

Localization of Antigens and Allergens in Thin Sections of the House Dust Mite, *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae)

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J. Med. Entomol. 27(3): 368-376 (1990)

ABSTRACT Cryostat sections of the house dust mite, *Dermatophagoides pteronyssinus*, were probed in fluorescent microscopy studies with rabbit polyclonal and mouse monoclonal antibodies specific for mite allergens including the major allergen, *Der p* I. Sections also were probed for allergens with sera from human mite-allergic subjects containing IgE antibodies to mite allergens and with lectins. Antibody binding was mainly to the gut lining and gut contents of the mite, although some specific labeling also was associated with the head region and cuticle. This is the first detailed localization of mite allergens in situ. The morphology of the mite was investigated using plastic embedded thin sections and was found to be similar to that previously described for *D. farinae*.

KEY WORDS Arachnida, *Dermatophagoides pteronyssinus*, allergen localization, microscopy

THE HOUSE DUST MITE, *Dermatophagoides pteronyssinus*, is found in the dust of carpets, beds, and clothing in tropical and temperate regions of the world and is a potent and common source of aeroallergens that may provoke asthma, rhinitis, and eczema (Platts-Mills & Chapman 1987, de Weck & Todt 1988).

Several studies have demonstrated the antigenic and allergenic complexity of mite extracts, but not the relative clinical importance of the allergens. Mite allergens have been isolated and identified in soluble extracts using immunoelectrophoretic (Lind 1980, Krilis et al. 1984, Lind et al. 1984) and Western blotting (Tovey & Baldo 1984, 1987) techniques, but have not been localized in situ. Extracts of mites have been shown to contain more than 30 allergens. The majority of mite-allergic subjects are allergic to six of these (Baldo et al. 1989). Many of these 30 allergens are shared by extracts of mite bodies, culture material, and feces, although their relative proportions differ in these sources (Tovey & Baldo 1987). Mite feces and fragmented bodies could become airborne and act as sources of allergen exposure. Because the mite allergen *Der p* I is found in the feces, it is presumed to have its origin somewhere in the digestive tract, however, its exact origin, or the origins of any of the other mite allergens is unknown.

The localization of mite allergens in situ is important for several reasons. Allergen localization may indicate the importance of secreted material, feces, and nonexcreted mite body components as allergen sources. Secondly, conventional studies of allergens usually are conducted with water soluble extracts. However, allergens of very low solubility

do occur, such as the allergens found in wheat endosperm (Walsh et al. 1985). Techniques for the identification of poorly soluble allergens could be developed from thin sectioning studies of allergen sources in situ. Third, localization studies may indicate the biological function of individual allergens as well as suggesting structural similarities between house dust mite allergens and allergens produced by other Acari, such as tick salivary proteins (Gauci et al. 1988).

The only previous report of mite antigen localization (Mumcuoglu & Ruflı 1979) used rabbit antiserum against whole mite extracts. However, the relevance of this study to human respiratory disease can be questioned. It is unknown whether rabbit IgG produced using an adjuvant and injection of antigen recognizes the same mite antigens as human IgE produced following prolonged natural exposure of the human respiratory system to inhaled antigen.

Since the early study using rabbit antisera of unknown specificity (Mumcuoglu & Ruflı 1979), both monoclonal and polyclonal antisera specific for the mite allergens *Der p* I and *Der p* II have become available. This study utilized some of these antisera, in addition to human IgE antibodies and lectins to probe thin sections of mites. A preliminary study of mite morphology also was made to facilitate the interpretation of the antibody-labeling results.

Materials and Methods

Preparation of Sections. For cryostat sections, the mites were grown and separated from culture

as previously described (Tovey et al. 1981a,b). Because of their minute size, the different developmental stages were not separated. Groups of 20–60 mites were embedded together to obtain a sample which included the different stages. Live mites were directly embedded in Tissue Tek (Miles Scientific, Elkhart, Ind.) and 4- μ m sections were cut with steel knives at -25°C on a Wild-Leitz model Kryostat 1720 microtome. Sections were mounted on clean glass slides and air-dried at room temperature.

For embedding in the plastic resin LR White (LRW) (medium grade) (Polaron, Watford, U.K.; batch 1243), clean mites were fixed in 4% paraformaldehyde with 2.5% sucrose in 0.05% phosphate buffer at 4°C for 2 h and washed in buffer. Mites were dehydrated successively for 1 h each in 20, 30, 40, 50, and 60% ethanol, overnight in 70% ethanol, and for 1 h each in 90, 95, and 100% ethanol. Infiltration was effected with 25, 50, and 75% LRW in ethanol for 1 h each and with 100% LRW for 24 h. Mites were embedded in gelatin capsules and hardened overnight in an oven at 60°C . Sections 1 μ m thick were cut with glass knives on an LKB ultramicrotome (model 5), floated on drops of distilled water onto glass slides, dried on a hot plate at 50°C , and stored at -20°C for later immunoprobings. During fixation, dehydration, and infiltration the mites were contained in capsules constructed from 1.5-ml Eppendorf tubes with a fine mesh stainless-steel screen over each end. During the procedures capsules were held in glass-stoppered tubes that were rotated at about 30 rpm at an angle of 45°C .

Staining and Immunoprobings of Sections. Mite sections were incubated briefly (5 min, 50°C) in toluidine blue Borate buffer (pH 4.5) as a general stain for tissues. For immunoprobings, mite sections were incubated for 2–4 h in the following antibody-containing solutions: rabbit antisera either against whole-mite body extract or a monospecific antiserum against the mite allergen *Der p* I prepared as described previously (Tovey & Baldo 1984, 1987); a monoclonal antibody against the mite antigen *Der p* I (CLBDER,P1) from a cell line established by R. Aalberse, Netherlands Red Cross Blood Transfusion Service, Amsterdam, and supplied by Commonwealth Serum Laboratories, Parkville, Victoria, Australia; and human sera containing mite-specific IgE antibodies from subjects, with a skin prick test wheal >5 mm with Bencard *Dermatophagoides pteronyssinus* mite extract obtained from the Allergy Department, Royal North Shore Hospital. Rabbit sera were used at a concentration of 1:50. The monoclonal antibody culture supernatant was used undiluted, and the human serum was used at a concentration of 1:10. IgE-binding was detected using MIAB rabbit antihuman IgE (Knivsta, Uppsala, Sweden) (batch number 40905) at 1:1,000. The binding of the rabbit or murine antibodies was detected by incubating with fluorescent-labeled sheep antirabbit or antimouse F(ab')₂ antibody

fractions (Silenus Laboratories Pty Ltd., Dandenong, Victoria) each at a dilution of 1:50 in phosphate buffered saline (PBS), pH 7.4, for 1–2 h. Sections were washed following each incubation in two changes of PBS with 0.005% Tween before being mounted in glycerol/water (1:1) for viewing under incident illumination on a Leitz Ortholux 2 microscope and photographed with a Leitz variograph camera system. Antibody controls were normal rabbit serum, Iscoves culture medium for monoclonal antibodies and IgE-free human cord serum. The fluorescent lectins were used at a concentration of 1 mg/ml, and have been described previously (Baldo et al. 1982).

Identification of Structures in Sections of Mites. The diversity in structures of mite internal organs (reviewed by Woodring & Galbraith 1976) is reflected, for example, in the variety of names and descriptions in the literature for the regions of the gut (Coons 1978). The identification of the organs and terminology follows the description of the closely related mite, *Dermatophagoides farinae* Hughes 1961 (Brody et al. 1972).

Results

Morphology Studies with Embedded Plastic Sections. Most organelles that were found to be fluorescing with immunostaining were identified in thin sections from different *D. pteronyssinus* mites embedded in LRW and stained afterward with toluidine blue. Identifications were easier on the larger mites, which were presumably late nymph or adult stages. Mite morphology appeared consistent, with small differences among individuals. A sagittal section, close to the midline, of two such mites is shown in Fig. 1 a and b. The preponderance of gut tissue is evident. Food enters via the pharynx and passes through the esophagus (star-shaped in cross section) into the large anterior midgut region. Two caecae which extend laterally from the anterior midgut are not seen in these sections. The anterior midgut varies in size depending on the state of engorgement and is connected to the posterior midgut by a narrow opening. Balls of food in the posterior midgut appear to be separated from the gut wall by an unstained region. These fecal pellets pass through the posterior hindgut before being excreted. Brain, salivary, reproductive and muscular tissues are identified in Fig. 1 based on their structure (in serial sections) and position in the body cavity (Brody et al. 1972).

The most striking morphological variability involves the midgut. In Fig. 1a, the walls of the engorged midgut appear to be lined with thin, flat, darkly stained cells which are covered with an inner lightly stained, filamentous layer (presumably microvilli). The lateral caecae of this mite were so distended as to appear continuous with the anterior midgut. In Fig. 1b the anterior midgut is less distended than in Fig. 1a, and large darkly stained

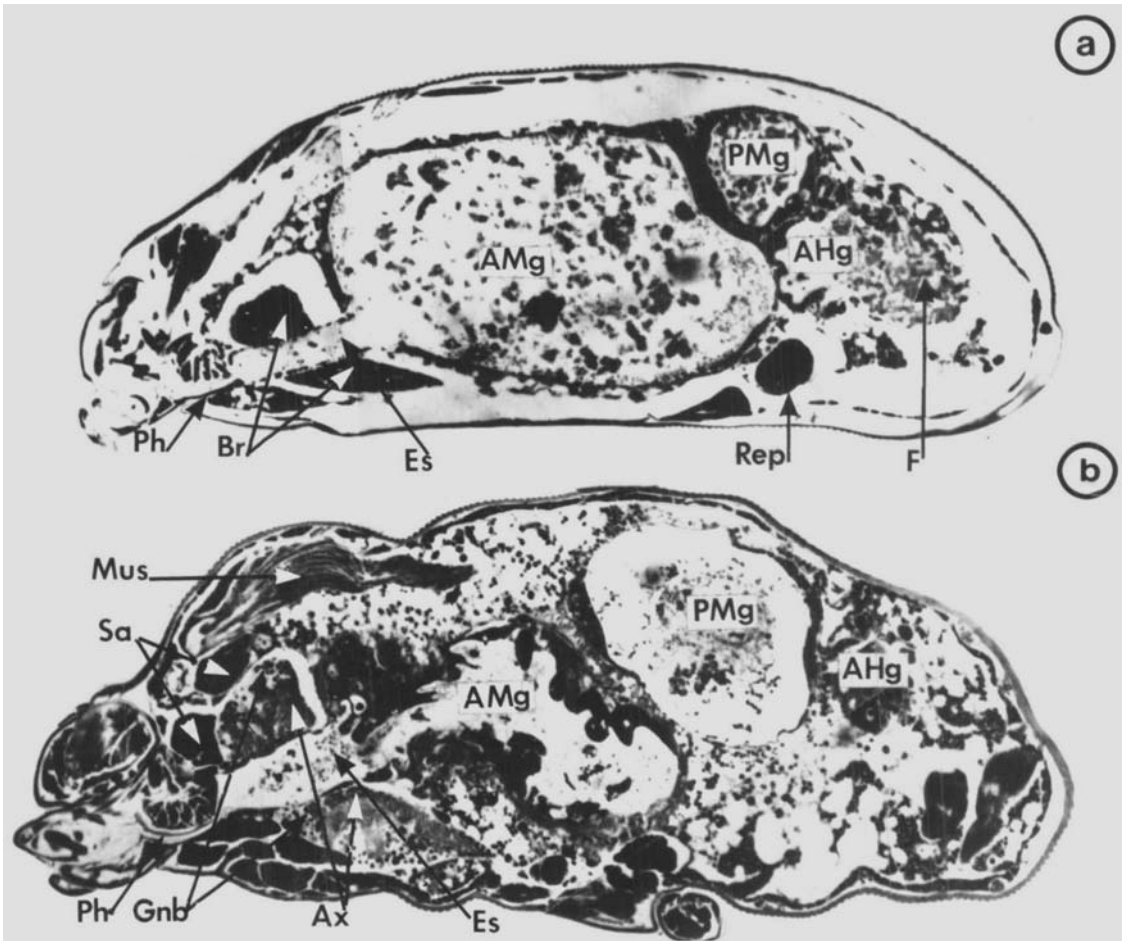


Fig. 1. Toluidine blue staining of sagittal thin sections of LRW embedded house dust mites. Fig. 1a shows the general features of the gut regions: pharynx (Ph), esophagus (Es), anterior midgut (AMg), posterior midgut (PMg), and the anterior hindgut (AHg). The brain (Br), a section of reproductive tissue (Rep) and a fecal particle (F) are also apparent. Fig. 1b, in addition, shows salivary tissue (Sa), large muscles (Mus) that probably are connected to the cheliceral shafts and the neural bodies (Gnb) and homogeneous staining axon (Ax) regions of the brain. Mite body length approximately 200 μ m in both figures.

cells are visible in the dorsal and ventral walls of the anterior midgut. The posterior midgut showed a range of forms, with the more typical appearance shown in Fig. 1a and a more distended form shown in Fig. 1b. The fecal pellet in Fig. 1a is shown surrounded by a thin, lightly stained region which is probably the peritrophic membrane.

In the anterior region of the mites, the esophagus can be seen passing through the offset center of the doughnut-shaped brain. Darkly stained globular salivary gland tissue is visible anterior to the brain in Fig. 1b. Dorsal to this, beneath the dorsal plate, are large bundles of muscle fibers probably connected to the cheliceral shaft.

Localization of Allergens. Immunofluorescent probing of cryostat-cut sections with antisera raised in rabbits against mite body extract showed labeling of almost all regions of the mite sections. The most intense fluorescence was associated with the gut, feces, epidermis closely associated with the

inner surface of the cuticle and interior regions which included the gnathostome and salivary glands (Fig. 2 a and b). Sections incubated with normal rabbit serum as controls demonstrated weak orange autofluorescence and only very weak, scattered fluorescent staining (Fig. 2c).

When a polyclonal rabbit antiserum specific for mite allergen *Der p I* was used for probing, the regions showing labeling were the anterior midgut, gut contents and posterior midgut (Fig. 3a). A similar pattern of labeling was observed when a mouse monoclonal antibody against *Der p I* was used, with additional labeling of the esophageal region (Fig. 3 b and c). With the monoclonal antibody, the labeling appeared sharper, although precise localization was restricted by the quality of the cryostat-cut sections. Control sections incubated with cell culture medium instead of the monoclonal antibody showed no immunofluorescent labeling (Fig. 3d). The lower resolution from the fluorescence

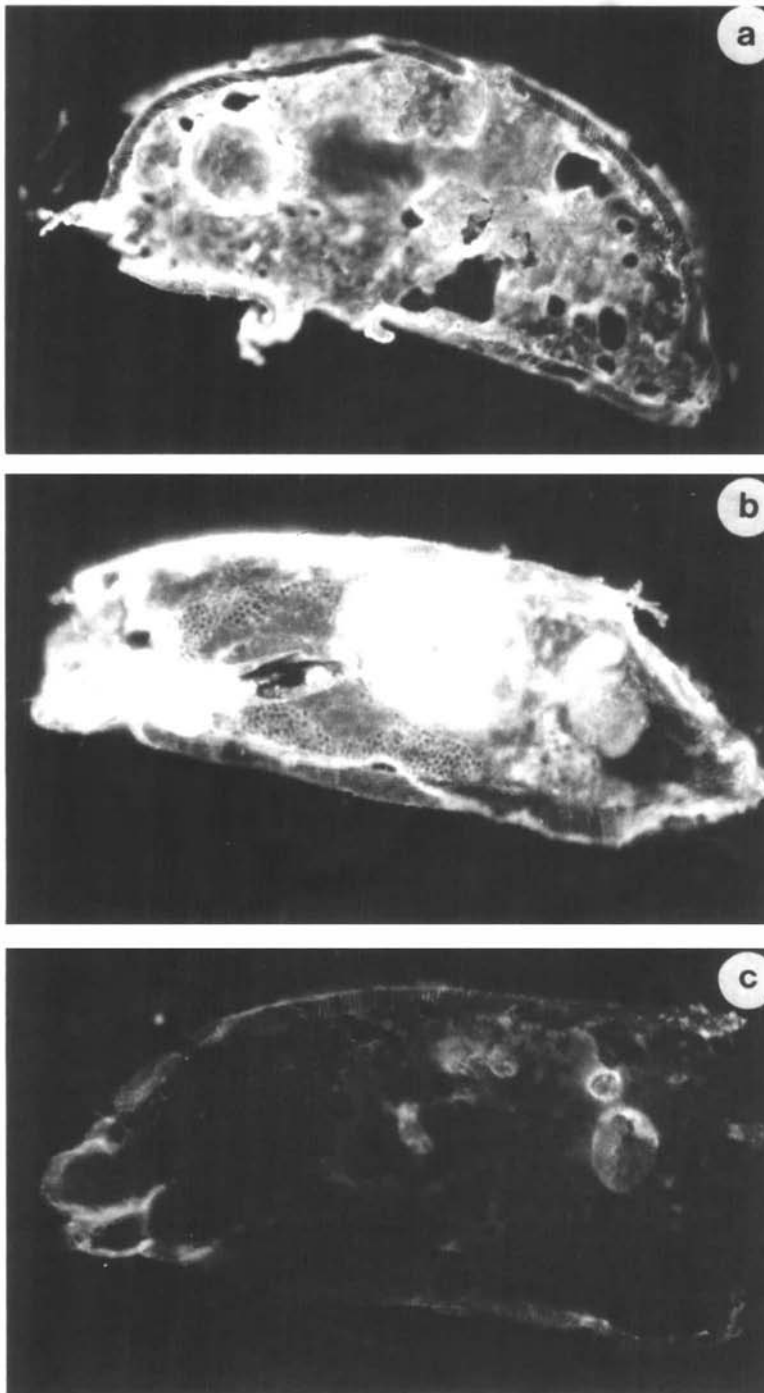


Fig. 2. Cryostat sections of mites probed with rabbit antisera against whole mite body extract or a control rabbit antiserum followed by fluorescent antirabbit antisera. Fig. 2a with the head of the mite to the right, shows intense labeling of a mite fecal particle and strong labeling of epidermal cells beneath the cuticle and the hemolymph. The strongest labeling in Fig. 2b (head of mite to the left) is in the region of the esophagus and gnathostome. Intense labeling is seen on the anterior midgut and again on the epidermal cells beneath the cuticle. Fig. 2c is a control using normal rabbit antiserum as the first antibody.

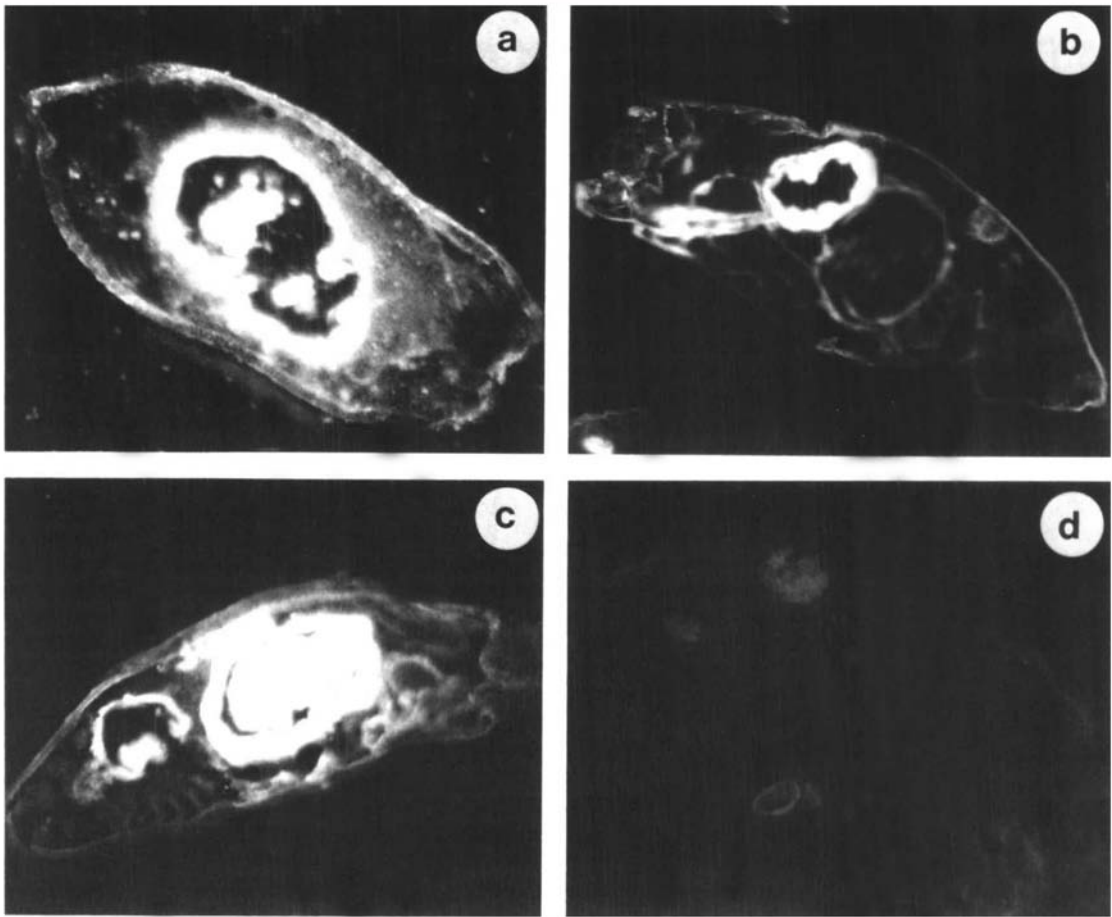


Fig. 3. Cryostat sections of mites probed with antisera against specific mite antigens or with a control antiserum. Antisera used: Fig. 3a, rabbit antiserum against *Der p* I; Fig. 3b and c, mouse monoclonal antiserum against *Der p* I; Fig. 3d, control medium as used for cell culture of monoclonal producing hybridoma. Detection of rabbit or mouse antibody binding was with the appropriate fluorescent-labeled antisera.

microscopy with frozen sections did not allow identification of organelles with the same degree of resolution seen in plastic thin sections.

When two separate human sera containing mite-specific IgE antibodies were used as the first antibody followed by rabbit antihuman IgE and then fluorescent-labeled antirabbit serum, the most prominently labeled areas were the anterior midgut, fecal pellet and possibly the midgut caecae and midgut cells (Fig. 4a and b, respectively). The weaker labeling of the head region and the inner surface of the mite cuticle was probably on the epidermal cells. In control section where IgE-free human cord serum was used as the first antibody, negligible fluorescent staining was observed (Fig. 4c). Probing of the cryostat sections with fluorescent-labeled lectins demonstrated strong binding to discrete regions of the mite. In particular soya bean lectin (specific for D-galactose) reacted with the gut contents and walls and the outer band around fecal balls (Fig. 5a and b). Wheat-germ

agglutinin (specific for *N*-acetyl-*D*-glucosamine) was more generally distributed with strong staining of the anterior midgut walls (Fig. 5c).

Specific identification of antibody-binding organelles by probing plastic embedded mites was not successful. When the LRW-embedded sections were probed first with specific rabbit antisera, mouse monoclonal antibodies, human allergic serum or saline and then followed by a labeled second antibody, speckled, weak fluorescent labeling was observed on the same locations irrespective of the antibody used. Similar nonspecific labeling also was observed with fluorescent-labeled lectins, but not with free unconjugated fluorescein. Labeling was confined to small foci in the hemolymph, cells lining the esophagus, areas associated with the mouthparts, areas of the inner and outer surface of the cuticle and a fine line around the outside of the fecal particles. The binding of the primary antibody on LRW thin sections also could not be detected using peroxidase staining.

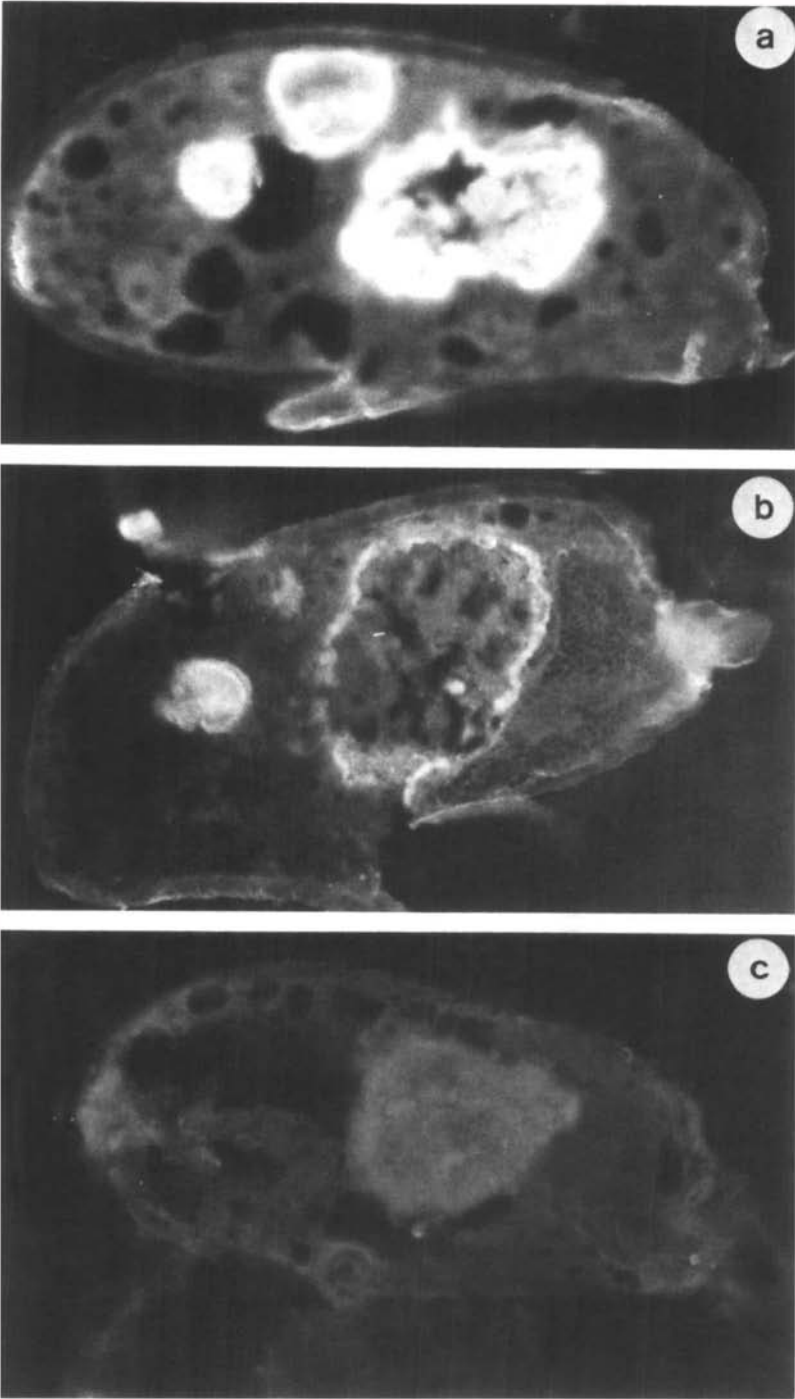


Fig. 4. Cryostat sections of mites probed with sera from mite allergic subjects or controls, showing detection of IgE binding using a rabbit anti-IgE followed by fluorescent-labeled antirabbit antisera. Fig. 4 a and b show labeling of anterior midgut regions and fecal particles by two different atopic sera. Weaker labeling of the epidermis beneath the cuticle is also apparent. Fig. 4c was probed with IgE-free human cord serum as the first antibody control.

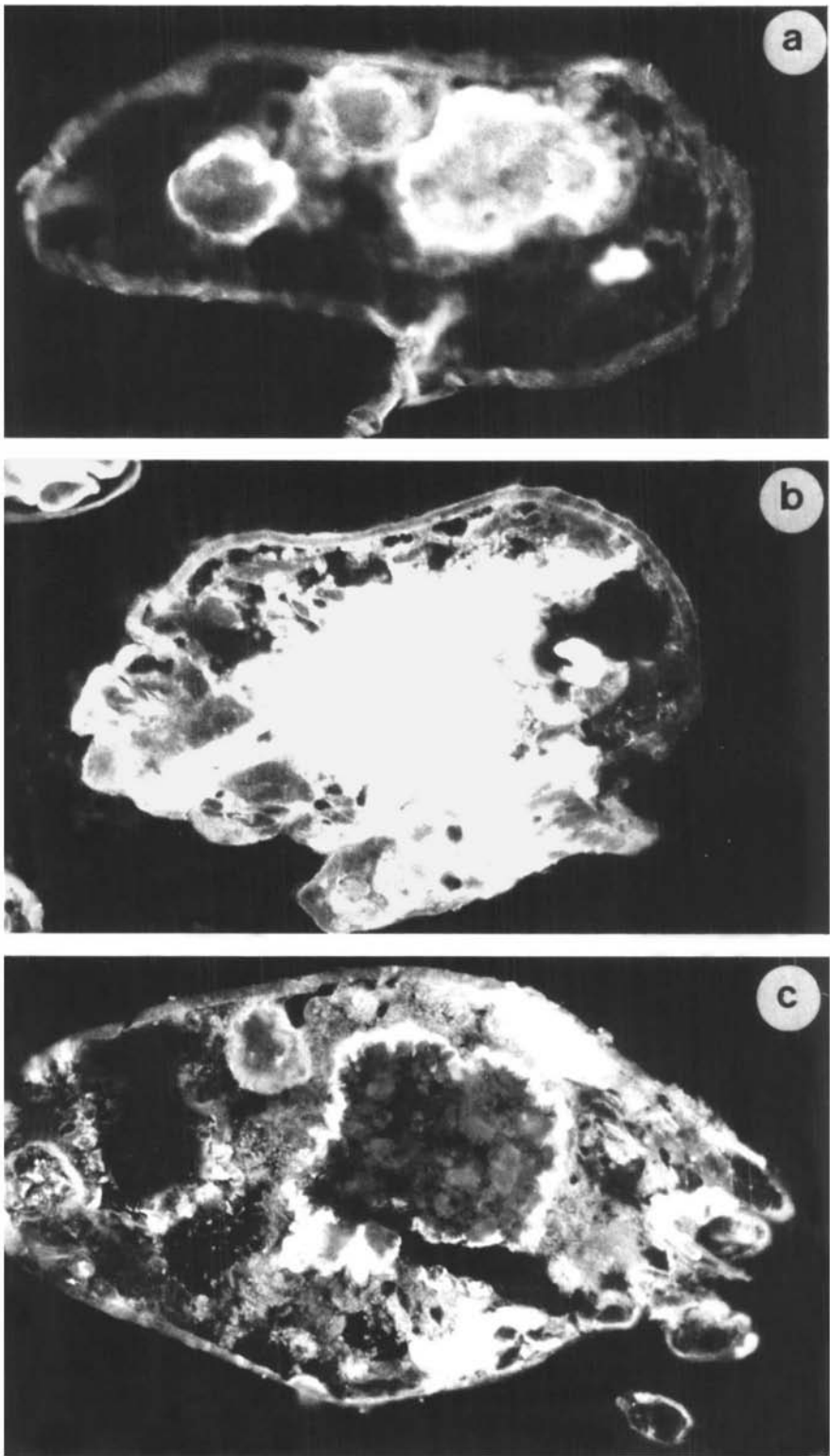


Fig. 5. Cryostat sections of mites probed with fluorescent-labeled lectins. Lectins used were: Fig. 5 a and b, *Glycine max* (soybean); Fig. 5c, *Triticum vulgare* (wheat-germ agglutinin).

Discussion

The morphology of *D. pteronyssinus* appears to be similar to that reported for *D. farinae* (Brody et al. 1972). Minor differences observed among individual mites, particularly in the structure of the caecum and anterior foregut, were attributed to differences in the degree of engorgement, stage of development or sex. The large dark-staining, pear-shaped cells associated with the anterior midgut wall or free in the gut contents were probably the same cells as those described in *D. farinae* by Brody et al. (1972). These authors suggested that the free-floating cells participate in intracellular digestion. Based on ultrastructural details, Coons (1978) suggested that the large cells in the wall of the anterior midgut of the mite *Macrocheles muscaedomesticae* are secretory and may be involved in extracellular digestion.

Immunoprobings of cryostat-cut sections with antiserum raised in rabbits against a whole mite body extract showed overall staining of virtually all regions of the mite except the brain. The strongest staining occurred in the gut regions and feces. These results were similar to a previous study which also used a rabbit anti-serum against a whole mite extract (Mumcuoglu & Ruffli 1979).

The use of a monospecific rabbit antiserum against the important mite allergen, *Der p* I, demonstrated that it was located principally in the walls of the anterior midgut and in the fecal pellets. This allergen originally was isolated from spent mite culture (Chapman & Platts-Mills 1980) and >95% of the total amount of this allergen in culture and house dust can be accounted for by the accumulated mite feces rather than mite bodies (Tovey et al. 1981b). The recent sequencing of this allergen and its homology to cysteine-dependent proteases (Chua et al. 1988) indicated that its biological function is a digestive enzyme. The present studies demonstrated that *Der p* I is secreted from the wall of the anterior midgut and becomes incorporated into the fecal pellet from which it may be extracted readily (Tovey et al. 1981b). The use of a monoclonal antibody against allergen *Der p* I produced a much finer staining than the polyclonal antisera of the same specificity, but the poor resolution of the cryostat-cut sections did not allow a precise localization of this antigen in the anterior midgut wall.

The localization of fluorescence in the gut region of mite sections probed with human antimite IgE antibodies indicated that the majority of allergens were associated with digestion and would accumulate in the feces which readily become airborne after defecation. However, some fluorescence also was associated with parts of the mite not directly associated with digestion, such as the epidermal layer beneath the cuticle. These studies only used sera from a few mite-allergic subjects. The use of more sera with a greater range of specificities for different allergens would probably increase the

number of organelles identified as allergen sources. Because whole mite bodies are too large to become airborne under normal circumstances, natural exposure to the allergens associated with these parts presumably occurs via fragmented parts of mite bodies, which become airborne and are inhaled.

Recent developments in blotting methodology may provide a method to determine the identity of the organelles in the mites associated with individual allergens. We have demonstrated the preparation of IgE antibody specific for individual allergens by eluting the antibody from the individual bands on Western blots which have previously been incubated with sera from mite-allergic subjects (Tovey et al. 1989). Antibody prepared in this manner would be suitable to probe for allergens in thin sections.

The binding of fluorescent-labeled lectins is of interest. Such studies demonstrate the potential of using lectins to both identify and isolate mite allergens and as a set of specific probes for studies of mite digestion. Specific immunoprobings of the LRW sections was not successful, since nonspecific staining by fluorescent-labeled antibodies occurred irrespective of the probe used. The mite components bound by the labeled antibodies, but not by fluorescence alone, were not identified. Other fixation conditions, resins, and labels should be investigated to enable specific antibody probing of thin and ultra-thin, plastic-embedded mite sections.

Acknowledgment

The excellent technical assistance of P. Boniface in preparing the sections and photography is acknowledged. D. Kemp (CSIRO, Long Pocket Laboratory, Indooroopilly, Queensland) greatly assisted with the description of the morphology. This work was supported by the National Health and Medical Research Council of Australia.

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Received for publication 31 December 1986; accepted 10 July 1989.