THE FINE STRUCTURE OF THE BASEMENT MEMBRANE IN EPIDERMAL TUMORS

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ABSTRACT

Epidermal tumors were induced in Swiss female mice by a topical application of 9,10-dimethyl-1,2-benzanthracene solution followed by repeated applications of croton oil solutions. Fourteen benign and malignant tumors were sampled 25 weeks after the treatment had begun, fixed in osmium tetroxide or potassium permanganate, and embedded in Epon. Sections stained with lead hydroxide were examined. In three tumors defects of the epidermal basement membrane were seen. These defects accompanied local invasion of the tumors. The possible mechanisms of the development of this unusual anatomical situation are discussed.

The basement membrane of the skin, as defined by the electron microscope, is "a continuous membrane covering the basal surface of the basal epidermal cells. This membrane is 200 to 300 A thick and is separated from the epidermal cell surface by a space of about the same width as the thickness of the membrane" (24). It has not been studied extensively in epidermal tumors. The report of Ashworth, Stembridge, and Luibel (1) has shown it to be intact in carcinoma *in situ* in the cervix, and to be absent in some portions of invasive carcinoma of the cervix, but not in others.

In the work reported here, the morphology of the basement membrane was studied in chemically induced experimental tumors in the skin of the mouse.

MATERIALS AND METHODS

Skin tumors were induced in adult Swiss albino virgin female mice by one or three cutaneous applications of 9,10-dimethyl-1,2-benzanthracene in mineral oil at various concentrations ("initiation"), followed by repeated applications of various dilutions of croton oil in mineral oil ("promotion") to the backs of the animals (4). Fourteen tumors harvested 25 weeks after the beginning of treatment were examined. Small pieces of these tumors were removed under ether anesthesia and fixed in either 1 per cent buffered osmium tetroxide (40) or 2 per cent buffered potassium permanganate (20) in an ice bath for 15 minutes to 1 hour. The tissue was embedded in Epon by the method of Luft (21) and sectioned on an LKB or a Porter-Blum microtome with a glass knife. Sections were mounted on carbon-coated Formvar film-covered grids and counterstained with lead hydroxide (23, 35) for 10 to 30 minutes. The sections were examined with an RCA EMU-3E microscope.

OBSERVATIONS

Thirteen of the tumors were histologically benign, although in some papillomata the tumor tissue reached below the level of the dermoepidermal junction. One tumor was distinctly malignant: it invaded the panniculus carnosus.

The fine structure revealed an intact basement membrane in most regions of most of the tumors examined. In three tumors, *i.e.*, two papillomata and one carcinoma, this was not so. In one of the papillomata a section showed the basement membrane to be intact except for a single small gap (Fig. 1). A part of the cytoplasm of the tumor basal cell, lying outside the boundary of the basement membrane in the region of the

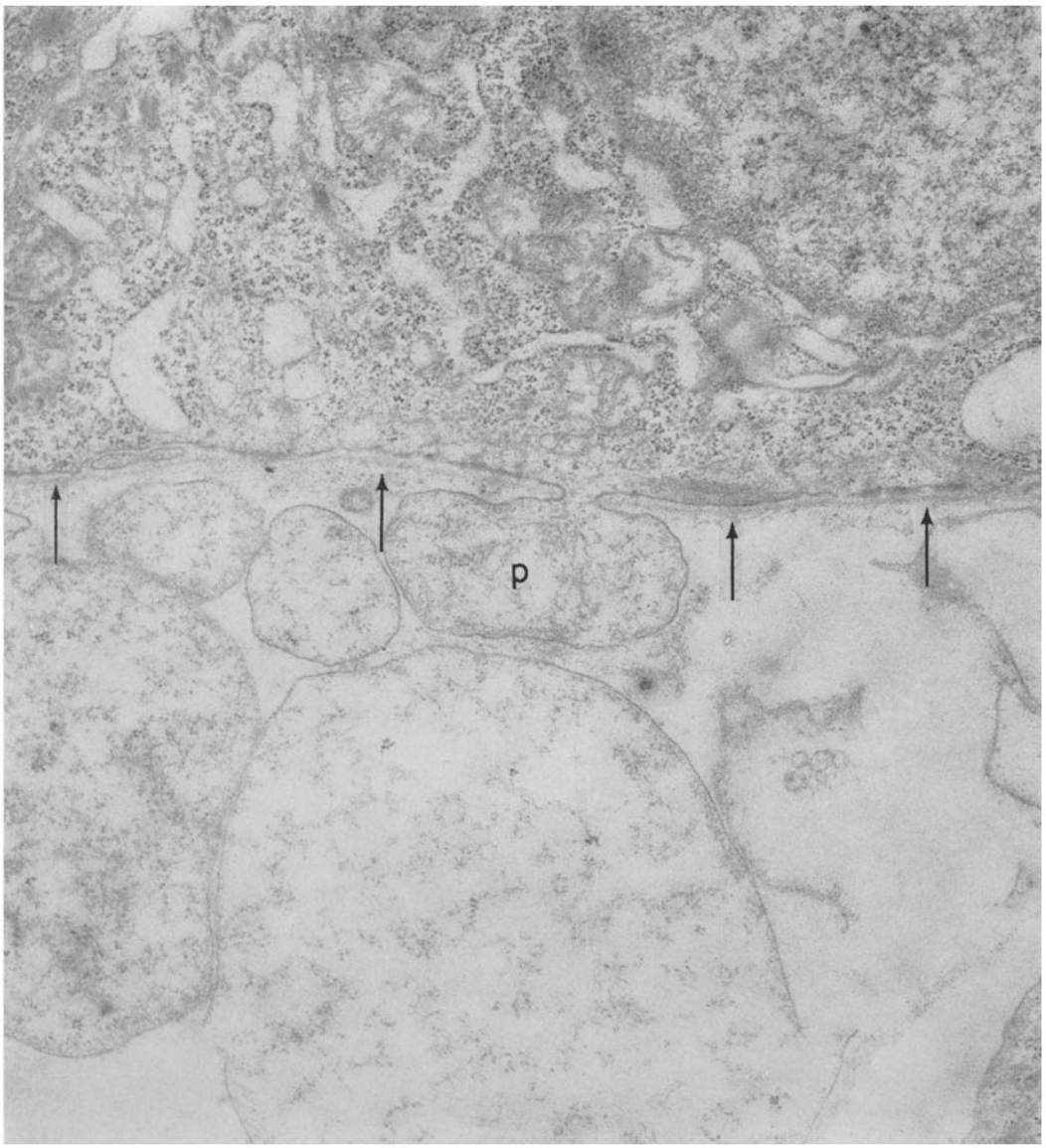


FIGURE 1

This micrograph shows a part of the dermoepidermal junction of a squamous cell papilloma. The basement membrane (arrows) separating tumor cells from stroma has a single gap through which protrudes a portion of the cytoplasm (p) of an epidermal tumor cell. This protrusion and similar structures lying in the dermis are not surrounded by a basement membrane. Osmium tetroxide fixation. × 22,000.

gap, was connected to the basal cell by a narrow stalk and was not covered by a basement membrane. This part had a less dense concentration of cytoplasmic particles than the rest of the tumor basal cell and contained no organelles surrounded by membranes. Similar larger and smaller ovoid cytoplasmic islands were seen in the surrounding dermis and were thought to be protrusions of basal cells through other gaps in the basement membrane, sectioned tangentially

so that their stalk was not in the plane of section. In another area of a papilloma (Fig. 2), there were two gaps in the basement membrane. Both of them were again fully occupied by protrusions of cytoplasm originating from tumor basal cells, similar to those seen in Fig. 1. Structures similar to these two protrusions, but again not connected to basal cells in the plane of section, were also seen in the dermis in this instance. The cytoplasmic sap of these ovoid masses of cytoplasm, not surrounded by a basement membrane, had varying densities of particles, which may indicate varying degrees of intracellular edema. Apart from being discontinuous, the basement membrane did not appear different from that seen in intact epidermis. These observations were made repeatedly in two papillomata.

An analogous situation was observed in the one carcinoma examined. In it, however, the discontinuities in the basement membrane were frequently more extensive and not always completely filled by cytoplasmic projections of tumor cells. Fig. 3 illustrates this observation in this tumor. An irregularly shaped portion of a tumor basal cell impinged against a portion of dermis which in this instance was rich in fibrillar material suggestive of collagen, though cross-striations were not clearly demonstrated. Portions of basement membrane were seen here only in a deep indentation in the cytoplasm of the basal cell. The intercellular space between this cell and a neighboring tumor basal cell was very wide and was not separated from the dermis by a basement membrane. Such observations were made repeatedly in this tumor.

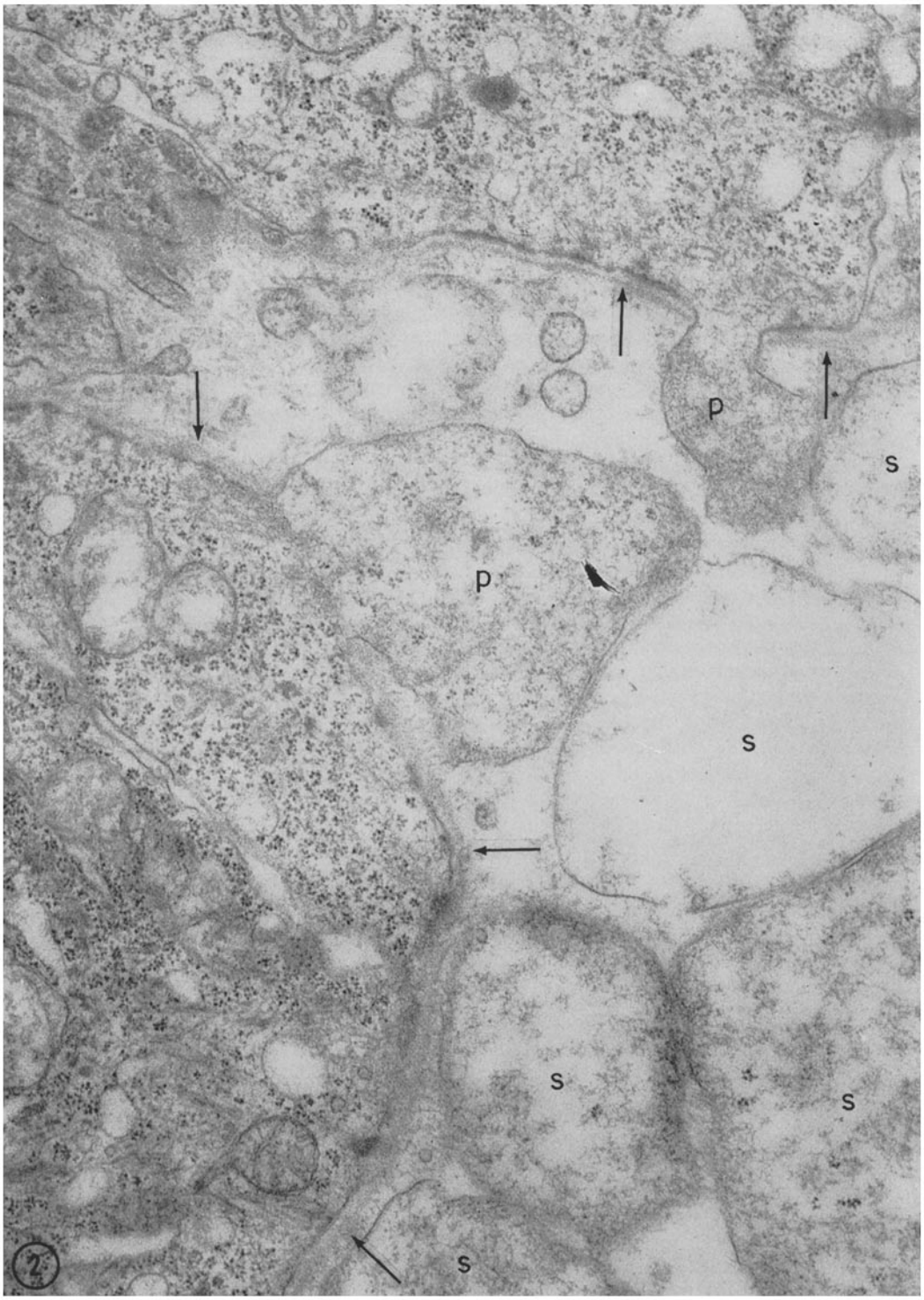
DISCUSSION

The basement membrane is an extracellular membranous structure about 300 A thick, internally homogeneous or with indefinite suggestions of a fibrillar substructure (8, 15, 17, 26, 37). An electron-translucent gap of about the same width separates the basement membrane from the cell membrane of the cells to which it is applied. This gap may be traversed by fibrils in certain instances (32). The function of the basement membrane has not been established as yet, since correlative physiological and fine structure studies are difficult. Circumstantial evidence suggests that it is a barrier between tissues (11, 16, 24) and that it may be involved in the process of differentiation (5).

A basement membrane is present between epithelia (1, 19, 22, 24–26, 29, 30, 33, 36), endothelia (13, 15, 33), and mesothelia (12) and the adjacent connective tissue. A basement membrane also surrounds Schwann cells and glial cells, but not neurons and their axons (3, 7). A similar structure surrounds individual fat cells (2) and smooth (13) and striated (10, 15) muscle fibers. It intervenes between the nervous and muscular elements of motor end-plates in vertebrates (38), but not in insects (34). In glomeruli its thickness is double that seen in other tissues (8, 17, 18, 27, 37), so that perhaps one-half of it belongs to epithelial cells and the other half to endothelial cells of the glomerulus. In all these situations the basement membrane is seen with present resolution as an intact plane without interruption. Fibroblasts, chondroblasts (31), osteoblasts (6), liver cells (9, 14), and liver (9, 14) and bone marrow sinusoids (39) are not surrounded by basement membrane. These observations also suggest that the basement membrane may be an important barrier between some tissues and some extracellular materials in a fully differentiated organism.

The deficiencies of the basement membrane and associated changes that were observed in epidermal tumors produced in the mouse by chemical means bear a great morphological resemblance to the changes observed in the basement membrane and the epidermal cells in the regenerating limb of the newt. Salpeter and Singer (28) reported that as the limb bud of *Triturus*, after it has first healed following amputation, begins to proliferate and grow, the epidermis is separated from the dermis by a basement membrane showing many gaps. The basal epidermal cells protrude through these gaps and the protruded cytoplasm appears swollen. This appearance gradually subsides with a reurn to the usual morphology of epidermis with an intact basement membrane, in a distal direction, as the new limb becomes anatomically normal.

In the epidermal tumors described in this presentation, similar morphological changes are suggestive of early steps in local invasion by neoplastic cells. In the malignant tumor examined, these changes were more marked and the appearance was often more like that seen in the groups of tumor cells of invasive carcinoma of the cervix which are devoid of basement membrane, while other groups of cells of the same carcinoma have an intact basement membrane (1).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There are at least two possible explanations of the development of gaps in the basement membrane of epidermal tumors. One is that the basement membrane of the epidermis has been penetrated and disrupted by the tumor cells, and the other that the neoplastic epidermal tissue grows faster than a basement membrane is formed beneath it. A choice between these two possibilities cannot be made on the morphological evidence available, but the similarity of behavior of the basement membrane in the limb bud of the newt (28), where there is no question of invasion, and in the chemically induced squamous tumors would favor the second explanation. This is also supported by the observation that some secondary deposits of a carcinoma of the cervix are surrounded by a basement membrane (1), presumably newly formed at the site of the metastasis.

Whatever the reason for the defects of the basement membrane surrounding neoplastic epidermal epithelium, their existence means that if the basement membrane provides a barrier between epithelium and stroma, and if it is concerned with the maintenance of differentiation, as suggested by the circumstantial evidence mentioned, the basement membrane may not function normally in these tumors. One may speculate that this may contribute to the abnormal differentiation of neoplastic cells, and that it may also give them a nutritional advantage, allowing them to grow faster than their normal counterparts. The basement membrane defects may also be responsible for changes in the tumor stroma, which is in more intimate contact with neoplastic epidermal cells through these gaps than it is with normal epidermal cells. This may be in part responsible for the well known differences between neoplastic and non-neoplastic stroma.

Note added in proof: Small cytoplasmic protrusions through the basement membrane of carcinoma of cervix were also recently described by Hinglais-Guillaud, N., Moricard, R., and Bernhard, W., *Bull. Cancer*, 1961, **48**, 283.

This work was supported in part by grant A-4422 from the United States Public Health Service, National Institute of Arthritis and Metabolic Diseases, and by a grant from the National Cancer Institute of Canada, Project 164.

The author would like to express his gratitude to Dr. H. Sheldon for many useful discussions. The technical assistance of P. M. Spicer, A. Strömblad, and B. Jönsson is also gratefully acknowledged.

Received for publication, June 4, 1962.

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Figure 2

This micrograph shows another part of the dermoepidermal junction of a squamous cell papilloma. The basement membrane (arrows) has at least two gaps. Protrusions (*p*) of the cytoplasm of tumor basal cells fill these gaps and reach into the dermis. They are not covered by a basement membrane. The dermis contains structures (*s*) similar to the protrusions (*p*), but not connected to tumor basal cells in the plane of section. Osmium tetroxide fixation. × 27,000.

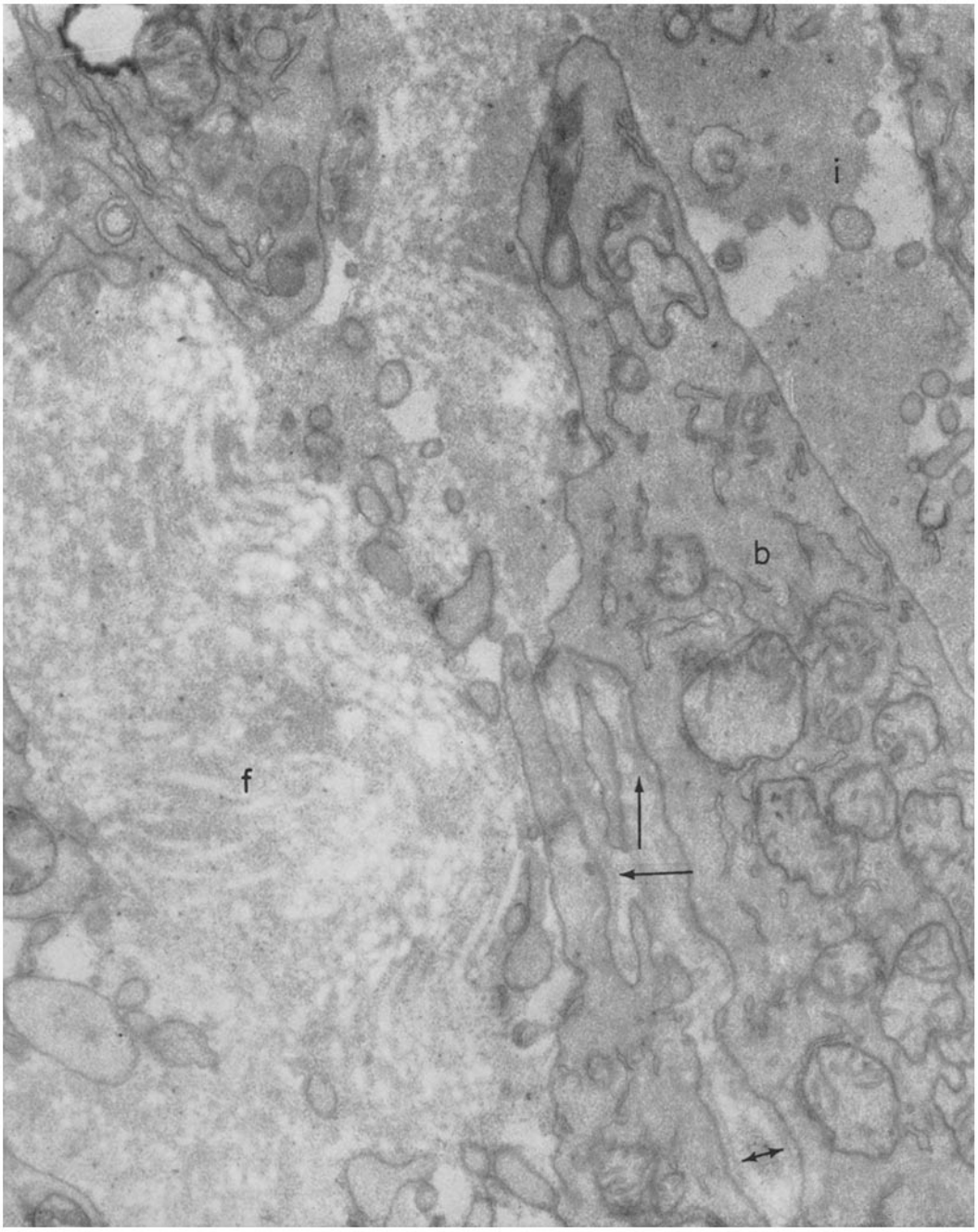

FIGURE 3

This micrograph shows a part of the dermoepidermal junction of a squamous cell carcinoma. A tumor basal cell (*b*) impinges on stroma containing bundles of fibrils (*f*) which may be collagen although they are not unequivocally cross-striated. No basement membrane separates the stroma from the tumor cell. Portions of basement membrane (arrows) are seen within a deep cleft in the cytoplasm of the tumor basal cell. The intercellular space (*i*) within the tumor is also not separated from the dermis by a basement membrane. Potassium permanganate fixation. × 22,000.

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