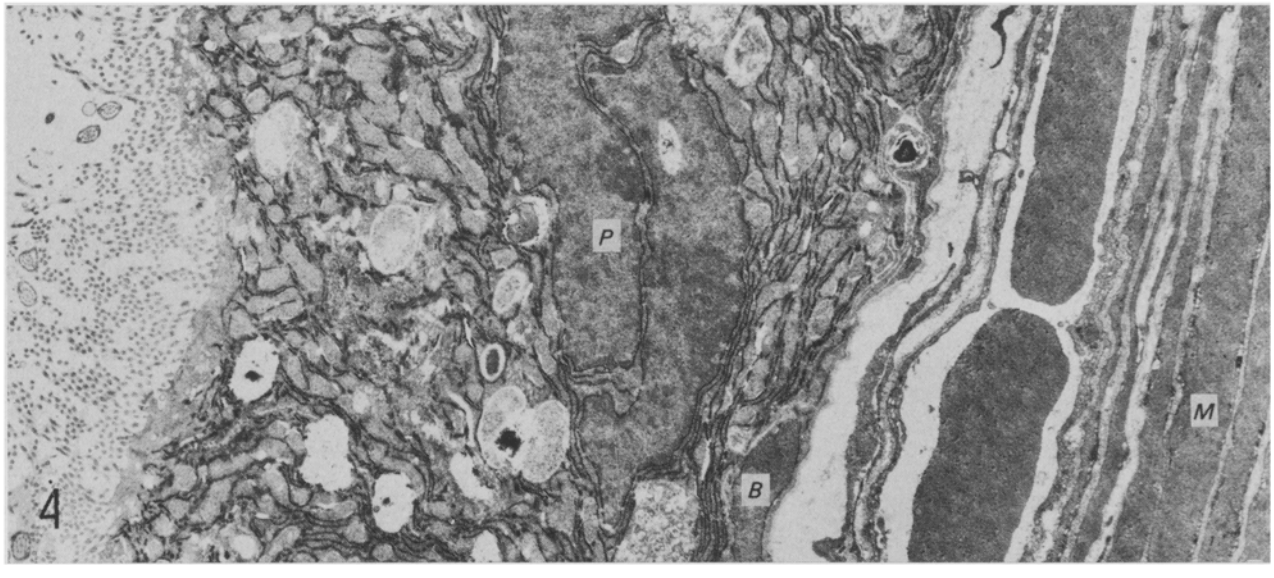


Fig. 2. Supranuclear portions of 2 principal cells in the initial segment of mouse epididymis. A small amount of reaction product is seen in the endoplasmic reticulum (E) and nuclear envelope (N). Dense materials (arrows) in the supranuclear vacuoles are not a deposition of lead. × 13,500. Fig. 3. Basal portions of 2 principal cells (P), and a basal cell (B) and smooth muscle cell (M) in part III of mouse epididymis. Reaction product is seen in the endoplasmic reticulum (E) and nuclear envelope (N) of these cells. Note that deposition of reaction product is apparently more abundant in principal cells than in basal cell and smooth muscle cell. × 9600. Fig. 4. Principal cells (P), a basal cell (B) and smooth muscle cells (M) in part IV of mouse epididymis. Abundant deposition of reaction product is seen in principal cells, while the amount of the product is scanty in basal cell and smooth muscle cells. × 7800.

pH 6.5. Sections were postfixed in buffered 1% osmium tetroxide for 2 h at 4 °C, dehydrated in a graded series of alcohols and embedded in epon. These sections were cut with glass knives on a LKB ultratome, stained with uranyl acetate and lead citrate and examined in a HU-12 electron microscope. Some of the sections cut at 30 µm were also incubated in the same medium but at pH 5.5 or in Allen's incubation medium[2] (pH 5.5). The sections were washed in distilled water, immersed in dilute ammonium sulfide and mounted in glycerine jelly for light microscope observations. The reaction product was present in the endoplasmic reticulum and nuclear envelope in all cell types of the epididymis, i.e. the principal cell, halo cell, basal cell, clear cell, smooth muscle cell and fibroblast (figures 2–4). The amount of the reaction product was scanty and was similar in all cell types in the initial segment and part I (figure 2), and apparently more abundant in the principal cell than in other cell types in parts II, III and IV (figures 3 and 4). The basal cell, halo cell, clear cell, smooth muscle cell and fibroblast showed similar amounts of deposits in all parts of the epididymis. The reaction product was also observed on the stereocilia of the principal cell and sometimes in the lysosomes in the principal and basal cells. The product on the stereocilia was abundant in the initial segment, in small quantities in part I and was not seen in the other parts.

In order to ascertain whether the reaction product is due to G6Pase activity, control experiments were carried out as described previously[9,10] at light and electron microscopic levels. Immersion of the glutaraldehyde-fixed sections in 0.1 M acetate buffer (pH 5.0) at 37 °C for 15 min before incubation in the reaction medium and the use of β-glycerophosphate in place of glucose 6-phosphate in the reaction medium, resulted in complete absence of the reaction product except on the stereocilia of the principal cell and in some lysosomes in the principal and basal cells. Pre-incubation of the sections in 0.25 M sucrose containing 1 mM Cu^{2+}, 10 mM Zn^{2+}, 10 mM F^- or 10 mM CN^-, and then incubation in the reaction medium containing equal moles of these ions, caused a loss of the histochemical staining except on the stereocilia, although no staining was seen on the stereocilia with CN^-. These results indicate that the reaction product in the endoplasmic reticulum and nuclear envelope is due to G6Pase activity, but the deposition of final product on the stereocilia of the principal cell and in the lysosomes in the principal and basal cells is probably related to non-specific phosphatase or acid phosphatase activity.

There were no deposits in the Golgi complex, mitochondria, plasma membrane and other organelles in these cells, including the supranuclear vacuoles of the principal cell. Under the light microscope the staining for the activity appeared more intense in sections incubated in the regular medium (pH 6.5) than in those incubated in the medium at pH 5.5 or Allen's medium (pH 5.5).

G6Pase is known to be present in almost all cell types in various organs[4,8,10–17]. The functional role of this enzyme in the liver and kidney is to release glucose into the blood by hydrolyzing glucose 6-phosphate produced via gluconeogenesis and glycogenolysis[3]. However, the role of the enzyme in other cell types is unknown. In the terminal segment and caudal half of the middle segment of epididymis, the activity of G6Pase is apparently higher in the principal cell than in other cell types. The epididymis is the site of maturation (initial and middle segments) and storage (terminal segment) of the spermatozoa[7]. The principal cell might, therefore, release glucose for use by the spermatozoa. On the other hand, the role of the enzyme in the basal cell, halo cell, clear cell, smooth muscle cell and fibroblast might be simply to regulate the concentration of glucose 6-phosphate in these cells, hydrolyzing any excess, as postulated for the enzyme in the tracheal epithelial cell[15].

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Cimetidine prevents the induction of unresponsiveness by feeding T-dependent antigens[1]

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Summary. Bovine γ globulin (BGG) or ovalbumin (OVA) given to mice orally induced virtually complete unresponsiveness to later parenteral immunization. When these antigens were given to mice orally, following cimetidine treatment, and the animals were parenterally immunized later, enhanced response to the challenge antigen occurred.

The receptor for histamine, designated H2, can be blocked with the drug cimetidine, which causes marked inhibition of gastric acid secretion[2–4]. Suppressor T cells (T_S) also bear this receptor and there is evidence that cimetidine may modify these cells and under some conditions produce an immunostimulatory effect [5,6]. Suppressor T cells have been shown to be involved in the specific unresponsiveness which follows the oral administration of certain antigens[7,8].

The present study was undertaken in order to investigate the influence of cimetidine on the humoral immune response to T-dependent and T-independent antigens given orally in mice.

Materials and methods. Randomly bred Swiss mice weighing 18–20 g were used. The antigens, *E. coli* lipopolysaccharide (LPS) - Difco Laboratories; Keyhole limpet hemocyanine (KLH) - A grade, (Calbiochem, San Diego, Calif.);