ture (Fig. 2C). Eventually, one sperm nucleus fuses with that of the egg and the other fuses with the central cell nucleus (Fig. 1), confirming Dahlgren's report (*4*) of double fertilization without the involvement of synergids.

Male cytoplasmic organelles can be identified in both the egg and central cell immediately after entry of the sperm nuclei. Sperm plastids are distinguished by their smaller size (0.5 to 0.7 μm wide), elongate shape (up to 2.2 μm long in section), inflated internal lamellae, and relatively electron-dense stromata (*9*). Such plastids are seen in Fig. 2A at the periphery of the sperm nucleus that entered the egg. Egg plastids are larger (about 1.2 by 2.0 μm), circular to elliptical in section, less electron-dense (Fig. 2B), and occasionally contain starch grains. Mitochondria of male origin can be distinguished from those of the female gametophyte by their size and cellular location (Fig. 2, C and D). Sperm mitochondria next to the sperm nucleus are usually circular in section and 0.2 to 0.3 μm wide. Mitochondria of the female gametophyte are elliptical and 0.5 to 0.8 μm wide. The sperm organelles appear normal in their ultrastructure and presumably are capable of replication under the right conditions. Meyer and Stubbe (*10*) reported the occurrence of ultrastructurally identifiable sperm plastids in zygotes of *Oenothera erythrosepala*. Chlorophyll mutations have been used to identify sperm and egg plastids in this and other species of *Oenothera*, and they are present throughout the life of the plant (*11*).

The presence of extranuclear sperm organelles in the cytoplasm of the egg and central cell supports the view that syngamy in angiosperms is initiated by cellular fusion (*12*). Therefore, both male organelles and nuclei enter the egg and central cell and may contribute to the development of the embryo and endosperm.

The genetic impact of male cytoplasmic inheritance during development on the mature plant depends on whether male organelles remain viable, reproduce, and transmit genetic information within the embryo. The region of the egg where gamete fusion occurs is destined to become part of the suspensor, but it seems unlikely that sperm organelles remain fixed and thus unincorporated in the embryo. Evidence suggests that angiosperms with plastids in the generative cell display biparental patterns of plastome mutation inheritance (*13*). However, full understanding of the impact of male cytoplasmic inheritance during later development in *P. zeylanica* must await research on the inheritance of mitochondrial or chloroplast genes in this plant.

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Cytostructural Localization of a Tumor-Associated Antigen

Abstract. *Tumor cell membrane glycoproteins may be involved in the induction of tumor immunity or in the escape of tumors from immunologic defense mechanisms. Forty-four benign and malignant breast lesions were examined for the presence of a carbohydrate precursor antigen (T antigen) of the human blood group system MN. T antigen was demonstrated by means of an immunohistochemical technique to detect tissue binding of peanut agglutinin, a plant lectin, with affinity for T antigen. Malignant breast lesions showed a pattern of T antigen expression different from that of benign breast tissues. A possible role for T antigen in the modulation of the immune response to breast carcinoma is suggested.*

Springer and colleagues have serologically identified an antigen that is present on malignant but not benign breast tissues (*1*). This antigen, termed T antigen, elicits delayed hypersensitivity reactions in patients with breast cancer but not in healthy controls (*2*). In additional studies, antibody present in normal human serums was used with an immunohistological technique to demonstrate that a similar or identical antigen may be detected on malignant but not benign breast epithelium (*3*). We have now further clarified the nature and specificity of this antigen and its relation to neoplastic transformation.

An immunoperoxidase technique was developed to detect binding of a plant lectin with affinity for T antigen to histological sections of breast tissue. This lectin is derived from peanuts (*Arachis hypogaea*) and is termed peanut agglutinin (PNA). The difference in the pattern of expression of T antigen between benign and malignant breast epithelium suggests that it may have a possible role in the induction of the immune response to neoplasia.

Tissues from breast carcinomas and benign breast lesions were selected, according to their recorded pathological diagnosis, from the surgical pathology files

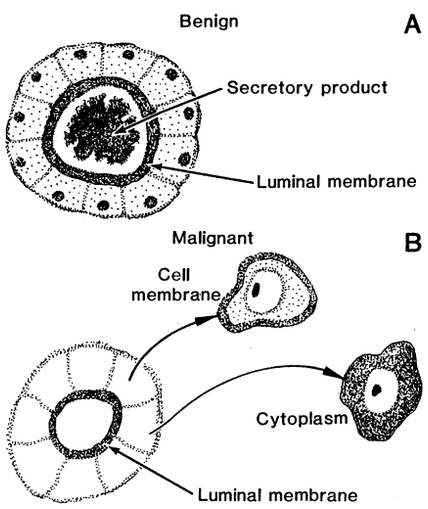

Fig. 1. Pattern of T antigen expression (dark stippled areas) in (A) benign breast tissues and (B) malignant breast tissues. (A) Peanut agglutinin binding occurs along the luminal cytoplasmic membrane with or without binding to intraductal secretory products. (B) Peanut agglutinin binding may occur along the luminal cytoplasmic membrane in the well-differentiated malignancies. Unique to neoplastic cells is the presence of T antigen in a diffuse pattern in the cytoplasm or along the peripheral cell membrane.

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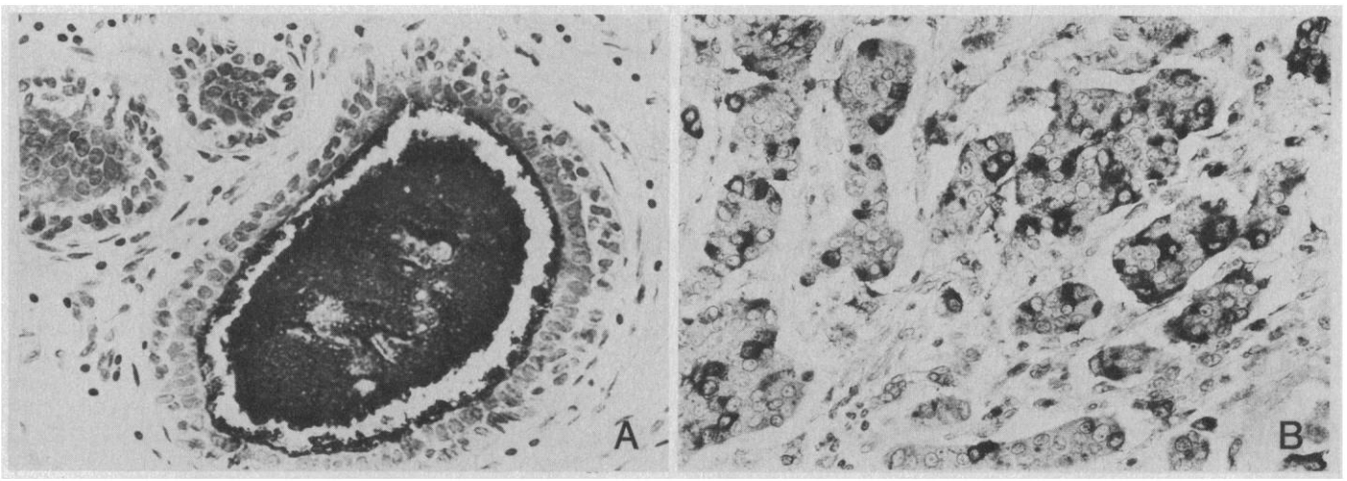

Fig. 2. Immunoperoxidase procedure was used to detect binding of PNA to breast tissue sections (black staining); the counterstain was hematoxylin. (A) Fibrocystic disease of breast (benign). T antigen occurs only on luminal cytoplasmic membranes and intraductal secretions (× 148). (B) Infiltrating ductal carcinoma of breast (malignant). T antigen is demonstrated within the cytoplasm of the malignant cells (× 124).

of the Maine Medical Center during 1977 to 1980. Hematoxylin and eosin sections were reviewed to confirm the diagnosis. The tissues had been prepared by routine laboratory procedures, including fixation in buffered Formalin solution and embedding in paraffin. The paraffin blocks were stored at room temperature. Serial sections were cut and used for the immunoperoxidase method of detecting tissue antigens (*3*, *4*). The steps in the procedure are summarized as follows. Formalin-treated paraffin sections were deparaffinized and incubated in hydrogen peroxide to destroy endogenous peroxidase activity. The tissues were then incubated sequentially in (i) peanut agglutinin, (ii) rabbit antibody against PNA, (iii) swine antibody against rabbit immunoglobulin, and (iv) peroxidase-antiperoxidase reagent. Sections were washed after each antibody application. Localization of the peroxidase label was visualized by adding diaminobenzidine in hydrogen peroxide, which produces a permanent brown coloration that contrasts with the blue hematoxylin counterstain. Sections were dehydrated and mounted in Permount before microscopic examination. Peanut agglutinin was prepared as described (*5*). All antiserums were obtained from Dakopatts (Accurate Corp., Hicksville, New York) except the antibody directed against PNA (Vector Laboratories, Burlingame, California).

A total of 44 benign and malignant breast lesions were examined for binding of PNA. The different patterns of T antigen expression are shown in Fig. 1. Of the 22 benign breast lesions examined (ten fibrocystic disease, ten fibroadenomas, one gynecomastia, and one lactating adenoma), 19 showed lectin binding (immunoperoxidase staining) along the luminal cytoplasmic membrane with or without staining of intraluminal secretions (Fig. 2A). As demonstrated by lectin binding, T antigen was confined to this region in all cases except one fibroadenoma, which also showed focal cytoplasmic positivity. Two of the lesions of patients with fibrocystic disease showed no PNA binding by this technique. In contrast, of 22 malignant lesions (21 infiltrating ductal and one lobular carcinoma), 17 showed diffuse cytoplasmic T antigen in a variable proportion of malignant cells (Fig. 2B). Of these 17, nine carcinomas also showed a pattern of PNA binding that occurred along the luminal membrane, similar to that seen in the benign lesions. This pattern, however, was seen only in the well-differentiated ductal carcinomas. Five carcinomas showed no evidence of T antigen expression as determined by PNA binding. These malignancies were the most poorly differentiated when judged by histological criteria (*6*).

Multiple controls of various types were performed to ensure specificity of the reaction. Controls for antiserums included tissue sections in which each of the above stages of the immunoperoxidase procedure was omitted in turn. In no instance was there staining of malignant or benign tissues when PNA or subsequent antiserums were sequentially omitted in the procedure. Absorption of lectin with erythrocytes, having exposed T antigen, eliminated all staining reactions (*5*). Peanut agglutinin possesses high affinity for glycoproteins containing the terminal sequence β-D-galactose(1→3)-*N*-acetyl-D-galactosamine and is inhibited by the simple sugar D-galactose, but not by other simple sugars, such as glucose. Peanut agglutinin, although not specific for the immunodeterminant structure of T antigen, has a higher affinity for it than for other carbohydrates (*7*, *8*). Incubation of PNA with galactose abolished all staining reactions. Glucose had no effect on the binding of PNA to tissue sections. Intrinsic controls for tissue were available in many instances. The staining pattern of benign or malignant tissues could often be observed in the same tissue section or within the same experimental run. The immunoperoxidase findings in our study corroborate and expand observations recently made on lectin binding by neoplastic and benign breast epithelial cells when studied by immunofluorescence techniques (*9*, *10*).

The different pattern of binding of PNA to benign and malignant cells suggests that the immune response to T antigen or a substance like T antigen may depend on cellular localization of the antigen. Presumably the luminal membrane localization in benign tissues is, in effect, "outside" the body or in an immunologically privileged site. Expression of the antigen in the cytoplasm of malignant cells, with the attendant cell death that invariably accompanied a neoplasm, might be expected to have better access to the host's immune defense system. The presence of cytoplasmic lectin binding in one benign breast lesion, although not explained, could suggest incipient neoplastic transformation. Its absence in five of the carcinomas appeared to be associated with a more aggressive histological type. It is possible that as a malignancy becomes more anaplastic, the site and nature of antigen expression is altered, with some antigens eventually lost. This loss may then be associated with failure to recognize the tumor as foreign and, consequently, the inability to mount an effective immune reaction.

T antigen may elicit delayed hyper-

sensitivity reactions in patients with breast carcinoma but not in healthy controls (*2*). The findings of previous studies in conjunction with the immunomorphological detection of a putative carcinoma-associated antigen (T antigen) described in our report suggest a possible link between neoplastic transformation and the development of tumor immunity.

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Photosynthesis of Previtamin D_3 in Human Skin and the Physiologic Consequences

Abstract. *Photosynthesis of previtamin D_3 can occur throughout the epidermis and in the dermis when hypopigmented Caucasian skin is exposed to solar ultraviolet radiation. Once previtamin D_3 is formed in the skin, it undergoes a temperature-dependent thermal isomerization that takes at least 3 days to complete. The vitamin D-binding protein preferentially translocates the thermal product, vitamin D_3, into the circulation. These processes suggest a unique mechanism for the synthesis, storage, and slow, steady release of vitamin D_3 from the skin into the circulation.*

About 60 years ago, exposure of children to radiation from a mercury-vapor quartz lamp (*1*) or to sunlight (*1*) was reported to prevent or to cure rickets. Subsequent investigations (*2*) culminated in the structural identification of vitamin D_3 (D_3) and its photochemical precursor 7-dehydrocholesterol (7-DHC) (*2*). It had been assumed that when skin is exposed to sunlight, part of the stores of 7-DHC in the epidermis undergoes a photochemical reaction resulting in the eventual formation of D_3. This concept was supported by the isolation of D_3 from human and rat skin (*3*). Until recently, however, the exact sequence of steps leading to the cutaneous photoproduction of D_3 was unknown, including the sites in the skin where D_3 formation occurs and the mechanism for entry of the vitamin into the circulation. Recently, we (*4*) established, in rat skin in vivo, that previtamin D_3 (preD_3) is the principal photoproduct that results from exposure of 7-DHC to a physiological dose of ultraviolet radiation. Because preD_3 is thermally labile and readily isomerizes in vitro by a temperature-dependent process to D_3 (*2*), we suspected that preD_3, once formed in the skin in vivo, would isomerize to form D_3.

We report on (i) the cutaneous sites where preD_3 formation occurs, (ii) the temperature dependence of the thermal equilibration of preD_3 to D_3 in vitro and in vivo, and (iii) the role of an α_1-globulin (the vitamin D–binding protein for translocating D_3 from the skin into the circulation.

We synthesized [3α-^{3}H]7-DHC, [3α-^{3}H]preD_3, and [3α-^{3}H]D_3, each with a specific activity of 4.8 Ci/mmole (*4*). Ten milligrams of 7-DHC was exposed to ultraviolet B radiation to generate sufficient quantities of preD_3 for the assay of binding protein. Chromatography of the photolytic products, skin lipid extracts, and thermal equilibration reactions was performed in 8 percent ethyl acetate in *n*-hexane on a high-performance liquid chromatography system (HPLC) equipped with a radial compression module containing a Radial-Pak-B (10 by 0.8 cm) coupled with an ultraviolet absorption detector at 254 nm (model 440, Waters Associates) and a printer-plotter data module integrator (Waters Associates). This chromatographic system permits complete resolution to baseline of 7-DHC, preD_3, and D_3. Quantitation of the concentrations of 7-DHC and preD_3 in skin lipid extracts was based on measurement of the integrated area under the appropriate peak and comparison with standard curves that were generated by plotting the integrated area under the peak as a function of a known concentration of pure compound. Identification of the skin lipid peak designated as 7-DHC was based on its ultraviolet absorption spectrum (maximum wavelength at 295, 282, and 271 nm), which is characteristic for $\Delta^{5,7}$-diene sterols, and its mass spectrum with M^+ at 384. Identification of the skin lipid peak designated as preD_3 was based on (i) its appearance only after skin containing 7-DHC was subjected to ultraviolet B radiation (spectral range, 290 to 320 nm), (ii) comparison chromatography studies with a preD_3 standard, and (iii) its thermal lability (*4*).

The location in human skin where preD_3 is initially formed after exposure to ultraviolet B radiation was determined by separating layers of skin without altering the integrity of the cellular structure. Surgically obtained hypopigmented Caucasian human leg skin with the subcutaneous fat removed was cut into samples (6.25 cm^2) and separated by incubation with staphylococcal exfoliatin [a substance that specifically cleaves the epidermis at the stratum granulosum–stratum spinosum interface (*5*)] (20 mg/ml) or by immersion in a 60°C water bath for 30 seconds (*6*) (this technique cleaves the epidermis at the stratum spinosum–stratum basale interface). After either treatment, but before physical separation of the skin layers, the stratum corneum side of the skin samples was exposed to ultraviolet B radiation (0.5 J/cm^2) in a narrow wave band centered at 295 nm with a 10-nm half-bandwidth. This radiation was obtained from a 6.5-kW xenon-mercury arc lamp coupled to a holographic grating monochromator (HL-300, Jobin-Yvon). Control skin samples were kept in an ultraviolet-free environment. Immediately after irradiation, the toxin-treated skin was separated into a top layer (stratum corneum and stratum granulosum) and a bottom layer (stratum spinosum, stratum basale, and dermis), whereas the heat-treated skin was differently separated into a top layer (stratum corneum, stratum granulosum, and stratum spinosum) and a bottom layer (stratum basale and dermis). The basal cells of the stratum basale were collected by mechanically scraping them off the dermis. Contents of all skin layers were confirmed by histological examination. The separated layers of skin were extracted with 8 percent ethyl acetate in *n*-hexane for 24 hours at −20°C. The extracts were centrifuged, the supernatant was dried under nitrogen and weighed, and a portion of each sample was chromatographed by HPLC in duplicate to determine 7-DHC and preD_3 concentrations.

To examine the thermal isomerization of preD_3 to D_3 in vitro, 1 μCi of [3α-^{3}H]preD_3 was dissolved in methanol, flushed with argon, and incubated in triplicate at 0° ± 1°, 25° ± 1°, or 37° ± 1°C. At various times, portions were removed

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