THE NATURAL HISTORY AND PATHOLOGY OF EPIDERMAL PAPILLOMAS OF FLATHEAD SOLE, *HIPPOGLOSSOIDES ELASSODON*, FROM EAST SOUND OF ORCAS ISLAND, WASHINGTON.

by

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To

MY MOTHER AND FATHER

and Especially to

CLAUDIA

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INTRODUCTION

STATEMENT OF THE PROBLEM

Neoplasia in fishes has received increasing attention in the past few years, not only with respect to the possible basic biological mechanisms involved, but also as regards the point of view of the possible significance of endemic neoplasia in those species of animals extensively consumed as food by humans and domestic animals. This thesis reports the occurrence of neoplasia in epizootic proportions in a species of marine flounder, *Hippoglossoides elassodon*. The tumors are microscopically epidermal papillomas, and their detailed morphology and natural history have not been heretofore described in this species. Some of the material presented herein has been previously reported in publications resulting from this work.

SURVEY OF THE LITERATURE

Tumors both benign and malignant have been recorded in at least 136 different species of fishes (1, 2, 3, 4, 5, 6, 7, 8, 9). Almost one-half of all the affected species, according to Lucké and Schlumberger (2), belong to only five families of teleost (bony) fishes: Salmonidae - salmon, trout; Cyprinidae - carp, dace, minnows, chubs, barbels; Gadidae - codfish; Bothidae - flatfish; and Pleuronectidae - flounders. It is suggested by these authors that the members of these families are no more susceptible to tumorigenic phenomena than any other; but that due to their economic importance, many more of these fish are caught and inspected during processing for food. Forty other families of teleosts, and four families of elasmobranchs (cartilaginous fishes), embracing nine species, are also susceptible to neoplasia.

Tumors of epithelial and mesenchymal origin are believed to be the commonest neoplasms of fish, as is true for mammals and birds (2). The entire spectrum of tumors of epithelial tissue are found: papillomas, epitheliomas, adenomas, dental neoplasms, adenocarcinomas, and thyroid neoplasms. Of these, the most common benign neoplastic process is the epidermal papilloma (1, 2). Adenomas and dental tumors are much less common, but when they do occur, they correspond closely to those of mammals. Epitheliomas (also called epidermoid carcinomas) are the more common type of epithelial malignancy, and arise primarily on the lips, oral mucosa, and skin. Thyroid tumors range from the non-neoplastic goiters, through the adenomas, to adenocarcinomas (2).

Benign epidermal papillomas are reported in at least 25 species of fish (Table 1) (1, 4, 5, 6, 7, 8, 9). In addition, twelve other species are reported as having epitheliomas (1). A similar type of lesion has been described in several additional species of fish under the names of *hyperplastic epidermal disease* and/or fishpox (*vide infra*). Many of the early reports of malignant epithelial tumors are open to question in view of the now standardized criteria for malignancy; for this reason, a brief review of the literature concerning this lesion is included.

Epidermal papillomas have been reported in eleven species of the order Pleuronectidae (flounders, flatfish) (Table 1) (1, 4, 6, 7, 8, 9).

TABLE 1: Papillomas on Fishes; Survey of the Literature.

Species	Common Name	Number of Fish	Sites of Tumor	Reference
Gobio (several species)	Gudgeon	4	Skin	Schlumberger & Lucké (1)
Barbus fluviatialis	Barbel	Several	Lips	Keysselitz (17)
Anabas scandens	Climbing perch	Several	Body surface	Fiebiger (15)
Osmerus eperlanus	Stint	37	Lips, fins	Breslauer (16)
Gobio nigronatatus	Gudgeon	2	Skin	Schlumberger & Lucké (1)
Gobio blennoides	Gudgeon	-	Skin	Schlumberger & Lucké (1)
Carassius auratus	Goldfish	2	Fins	Schlumberger & Lucké (1)
Carassius carassius	Crucian carp	1	Skin of operculum	Schlumberger & Lucké (1)
Anguilla anguilla	Eel	3	Lower lip	Schlumberger & Lucké (1)
Salmo trutta	Brown trout	-	Skin of back	Schlumberger & Lucké (1)
Electrophorus electricus	Electric eel	-	Skin	Schlumberger & Lucké (1)
Iridio bivittata	Slippery Dick	30	Skin	Schlumberger & Lucké (1)
Catostomus commersonnii	Sucker	-	Skin near tail	Schlumberger & Lucké (1)
Genyonemus lineatus	Croaker	10	Body surface, fins	Russel & Kotin (18)
PLEURONECTID FISHES:				
Hippoglossus hippoglossus	Halibut	1	Snout	Johnstone (10)
Limanda limanda	Dab	3	Body surface	Johnstone (11)
Pleuronectes platessa	Plaice	1	Body surface	Johnstone (11)
Solea solea	Sole	2	Skin of trunk	Thomas (12)
Parophrys vetulus	English sole	Epizootic	Body surface, fins	Good (4); Wellings, *et al.* (6, 7); Chuinard, *et al.* (8)
Hippoglossoides elassodon	Flathead sole	Epizootic	Body surface, fins	Wellings, *et al.* (6,7,13); Chuinard, *et al.* (14)
Psettichthys melanostictus	Sand sole	Epizootic	Body surface, fins	Wellings, *et al.* (6,7); Nigrelli, *et al.* (9)
Platichthys stellatus	Starry flounder	Epizootic	Body surface, fins	Chuinard, *et al.* (8)
Glyptocephalus zachirus	Rex sole	3	Body surface, fins	Wellings, *et al.* (6, 7)
Lepidopsetta binneatta	Rock sole	5	Body surface, fins	Nigrelli, *et al.* (9)
Microstomus pacificus	Dover sole	1	Body surface	Wellings, *et al.* (66)

The earliest report appears to have been by Johnstone (10) in 1914 in which he described a "cauliflower-shaped" lesion cut from the snout of a halibut, *Hippoglossus vulgaris, sic, hippoglossus*. The lesion was from the pigmented side of the fish and in places was somewhat darker than the normal epithelium. The gross appearance was highly irregular, with the surface thrown into large, lobulated, closely packed folds in some places; elsewhere the surface of the tumor was only minutely papillated. Its color varied from grey-white to black. Microscopically, the tumor consisted of coarse, branching and anastomosing fibers, sometimes swollen and degenerate, which extended into the interior of the papillae. No epidermis or epithelial tissue was noted, and the limiting membrane was indistinct. The papillae, consisting of fine areolar networks with numerous nuclei and bundles of coarse fibers, were very vascular with branching and anastomosing capillaries most numerous directly below the surface of the tumor. Scattered throughout the neoplasm were nodular proliferations composed of numerous small, round cells, possibly indicating a tendency toward local invasion.

In 1925 Johnstone (11) reported the occurrence of "cutaneous warts" on the epithelial surfaces of three dabs, *Limanda limanda*. One individual (a mature female) had a large, white, corrugated lesion on the pigmented (ocular) side which measured 6 x 5 centimeters. It was raised above the level of the normal epidermis, and thinned out toward the periphery. A second fish (a mature female) had the pigmented side covered with discrete, white, 2 to 3 millimeter nodules, which became confluent in two places near the center of the body on either side of the lateral line. The third dab (a mature male) had two white tumors:

one measured 10 x 5 millimeters and covered the right eye; the other measured 2 centimeters in length and was located on the middle part of the dorsal fin. Microscopically, the tumors were all similar. The main tissue element was an epidermal cell supported by a fine connective tissue stroma. In this same report (11) brief mention is made of an identical lesion occurring on the right pectoral fin of a small plaice, *Pleuronectes platessa*.

Thomas (12) reported in 1930 the occurrence of similar tumors on two sole, *Solea solea*. The tumor on the first fish was located behind and below the opercular opening of the pigmented side, and measured 13 x 17 millimeters; the tumor on the second sole was on the pigmented side of the fish at the junction of the anterior two-thirds with the posterior one-third and measured 35 x 20 millimeters. Both tumors were sessile and grossly lobulated; coloration varied from blue-grey to brown. Microscopically, long epithelial pegs limited by an intact basement membrane extended into the subcutaneous tissue. The author ascribed the etiology of these tumors to chronic irritation of the postopercular skin by sand particles drawn through the mouth and expelled through the gill vent in the act of respiration.

In 1940, Good (4) presented his Master's thesis on the incidence of a similar lesion found on the English sole, *Parophrys vetulus*. Sixty-one tumor fish out of a total collection of 1355 were recorded, for a tumor incidence of 4.50 percent. These fish were collected in the Seattle, Washington, area over a two year period by means of a beach seine set from 100 to 200 feet from shore and hauled in by hand. The fish with tumors ranged from 6.8 to 19.0 centimeters in total body length. There were a total of 89 tumors present on 61 diseased fish. Seventy-five percent of the involved fish had only one tumor; the rest had two or three, with one fish having eleven. Good stated that more of the tumors were located on the upper (pigmented, ocular) side of the fish, but no statistical evaluation of tumor location was presented. Grossly, the tumors were similar to the papillomas described by Johnstone and Thomas. Microscopically, the tumors were composed of atypical epithelial cells with dark staining, pleomorphic nuclei which, in some instances, were seen to be "invading" the underlying loose connective tissue stroma. Coarse fibrous connective tissue, often replete with small capillaries, formed the supporting stroma for the tumor; sometimes this tissue appeared to be also invaded by the atypical epithelial cells. Of eleven tumor-bearing fish kept up to fifteen weeks in the laboratory under continuous observation, six had died by the end of the fifteenth week; however, accurate records of the size of the tumors were maintained. Only two of the twelve original tumors (one fish had two tumors) regressed, while the remainder continued to enlarge. No new tumors occurred. Attempts were made to transplant tumor and to initiate tumorigenesis with methylcholanthrene in uninvolved fish of the same species; however, these experiments were unsuccessful.

Epithelial neoplasms, thought to be of a benign nature, have recently been reported for four other species of Pleuronectidae: the flathead sole, *Hippoglossoides elassodon*, the sand sole, *Psettichthys melanostictus*, the rex sole, *Glyptocephalus zachirus*, and the starry flounder, *Platichthys stellatus* (6, 7, 8, 9, 13, 14). Three of these reports also contained discussions of the same neoplastic process on the English sole, *P. vetulus* (6, 7, 8). The epidermal papilloma of the flathead sole, *H. elassodon*, is the subject of this thesis, and will be discussed in detail below.

Of the two sand sole, one fish had two tumors, and the other had one. Grossly, the tumors varied from grey-white to brown in color, and from a sessile, sharply limited, cauliflower-like mass without spreading edges to a granular, plaque-like formation consisting of multiple small polypoid lesions. Microscopically, these lesions were composed primarily of dermal connective tissue elements and vascular tissue covered by an apparently normal, to mildly hyperplastic, overlying epidermis containing numerous mucous cells. The tumors were composed of two distinctly different epithelial cells: one was large, about 30 microns in greatest diameter, with finely granular, acidophilic cytoplasm. Nuclei were large and moderately pleomorphic; one to three nucleoli were present. There were occasional nuclear chromatin clumps which stained with basic dyes. The other cell type was smaller and about 10 microns in greatest diameter; the nucleus was small, intensely basophilic and without apparent nucleoli. At times cells morphologically similar to the smaller epithelial cell were noted surrounding the vascular channels of the connective tissue stroma. It was thought that this might represent local invasion (6, 7).

Three rex sole had one tumor each. These were all described as flat, plaque-like tumors which were thickest in the center. The edges thinned out into the surrounding normal epidermis. The tumor was grey-white to black in color, and occurred on both the non-pigmented and the pigmented sides of the fish. Microscopically, the cellular elements were uniform throughout, resembling the large epithelial cells of the sand sole tumor described above (6, 7).

The gross appearances of the tumors of the English sole (two fish with one tumor each) were entirely similar to those of the rex sole. Cytologically, the tumor cells resembled the large epithelial cells of the sand sole tumors and the cells of the rex sole tumor (6, 7).

In 1965, Nigrelli, Ketchen, and Ruggieri (9) reported the occurrence of similar lesions on 231 of 1626 sand sole, *P. melanostictus*. The gross and microscopic patterns were similar to those described above. In some instances an extensive, proliferative angiomatous-like development of the dermal tissue was noted. This was thought to be in response to encysted parasites (echinostome). Periarteritis, angiitis, and lymphangiectasis of the cutaneous and submuscular lymph vessels were frequently noted in association with the encysted parasites. Similar epithelial tumors were occasionally found on young rock sole, *Lepidopsetta bilineata*.

At this time data are being gathered regarding the occurrence of similar epidermal tumors on another pleuronectid fish, the starry flounder, *Platichthys stellatus*. This study also includes a high incidence of English sole affected with epidermal papillomas (8).

Somewhat similar lesions have been described on several other species of fish. In 1909 Fiebiger (15) reported the occurrence of multiple small, wart-like papillomas that developed on the epithelial surfaces in a group of climbing perch, *Anabas scandens*, which had been kept in the same aquarium for two years. Microscopy demonstrated marked epidermal hyperplasia and connective tissue papillary proliferation. Fiebiger pointed out the resemblance of the perch tumors to those of infectious warts of mammals. Nigrelli (5) subsequently reported a similar occurrence in the same species kept in the tanks of the New York Aquarium. He reported that no inclusion bodies were found in the diseased cells, and that attempts to transmit the tumor by "recognized viral methods" were unsuccessful.

In 1916 Breslauer (16) described a papillary tumor which occurred primarily on the lips, and sometimes on the oral mucosa or fins of smelt, *Osmerus eperlanus*, taken from the brackish water of an inlet of the Baltic Sea. Occasionally the arrangement of the cells suggested early, local malignancy. Cytoplasmic inclusions were occasionally found in the tumor cells.

Keysselitz (17), in 1908, found that papillary tumors of the lip were common on barbels, *Barbus fluviatialis*, caught in the Mosel River. He originally described this tumor as an epithelioma, but Schlumberger and Lucké (1) subsequently interpreted it as a papilloma. Microscopically, these lesions appeared as papillomas consisting of tightly packed epithelial cells supported by a coarse fibrous connective tissue stroma. The author described one or more intranuclear inclusions in many of the epithelial cells which appeared to be surrounded by a clear zone; these were easily distinguished from nucleoli. Keysselitz traced the development and disappearance of these inclusions: they appeared dense and compact in the early stages of the disease, but later became indistinct. He believed these inclusions represented cellular reaction products to the invasion of an etiologic virus. He also compared these inclusions with those of vaccinia, epitheliomas of fowl, and other virus diseases.

Takahashi (1) in 1929 reported observing for 16 months a sharply circumscribed, greyish-white, 3 x 2 centimeter, papillomatous tumor located near the posterior margin of the left operculum on a crucian carp, *Carassius carassius*. It was noted that the tumor appeared to be regressing, so a biopsy was taken and pieces were transplanted into other fish without success. Microscopically, this tumor showed evidence of active epithelial proliferation with numerous mitoses and epithelial "pearls". Stromal connective tissue was minimal. No evidence of local invasion or distant metastasis was found.

Lip papillomas in the dwarf gourami, *Colisa lalia*, reported by Nigrelli (5), revealed the typical papillary architecture described above, except that in some cases the supporting connective tissue became heavily chondrified or ossified. Rarely, the tumors resembled a malignant papillary epithelioma. These fish were raised in outdoor pools for sale as tropical fish; under these conditions they exhibited a high incidence (0.5 to 1.0 percent) of tumorigenesis. The author stated that there was some evidence for the presence of inclusion bodies in the affected epithelial cells, but that all attempts at transmission under laboratory conditions were negative, although under natural conditions there seemed to be epidemiologic evidence indicating the existence of an infectious process.

In 1957 Russel and Kotin (18) reported squamous papillomas on the lips and about the mouths of ten white croakers or kingfish, *Genyonemus lineatus*, caught with trawling gear off the Southern California coast;

these ten fish were included in a total collection of 353 croakers. Most diseased fish were found to have multiple small, firm, white lesions, microscopically characterized by hyperplastic epithelium contained within the intact basement membrane. Occasional isolated "pearls" of nonkeratinized epithelial cells were seen in the sub-epithelial tissue; however, no other evidence of neoplastic degeneration or invasion was found.

Schlumberger and Lucké (1) have pointed out the great difficulty in classifying and differentiating certain of the epithelial neoplasms of fishes, particularly the benign papilloma and the malignant epithelioma. They cited the report by Anitschkov and Pavlovsky, in 1923, of tumors in several species of gudgeon (*Gobio*). Schlumberger and Lucké felt that the three tumors which the original authors described as distinct types, merely represented the same tumor at different developmental stages. The first case was described as multiple small nodules and flattened plaques, the larger of which were papillomas with marked inflammatory reaction about their bases. Anitschkov and Pavlovsky compared these growths to chronic inflammatory papillary hyperplasia of the skin and mucosa in man. In the second case, a large, flat, solitary tumor, which was microscopically a benign papilloma without associated inflammation, was located on the dorsum of the fish, *Gobio nigronatatus*. The third case concerned a 2 x 1 x 1 centimeter sessile, cauliflower-like mass on the dorsum of a small *Gobio blenniodes*. Microscopically, this last tumor differed from the second in the markedly irregular hyperplasia of the overlying epithelium, and the paucity of supporting stromal connective tissue elements. The original authors regarded the last case as a papillary epithelial carcinoma, although there was no infiltration of the dermis and other evidence for malignancy was tenuous. It appears that this lesion, which Anitschkov and Pavlovsky considered to be three, undergoes much the same morphological changes as those to be discussed in this thesis for the flathead sole.

A non-neoplastic disease known as fishpox or carppox has been reported as occurring in several species of fish, especially the cyprinids (carps, tench), and it has attained epidemic proportions (1, 5). It is noted that the disease has no relation to the various pox diseases of mammals or birds. This disease can involve any area of the epithelial surface, including that over the eyes. It is characterized by numerous grey to white, flat, raised, or papillary growths. Microscopically, the lesions are seen as a marked proliferation of the epithelium with little or no involvement of the underlying dermis. No stromal elements are found in the affected epithelium; and inflammatory changes, if present, are minimal. According to Schlumberger and Lucké (1), "On histologic grounds no sharp distinction can be drawn between these lesions and true neoplasms, but clinically the lesions of pox, unlike true tumors, usually regress and disappear entirely."

Nigrelli (5) cited the papers of Loewenthal (1907) and Plehn (1910) as describing intranuclear and intracytoplasmic inclusions during certain stages of fishpox, which were absent after the epidemic had passed its peak and was apparently no longer infectious. Plehn was noted to have recognized the infectious nature of the disease as probably caused by an ultramicroscopic organism transmitted by fish lice (argulids). Using touch preparations of carppox, Roegner-Aust and Schleich demonstrated inclusions which were felt to be the same as some elementary bodies seen with the electron microscope (5). The particles, 70 to 220 millimicrons in diameter, were comparable in size to the inclusions found with pox disease of higher vertebrates. Goncharov (19), using phase-contrast and dark field light microscopic techniques, confirmed the findings of Roegner-Aust, again noting the great similarity between the elementary bodies of fishpox virus and the poxviruses of warm-blooded animals.

As yet, fishpox has not been experimentally transmitted, although epidemiologic evidence indicates the involvement of an infectious process. Epidemics have been reported on the European continent, although sporadic outbreaks are more common. In some lakes or ponds yearly occurrences in the same species of fish with eventual regression and recurrence have been reported (5).

Nigrelli (5) has used the term *hyperplastic epidermal disease* interchangeably with fishpox. In 1948 (20) and again in 1952 (5), he described what he called "An epidermal hyperplasia, pathologically and epidemiologically similar to carp pox..." (5) in the bluegill sunfish, *Lepomis macrochirus* Rafinesque, found in a lake near New Preston, Connecticut. Grossly, the tumors were morphologically similar to the lesions described as either papillomas or fishpox. Microscopically, the lesions most closely resembled papillomas. Nigrelli (20) stated "Histologically, the growth shows a great hyperplasia of epithelium... The hyperplasia consists of closely packed epithelial cells and epidermal elements around a central core originating from the corium... Melanophores, capillaries, nerve elements and fibrous material are found in this core." In every preparation studied, Nigrelli (5) found, associated with the lesion, the ectoparasitic ciliates, *Trichodina* and *Ichthyophthirius*; the latter was encysted in the growth, and the former was found free on the surface.

Smith (21) also described a lesion which he called hyperplastic epidermal disease occurring in the winter flounder, *Pseudopleuronectes americanus*, in association with an encysted metacercariae of the trematode, *Cryptocotyle lingua*, belonging to the family Heterophyidae. In this instance the epithelium was hyperplastic, the corium was edematous, and fibrous bands extended from it into the epithelium. There was a rich capillary vascular bed located in the corium, and some mucous cells were present in the epidermis, primarily in the peripheral areas of the neoplasm. Morphologically, this lesion seemed entirely similar to the one described above by Nigrelli (20).

Nigrelli and Smith (22, 23) have reported the association of various related cnidosporidians (myxosporidians and microsporidians) with epidermal changes or epithelial hyperplasia in two other species of fish. In 1948, Nigrelli (24) reported a different morphologic epidermal tissue response to a myxosporidian in the redhorse sucker. In this case, the infecting organism, *Mysobolus moxostomi*, was localized in the deeper layers of the dermis, and stimulated the development of a delicate fibrous network which surrounded the trophozoite and spore masses; inflammatory response was mild. However, above and adjacent to the site of infection, the tissue reaction was quite marked. Fibrous growth was considerably thickened, with increased vascularization. In some areas, stromal papillae formed from this hypertrophied connective tissue, and appeared to support the overlying hyperplastic epithelium. This epithelial reaction was composed primarily of prickle cells with prominent intercellular bridges; less frequently observed were normal appearing polygonal-shaped cells which occasionally contained several deeply stained, peripheral cytoplasmic granules of various sizes. In some areas, the prickle cells had destroyed the basement membrane and "invaded" the subjacent corium, although not extensively.

Nigrelli (25) states that fish are constantly subject to infection by ectoparasites which gain their nourishment by feeding on the host tissue cells. Host susceptibility is increased during periods of temperature variation, at which time these Protozoa enter the skin, thereby stimulating the hyperplastic tissue response in both the corium and the epithelium (23). The presence of a parasite, either bacterial, protozoan, helminthic or arthropodic, is a consistent finding in all reported cases of hyperplastic epidermal disease. Nigrelli (24) states, "Intercellular and intracellular cnidosporidians elaborate proteolytic enzymes and other chemical substances which may be responsible for considerable cellular degeneration, cell hypertrophy and other tissue responses noted in these infections."

As indicated above, it is often quite difficult to discern from the older literature if the reported lesion is a true epithelioma (epidermoid carcinoma) or a benign papilloma. Schlumberger and Lucké (1) reclassified certain tumors, reported as epitheliomas, as papillomas "because of their structure and absence of invasion." Although in terms of structure a tumor might have the general appearance of a papilloma, if any epithelial cells penetrated into the dermis or the deeper tissues, this lesion was classified as malignant, even though no metastases were found at the time of examination. In the twelve species of fish (Table 2) for which epitheliomas have been described, the skin, the lips, or the oral mucosa have been the tissues of origin in all but one case: a goldfish, *Carassius auratus*, in which the bladder was involved (1). Morphologically, these tumors were similar to epidermoid carcinomas of mammals; in some cases, the resemblance to squamous cell carcinoma was striking. Fish epidermis does not keratinize; however, collections of concentrically arranged, nonkeratinized cells are often seen.

The first reported fish epithelioma, according to Schlumberger and Lucké (1), was found on the lower lip of a catfish, *Ictalurus catus*. It was reported by McFarland in 1901 in the Proceedings of the Pathology Society of Philadelphia, Pennsylvania. The tumor had the gross appearance of a lobulated papilloma, 4 centimeters in diameter; the mucosa adjacent to the lower lip, the upper lip, and the surrounding skin was also affected with multiple small nodules. Microscopically, the large tumor was papillomatous, composed of epithelial cells supported by a vascular connective tissue stroma, and was invasive.

In 1924, Johnstone (26) reported the occurrence of a 1 x 1 centimeter epithelioma, located on the right side of the mandible (with a short extension into the mouth) of a whiting, *Merlangus merlangus*. Microscopically, it was noted that the tumor had completely destroyed

TABLE 2

Epitheliomas (Epidermoid Carcinomas) on Fishes; Survey of the Literature

Species	Common Name	No. of Fish	Sites of Tumor	Reference
Icatalurus catus	Catfish	---	Both lips	Schlumberger & Lucké (1)
Cyprinus carpio	Carp	2	Skin of head, fins, operculum	Schlumberger & Lucké (1)
Spinachia spinachia	Stickleback	---	Skin of trunk	Schlumberger & Lucké (1)
Tinca tinca	Tench	2	Lips	Schlumberger & Lucké (1)
Carassius auratus	Goldfish	---	Bladder	Schlumberger & Lucké (1)
Barbus vulgaris	Barbel	---	Lower lip	Schlumberger & Lucké (1)
Chondrostoma soetta	LeNez	---	Oral mucosa	Schlumberger & Lucké (1)
Pogonias chromis	Croaker	2	Lips	Schlumberger & Lucké (1)
Merlangus merlangus	Whiting	---	Mandible	Johnstone (26)
Pollachius virens	Codfish	---	Lower lip	Williams (27)
Cichlasoma tetracanthus	Biajaca	---	Skin about orbit	Schlumberger & Lucké (1)
Ameiurus nebulosus	Catfish	166	Lips, oral mucosa	Lucké & Schlumberger (28)

Meckel's cartilage, reduced the mandibular bone size markedly, and replaced all but a few traces of the normal jaw musculature. The tumor was primarily composed of a dense mass of small globular bodies which were not unlike nonkeratinized epithelial pearls. Surrounding each pearl there was a rather loose connective tissue capsule which was distinct from the fine fibrous tissue which comprised the vascular supporting stroma. The cells within the pearls were small and closely apposed. In some, the central tissue had broken down, leaving irregular colloidal spaces. Occasional multinucleated cells were seen. Although the structure is fairly classic for an epithelioma, the basement membrane was found to be intact and no metastases were noted on examination.

Williams (27) reported in 1929 the occurrence of a bilobed tumor which had spread over the anterior portion of the lower jaw of a large codfish, *Pollachius virens*. The anterior lobe of the tumor involved the point of the jaw, and the posterior lobe extended about 7 centimeters along the right side; the latter lobe projected 2.2 centimeters beneath the dental line. Both lobes extended into the mouth. Grossly, the tumor had a pitted appearance and was somewhat darker than the surrounding epidermis. A watery, mucus-like material could be expressed from the surface. Microscopically, the tumor was composed of glandular and epithelial cells grouped about cyst-like cavities and supported by a well developed connective tissue stroma which contained numerous aggregates of black pigment and was relatively avascular. The cells which lined the cystic spaces were columnar and tended to be arranged in a palisade-like manner. The author felt that the tissue of origin was probably the mucous glands, as many of the cystic spaces were filled with a mucoid secretion. Dr. Howel Evans, of the Pathology Department of Liverpool University, felt this tumor to be comparable to the human epithelioma adenoides cysticum. The main point of difference was the abundance of connective tissue stroma in the fish tumor compared to the human analogue.

In 1941, Lucké and Schlumberger (28) reported the occurrence of a transplantable epithelioma on the lip of the catfish, *Ameiurus nebulosus*, taken from rivers near Philadelphia, Pennsylvania. The tumors appeared as single or multiple, large, red, broadly sessile masses, with a smooth or coarsely nodular surface, and were most often located on the lips or dental plate. The average size of the tumors was 1 to 2 centimeters, but some attained such size as to interfere with normal closure of the mouth. Microscopically, the tumors were composed of closely packed masses of epithelial cells growing about a delicate, highly vascularized connective tissue stroma. The smaller tumors did not appear to invade; the larger tumors commonly sent broad bands of epithelial cells deep into the subjacent tissue. Some of these epithelial pegs were limited by an intact basement membrane, but in others this was lost and the underlying tissue was invaded by flame-shaped processes of loosely arranged cells. Emboli of neoplastic cells were often found in blood vessels in tumors which were deeply invasive, although no distant metastases were found.

Lucké and Schlumberger did extensive laboratory investigation into the natural history of this tumor. Explants from these tumors were placed in the anterior chamber and between corneal layers in the eye;

autologous and homologous transplants were successful. In the anterior chamber of the eye the tumor formed dense membranes which spread over the posterior surface of the cornea, and continued to grow until the entire anterior chamber was filled. Within the cornea, growth occurred by expansion, with further dissection of corneal layers by the enlarging tumor. Heterologous transplantation of the tumor to two other species of fish and to frogs was not successful. In the case of the leopard frog, an abundant leukocytic exudate immediately formed about the explant; this did not occur in the two, more closely related species of fish in which this was attempted, the goldfish and the sunfish (28).

Because the skin of fish remains nonkeratinized, even in a diseased state, it was possible for Lucké and Schlumberger to make direct microscopic observations on the growth and development of the catfish epithelioma *in vivo*. It was found that the occurrence of a tumor was preceded by intense hyperemia of the epithelium at the subsequent site; no abnormal epithelial changes were identifiable at this time. The hyperemia was due to the formation of irregular, wide-meshed networks of underlying capillaries; the number, arrangement, and structure of these vessels were strikingly atypical. Within two weeks the overlying epithelium began to thicken and become elevated. By two months the lesion progressed to the formation of a definite nodular or plaque-like growth (28).

It should be noted that no malignant epitheliomas have as yet been reported in the literature as occurring on flatfish or flounders of the family Pleuronectidae.

Typical cutaneous papillomas have been reported in several other poikilothermic species, although this lesion has not been reported in any of the species of amphibia. Smith and Coates (29), in 1938, reported the occurrence of fibroepithelial growths in six specimens of the edible green turtle, *Chelonia mydas*. These were described as usually typical papillomas, but occasionally the underlying fibrous tissue was also hyperplastic. These investigators removed a total of 250 tumors from six turtles, and found ova of the parasitic trematode, *Hapalotrema constrictum* (reclassified as *Distomum constrictum*), in more than one-half of them (30). They felt, however, that the ova were probably deposited in the preexisting vascular tissues of the tumor, and had no effect on the development or growth of the tumor.

Lucké (1) reported, in the same year, similar lesions on the tail, flippers, the axillae, neck, eyelids, and corneas of the same species of turtle. The tumors were sessile or broadly pedunculated and ranged in size from small warts of a few millimeters to lesions of 20 centimeters in diameter. Microscopically, the numerous connective tissue papillae were covered by several layers of keratinized squamous epithelium.

In 1941, Smith, Coates and Nigrelli (31) described a papillomatous disease of the gallbladders removed from 100 marine turtles of the same species. All were found to contain the trematode, *Rhytidodoides similis*. The gallbladder mucosa was usually hyperplastic, and the underlying connective tissue and capillaries had undergone proliferation. The authors felt this disease was caused by mechanical or chemical factors related to the presence of the parasite.

Schlumberger and Lucké (1) report the presence of three papillomas on two musk turtles, *Sternothaerus odoratus*, obtained from the Philadelphia Zoological Gardens in 1948. Microscopically, these tumors closely resembled the common human wart: a loose, vascular connective tissue papilla was covered by stratified squamous epithelium which demonstrated hyperkeratosis and parakeratosis. The lesions were excised and transplanted to other species of turtle; the explants did not grow, and the papillomas did not recur at the site of excision.

Papillomas have been reported on three species of lizard, *Lacerta agillis, Lacerta muralis fiumensis,* and *Lacerta viridis* (1). Grossly and microscopically they are in every way consistent with the picture already described for other papillomas. In the same paper, Schlumberger and Lucké (1) reported two occurrences of squamous cell carcinoma in two different species of tegu (a lizard), *Tupinambis teguixin* and *Tupinambis nigropunctatus*. In the former case, the authors cited Schwarz's description of a 3 centimeter tumor located on the right forefoot which had not metastasized, although it was obviously malignant microscopically. The second case was from their own experience. They observed the growth and development of two tumors on one animal over a 32 month period. These tumors were microscopically similar to the first tumor and had similarly not metastasized.

Tumors of the warm blooded animals, birds and mammals, have received the greatest share of experimental investigation and attention. Domestic birds, such as chickens, pigeons, ducks and geese, and caged or captive wild birds have been the primary source of information about avian neoplasms (3). Reports of papillomas in birds are infrequent, and are usually found incidentally.

In contradistinction to the meager data available regarding neoplasia in the other classes of vertebrates, the amount of descriptive literature about mammalian tumors is enormous. Nevertheless, Schlumberger (3) states that "... of the more than 10,000 mammalian species only about ten are represented by more than a few reports of isolated cases." Domesticated and laboratory animal species provide the greatest and most complete information regarding mammalian neoplasia. There is definite evidence that certain of these tumors possess various degrees of species-specificity. Schlumberger (3) feels, that while the terms "species-specificity" and "species characteristic" carry implications of constitutional susceptibility, this "... need not be to neoplastic growth, *per se*, but rather to a particular environmental factor or even a behavior pattern that, in turn, initiates the tumors."

A good example of the above statement is the virus-caused papilloma found on the wild cottontail rabbit, *Sylvilagus floridanus*, first reported by Shope in 1932 (32) and 1933 (33). In some areas of their natural habitat in Kansas, as many as 25 percent of the cottontail population may be affected with papillomas. It is quite rare for these to become cancerous in the natural host; however, when the disease is transmitted to the domesticated rabbit by inoculation with the virus, the resultant papillomas usually undergo malignant transformation (34, 35, 36).

In 1936 and 1943, Parsons and Kidd (37, 38) reported an infectious papillomatous disease which was confined to the oral mucosa of rabbits. As in the Shope rabbit papilloma, animals of all ages were susceptible and the lesions produced were typical papillomas, grossly and histopathologically. Richter, Shipkowitz and Rdzock (39) described the virus-like particle associated with this disease in 1964. A similar type of infectious neoplastic disease was discussed by DeMonbreun and Goodpasture (40) in 1932, which involved the oral mucosa of dogs. The morphology of the viral etiologic agent of this lesion was reported by Cheville and Olson (41) in 1964. A 1946 communication from the United States Department of Agriculture discussed the morphology and the evidence for a viral etiology of the cutaneous papillomas of cattle (42). These morphologically typical epidermal papillomas occur about the shoulders, head and neck of young animals, and last for about two months before they begin to regress. Cutaneous papillomas, usually located about the nose and lips of young horses, were reported by Cook and Olson (43) in 1951.

All of the above cited mammalian papillomas are epidemiologically infectious and can be transmitted by cell-free filtrates. Most are species-specific, although the Shope virus can be introduced into other species of rabbits, in which case the natural history of the resultant disease is altered (34, 35, 36). Most infections are of short duration, two to five months, and infect only the younger animals. Both rabbit papilloma diseases, however, are exceptions - they persist for longer than six months, and animals of any age are susceptible. In all cases, once the animal has been infected, immunity is strong and permanent.

The occurrence of an epidermal papilloma on the tongue of a blue whale has been reported. This was a coarse, pedunculated, 8 centimeter papilloma described by Rewell and Willis (44) in 1949. An unusual microscopic feature of this otherwise typical, benign epithelial papilloma was the prominence of the dendritic melanophores in the fibrous supporting stroma.

The human papilloma, the verruca vulgaris or common wart, was demonstrated to be infective in 1919 by Wile and Kingery (45). In 1948, Goldschmidt and Kligman (46) injected a cell-free extract of ground-up condylomata accuminata (the human venereal wart) into the skin of adult human volunteers, producing typical lesions of verruca vulgaris at the site of inoculation. Williams, Howatson and Almeida (47, 48), and Chapman, Dawson and Todd (49) have studied human warts with the electron microscope in order to ascertain the sites of virus production and the nature of the intranuclear inclusion bodies. They reported that the virus was first seen in nuclei of the cells of the stratum spinosum. The virus then appeared to spread throughout the nuclei of the cells of the stratum granulosum. In the stratum corneum there was a complete dissolution of all cell constituents, leaving only crystalline aggregates of the virus particles embedded in the keratin. This process is identical to that described for the virus of the Shope papilloma (50, 51).

The possibility of a viral etiologic agent for the epidermal papilloma of the flathead sole has been presented and discussed briefly in two recent publications (7, 52). This thesis will delineate the natural history and morphology of this tumor, and present and discuss possible etiologic factors.

MATERIALS AND METHODS

All tumor-bearing flathead sole, *H. elassodon*, were collected in East and West Sounds of Orcas Island, and the surrounding waters, near Friday Harbor, Washington (Chart 1), from June, 1962, through January, 1965. The great majority of specimens was obtained from a standardized area of East Sound, Orcas Island (Chart 2). Fish were collected by means of an otter trawl (net mesh size of 1 1/2 inches) operated from the fishing vessel *Hydah*. The length of tow averaged 1000 yards and lasted approximately 30 minutes; occasional hauls of shorter and longer lengths (and durations) were made. Water depth, obtained from soundings at mean low water, on Coast and Geodetic Survey maps, and from measurements with a bathythermograph, was about 15 fathoms. The water temperature at the bottom, measured with a bathythermograph, was a maximum of 12° C in the summer and a minimum of 7° C in the winter. The bottom was constituted primarily of a mud-sand combination; the shoreline was rocky and heavily forested, with occasional private dwellings. The only proximate commercial enterprise is a resort hotel, called Rosario, located northeast of the standardized collecting area (Chart 2); however, a small village is located at the head of East Sound, about one mile north of this area (Chart 1).

Four to five hauls were made in the course of a day's collection, which comprised several thousand pounds of fish. The fish were deposited on board ship in live-tanks filled with circulating sea water, and sorted according to species. All flathead sole were separated, counted, and examined for surface tumors. Fish of any species which possessed

CHART 1: San Juan Islands, Washington, Showing the University of Washington Friday Harbor Laboratories, Friday Harbor, Washington, and East Sound, Orcas Island, Washington.

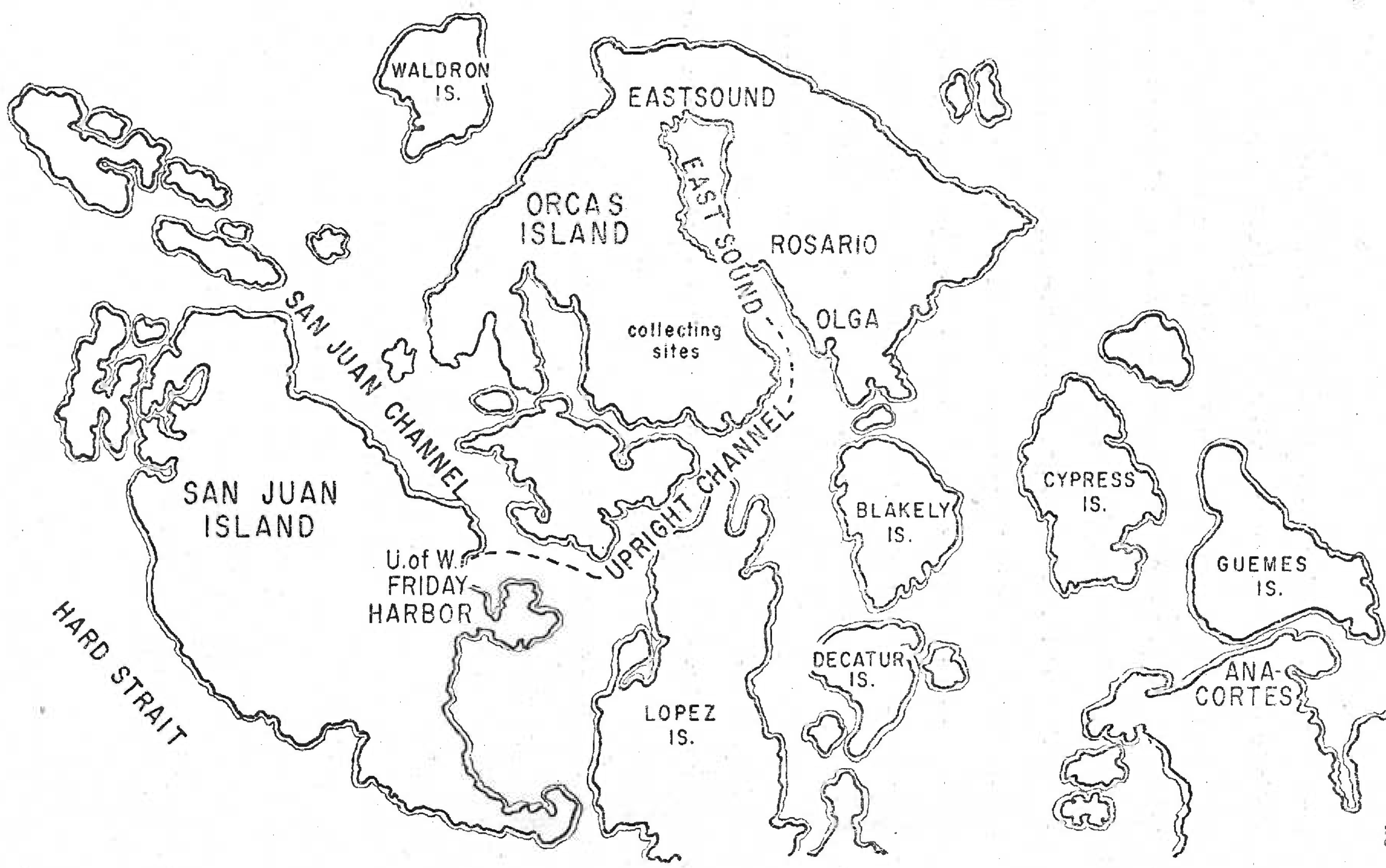
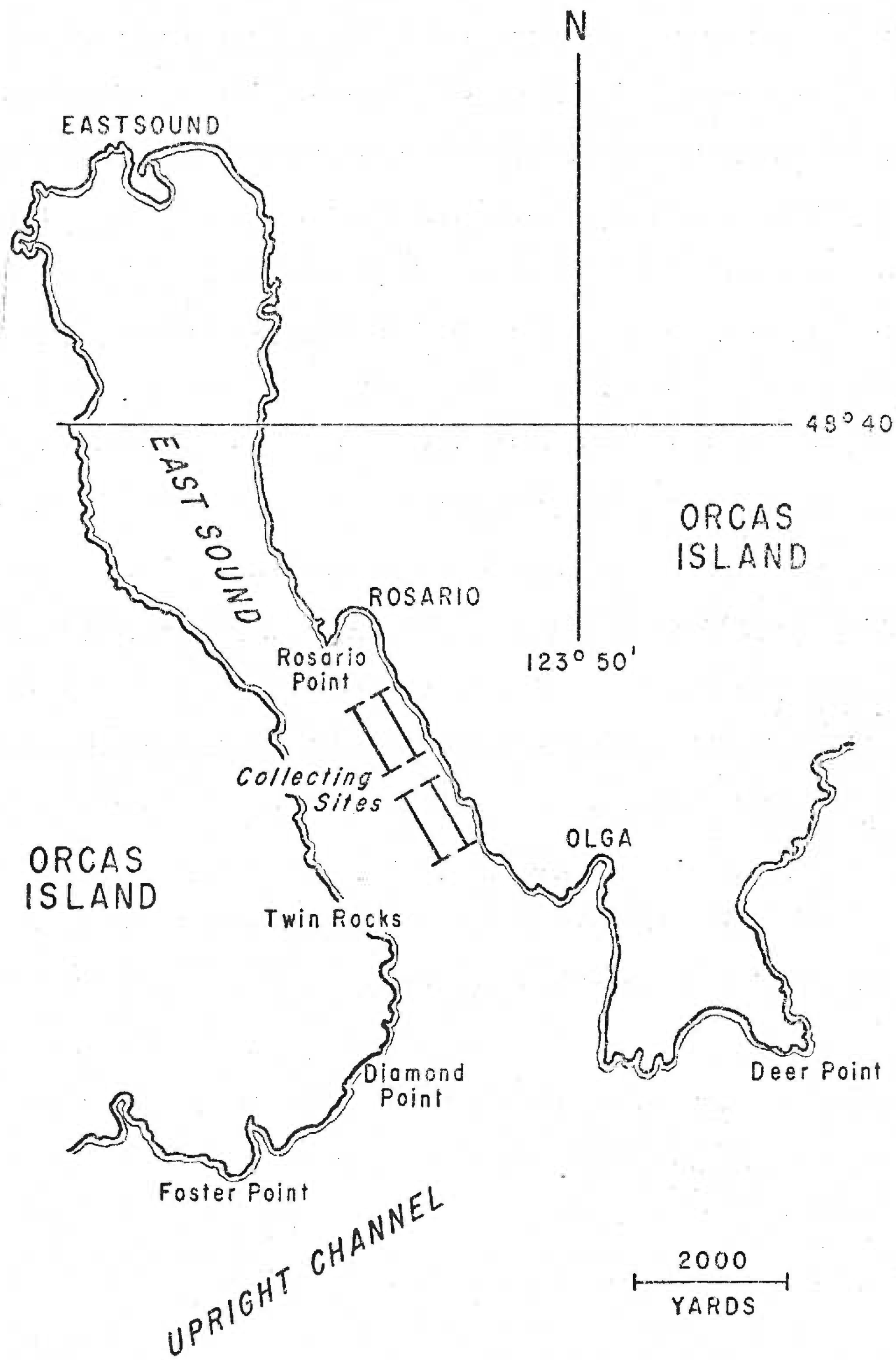


CHART 2: Collecting Sites in East Sound, Orcas Island, Washington, from Coast and Geodetic Survey Chart No. 6380.

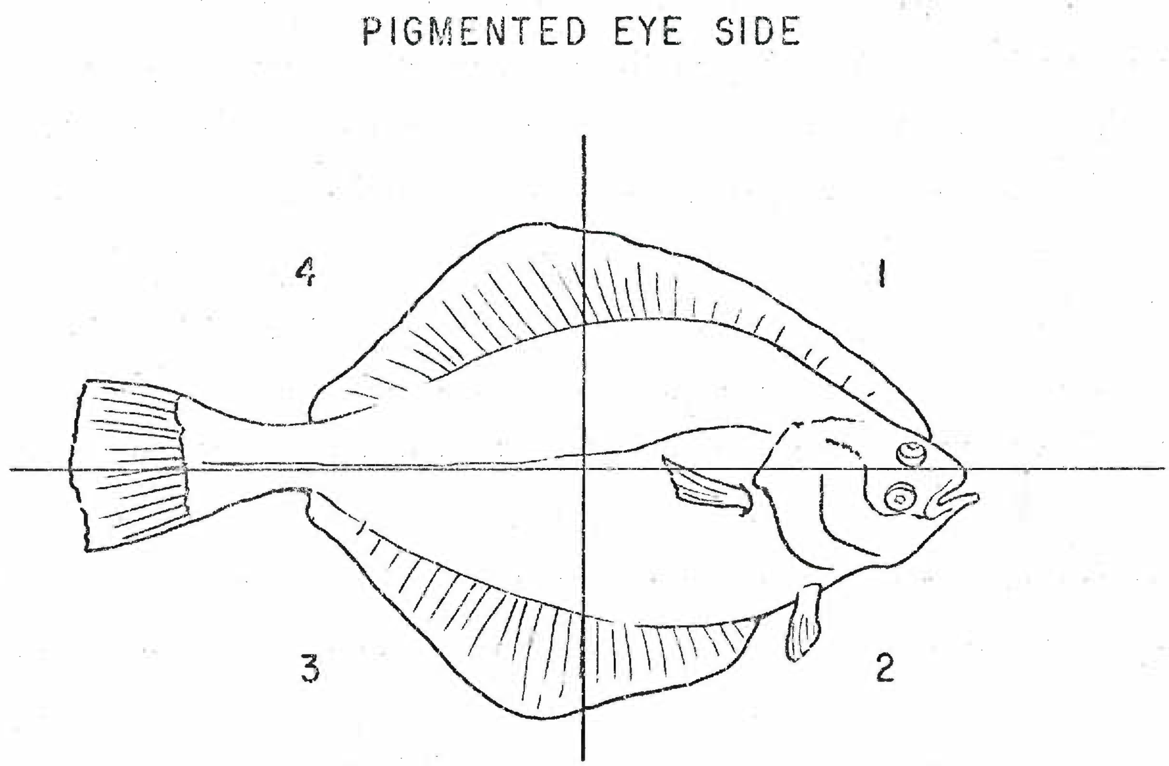


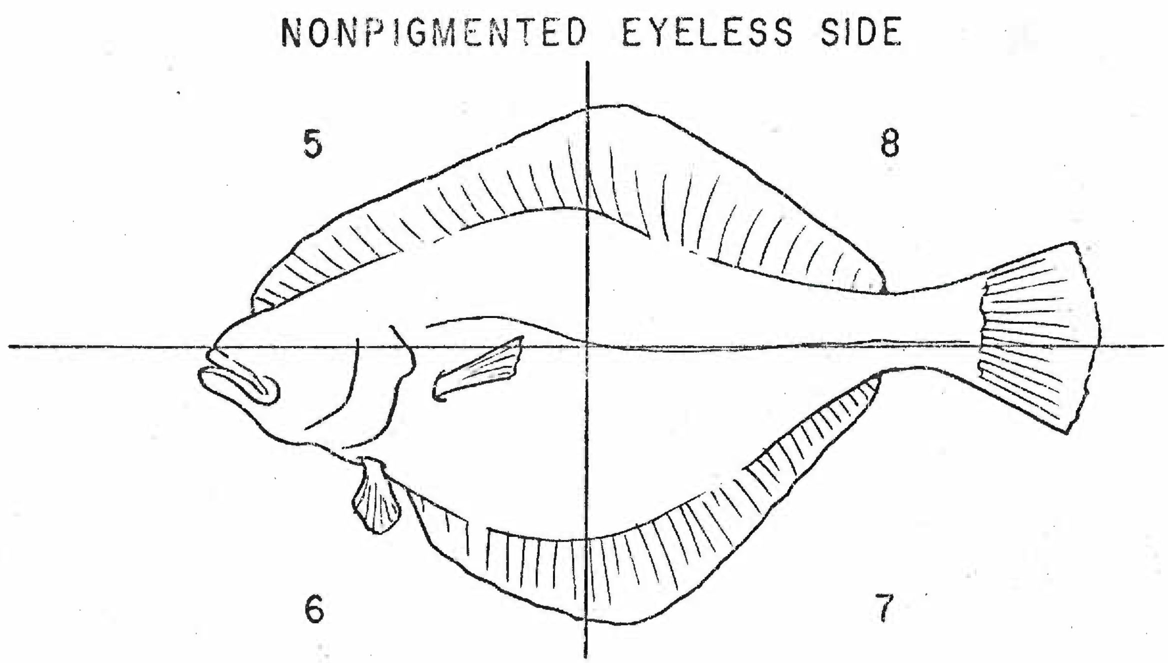
visible surface lesions were also retained and placed, along with diseased flathead sole, in separate live-boxes for subsequent examination as described below. Some grossly normal flathead sole were also placed in separate live-boxes for transportation back to the Friday Harbor Laboratories of the University of Washington, located at Friday Harbor, Washington (Chart 1). There they were transferred to floor or bench tanks, supplied with running sea water, for long term observation and experimentation. These fish were maintained on a diet of chopped fish, clams and brine shrimp for periods of up to twelve months. Canned clams and brine shrimp were obtained through commercial outlets; the fish, mostly herring and smelt, were caught during the collections; all were enthusiastically eaten by the sole.

All fish possessing surface tumors were measured for total length and standard length (53), and their age was ascertained by a count of growth rings (annuli) on the ear ossicles, interopercular bones, and subopercular bones (54). All methods to determine age were in agreement. Fish were then grouped according to the following age designations: age group 0 indicated fish in the first year of life (0 to 1), with no annual growth ring; age group I indicated fish in the second year of life (1 to 2), with one annual growth ring; age group II indicated fish in the third year of life (2 to 3), with two annual growth rings, etc.

Each side of the fish was divided into four more or less equal quadrants (Chart 3), and the size, gross morphology, and location of 782 tumors on 264 diseased fish were recorded according to quadrant location on the pigmented (ocular, upper, right) or non-pigmented

CHART 3: Quadrants for Recording Location of Tumors on Pigmented and Nonpigmented Sides of Flathead Sole, *Hippoglossoides elassodon*.





(eyeless, lower, left) side, or on both sides, if continuous. The number of tumors occurring on each side, both sides, and within the individual quadrants was tabulated and compared.

Normal and tumor-bearing flathead sole of all age groups were observed for periods of one to twelve months in the laboratory. Diseased fish were usually, but not always, placed in separate tanks. Records were maintained indicating the number, location, and size of all skin tumors; in this way, the progression or regression of existing lesions, or the appearance of any new ones was detected.

For light microscopy, normal appearing fin web and skin from involved and uninvolved fish, and neoplastic tissue from all areas within and around the tumor were fixed in Bouin's fluid or 10 percent formalin. Blocks of these tissues were embedded in paraffin, sectioned at 5 microns, and stained as follows: hematoxylin and eosin, Masson's trichrome, Van Gieson's stain, Gridley's reticulum stain, periodic acid-Schiff (PAS) reaction, Feulgen reaction, phosphotungstic acid stain, Brown and Brenn stain for bacteria in tissue, Giemsa stain (Wolbach's modification), Gram stain, and Schorr's stain for inclusion bodies (55). Microscopic data are based on studies of 156 tumors occurring on 104 flathead sole.

For electron microscopy, similar tissue samples were fixed for one hour in 1 percent OsO_4 buffered with veronal acetate and 0.45 gm/cc sucrose added, or 1.33 percent OsO_4 buffered with s-collidine (56), embedded in Epon 812 (57) or Swiss araldite, and thin sectioned on an LKB ultrotome equipped with a diamond or glass knife. Sections were stained with uranyl acetate and/or lead citrate (58) at high pH and examined by means of an RCA EMU 3G electron microscope. Electron microscopic data are based on studies of 127 blocks from 86 tumors occurring on 61 flathead sole.

Tumor-bearing fish were dissected for the presence of visceral tumors and/or metastases; none were found.

RESULTS

GENERAL

During the 32 months of this investigation, many different species of fish (Table 3), and numerous invertebrates were collected. Ten species of the family Pleuronectidae and one species of the family Bothidae comprised the types of flatfish collected (Table 4); tumors were observed only in the Pleuronectidae. This report is based on 350 tumor-bearing flathead sole obtained from a total collection of 5405 fish of this species, a tumor incidence of 6.48 percent (Table 5).

Individual fish of age group 0 had from one to 53 tumors. Those of age group I had one to fourteen tumors, while fish of higher age groups usually had solitary tumors (Table 6). Tumors were not observed, however, in fish of age group IV or greater. A total of 782 tumors were recorded on 264 of the 350 diseased fish, for an overall average tumor incidence of 2.96 per diseased fish (Table 6). Tumors were located anywhere on the external surface, including the body, fins, eyes, and gills. In two additional instances, tumors were present on the mucous membranes of the palate. In fish of all involved age groups, tumors tended to occur more frequently on the pigmented side than on the non-pigmented side. This proved to be highly significant statistically by the chi-square test (Table 6). Tumor distribution was, however, random with respect to the individual quadrants (Chart 3) on both the pigmented and non-pigmented sides.

The peak of the spawning season for the flathead sole was probably in early April since mature ova and sperm were present in the highest

TABLE 3: Fishes Without Apparent Neoplasms of the Skin Collected in East Sound, Orcas Island, Washington. June 1962 to January 1965*

Family Hexanchidae (cow sharks)
Hexanchus griseum

Family Squalidae (dogfish sharks)
Squalus acanthias

Family Rajidae (skates)
Raja binoculata
Raja rhina

Family Chimaeridae (chimaeras)
Hydrolagus colliei

Family Clupeidae (herring)
Clupea harengus pallasi

Family Osmeridae (smelts)
Allosmerus elongatus
Spirinchus thaleichthys
Thaleichthys pacificus

Family Gadidae (cod & hakes)
Gadus macrocephalus
Merluccius productus
Microgadus proximus
Theragra chalcogrammus

Family Embiotocidae (surfperches)
Cymatogaster aggregata
Rhacochilus vacca

Family Gobiidae (gobies)
Lepidogobius lepidus

Family Scorpaenidae (rockfishes)
Sebastodes caurinus

Family Anoplopomatidae (sablefishes)
Anoplopoma fimbria

Family Hexagrammidae (greenlings)
Hexagrammos stelleri
Ophiodon elongatus

Family Cottidae (sculpins)
Blepsias cirrhosus
Chitonotus pugetensis
Dasycottus setiger
Enophrys bison
Leptocottus armatus
Myoxocephalus polyacanthocephalus
Radulinus asprellus
Scorpaenichthys marmoratus

Family Agonidae (poachers)
Agonopsis emmelane
Agonus acipenserinus
Asterotheca infraspinata

Family Cyclopteridae (lumpfishes & snailfishes)
Liparis pulchellus

Family Trichodontidae (sandfishes)
Trichodon trichodon

Family Stichaeidae (pricklebacks)
Delolepis gigantea
Lumpenus maculatus
Lumpenus sagitta
Lyconectes aleutensis

Family Zoarcidae (eelpouts)
Lycodes brevipes
Lycodopsis pacifica

Family Stromateidae (butterfishes)
Palometa simillima

Family Batrachoididae (toadfishes)
Porichthys notatus

* This table was compiled with the assistance of Mr. Bruce Miller, graduate student in the College of Fisheries, University of Washington, Seattle.

TABLE 4: Flatfish Collected in East Sound, Orcas Island, Washington. June 1962 to January 1965*

Family Bothidae (sanddabs)
Citharichthys sordidus

Family Pleuronectidae (flounders)
Atheresthes stomias
Glyptocephalus zachirus
Hippoglossoides elassodon
Isopsetta isolepsis

Family Pleuronectidae (continued)
Lepidopsetta bilineata
Microstomus pacificus
Parophrys vetulus
Platichthys stellatus
Pleuronichthys coenosus
Psettichthys melanostictus

* This table was compiled with the assistance of Mr. Bruce Miller, graduate student in the College of Fisheries, University of Washington, Seattle.

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percentage of individuals at that time. Ripe fish of both sexes, however, were observed during March, April and May. It was also noted that new annular growth rings on the ear ossicles and the interopercular and subopercular bones were formed at this same time. Therefore, April 1, as the approximate midpoint of the spawning season, was arbitrarily designated as the end of the "collecting" year. All fish caught after this date were arbitrarily placed in age group I or greater. Although for some fish in each age group annular growth-ring development was not entirely complete at that time, the standard lengths of such fish were comparable to the majority within each group whose growth rings were complete and whose ages could thus be definitely determined.

In order to properly understand the distribution, morphology and probable evolution of the tumors in this species, one additional point requires explanation. Fish of age group 0 (0 to 1 year old) were obtained only in collections made during the months of October through March (collections 9 to 12 and 16 to 18, Table 5). Fish of a size

TABLE 5: Occurrence of Skin Tumors in *Hippoglossoides elassodon* Collected from June 1962 to January 1965.

Collection Number	Date	Age Group 0 (0-1 yr.)	Age Group I (1-2 yrs.)	Age Group II (2-3 yrs.)	Age Group III 3-4 yrs.)	No. Fish with Tumors, Age Determined	Total No. Fish with Tumors	Total No. Fish
1	6-23-62	-	4	-	-	4	4	59
2	7-6-62	-	15	1	-	16	20	311
3	7-11-62	-	13	-	-	13	13	212
4	9-5-62	-	7	2	-	9	9	116
5	7-15-63	-	21	6	-	27	36	350
6	7-24-63	-	4	-	1	5	30	341
7	7-29-63	-	8	1	-	9	15	273
8	9-27-63	-	-	2	-	2	7	363
9	11-8-63	7	1	-	-	8	12	224
10	12-13-63	18	1	-	-	19	22	421
11	1-17-64	12	-	-	-	12	22	161
12	2-21-64	15	-	-	-	15	24	308
13	4-3-64	-	11	3	-	14	22	313
14	5-2-64	-	29	1	-	30	30	587
15	6-5-64	-	57	2	-	59	62	1023
16	10-23-64	1	2	-	-	3	3	47
17	12-11-64	4	5	-	-	9	9	141
18	1-8-65	5	5	-	-	10	10	155
Totals		62	183	18	1	264	350 or 6.48%	5405

TABLE 6: Location and Incidence of Tumors by Age Groups; 264 Fish with 782 Tumors.

Age Group	Number of Fish with Tumors	Number of Tumors on Pigmented Side	Number of Tumors on Non-Pigmented Side	Number of Tumors Involving Both Sides	Total Number of Tumors	Range of Number of Tumors	Average Number of Tumors
0	62	257 (66.24%)	122 (31.44%)	9 (2.32%)	388	1-53	6.26
I	183	168 (45.65%)	120 (32.61%)	80 (21.74%)	368	1-14	2.01
II	18	10 (43.48%)	7 (30.43%)	6 (26.09%)	23	1-4	1.28
III	1	2 (66.67%)	0	1 (33.33%)	3	3	3.00
TOTAL	264	437* (55.88%)	249* (31.84%)	96 (12.28%)	782		2.96

* χ^2 determination: In the determination of the statistical significance of the difference in occurrence of the tumors on the pigmented versus the non-pigmented side of the fish, all tumors on both sides of the fish were disregarded since it was not possible to ascertain the site of origin. Therefore, the calculation was based on the sum of the tumors occurring on the pigmented and non-pigmented sides: 437 + 249 = 686.

$$\chi^2 = \Sigma \frac{(437-343)^2}{343} + \frac{(249-343)^2}{343} = \frac{(94)^2}{343} + \frac{(94)^2}{343} = 51.52$$

This value for χ^2 exceeds the significance level of 0.1 percent (10.83) with one degree of freedom.

comparable to those called age group 0 in March were, as indicated above, automatically placed in age group I if taken after April 1st. Age group 0 fish did not subsequently reappear in collections until October of the year in which they were spawned. Unpublished data (59) indicate that the failure to obtain age group 0 fish during the period April to September of each year was probably the result of the mesh size of the otter trawl being too large to trap the small fish.

GROSS MORPHOLOGY AND LIGHT MICROSCOPY

NORMAL SKIN:

Histologically, the normal skin of the flathead sole (Figure 6) is composed of a stratified squamous epidermis separated from a dermis (also called the corium) by a basement membrane. The basal cell layer of the epidermis is the *stratum germinativum* (60) and is composed of round to oval cells. The more superficial cell layers are flattened and more squamous in appearance. Scattered within the epithelium are numerous oval to flask-shaped mucous cells. Fish skin does not keratinize (60) and, in this sense, resembles human oral mucosa (61).

The dermis is composed of a relatively acellular fibroelastic connective tissue containing nutrient blood vessels, nerves and cutaneous sense organs, and pigment cells. VanOosten (60) divides the dermis into the *stratum vasculare*, a thin, loose upper layer, and the *stratum compactum*, a thick, dense lower layer. The most prominent dermal appendages are the scales, which comprise the so-called "dermal skeleton". The entire integument is bound to the underlying muscle (and bone) by a strong, but loosely organized band of connective tissue called the *subcutis* (60).

Two morphologically distinct types of epidermal tumors are encountered in the flathead sole, along with a continuum of transitional stages between them. These lesions differ both grossly and microscopically.

ANGIOEPITHELIAL NODULE:

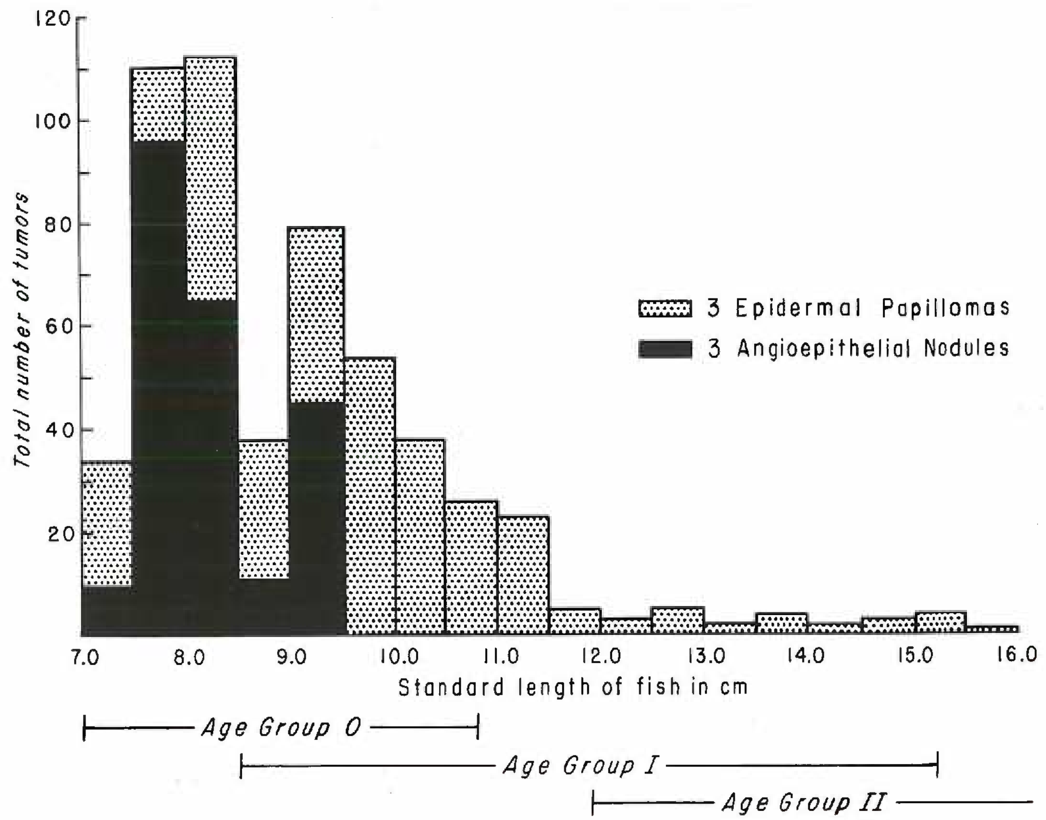
In fish of age group 0, most tumors are small, sharply circumscribed, hemispherical, sessile nodules measuring 1 to 2 millimeters in diameter (Figure 1). The overlying surface is smooth, and the color varies from white to pink to red. Although sometimes found in close proximity to the eyes and gills, they are never seen directly on them. All other body surfaces, however, are affected (*vide supra*, Table 6). Microscopically, these nodules are angiomatous proliferations of vascular tissue of dermal origin capped by a layer of mildly hyperplastic and pleomorphic epidermis. The epithelial and dermal components are separated by a prominent, intact basement membrane (Figures 7, 8, 9, 10, 11). Mucous cells are sparse in the hyperplastic epidermis, in contrast to their relative abundance in normal skin (Figure 6). Cytologically, the vascular endothelial cells have relatively uniform, diffusely granular, acidophilic cytoplasm. The nuclei are small, mildly pleomorphic, and stained deeply with hematoxylin. The cytoplasm of the hyperplastic epithelial cells is also relatively uniform, and stains homogeneously with eosin. The nuclei of some of the epithelial cells are relatively larger than those of the normal epidermis, are moderately pleomorphic, have prominent nucleoli, and exhibit mild variability in the intensity of staining with hematoxylin.

The angiomatous component shows possible invasive tendencies (Figures 7, 8), since it sometimes extends down into the subdermal connective tissue and surrounds individual skeletal muscle fibers or fin rays. Because of the histopathologic appearance of these early lesions, they are descriptively named *angioepithelial nodules*.

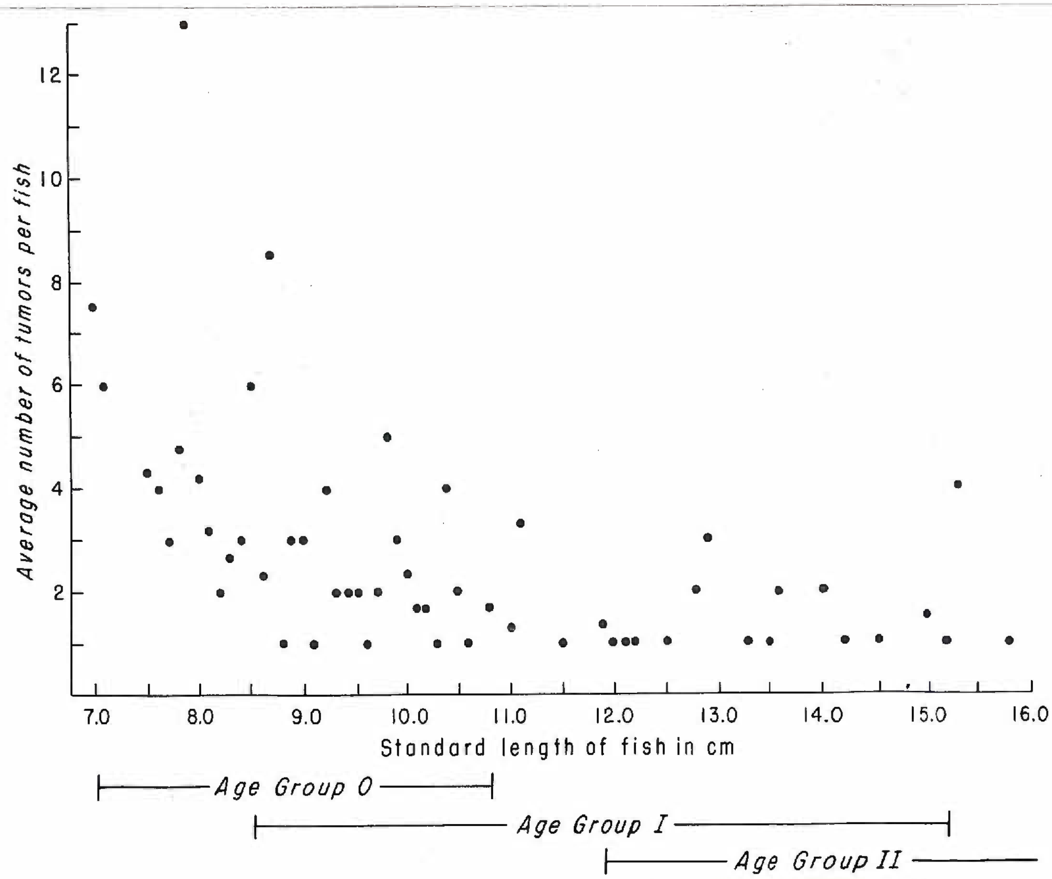
These nodules are never observed on fish older than one year of age; however, transitional forms (Figure 2) between this lesion and the mature papilloma are observed on fish as early as approximately ten months of age, and as late as fourteen months of age. The data have been compiled into a histogram (Graph 1) which demonstrates the marked decrease in the total number of tumors observed on flathead sole as age group and standard length increase. These data also show the disappearance of the angioepithelial nodule before the end of the first year of life. In this tabulation, it should be noted that the transitional stage is defined as beginning with the appearance of a spreading edge around the central angioepithelial nodule; however, for the sake of clarity, all transitional tumors are considered within the category of epidermal papilloma. The data were also tabulated by comparing the average number of tumors per fish against the standard length (Graph 2) to further illustrate the decline in number of tumors per fish, as well as the decrease in number of involved fish, as the age group increases.

GRAPH 1: Histogram illustrating total number of tumors found on flathead sole within standard length groups of 5 mm (based on 264 fish with a total of 782 tumors). At the bottom, standard length is related to age group. As age and length increase, the number of fish collected with tumors decreases. *Note* that the angioepithelial nodules, represented in black, disappear by the end of the first year of life, possibly as a result of their progression to epidermal papillomas.

GRAPH 2: Average number of tumors per individual flathead sole at each millimeter of standard length (based on 264 fish with a total of 782 tumors). At the bottom, standard length is related to age group. The average number of tumors per fish decreases with increasing age and length. This is partly due to confluence of smaller lesions, and also could be due to earlier death of fish with great number of tumors.



120
100
80
60
40
20
Total number of tumors
3 Epidermal Papillomas
3 Angioepithelial Nodules
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
Standard length of fish in cm
Age Group O
Age Group I
Age Group II



12
10
8
6
4
2
Average number of tumors per fish
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
Standard length of fish in cm
Age Group O
Age Group I
Age Group II

EPIDERMAL PAPILLOMA:

The lesions of older flathead sole (Figures 3, 4, 5) of age groups I, II, and III are, by contrast with the angioepithelial nodules, much larger. Grossly, they appear as grey-white to brown or black cauliflower-like protrusions of up to 1.5 centimeters in height, and 1 to 6 centimeters in greatest diameter. The centers of the tumors are most often raised and warty; the edges are most often flat and plaque-like, grading imperceptibly into the surrounding normal epidermis. Occasional tumors are polypoid with a highly convoluted surface and are attached by a connective tissue stalk.

Microscopically (Figures 12, 13, 14, 15, 16, 17, 18, 19), the dermal angiomatous component, which is the most prominent part of the angioepithelial nodule, is usually quite inconspicuous in the papilloma (Figures 12, 13, 14), while the epidermal portion consists of broad, closely spaced, papillary folds of epithelial cells supported by thin strands of connective tissue. An intact basement membrane provides a sharp junction between the hyperplastic epithelium and the fibrous stroma. A rich vascular network and occasional melanophores are observed in the stroma of the papilloma.

Cytologically, the epithelial cells of the tumor are ovoid to polygonal in shape and relatively uniform in size. Their cytoplasm is finely granular and stains with acid dyes such as eosin. The nuclear-cytoplasmic ratio is markedly increased over the normal, and the nuclei are large and moderately pleomorphic with one to three prominent nucleoli and occasionally clumped chromatin. They exhibit striking variability in the intensity of staining with basic dyes such as hematoxylin. Staining characteristics are normal in the basal layer; however, those cells near the surface of the epithelial folds of the papilloma lack basophilia, stain with eosin, and prove negative to the Feulgen reaction. No intracellular inclusion bodies are seen.

Many histologic preparations have been stained by the methods outlined above; no definite evidence has been obtained by light microscopy, which indicates an association or presence of any casual or causal biological agent. The PAS stain confirms the absence of mucous cells in most tumors. The cytoplasm of many tumor cells, however, stains faintly by the PAS method.

These tumors appear to be neoplasms, since they are capable of progressive growth and are at least partially independent of local tissue organizers. Because definite invasion of the subjacent corium is never seen, and mitotic figures are rarely observed, they seem to be benign and as such are called *epidermal papillomas.*

As stated above, transitional stages between angioepithelial nodules and epidermal papillomas are frequently seen in fish late in the first and early in the second years of life (Figure 2). The nodules gradually change in color from red or pink to grey or white, and this is followed by a loss of the sharp demarcation between the nodule and the surrounding normal skin. The transition becomes apparent as the grey-white, plaque-like border spreads out irregularly into the adjacent normal skin. (This process is much more apparent on the pigmented side of the fish where the grey-white neoplastic tissue contrasts markedly with the normal brownish skin.) At the same time, the central nodular part often becomes more prominent with the development of multiple, compact, wart-like papillae.

The above changes in gross morphology were followed in the laboratory for up to eight months in ten flathead sole of age group 0 with surface angioepithelial nodules. In an eleventh fish of age group 0 to I, however, the spreading edges of several early epidermal papillomas regressed, leaving a central angioepithelial nodule. This exceptional phenomenon may have been related to the poor nutritional state of this individual, since the fish refused to eat, and expired within two months. Over 30 fish of age groups I and II with epidermal papillomas have been observed in the laboratory for periods of two to ten months. In all cases, the epidermal papillomas already present either remained the same, or in the majority continued to enlarge; however, no new tumors of any sort appeared. Numerous normal flathead sole of all age groups have been maintained in live-tanks and periodically examined during the same periods of time; no tumors developed in these fish.

ELECTRON MICROSCOPY

NORMAL SKIN:

The dermis of the fin web is composed of dense collagenous connective tissue containing scattered melanophores and capillaries. It is separated from the epidermis by a thick, 200 millimicron basement membrane. The epidermis of the fin web is a stratified squamous epithelium composed of five to ten layers of epithelial cells of three morphologically distinct types: basal cells, intermediate cells, and superficial cells (Figure 20). The basal cells are ovoid to elongate or polygonal with proportionately large nuclei (Figure 21). They are separated from the basement membrane by a narrow space measuring approximately 50 millimicrons, which is filled with a finely granular substance of low density. Microvillous surface projections interdigitate with similar structures present on the surface of adjacent basal cells and of the lowermost cells of the intermediate cell layer. Desmosomes are observed between adjacent cells. The cytoplasm appears to be separated into morphologically distinct perinuclear and peripheral zones. The perinuclear zone contains a relatively small Golgi complex and most of the mitochondria, ergastoplasmic sacs, and free ribosomes. A few bundles of cytoplasmic filaments are located near the desmosomes and at the junction between the peripheral and the perinuclear cytoplasmic zones in association with numerous ribosomes. Numerous small vessicles, with a single limiting, smooth-surfaced membrane, are found in the peripheral zone, especially near the cell membrane. The nuclei are round to oval and are finely granular.

The intermediate cell layer varies in thickness from three to eight cells. Interspersed throughout this layer are numerous mucous cells (Figure 22). They are round to oval with eccentrically placed nuclei which are relatively more dense than those of the epithelial cells. No desmosomes have been observed connecting mucous cells to the intermediate squamous cells; moreover, there are no cytoplasmic filaments in the mucous cells. The ergastoplasm is composed of elongated sacs, oriented in parallel, which are surfaced by numerous ribosomes. The mucus is contained within single-walled, smooth-surfaced, membranous sacs, possibly of Golgi origin. Golgi apparatus, composed of small flattened sacs and vesicles, are topographically related to the mucous sacs.

The unit membranes of the intermediate cells are more convoluted than those of the basal cells, with an increase in the size and number of microvilli (Figure 23). The desmosomes are larger and more numerous. The separation of the cytoplasm into perinuclear and peripheral zones is more pronounced, and the peripheral zone is broader than in the basal cells and contains numerous cytoplasmic filaments which are more abundant, are closely packed in parallel array, and appear to surround the perinuclear zone. Cytoplasmic organelles are condensed about the nucleus within a perinuclear zone: the Golgi apparatus is more complex, with many cisternae and associated vesicles; the ergastoplasm is more voluminous; the mitochondria are increased in size and number. Free ribosomes, however, are decreased in number. Single-walled, empty appearing vesicles of different sizes and shapes are present. The nuclei are less homogeneous than those of basal cells, and coarse clumps of chromatin are located in the interior of the nucleus and at the inner surface of the nuclear membrane.

The cells of the intermediate layer which are located closest to the surface are flatter than the deepest cells; moreover, the long axes of these more superficial intermediate cells are oriented parallel to the basement membrane. Their nuclei are smaller and more granular, and the peripheral zone of the cytoplasm is filled with closely spaced cytoplasmic filaments, many of which appear to attach to abundant desmosomes. A condensation of relatively dense, finely granular material is observed immediately adjacent to the cytoplasmic membrane. Free ribosomes are more dispersed and fewer in number than in the cells of the basal layer. Vesicles are less numerous than in the deeper intermediate cells, but are more irregular in shape and size. The Golgi complex appears less conspicuous in cells near the surface of the epidermis. There is a decrease in the number of mitochondria. The sacs of the rough-surfaced ergastoplasm are variable in size and shape.

The superficial (surface) cell layer is composed of moderately flattened cells. The complex cellular interdigitations of the subjacent layers are absent, and the cell membranes are relatively evenly contoured (Figure 24). The nuclei are eccentrically located near the base of the cell. They are small, irregular in outline, and dense chromatin clumps often obscure the nuclear membrane. The cytoplasmic components are tightly packed about the nucleus, and the mitochondria and ergastoplasm are reduced in amount. The Golgi complex is inconspicuous, and the free ribosomes are dense and are aggregated in the perinuclear area. The peripheral cytoplasmic zone contains a few remnants of ergastoplasm, and occasional ribosomes are randomly scattered throughout this area. Most of the cytoplasmic filaments are located above the nucleus and are similar in morphology to those previously described. The outer (free) surface membrane of the superficial cells possesses regularly spaced, broad-based microvillous projections which average 450 millimicrons in length and 175 millimicrons in width (Figures 24, 25). Thin, filamentous strands, 50 Å in width and up to 125 Å in length, are often seen attached to the cytoplasmic membrane of these projections. A finely granular cytoplasmic condensate, which is more electron dense than the adjacent cytoplasm, fills the microvilli, and continues between them. It averages 100 millimicrons in width and continues irregularly around the lateral and basal portions of the cells.

Numerous desmosomes are present along the apposed membranes of the cells of the superficial layer and of the superficial and intermediate cells. These desmosomes are longer than those seen in deeper layers. With high magnification, the parallelism of the apposing cell membranes is characteristic (Figure 26), and it is also apparent that the dense material in the vicinity of the desmosome is composed of highly compacted 100 Å cytoplasmic filaments which appear to attach perpendicularly to the desmosomes. Scattered throughout the filaments, especially around the desmosomes, are numerous dense granules measuring 100 to 200 Å in diameter.

Terminal bars (*zonula occludentes* [62]) are observed at the superior juncture of the lateral walls of the superficial cells (Figure 25). In this area, the outer leaflets of the apposing membranes fuse into a single, dense band of 150 millimicrons in thickness and 200 to 500 millimicrons in length. In amphibian skin, Farquhar and Palade (62) feel that these structures are continuous around the cells, and act to close the intercellular space from the surrounding environment.

ANGIOEPITHELIAL NODULE:

A limited number of thin sections from angioepithelial nodules have been observed by electron microscopy.

Some cells of the hyperplastic epidermis of the nodule contain cytoplasmic inclusions of unknown type (Figures 27, 28). They are intensely osmophilic particles of circular or angular outline, varying in size from 200 to 600 millimicrons, and averaging about 350 millimicrons. These particles are usually found in fairly compact aggregates, but may also occur singly in close proximity to the cell membrane.

A second type of inclusion, observed on only one occasion, was much smaller and less variable in size, averaging 44 millimicrons in diameter (Figure 29). These particles had a double-walled limiting membrane and a fine, granular core. Several contained a dense, 60 Å nucleoid-like particle. They are identical in morphology to the *a particles* found in the epidermal papillomas.

EPIDERMAL PAPILLOMA:

Cellular Morphology: The basal layer of the papillary fronds is composed of small, round cells which are limited from the underlying connective tissue stroma by an intact basement membrane. The cell membranes lack microvilli; however, delicate undulations of irregular periodicity are present. Desmosomes and their associated cytoplasmic filaments are absent. The cytoplasmic stratification noted in normal fin web is not present, and cytoplasmic components (mitochondria, Golgi apparatus, ergastoplasmic sacs, and ribosomes) appear decreased in amount. The nuclei are generally ovoid to slightly irregular and nuclear "pores" are seen in the nuclear membranes. The nucleoplasm is finely particulate and usually contains one homogeneous nucleolus composed of a dense aggregate of particles morphologically similar to the free ribosomes of the cytoplasm.

The epithelial cells of the more superficial layers of the papilloma are similar to the basal cells except that they tend to be larger (Figure 30). They are ovoid to polygonal in shape, but tend to flatten toward the surface. They have the same finely convoluted cell membranes as the basal cells, and desmosomes are absent. Occasional short, broad-based interdigitations are observed between closely apposed cells. The nuclei are larger, and the nuclear membranes are sometimes indistinct, or obscured by aggregates of condensed chromatin. The mitochondria are large, with fewer, indistinct, randomly oriented cristae. The ergastoplasm is reduced in amount, vesicular to slightly irregular, and is randomly distributed in the cytoplasmic matrix. The Golgi complex is generally inconspicuous. Small, dense particles of 100 to 150 Å in diameter, interpreted as ribosomes, are scattered throughout the cytoplasm. Smooth-surfaced vesicles are numerous and often contain finely granular, *ca.* 50 Å, material of moderate electron density. Multivesicular bodies, similar to those described by Farquhar and Palade (62) in keratinizing amphibian skin are present (Figure 33).

Cellular Inclusions: The cytoplasm of most of the epithelial cells of the epidermal papilloma contains numerous inclusions of at least three different morphologies. No definite inclusions are seen in the nuclei.

One type of inclusion body, previously designated *particle a* (7, 52), is a vesicular, virus-like particle averaging 44 millimicrons in diameter (Figures 32, 36, 37, 42). Most of these virus-like particles possess a small, very dense, central nucleoid, about 60 Å in diameter, surrounded by a less dense, homogeneous material. The entire particle is contained within a dense, double-walled, 60 to 70 Å membrane. The *a particles* are located throughout the cytoplasm, outside the dilated sacs of the ergastoplasm.

These *a particles* are also topographically associated with the second morphologic type of cytoplasmic inclusion, previously referred to as *particle b* (7, 52). These are irregular in shape, homogeneous in appearance, and of varying electron densities (Figures 31, 32, 33). They are found both free in the cytoplasm and adjacent to or contiguous with large, single-walled sacs which resemble "mitochondrial precursors" such as described in association with the replicating virus of avian myeloblastosis (63, 64) (Figures 33, 42, 43). These particles are most often stellate or arboral in profile, with the intensely osmiophilic core becoming less dense at the edges. They measure from 100 to 225 millimicrons, and average about 150 millimicrons in greatest dimension. The associated *a particles* are found within the matrix or the less dense edges of the *b particles* (Figures 31, 38, 42). Often they are located at the end of an irregular projection of the *b particle*, as if on a stalk, and the limiting membrane of the *a particle* appears to be continuous with the dense matrix of the *b particle* (Figures 32, 33, 41). The *a particles* within the matrix are smaller than those at the ends of the projections, which, in turn, are often smaller than those found free in the cytoplasm (Figure 42).

The third principal type of particle found in the neoplastic cells of the papilloma has been descriptively designated the *granular body*, or *particle c* (7, 52). This inclusion is roughly spherical, measures from 160 to 200 millimicrons in greatest diameter and is usually enclosed within a single-layered, 60 to 80 Å membrane of possible ergastoplasmic origin (Figures 32, 38, 39, 43). The central core is homogeneous, moderately electron dense, and averages about 100 millimicrons in diameter. It is surrounded by an osmiophilic aggregate of small, ovoid granules which appear in occasional micrographs to be radially oriented rods of about 40 to 50 millimicrons in length, which project from the core to the outer surface (Figure 44). These rods are suggestive of capsomeres, but are poorly demonstrated. *Granular bodies* (*c particles*) of the same, or slightly smaller size, but without the complex internal structure of the dense particles, are also seen (Figures 31, 35, 36); these may represent transitional forms between the *a particle* and the *c particle*.

DISCUSSION

During the first year of life, many flathead sole in the waters of the San Juan Islands, Washington, develop single or multiple, 1 to 2 millimeter, raised, red to pink skin tumors. These lesions are first observed on fish of an estimated six to eight months of age (*i.e.*, post-metamorphosis, age group 0, and less than one year of age). Microscopically, the tumors are characterized by angiomatous proliferations of dermal connective tissue which are capped with a layer of mildly hyperplastic epidermis. Observations in the laboratory indicate that the angioepithelial nodule develops into a progressively enlarging epidermal papilloma. The angiomatous component apparently regresses in most instances. The early angioepithelial nodules resemble, in part, the descriptions of skin tumors in *Hippoglossus hippoglossus* (10), and *Platessa platessa* (11), and are indistinguishable from similar nodules found on *Parophrys vetulus* (8) and *Platichthys stellatus* (8).

In flathead sole of age groups I (one to two years old) and greater, the skin tumors are morphologically typical epidermal papillomas. The epidermal component of these lesions predominates, and the supporting connective and vascular tissue is relatively inconspicuous. In the laboratory, the tumors progressively enlarge in most instances, although metastases have not been found. The morphological characteristics and progressive nature of the papillomas suggest that they are true neoplasms, and therefore at least partly and permanently independent of local tissue organizers. Similar papillomas have been described for *Parophrys vetulus* (4, 7, 8), *Glyptocephalus zachirus* (7), *Psettichthys melanostictus* (9), *Limanda limanda* (11), and *Solea solea* (12).

Since the epidermal component of the papillomas found on the flathead sole of age groups I and higher is apparently noninvasive and does not metastasize, the papillomas are considered biologically "benign". Although the vascular connective tissue of the angioepithelial nodules demonstrates possible infiltrative tendencies, many benign angiomatous processes have such patterns and yet are known to be benign and self-limiting. Since the angiomatous component of the angio-epithelial nodules has not as yet been observed in the absence of overlying epidermal hyperplasia or neoplasia, it is not possible to determine which component, if either, precedes and perhaps induces the formation of the other.

The progression of the angioepithelial nodule to a typical epidermal papilloma might explain the absence of the nodules in fish of age groups I and greater. No new angioepithelial nodules (or epi-dermal papillomas) appear *de novo* on normal or tumor-bearing fish of any age group kept in the laboratory for various periods of time. However, it is recognized that the laboratory isolates the fish from its natural environment, thereby altering or eliminating one, or perhaps several, etiologic factors necessary in the production of the disease. The youngest fish collected (age group 0) possess the greatest number of tumors per fish, and these tumors are already present on the youngest fish obtained in the collections (*i.e.*, approximately six to eight months of age). These observations suggest that the events which cause the formation of the angioepithelial nodules take place prior to six to eight months of age.

The biology of the tumors can best be interpreted in the light of our limited knowledge of the natural history of the flathead sole. The fertilized eggs and larvae are apparently planktonic (65). The planktonic larvae are bilaterally symmetrical and swim with the dorsal aspect uppermost; and therefore, the right and left sides are mirror images of each other. The larvae probably remain in the plankton until two to four months of age. During metamorphosis, the fish rotates on its long axis so that the right side becomes the upper side, and the left eye migrates onto the right side. The right side also becomes pigmented, as opposed to the left side which has little or no pigment. The fish then leave the planktonic environment and drop to the bottom, presumably on a suitable mud-sand substrate. The youngest post-metamorphosis fish first appear in the otter trawl collections in October. At this time they are already afflicted with the early angioepithelial nodules. The difference in tumor incidence on the two sides (Table 6) suggests that the major tumorigenic event(s) occurs after metamorphosis, since prior to metamorphosis there are no apparent significant differences between the two sides in morphology, orientation to the planktonic environment, or other definable characteristics. Therefore, the formation of the angioepithelial nodule must occur sometime between metamorphosis and the time of first appearance of the young fish in the collections, *i.e.* between two to four and six to eight months of age.

The high frequency of neoplasms in flathead sole is a striking feature of the data (6.48 percent of the collected population, Table 5). The occurrence of these tumors in the flathead sole may be a generalized phenomenon in Puget Sound, since tumor-bearing flathead sole are found at several widely spaced collecting sites (8). Moreover, similar neoplasms have been observed in other species of Pleuronectidae: English sole, *P. vetulus* (4, 7, 8), rex sole, *G. zachirus* (7), sand sole, *P. melanostictus* (7, 9), starry flounder, *P. stellatus* (8), rock sole, *L. bilineata* (9), and dover sole, *Microstomus pacificus* (66). Tumors were observed only in the Pleuronectidae, suggesting that this toxonomic group is unusually susceptible. The ship's log of the vessel *Hydah* records the casual occurrence of flathead sole with skin tumors on several occasions during the past ten years, indicating that the disease is an extended one in the temporal sense.

The data do not make it possible to compare the *rate* of disappearance of tumorous fish from the total population of flathead sole with the *rate* of disappearance of normal fish from the same population. It is not known whether a tumor-bearing fish recovers from the lesion or dies as a result of it. However, certain observations suggest that most tumors do not regress, and that at least some tumor-bearing fish die as a direct or indirect result of their neoplasms. Both types of tumors usually enlarge progressively when observed on fish maintained in the laboratory. Moreover, the number of diseased fish in each age group decreases as the age group increases (Table 5). Finally, it is reasonable to assume that the presence of a papilloma would decrease the probability of survival of the fish, especially since fins, eyes, and sometimes mouth and gills are involved. Thus, the ability of the fish to avoid and escape predators, to feed, and to perform other functions necessary for survival might be compromised.

Further evidence suggesting that tumor-bearing fish are adversely affected by their lesions is contained in Graph 1 and Graph 2. The *total* number of tumors per involved fish declines rapidly during the second year of life (age group I). Concurrently, the *average* number of tumors per involved fish (except for one fish of age group III) is highest in age group 0 and progressively decreases as the age group increases. Also, the average size of the tumors per age group is smallest for age group 0 and increases in size for each subsequent age group. In part, these phenomena may be due to the coalescence of small epidermal papillomas which develop from two or more closely approximated angioepithelial nodules. Conclusive evidence that the presence of a tumor decreases the probability of survival as compared to the normal may soon be forthcoming from data now being gathered during the course of an investigation of the life history of the flathead sole (59). In this study, thousands of normal fish are being compared to tumor fish as regards age, sex, length, and weight. It may thus be possible to determine the effects of the tumor on individual fish and on the total population of flathead sole in the collecting area.

Hyperplastic epidermal disease and some instances of papilloma, reported for other species of fish, have at times all been associated with a parasitic infection (5, 20, 21, 22, 23, 24, 25). Also, during the course of this study, papillomatous lesions have been observed on the lips of dogfish, *Squalus acanthias*, which appeared to be reactive hyperplastic changes due to the presence of nematode larvae in the underlying connective tissue (67). It should be stressed, however, that no parasitic nematodes, copepods, *etc.*, have been found in, or in proximity to any of the hundreds of papillomas which were dissected and sectioned.

The preponderance of tumors on the pigmented side as opposed to the non-pigmented side in the flathead sole agrees with the observations of Smith (21) who reported the occurrence of a papilloma-like hyperplastic epidermal disease on the winter flounder. This author noted that the lesions were associated with a trematode infection and were predominantly located on the pigmented side. Nigrelli (20) found that the nodules of hyperplastic epidermal disease in the blue gill sunfish occurred more frequently on the dorsolateral surfaces, which are also the more pigmented areas of the fish. Moreover, Johnstone (11) reported nodules and tumors on three plaice, all of which occurred on the pigmented side.

Local environmental conditions such as the relatively low salinity and higher water temperature in the bays of the San Juan Islands at certain times of the year could affect tumor incidence. In this regard, it has been pointed out by Nigrelli (25), that the susceptibility of fish to parasitic infection is increased during periods of temperature variation and that this is especially true when the temperature is rising during the late spring.

Various ectoparasitic biologic agents are known to be vectors for viral transmission in higher vertebrates: swine influenza virus is transmitted by the larvae of the nematode, *Metastrongylus elongatus*, and the virus which causes salmon poisoning in men and dogs is transmitted by the metacercarial larvae of the fluke, *Troglotrema salmonicola*. Ectoparasites (*i.e.* copepods, "fish lice") are sometimes found on the external surfaces of flathead sole. It seems possible that the angioepithelial nodule represents a local tissue response at the site of entry or injury in the skin by a parasite. The reactive tissue changes might resemble or progress to neoplasia, either with or without the intervention of a tumorigenic virus or other agent carried by the parasite.

Other factors of possible etiologic significance must also be considered. Schaperclaus (as cited by Nigrelli [20]) postulated that a nutritional deficiency was at least partly responsible for a hyperplastic epidermal growth found in European cyprinid fish (carp, *etc.*) but presented no supportive evidence. However, Wolf (68) found that the susceptibility in fingerling trout to "gill disease" (hyperplasia of the epithelial covering of the gills) was increased in fish maintained in the hatchery on a diet deficient in pantothenic acid. Wolf felt, moreover, that the hyperplasia was in response to chemical irritants produced in the hatchery waters during the breakdown of fish waste products (68). The tidal flow in the shallow waters of the narrow bays in the San Juan Islands is much reduced as compared with the flow in the more open channels into which they empty. Therefore, chemical irritants of all kinds, including possible carcinogenic compounds from the various decomposition products of sewage, petroleum products, industrial waste products, *etc.*, might be present in higher concentrations in the relatively shallow waters found in East Sound, the main collecting site in this study.

It is possible that the initial event may not involve a biologic agent at all. Mechanical disruption of the epidermis, by abrasion or penetration of an infinite variety of objects found on or within the mud-sand bottom, is always possible. The tissue response to such a mechanical event, with or without the presence of an associated viral agent or a chemical carcinogen, might be the same as described.

The disease could have some genetic basis, and might begin as a congenital angiomatous process involving the dermis which subsequently induces the formation of an epidermal papilloma from the overlying epidermis.

Good (4) attempted to experimentally induce epidermal neoplasia in normal specimens of English sole by subcutaneous implantation of methylcholanthrene. Although this chemical is highly tumorigenic for many higher vertebrate species, Good's results were uniformly negative: the skin became inflamed and ulcerated at the site of inoculation, many of the fish died within 24 hours, and none survived over six weeks. Li and Baldwin (69) have reported the only chemical induction of a tumor in fish. They produced interstitial cell tumors in the testes of three out of 40 adult swordtails, *Xiphophorus hellerii*, with repeated retroperitoneal injections of sesame oil. Tumor induction with carcinogenic compounds has not as yet been attempted in the flathead sole.

Homologous transplantation of the epidermal papilloma in the flathead sole, using the method of Lucké and Schlumberger (70), has been attempted both subcutaneously, intraperitoneally, and into the anterior chamber of the eye. To date, the results are all negative. Good (4) also reported negative results for twenty subcutaneous transplantations in English sole.

The epidermal papillomas described herein have no morphologic similarity or relationship to lymphocystis disease of marine and fresh water fish. The characteristic lesion of lymphocystis disease consists of a mass of ovoid or spherical cells measuring up to 5000 microns, contained within a large connective tissue sac which may be found anywhere on the body surface, the gills, and within the body cavities (5, 71, 72). Although the viral etiology of this disease has been established by Wolf (73), the lymphocystis virus, as characterized by Walker (74), bears no morphologic resemblance to the virus-like particles described above.

There is some histologic resemblance between the epidermal papillomas of the flathead sole and so-called "fishpox", "carppox", or "hyperplastic epidermal disease" (5, 9, 20, 21). The relationship of these various diseases is uncertain. Goncharov (19) has confirmed the work of Roegner-Aust, as cited by Nigrelli (5), establishing the presence and morphology of virus-like particles associated with carppox. The virus-like particles of carppox, however, seem to be dissimilar to those found in the tumors of the flathead sole.

By electron microscopy, the ultrastructure of the normal squamous epithelial cells of the flathead sole are similar in several respects to previous descriptions of epidermal cells of amphibian skin (62). There are complex microvillous interdigitations of adjacent cellular membranes and numerous desmosomes. The cytoplasm of the cells of the intermediate and superficial layers of the epidermis contains an abundance of 100 Å cytoplasmic filaments. The mucous cells of the normal epidermis have morphologic similarities with other types of glandular cells which synthesize visible, protein-rich secretory products. These include a well developed ergastoplasm consisting of numerous flattened sacs oriented parallel to one another and surfaced by abundant ribosomes, an active Golgi apparatus, and large secretory droplets, interpreted in this case as mucus. In contrast to the squamous cells, the mucous cells lack the microvillous cell surface, desmosomes and cytoplasmic filaments.

The cells of the epidermal papilloma differ in most respects from normal mucous and squamous epithelial cells. The tumor cells lack cytoplasmic filaments, desmosomes, a well developed ergastoplasm and Golgi complex. The tumor cells, however, possess several features not seen in normal epidermal cells, including conspicuous nuclear "pores", very large nucleoli and virus-like cytoplasmic particles.

As detailed in the Introduction, several papillomas and papilloma-like lesions are of proven or suspected viral etiology (33, 38, 40, 42, 43, 45). In some of these, notably the Shope papilloma and the human wart, the morphology of the virus and its site and mode of replication are well known (47, 48, 49, 50, 51). Andrews (75) classifies both of these viruses as belonging to the papilloma-polyoma (papovavirus) group of ether-resistant, DNA-viruses, which range from 25 to 45 millimicrons in diameter and are thought to have 42 or 92 surface capsomeres. Among all papilloma-forming viruses of the papova group, viral replication takes place in the nucleus, and the particles subsequently form crystalline aggregates in the cytoplasm. There is some question in the case of the human wart as to whether the virus passes from the nucleus or "leaks" out through nuclear membrane discontinuities (49).

According to Andrews (75), the criteria for classifying a virus within the poxvirus group include: that it is composed of DNA, probably contained within the nucleoid, that the development of the virus is confined to the cytoplasm, that it begins as an oval or spherical particle which undergoes progressive "maturation", and that development of the infective particle is completed within the cell. Most of the poxviruses are also relatively resistant to heat and to 20 percent ethyl-ether inactivation. Most produce skin lesions in susceptible hosts. The poxviruses range in size from 150 to 300 millimicrons; however, the capsomere number is not known. During the maturation process, all of the members of this group of viruses form, or are associated with, one or more membranes which surround the capsomeres.

Molluscum contagiosum, although similar in gross appearance to the human wart and dominated by epidermal proliferation, is caused by an entirely different virus which Andrews (75) classifies among the miscellaneous poxviruses. Banfield and Brindley (76), Dourmashkin and Bernhard (77), and Charles (78) have described the development of this virus within the cytoplasm of epithelial cells. All three articles present a schema outlining a series of developmental stages for the virus from an immature to a mature particle.

The etiologic factor(s) responsible for the epidermal papillomas of flathead sole is presently obscure. Although virus-like particles, not seen in normal epidermal cells, are observed by electron microscopy in the cells of the epidermal papillomas, this is only presumptive evidence of a viral etiology; moreover, the appearance of the particles is unlike that of any virus previously described. As yet the physicalchemical properties of these virus-like particles have not been established; however, certain morphological observations indicate that these cytoplasmic inclusions could possibly be a hitherto unclassified member of the poxvirus group. Using molluscum contagiosum as a viral model (76, 77, 78), some morphologic considerations are discussed below.

The three types of osmiophilic particles found in the cells of the epidermal papilloma and described in the present study may be considered as related developmental forms. This possibility is schematically presented in Figure 45. The irregular, osmiophilic body, the *b particle*, might be the locus of virus formation. As such, the *b particle* could represent "viroplasm", such as is described in association with the appearance of the earliest forms of the molluscum contagiosum virus (76, 77, 78), and other poxviruses (79, 80, 81). The *b particle* is often found in close association, or contiguous with, a large, single, membrane-bound vacuole with a finely granular content (Figures 33, 39, 41, 42), which resembles the mitochondrial precursor or so-called "gray body" described by Bonar *et al.* (63, 64), in virus-infected cells of avian myeloblastosis. These authors feel that this mitochondrial precursor is closely allied with the replication of the causative virus of this disease. The significance of the possible association between the suspected viroplasm and the apparent mitochondrial precursor in the cells of the epidermal papilloma is unknown.

It is apparent that the *b particle* often contains, within its matrix, the vesicular, virus-like *a particle*. The latter particle is also seen in close proximity to the presumed "viroplasm" (*particle b*), and is often located at the tip of a pseudopod-like projection of the *b particle*, as if in the process of separation from it. The *a particle* often appears to contain a prominent nucleoid and, although of smaller size, it is comparable to the earliest nucleoid containing cytoplasmic inclusion found in cells infected with molluscum contagiosum virus (76, 77, 78).

Thus, the *a particle* appears to be the most immature form of the virus-like particles observed in the epithelial cells of the epidermal papillomas of flathead sole. Numerous suspected transitional forms between the *a particle* and the *c particle*, or *granular body*, are observed (Figures 32-41, 43, 45).

Although the morphology of the three cytoplasmic inclusion particles is different than that of all the other poxviruses, they appear to represent stages of development which are analogous to the maturation process designated by Andrews (75) as a criterion for classification of a virus within this group. Moreover, each particle appears to have at least some of the structural components observed in the various transitional forms of the molluscum contagiosum virus (76, 77, 78).

Finally, it is known that multiple factors operate in the causation of neoplasia. Porter (82) lists the following multiple and interrelated factors as operable in conditions where a virus-tumor relationship obviously exists: genetic constitution, hormonal status, age, immunity, dose, and presence of other viruses. In regard to this last factor, Riley (83) has demonstrated that tumor growth can be enhanced by the presence of a virus unrelated to tumor etiology regardless of whether or not tumorigenesis is virus-induced in the first place. In light of this, it is recognized that the virus-like particles may bear no causal relation to the epidermal papillomas, and may be present only incidentally or in a system of virus-tumor synergism.

SUMMARY

From June, 1962, through January, 1965, 350 tumor-bearing flathead sole, *Hippoglossoides elassodon*, were obtained from a total collection of 5405 fish of this species (6.48 percent tumor incidence) in the waters around the San Juan Islands, Washington. The size, anatomic location and gross appearance of 782 tumors occurring on 264 of the tumor fish were described. One hundred and fifty-six tumors on 104 fish were examined by light microscopy; 127 blocks from 86 tumors on 61 fish were examined by electron microscopy. The tumors were observed first on fish of age group 0 (0 to one year old) as small, 1 to 2 millimeter, angiomatous proliferations of dermal connective tissue capped by a layer of hyperplastic epidermis. These lesions were descriptively designated as angioepithelial nodules. Epidermal papillomas characterized by complex folds of epithelium and a scanty stroma were observed on fish of age group I (one to two years old) and older. Transitional stages between the nodule and papilloma were observed near the end of the first year and early in the second year of life. The epidermal papillomas spread to cover extensive portions of the body surface and fins, and occasionally involved the eyes, gills and oral mucosa. Although tumors occurred on any part of the outer surface, there was a significant tendency for them to be located on the pigmented (right, eye) side. The number of tumors per fish was highest in the first year of life and decreased each subsequent year through the third year of life. No tumors were observed on fish older than age group III, while angioepithelial nodules were not seen on any fish

older than age group I. New tumors of either kind never appeared *de novo* on numerous normal and tumor-bearing fish maintained in the laboratory. Light and electron microscopic examination demonstrated distinct differences between normal epithelium and the epithelial investments of both the angioepithelial nodules and the epidermal papillomas. In the papillomas the most important findings, as compared to normal, included a lack of mucous cells, desmosomes and cytoplasmic filaments, and the presence of prominent nucleoli and nuclear "pores". Although by light microscopy there was no demonstrable biological etiologic agent, electron micrographs demonstrated virus-like cytoplasmic particles in the cells of the epidermal papillomas; these particles were not seen in normal fish epidermis. Three types of particles were present, two of which were morphologically similar to those seen with poxviruses. The possible significance of these particles and of other etiologic factors in the production of angioepithelial nodules and epidermal papillomas in the flathead sole was discussed.

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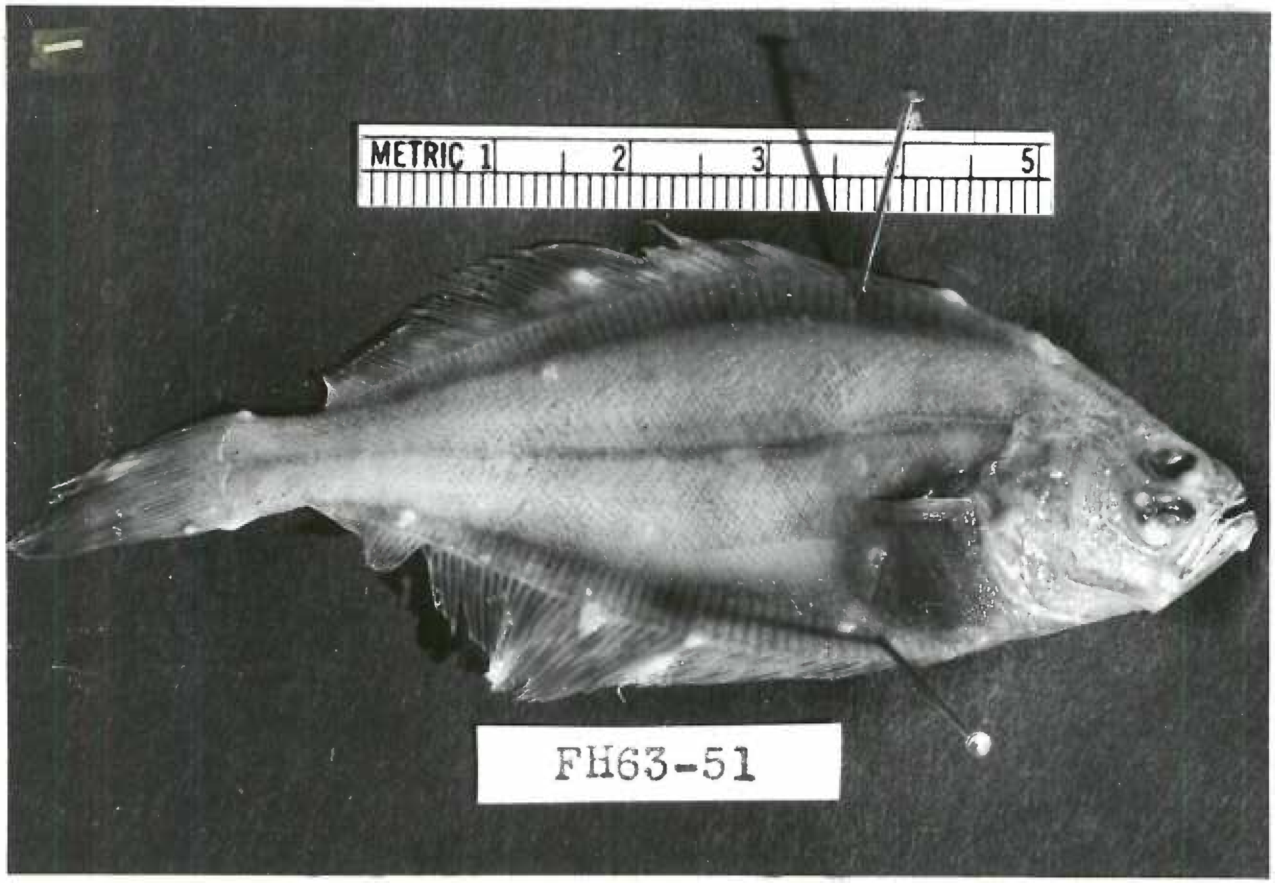
ILLUSTRATIONS

GLOSSARY OF ABBREVIATIONS FOR ILLUSTRATIONS

BC: Basal cell
BM: Basement membrane
BV: Blood vessel
CF: Cytoplasmic filament
CG: Cytoplasmic granule
CM: Cell membrane
CT: Connective tissue (stroma)
DC: Dermal collagen
E: Epidermis
ER: Ergastoplasm
FIL: Extracellular mucous filament
G: Golgi apparatus
GB: "Mitochondrial precursor" ("gray body")
IC: Intermediate cell
IS: Intercellular space
M: Mitochondrion
MC: Mucous cell
MV: Microvillous
N: Nucleus
NM: Nuclear membrane
N_1: Basal cell nucleus
N_2: Intermediate cell nucleus
N_3: Superficial cell nucleus
N_4: Mucous cell nucleus
RNP: Ribonucleoprotein
S: Scale
SC: Superficial cell
SDL: Submembranous dense layer
SM: Skeletal muscle
TB: Terminal bar
V: Vacuole
V_M: Mucous vacuole
a: *a particle*
b: *b particle*
c: *c particle*
d: Desmosome
nc: Nucleolus

FIGURE 1: Flathead sole of age group 0 (0-1 year old), estimated age 6 to 8 months. Numerous angioepithelial nodules, light in color, are scattered over the pigmented side. *Note* nodules on fins, body, mandible, and near right eye.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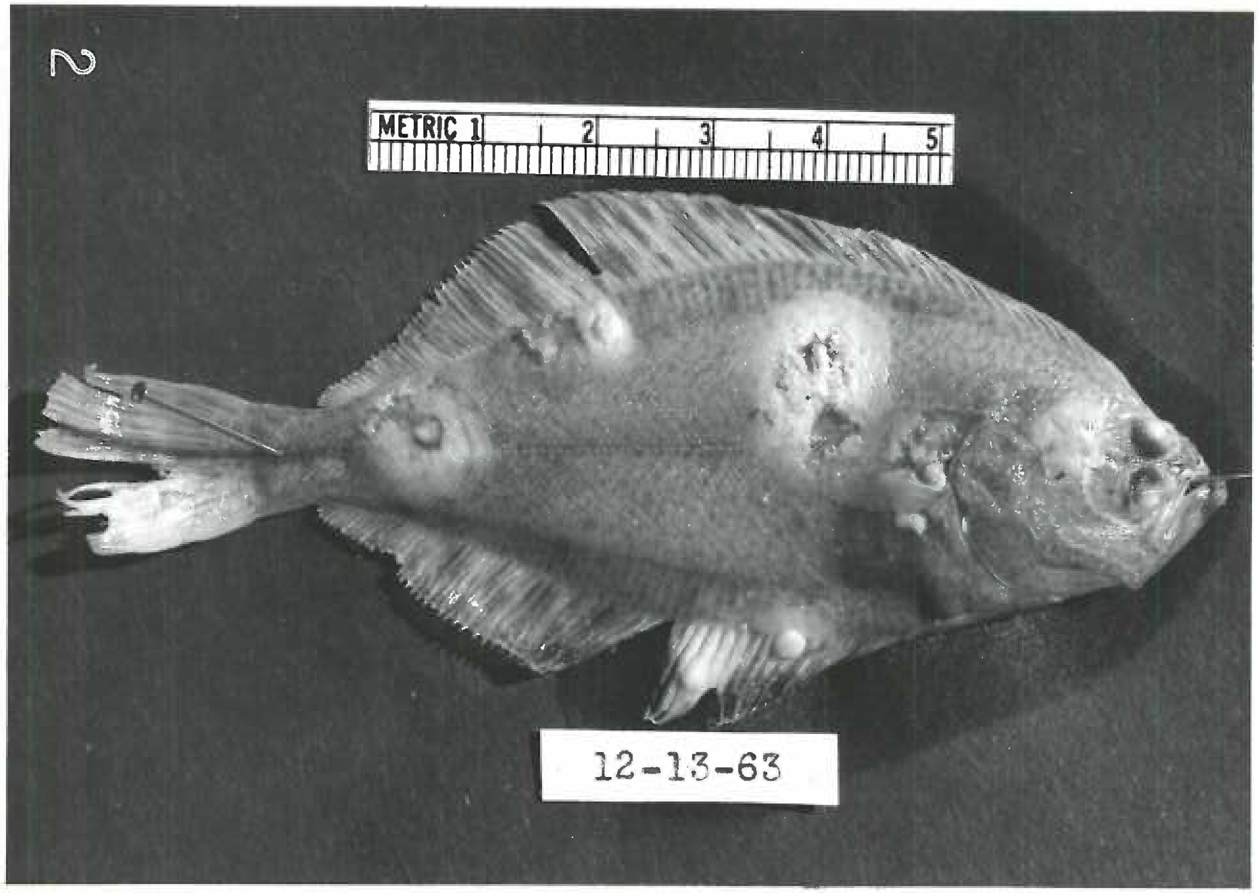
FH63-51

FIGURE 2: Flathead sole of age group 0 (0-1 year old), estimated age 9 to 11 months. Eight tumors are either early epidermal papillomas, or represent transitional stages between the angioepithelial nodule and the papilloma. The central raised areas are interpreted as the location of the original angioepithelial nodules. *Note* the spreading, plaque-like edge surrounding the central raised areas. On the anal fin, 2 of these spreading edges are becoming confluent. Microscopically, these tumors were epidermal papillomas.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METRIC 1 2 3 4 5
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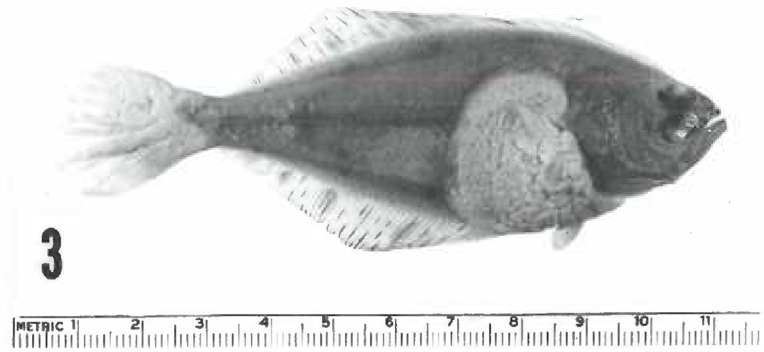
FIGURE 3: Flathead sole of age group I (1-2 years old) with 2 well developed epidermal papillomas, 1 on the caudal fin and 1 on the anteroventral aspect of the pigmented (right) side of the fish, and involving the anal and right pectoral fins. Both tumors extended onto the non-pigmented (left) side of the fish.

FIGURE 4: Flathead sole of age group I (1-2 years old) with 2 well developed epidermal papillomas on the pigmented (right) side of the fish. *Note* that the anterior tumor covers both eyes.

FIGURE 5: Flathead sole of age group I (1-2 years old) with 1 epidermal papilloma on the non-pigmented (left) side of the fish. The tumor has more pigment than the surrounding normal tissue, and therefore is prominent. This was true for most tumors located on the non-pigmented side.

FIGURE 6: Normal skin of flathead sole. The epidermis (E) is approximately 6 cell layers thick and contains numerous mucous cells (MC). The dermis is composed of loose areolar layer containing the scales (S), and vascular elements and a dense collagenous layer (DC) that is attached to the underlying skeletal muscle (SM). Van Giesen stain. X200.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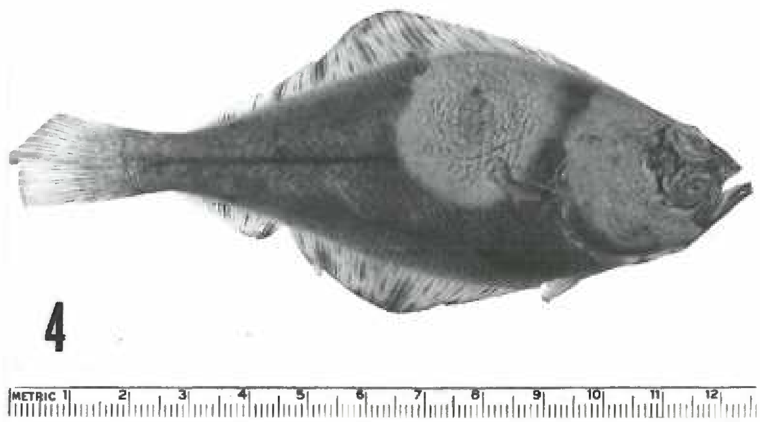
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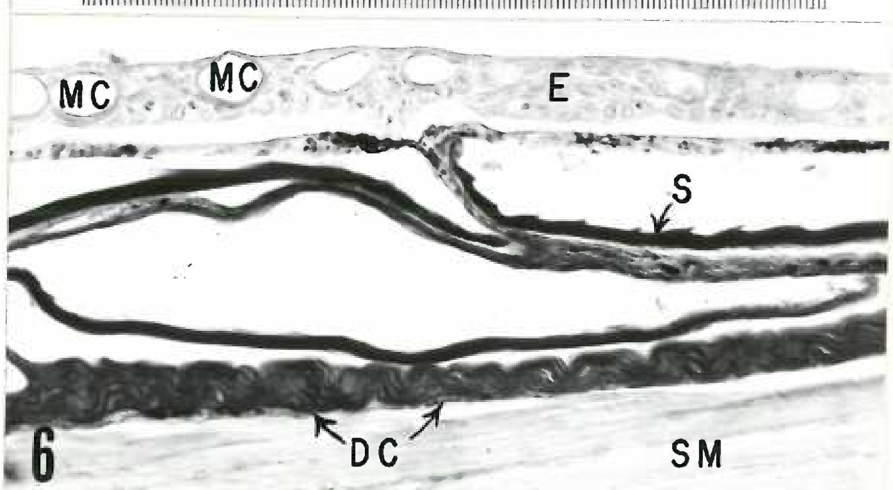

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FIGURE 7: An angioepithelial nodule which has developed on both sides of a fin of an age group 0 (0-1 year old) flathead sole. *Note* the fin rays in the center of the lesion. *Arrows* indicate the junction between the hyperplastic epithelial cap and the vascular tissue beneath. Hematoxylin and eosin. X35.

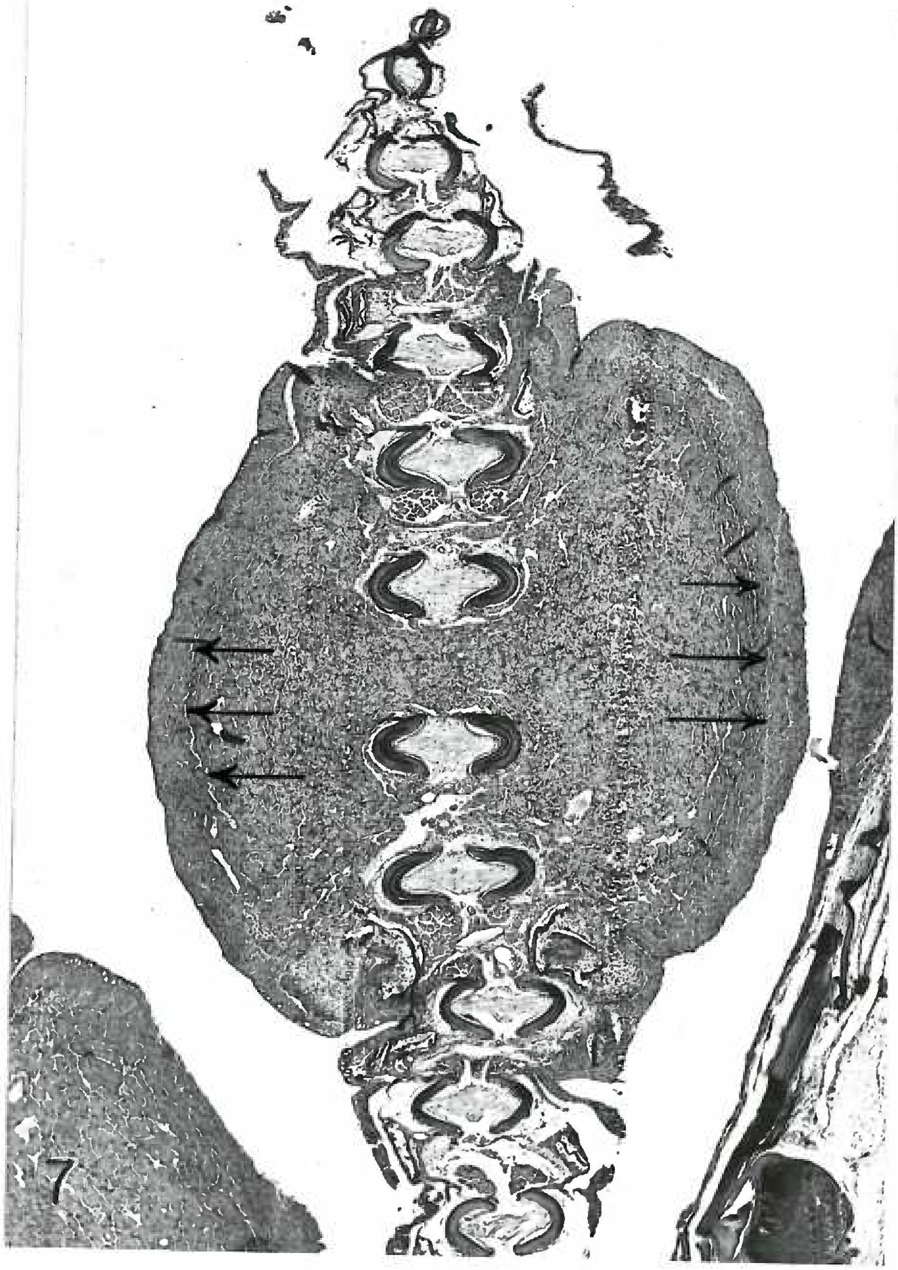
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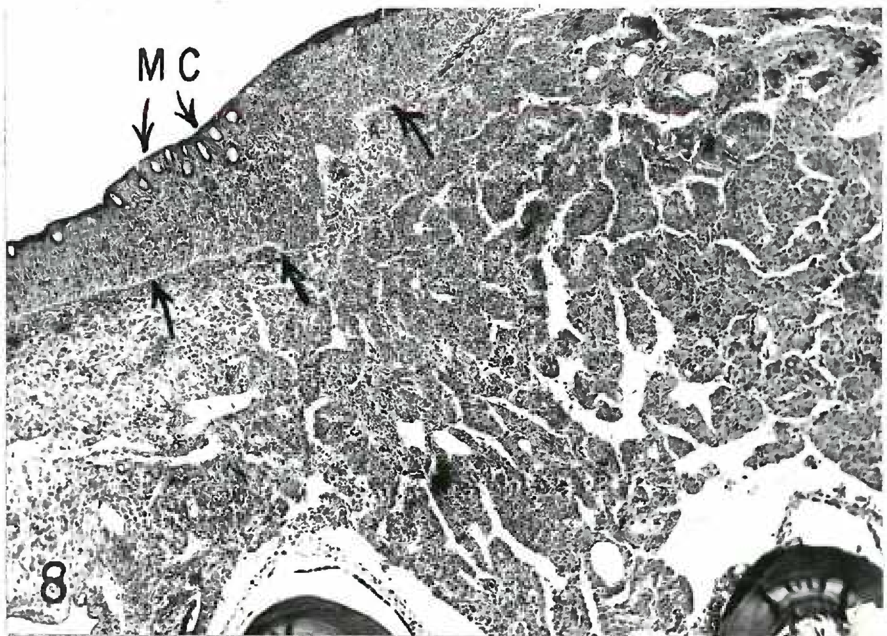
FIGURE 8: An angioepithelial nodule on the fin of a flathead sole of age group 0 (0-1 year old). *Note* the fin rays at the bottom of the micrograph. The hyperplastic epidermis, containing mucous cells (MC), overlies the angiomatous component. *Arrows* indicate the location of the basement membrane. Masson's trichrome stain. X150.

FIGURE 9: An angioepithelial nodule of a flathead sole of age group 0 (0-1 year old). The vascular connective tissue component of the lesion has numerous blood vascular channels (BV), many of which are collapsed. Hematoxylin and eosin. X250.

FIGURE 10: An angioepithelial nodule of a flathead sole of age group 0 (0-1 year old). The intact basement membrane (BM) separates the hyperplastic epidermis from the angiomatous component. Blood vascular channels (BV). Hematoxylin and eosin. X500.


MC
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BV
BV
BV
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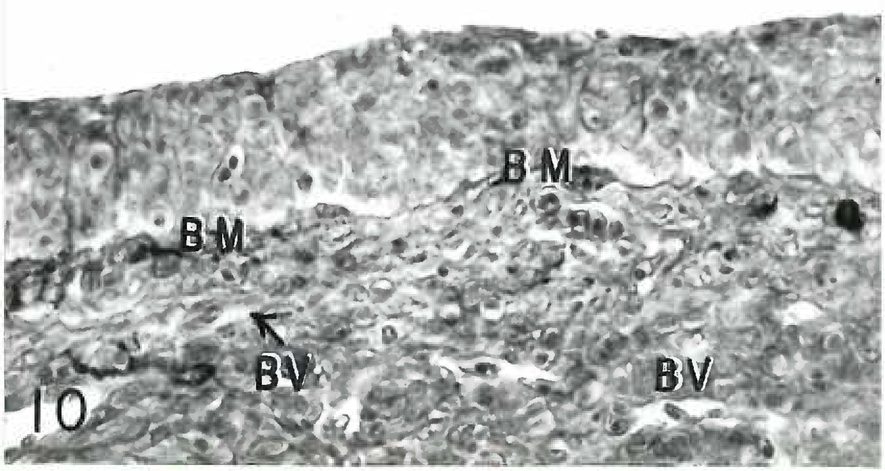
BM
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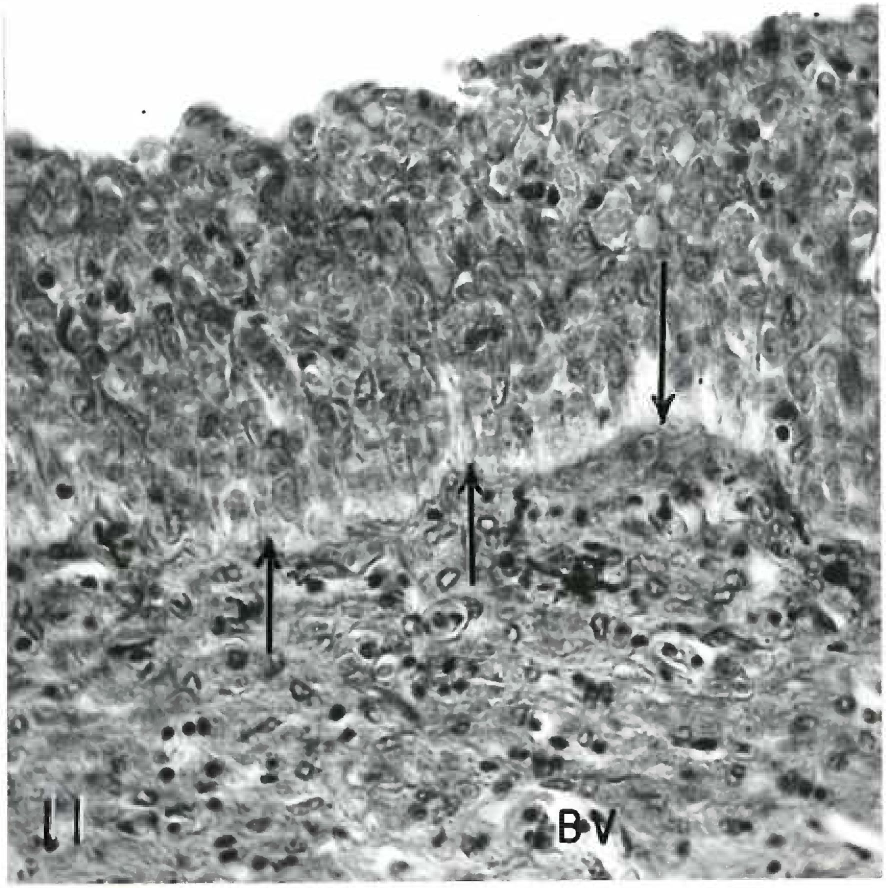
FIGURE 11: An angioepithelial nodule of a flathead sole of age group 0 (0-1 year old). Pleomorphism of the epithelial cells is prominent. Mucous cells are absent. *Arrows* indicate the intact basement membrane separating the vascular tissue from the epidermal cap. Blood vascular channels (BV). Hematoxylin and eosin. X550.

FIGURE 12: An epidermal papilloma of a flathead sole of age group I (1-2 years old). The section is through the body of the fish with the fin in the lower left and the vertebral column to the right. The thick, darkly staining papilloma is supported by a thin connective tissue stroma (indicated by black lines). The normal epidermis of the non-pigmented side of the fish is so thin as to be hardly visible at this magnification. Hematoxylin and eosin. X5.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BV


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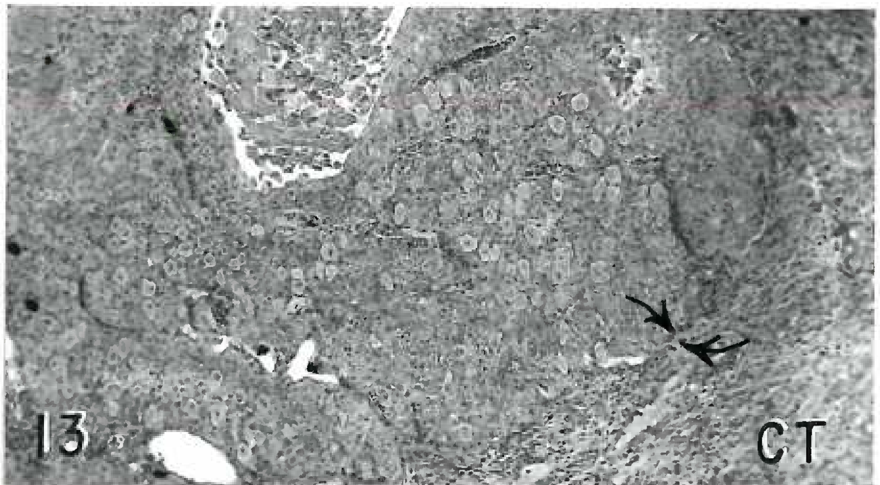
FIGURE 13: An epidermal papilloma of a flathead sole of age group I (1-2 years old). *Note* the group of necrotic cells at the top and the vascular connective tissue stroma (CT) at the bottom. *Arrows* indicate the location of the basement membrane. Hematoxylin and eosin. X200.

FIGURE 14: An epidermal papilloma of a flathead sole of age group I (1-2 years old). The delicate supporting connective tissue stroma is separated from the hyperplastic epidermis by the intact basement membrane (BM). Hematoxylin and eosin. X200.

FIGURE 15: An epidermal papilloma of a flathead sole of age group I (1-2 years old). Hematoxylin and eosin. X200.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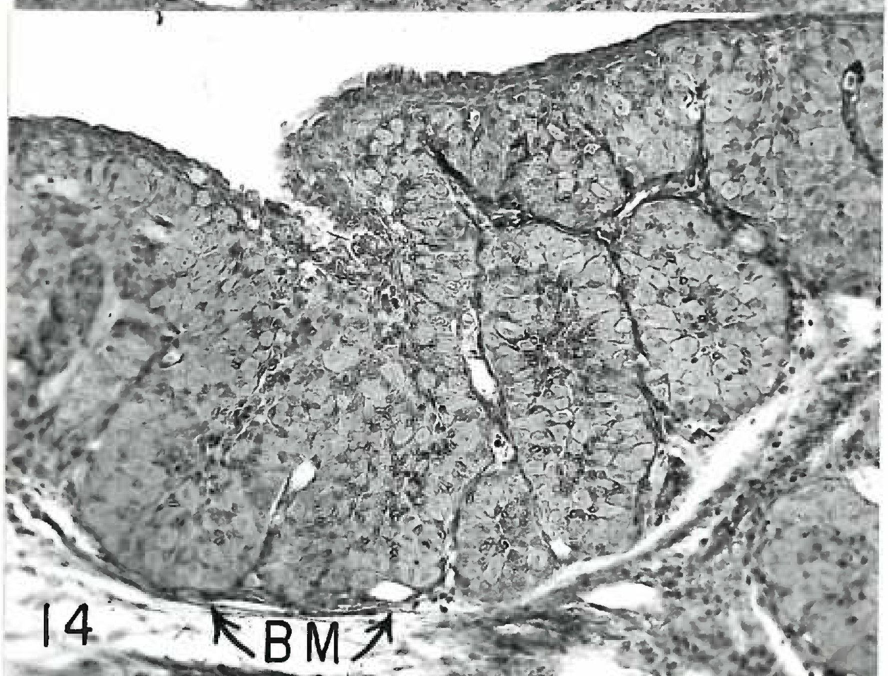
CT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BM

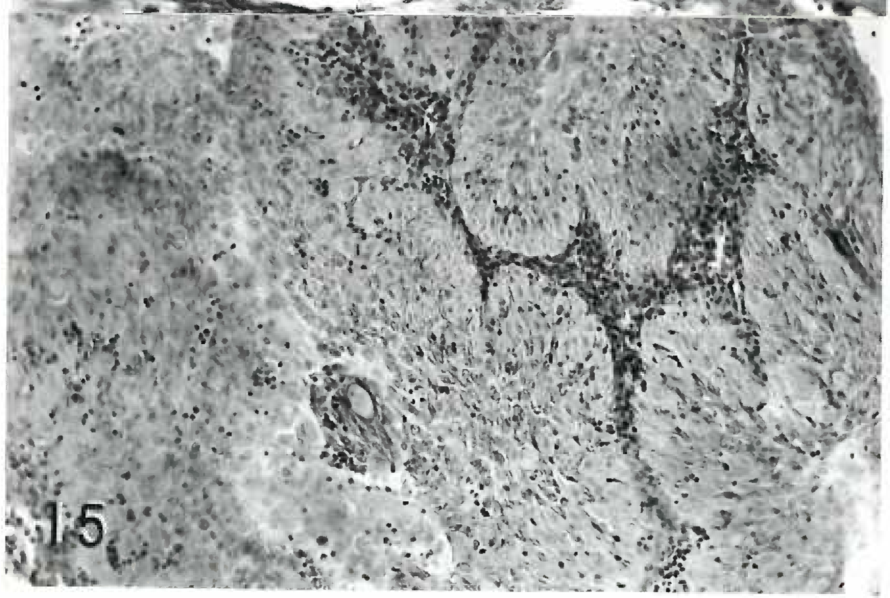
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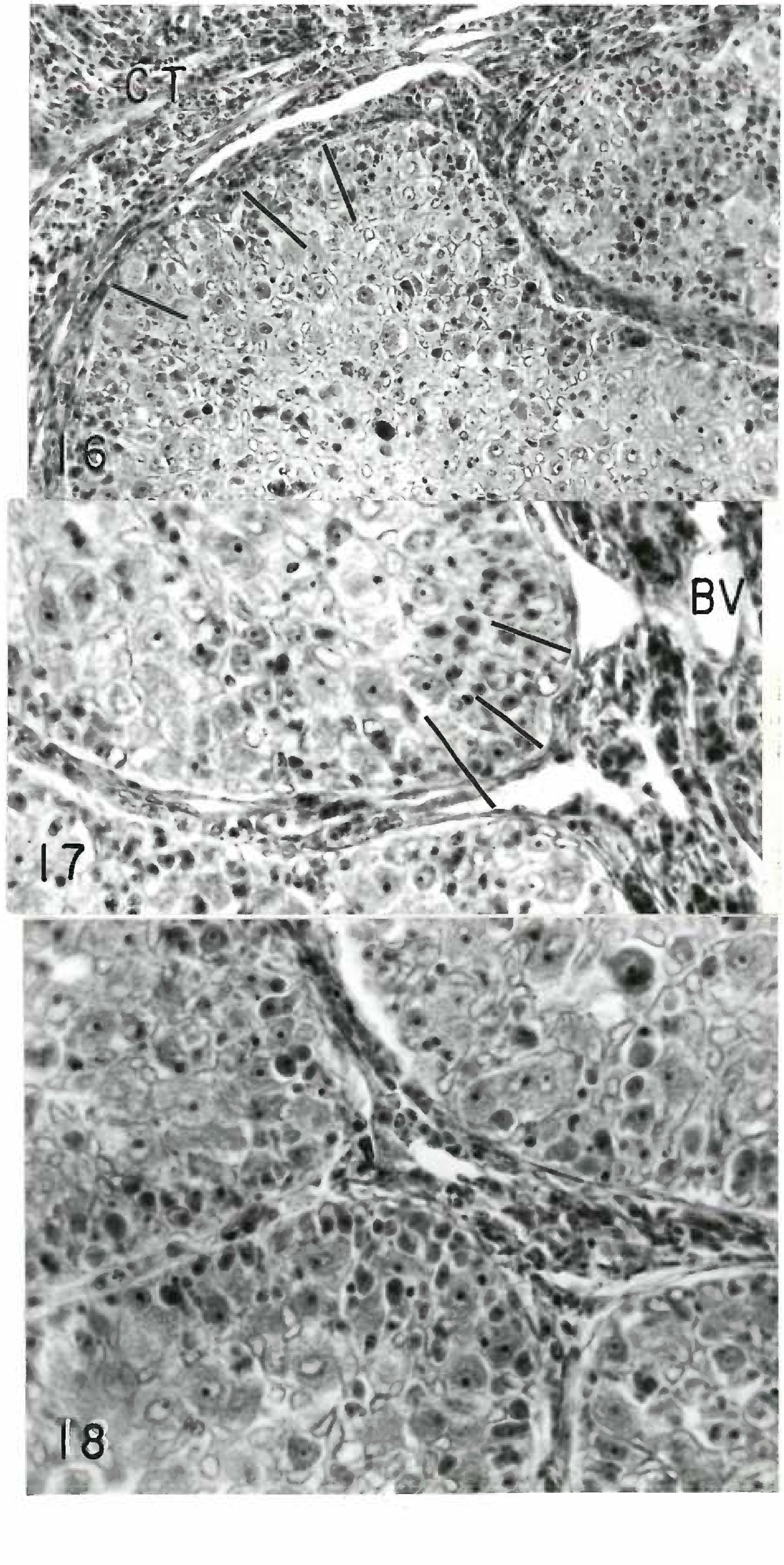
FIGURE 16: An epidermal papilloma of a flathead sole of age group I (1-2 years old). The stroma (CT) at the base of the papilloma gives rise to the supporting connective tissue of the hyperplastic epidermis. The basement membrane is indicated by the black lines. Masson's trichrome stain. X300.

FIGURE 17: An epidermal papilloma of a flathead sole of age group I (1-2 years old). *Note* the prominent blood vascular channels (BV) in the basal connective tissue stroma. Black lines indicate the intact basement membrane. Masson's trichrome stain. X400.

FIGURE 18: An epidermal papilloma of a flathead sole of age group I (1-2 years old). Four groups of hyperplastic epithelial cells surround a connective tissue stalk. *Note* the pleomorphism of the epidermal cells. Masson's trichrome stain. X400.


CT
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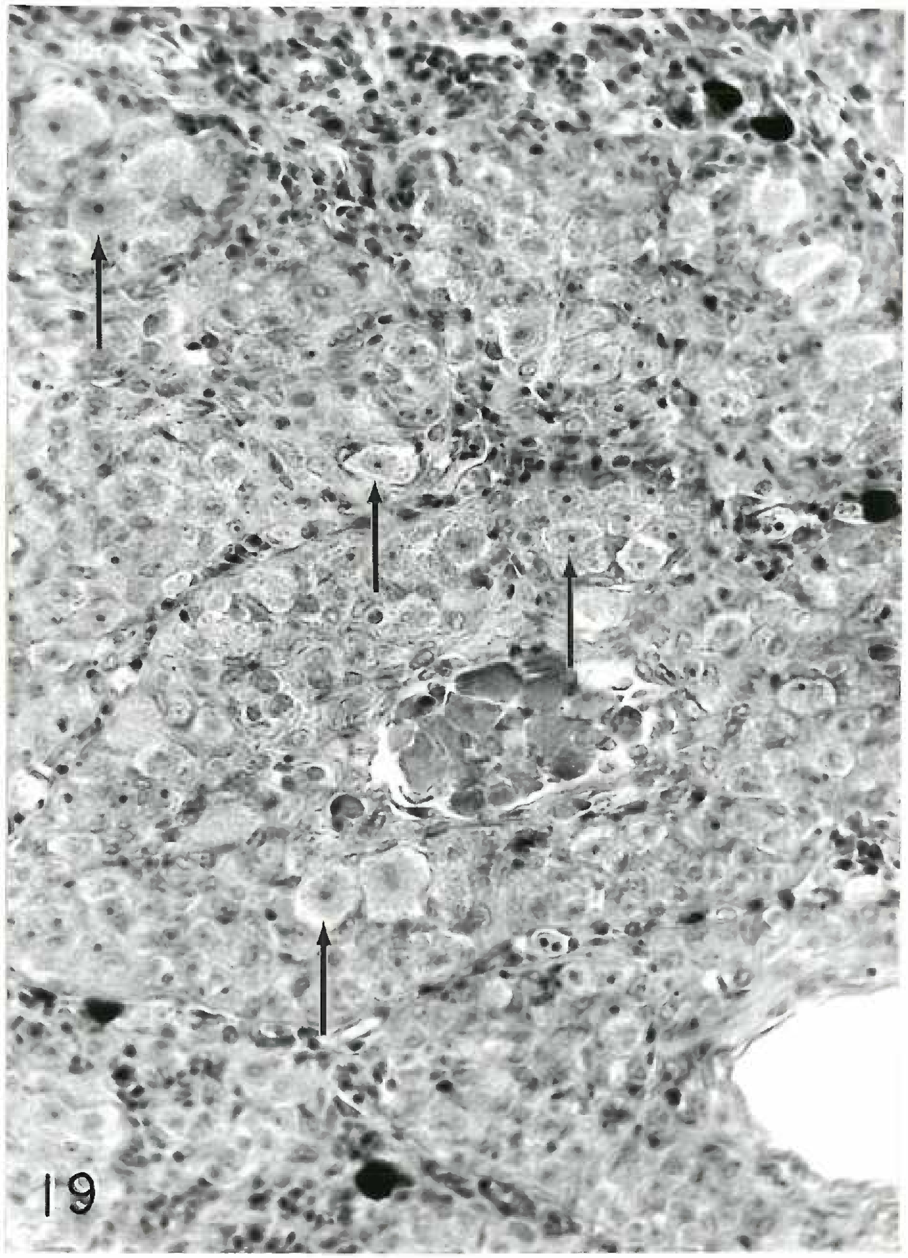
17
18

FIGURE 19: An epidermal papilloma of a flathead sole of age group I (1-2 years old). *Note* the pleomorphism of the epidermal cells. Numerous cells have nuclei with prominent nucleoli (*arrows*). Near the center is a group of eosinophilic, hyaline necrotic cells, and in the lower right corner is a cystic area where central necrosis has previously occurred. Hematoxylin and eosin. X550.



19

FIGURE 20: An electron micrograph of full thickness of normal fin web epidermis. The dermal collagen (DC) is separated from the epidermis by the basement membrane (BM). The epidermis has three cell layers: basal cell layer (BC), intermediate cell layer (IC) and superficial cell layer (SC). Mucous cells (MC) are located in the intermediate cell layer and have small dense nuclei (N_4). Numerous desmosomes (d) are present. The microvilli normally present on the outer surface of the superficial cells are artefactually distorted (compare with Figures 24 and 25). Basal cell nucleus (N_1); intermediate cell nucleus (N_2); superficial cell nucleus (N_3). Lead citrate stain. X6,000.

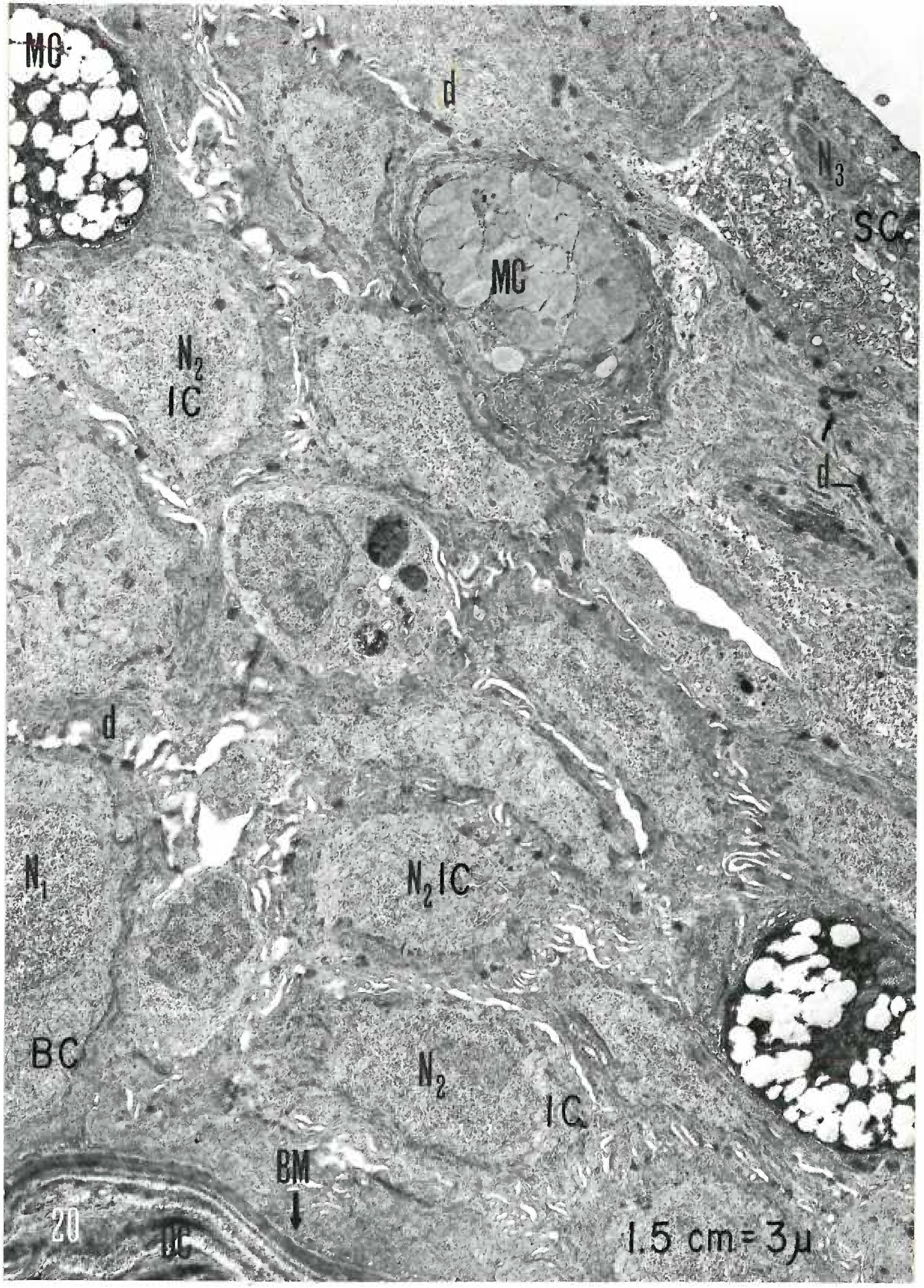

MC
d
N3
SC
MC
N2
IC
d
d
N1
N2IC
BC
N2
IC
BM
20
OC
1.5 cm = 3μ

FIGURE 21: An electron micrograph of a basal layer squamous cell of normal fin web epidermis. *Note* the prominent intercellular spaces (IS). Dermal collagen (DC); basement membrane (BM); cell membrane (CM); desmosomes (d); nucleus (N_1); nuclear membrane (NM); mitochondrion (M); ribonucleoprotein (RNP); Golgi apparatus (G); ergastoplasm (ER). Lead citrate stain. X16,000.

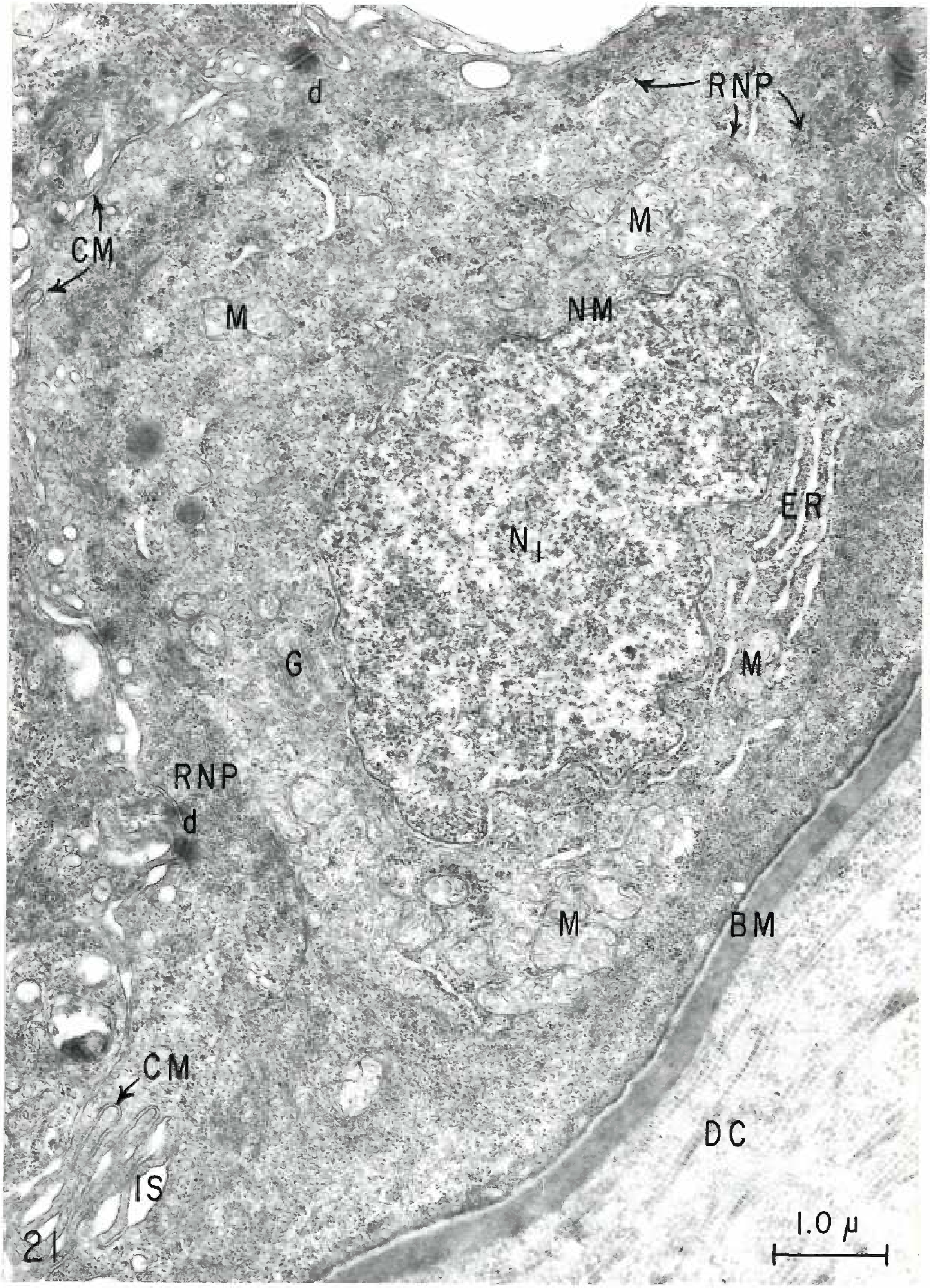
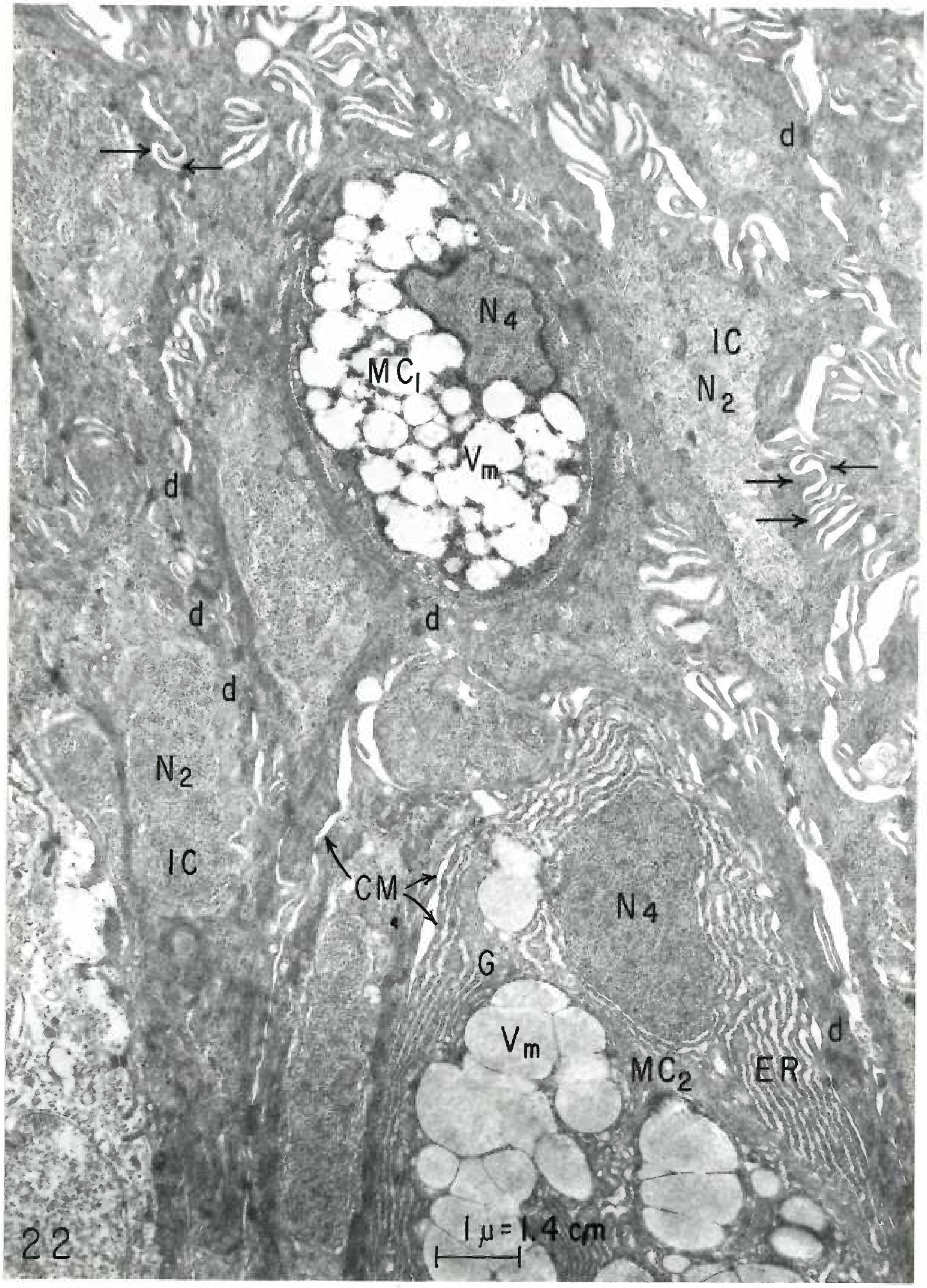

d
RNP
CM
M
NM
ER
N1
G
M
RNP
d
M
BM
CM
DC
IS
1.0 μ
21

FIGURE 22: An electron micrograph of intermediate cell layer of normal fin web epidermis. Mucous cells (MC_1 and MC_2) are surrounded by intermediate layer squamous cells (IC). MC_2 displays prominent ergastoplasm (ER), Golgi apparatus (G) and vacuoles (V_M) with smooth unit membranes containing relatively homogeneous material of low electron density, presumed to be mucus. Intermediate squamous cells show interdigitating microvilli (*arrows*), desmosomes (d) and dense peripheral cytoplasm. Intermediate cell nucleus (N_2); mucous cell nucleus (N_4); cell membrane (CM). Lead citrate stain. X12,000.



d
N4
IC
N2
MC1
Vm
d
d
d
d
N2
IC
CM
N4
G
Vm
MC2
ER
d
1 μ = 1.4 cm
22

FIGURE 23: An electron micrograph of part of an intermediate layer squamous cell of normal fin web epidermis. The nucleus (N_2) is irregular in outline and contains dense chromatin clumps. The perinuclear cytoplasmic zone contains the Golgi apparatus (G), mitochondria (M), empty-appearing smooth surfaced vacuoles (V) and dilated sacs of ergastoplasm (ER). *Note* distinctive periodic structures of unknown significance (*arrows*). Peripheral cytoplasmic zone is occupied by closely packed cytoplasmic filaments (CF). Nuclear membrane (NM). Lead citrate stain. X23,000.

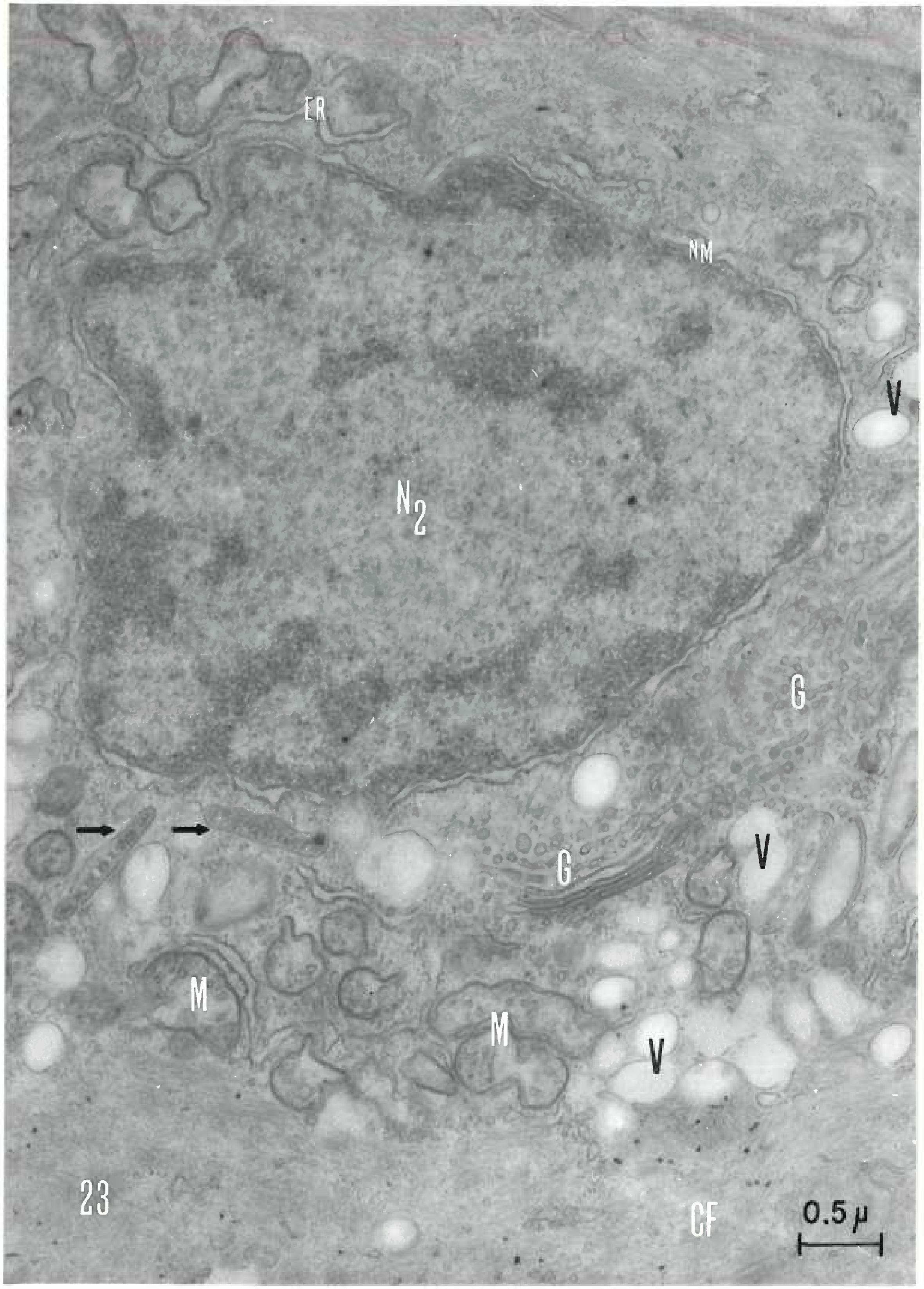
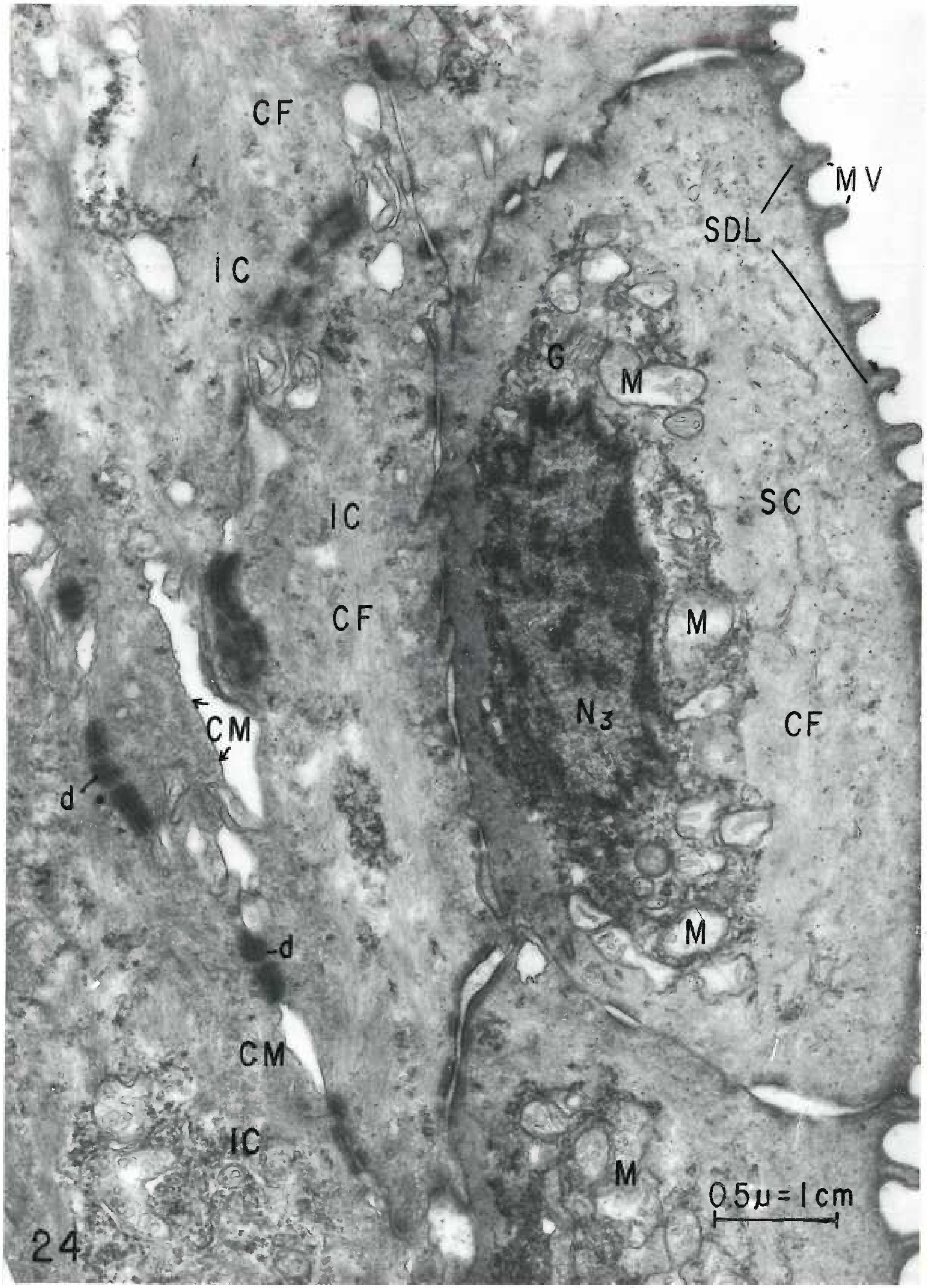

ER
NM
V
N2
G
G
V
M
M
V
23
CF
0.5 μ

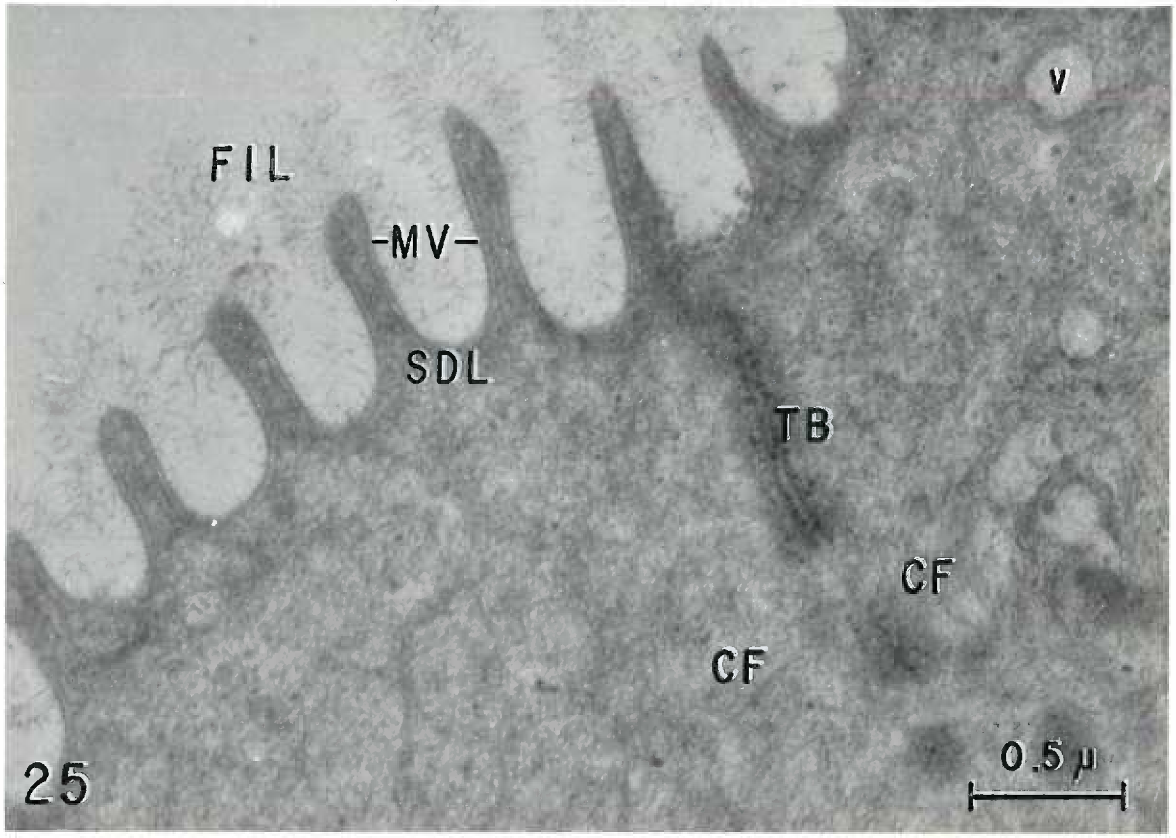
FIGURE 24: An electron micrograph of squamous cells of the intermediate layer (left) and the superficial layer (right) of normal fin web epidermis. *Note* superficial cell (SC) with differentiated outer surface characterized by microvillous projections (MV). There is a dense layer of cytoplasm (SDL) beneath the outer cell membrane; this material follows the contours of the microvilli. The cytoplasm deep to the dense outer layer contains cytoplasmic filaments (CF) similar to those seen in the peripheral cytoplasmic zone of the intermediate layer squamous cells (IC). The nucleus (N_3) of the superficial cell is irregular in outline with dense chromatin aggregates. The perinuclear cytoplasmic zone contains the Golgi apparatus (G), mitochondria (M), and most of the ribonucleoprotein. Cell membrane (CM). Desmosome (d); intermediate cell nucleus (N_2). Lead citrate stain. X16,000.



CF
IC
IC
CF
CM
d
d
CM
IC
G
M
M
N3
M
M
SC
CF
SDL
MV
0.5μ = 1cm
24

FIGURE 25: An electron micrograph of portions of two adjacent superficial layer squamous cells of normal fin web epidermis. This shows the detail of the outer surface with microvilli (MV), the submembranous dense layer of cytoplasm (SDL), and the terminal bar (TB) between the adjacent cells. The cytoplasm contains abundant cytoplasmic filaments (CF). *Note* the fine, extracellular filaments (FIL) which are apparently attached to the outer surface of the cell membrane. These are interpreted as strands of mucus. Lead citrate and uranyl acetate stains. X32,000.

FIGURE 26: An electron micrograph of portions of two adjacent intermediate layer squamous cells of normal fin web epidermis. The desmosomes (d) appear to be composed of thickened portions of adjacent cell membranes, and do not have obvious internal structure. The cytoplasmic filaments (CF) appear to attach to the desmosomes. Ribonucleoprotein (RNP); vacuole (V). Lead citrate and uranyl acetate stains. X47,000.


FIL
-MV-
SDL
TB
V
CF
CF
25
0.5 μ

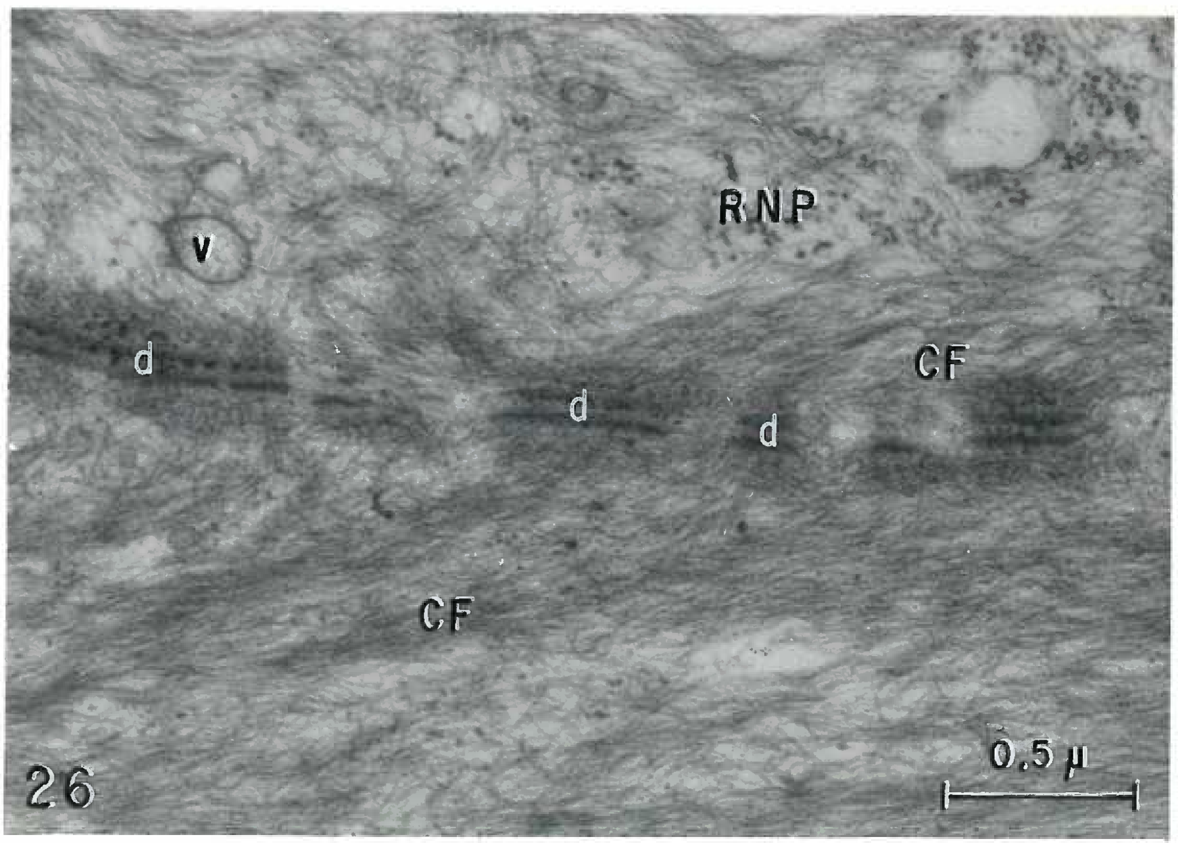

RNP
V
d
d
d
CF
CF
26
0.5 μ

FIGURE 27: An electron micrograph of portions of several epithelial cells from the hyperplastic epidermis of an angioepithelial nodule. Two adjacent cells contain aggregates of large dense particles (CG) which are not found in normal fin web epidermal cells. These cytoplasmic granules are sometimes pentagonal or hexagonal in profile, suggesting that some are icosahedrons. These bodies of unknown type are unlike the dense granular bodies found in the epithelial cells of the epidermal papillomas. However, the cells containing these particles resemble the epithelial cells of the papilloma as regards the appearance of the nucleus (N), nuclear membrane (NM), nucleolus (nc), mitochondria (M), and cell membranes (CM). Lead citrate stain. X16,000.

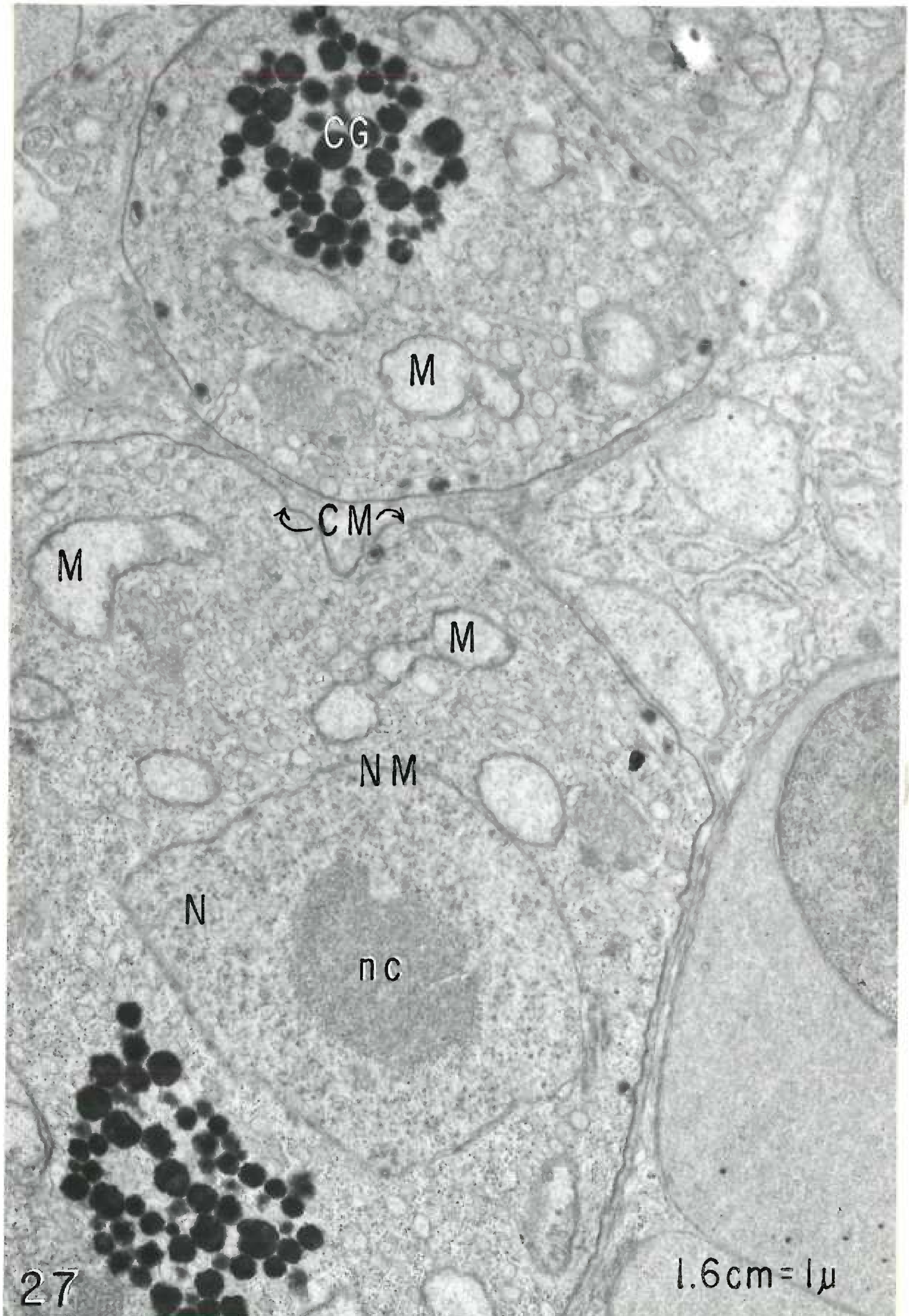

CG
M
CM
M
M
NM
N
nc
27
1.6cm=1μ

FIGURE 28: An electron micrograph of part of an epithelial cell from the hyperplastic epidermis of an angioepithelial nodule. *Note* that some of the cytoplasmic granules (CG) possess internal vacuoles. The nucleus (N) is irregular in shape, the nuclear membrane (NM) is largely indistinct, and there is a nucleolus (nc). Mitochondrion (M); ergastoplasm (ER); cell membrane (CM). Lead citrate stain. X32,000.

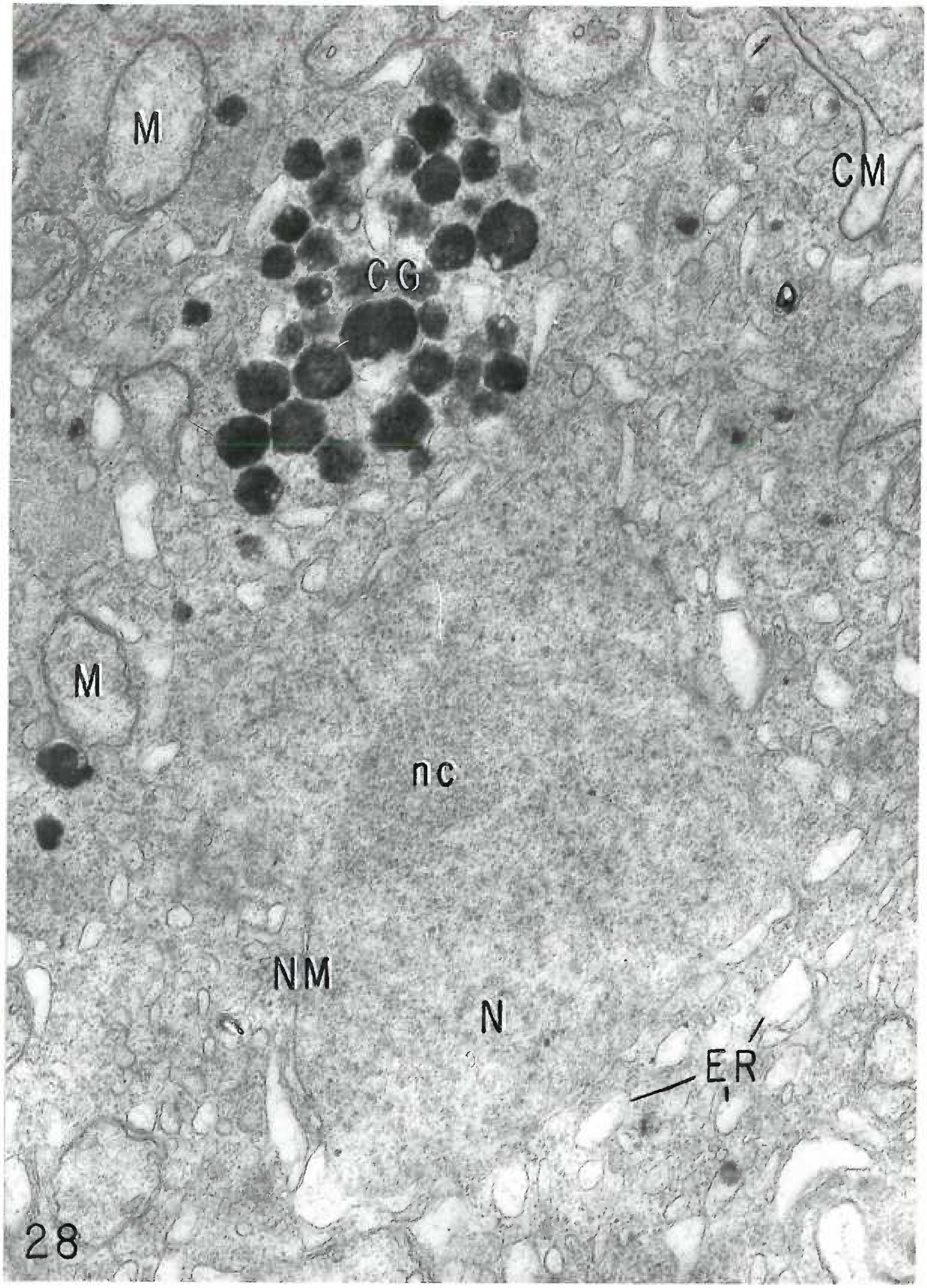

M
CM
CG
M
nc
NM
N
ER
28

FIGURE 29: An electron micrograph of part of a cell from the dermal portion of an angioepithelial nodule. The nucleus (N) shows some clumped chromatin adjacent to the irregular nuclear membrane. The cytoplasm contains altered mitochondria (M), vacuoles (V) and ribonucleoprotein (RNP). *Note* the numerous vesicular, membrane limited particles (*arrows*), several of which contain a nucleoid; these are identical in morphology to the small virus-like *a particles* observed in epithelial cells of the epidermal papilloma (see figures 31, *ff*). Lead citrate stain. X32,000.

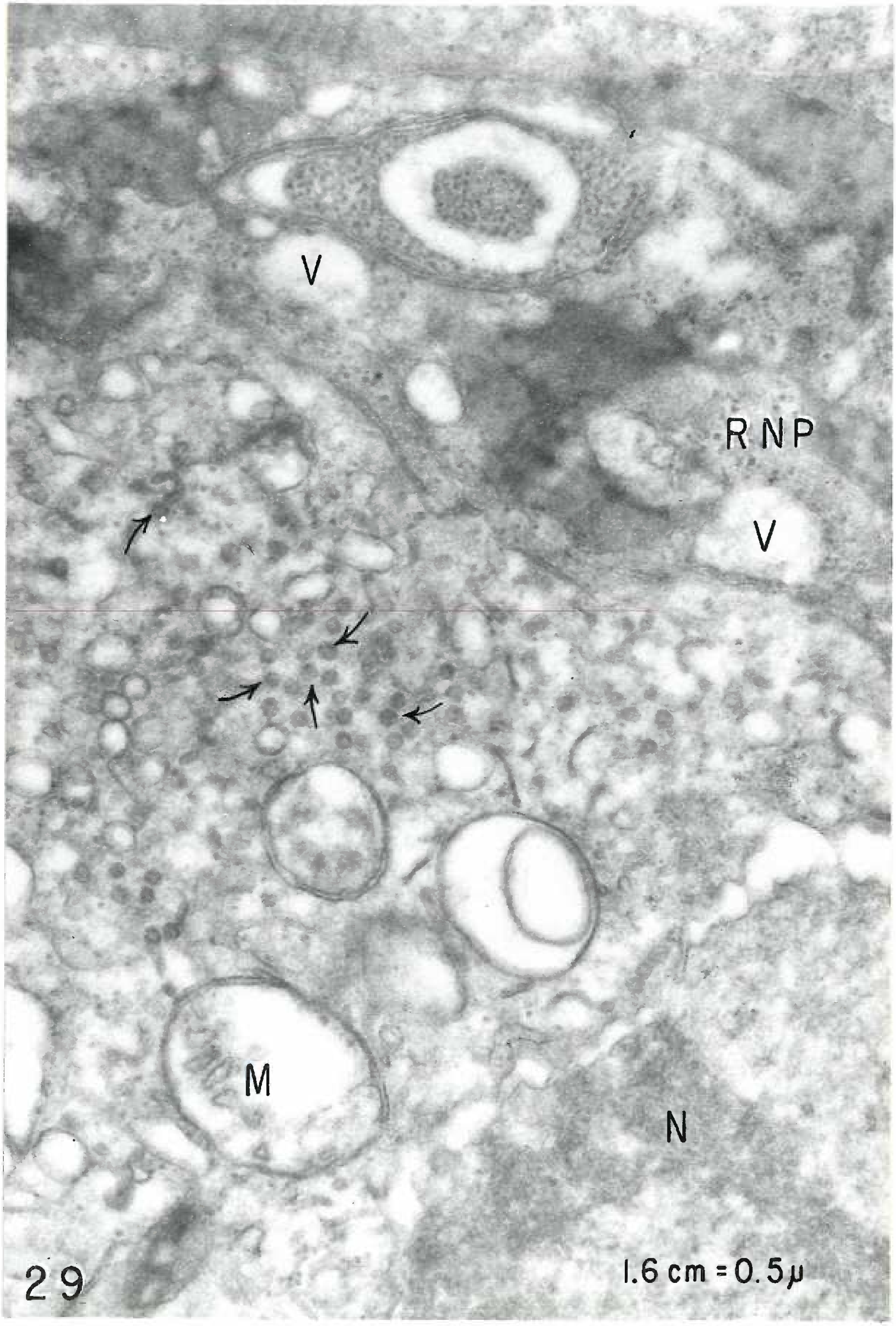

V
RNP
V
M
N
29
1.6 cm = 0.5μ

FIGURE 30: An electron micrograph of an epithelial cell of an epidermal papilloma. The nucleus has a prominent nucleolus (nc) and an irregular nuclear membrane (NM) with numerous nuclear "pores". The cytoplasm contains altered mitochondria (M), ergastoplasm (ER) and numerous cytoplasmic particles *(arrows)*. *Note* the relative regularity of the cell membrane (CM) and the lack of microvillous interdigitations and desmosomes between adjacent cells. Lead citrate stain. X12,000.

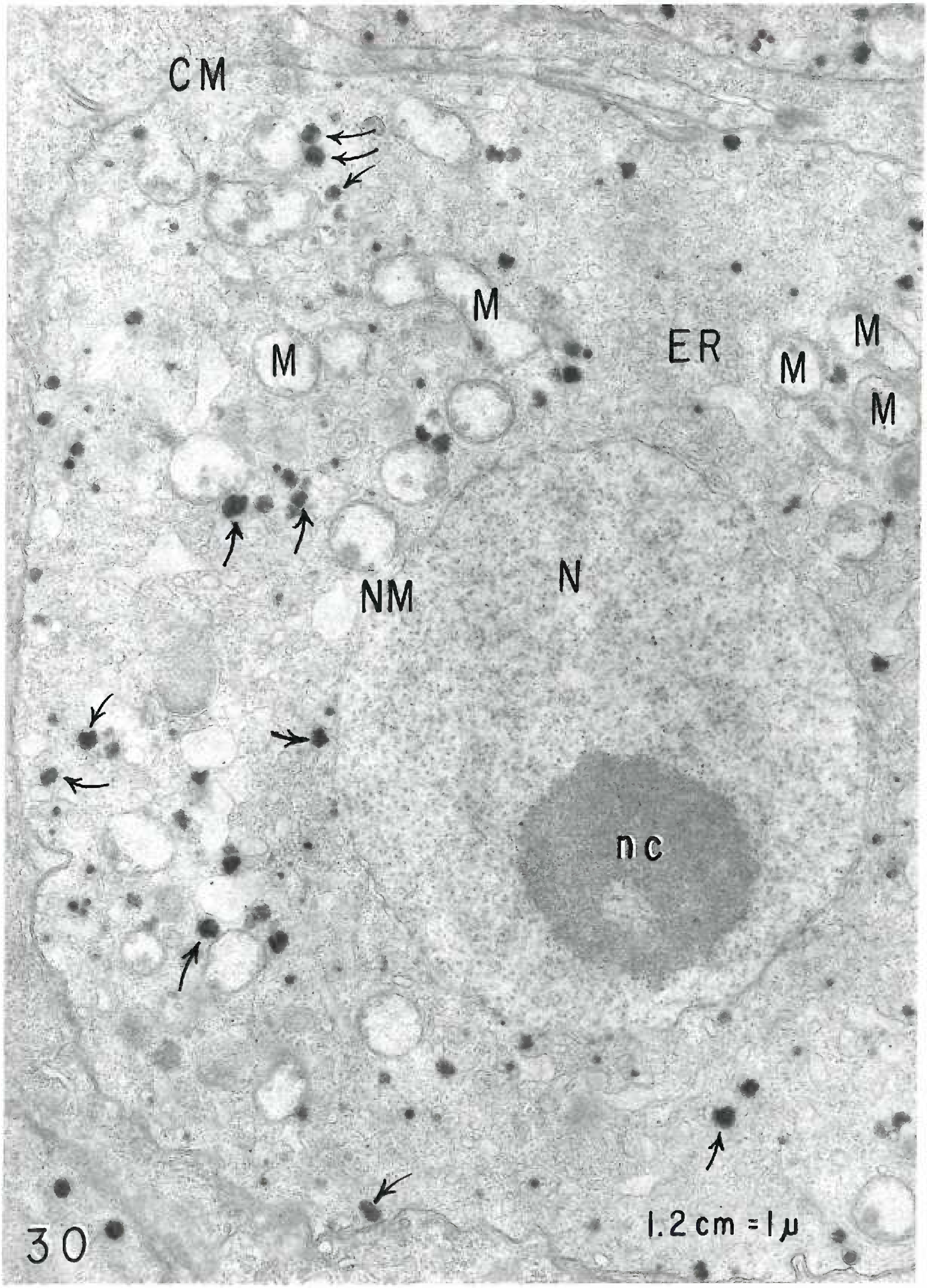
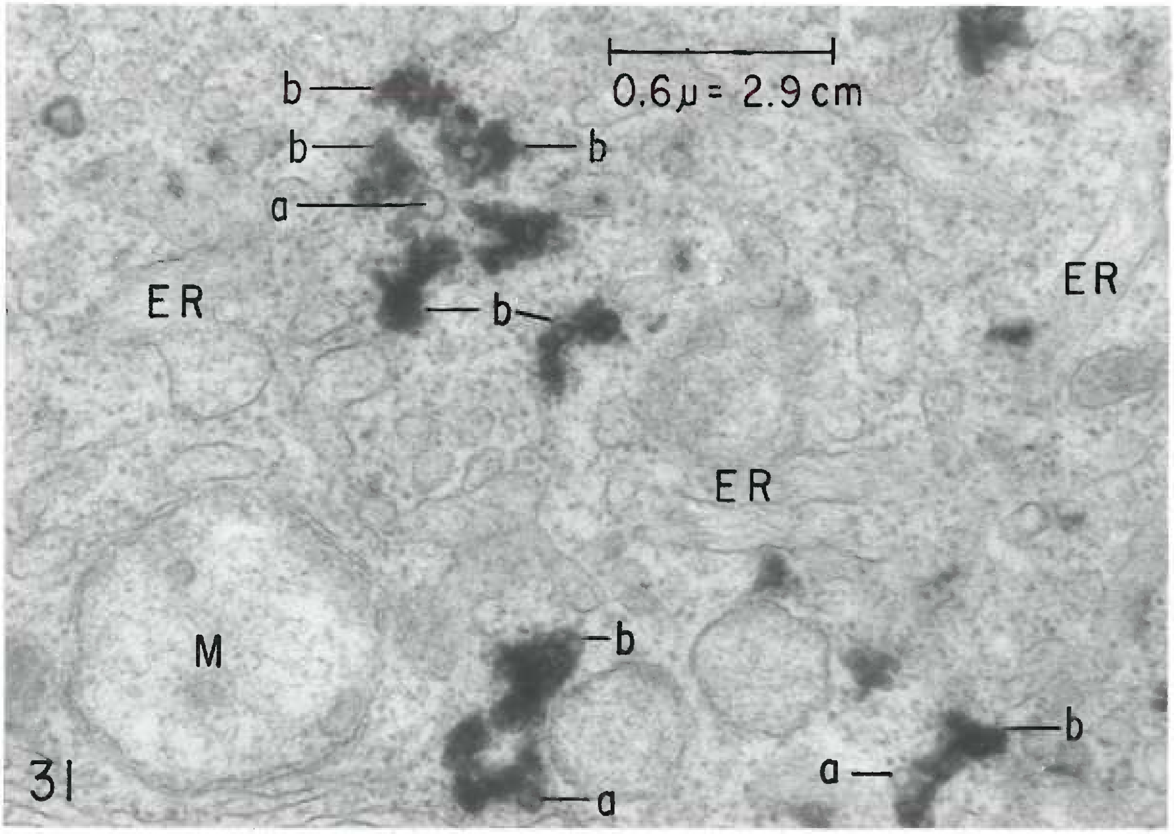
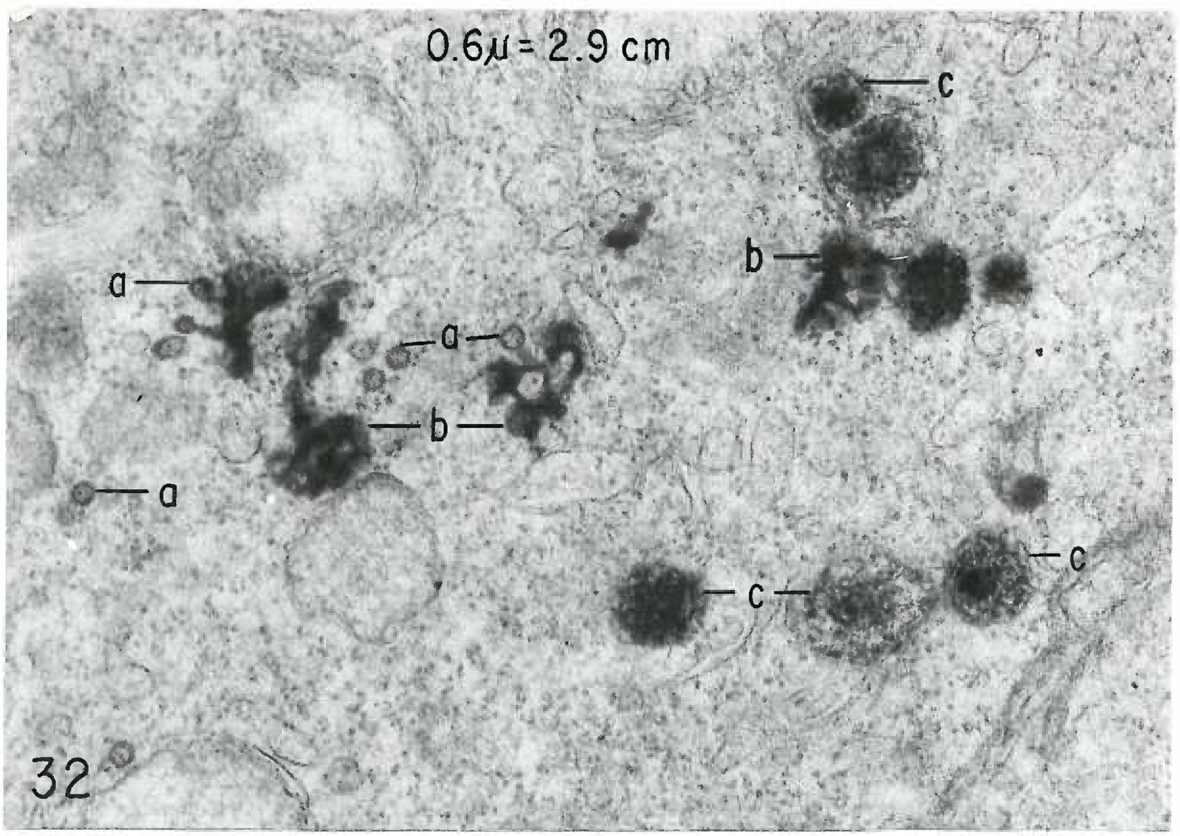

CM
M
ER
M
M
M
M
NM
N
nc
1.2 cm = 1 μ
30

FIGURE 31: An electron micrograph of part of an epithelial cell of an epidermal papilloma. The cytoplasm contains a mitochondrion (M), ergastoplasm (ER) and numerous particles of ribonucleoprotein. The amorphous *b particles* (b) appear to contain the virus-like *a particles* (a). Lead citrate and uranyl acetate stains. X47,000.

FIGURE 32: An electron micrograph of part of an epithelial cell of an epidermal papilloma. The *a particles* (a) are seen free in the cytoplasm or at the ends of pseudopod-like projections from the *b particles* (b). Several forms of *c particles* (c) are seen. Lead citrate stain. X47,000.



0.6μ = 2.9 cm
b
b
b
a
ER
b
ER
ER
M
b
b
a
a
31



0.6μ = 2.9 cm
c
b
a
a
b
a
c
c
c
32

FIGURE 33: An electron micrograph of part of an epithelial cell of an epidermal papilloma. Adjacent to the altered mitochondrion (M) is a multivesicular body of unknown origin and function. Numerous *a particles* (a), *b particles* (b), and *c particles* (c) are present. *Note* the granular bodies enclosed within unit membranes (GB) which are contiguous with two *b particles*. These are similar to the "mitochondrial precursors" or "gray bodies" described by Bonar *et al.* as occurring in avian myeloblastosis. Lead citrate stain. X47,000.

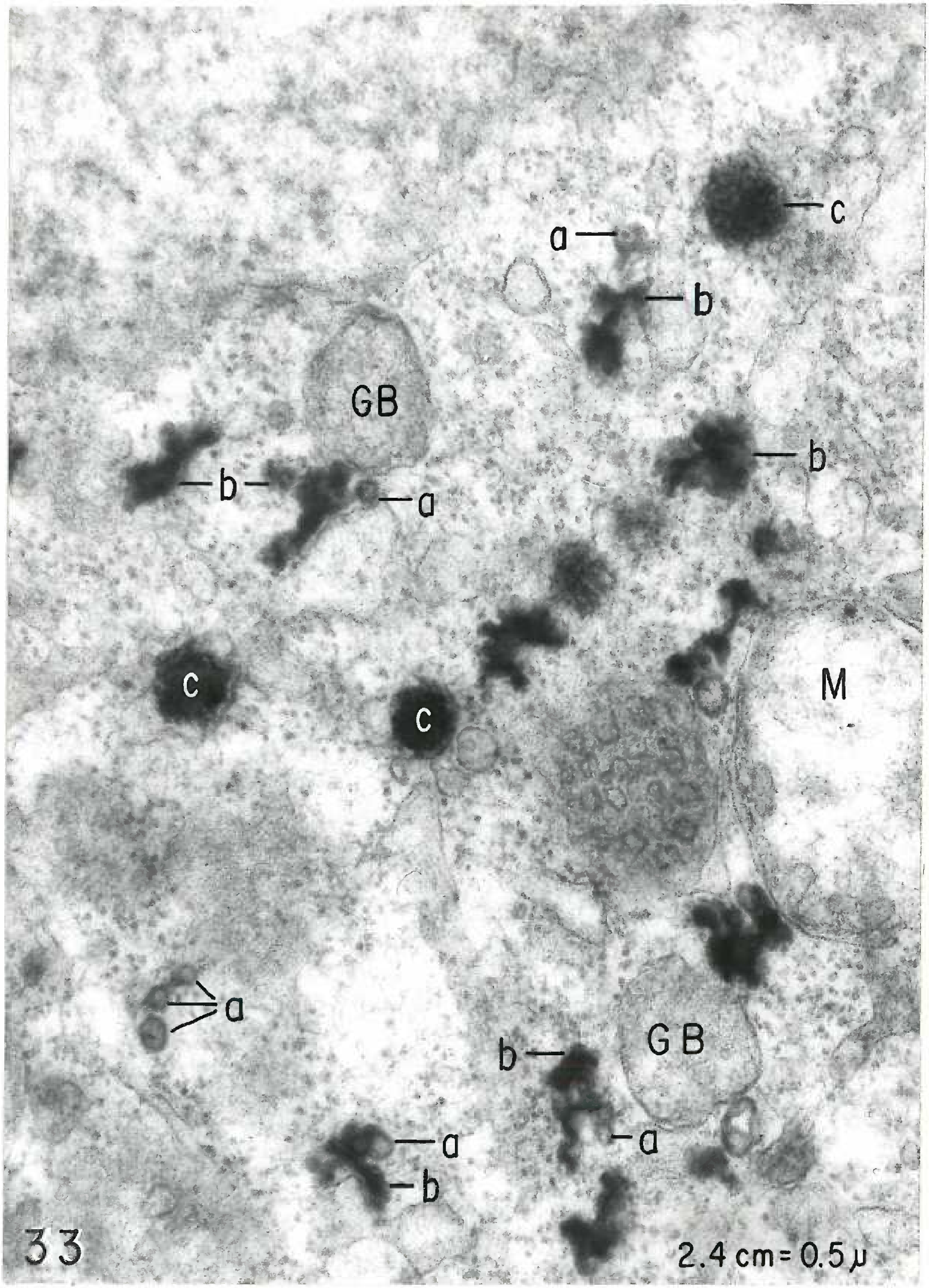

c
a
b
GB
b
a
b
c
c
M
a
GB
b
a
a
b
33
2.4 cm = 0.5 μ

FIGURE 34: An electron micrograph of portions of two adjacent epithelial cells of an epidermal papilloma. The prominent *a particle* (a) shows an outer unit membrane and the unit membrane of the nucleoid. *Note* the fine irregularity of both the cell membranes (CM) and the nuclear membrane (NM). *b particle* (b); ergastoplasm (ER). Lead citrate stain. X59,000.

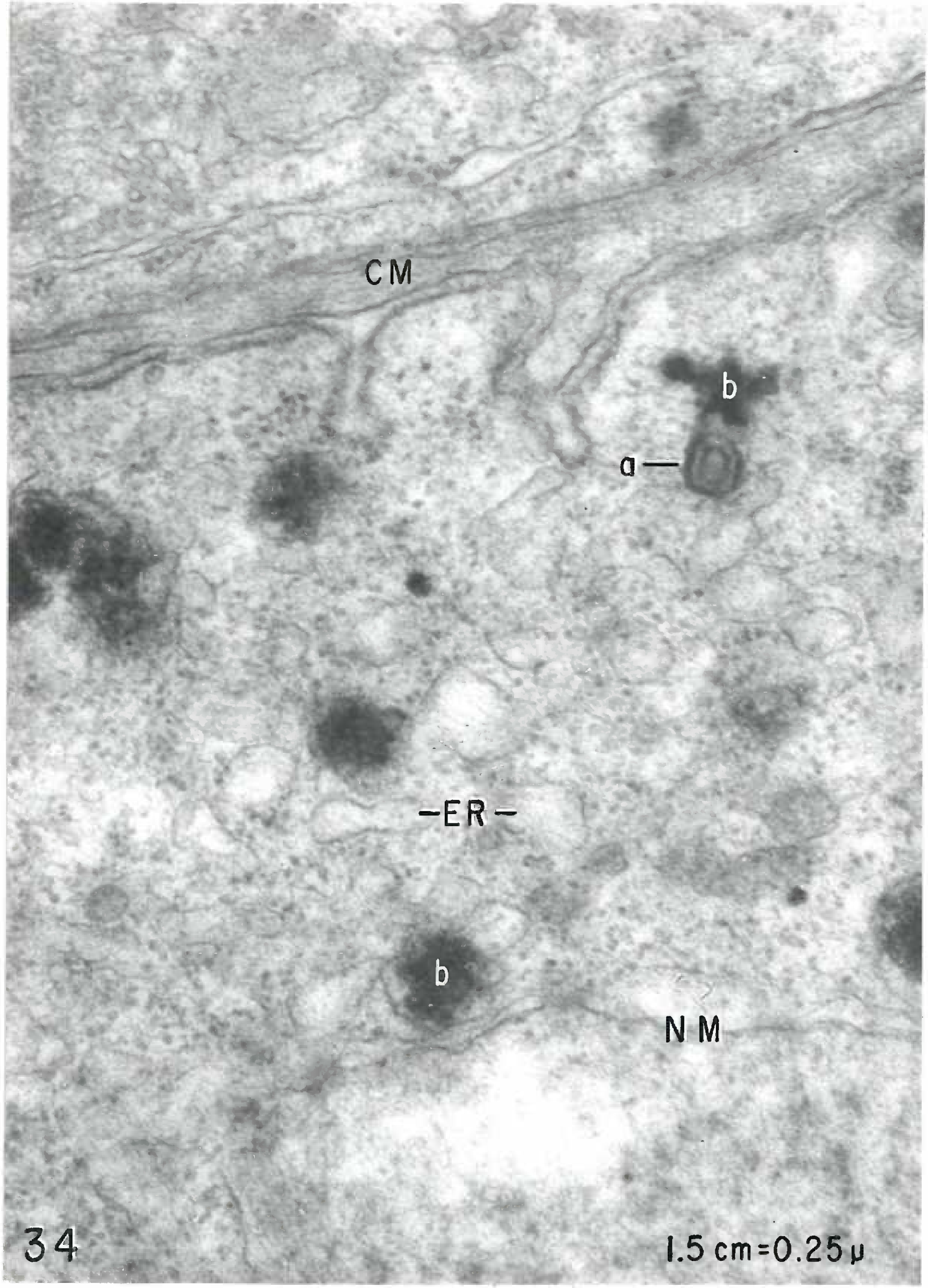
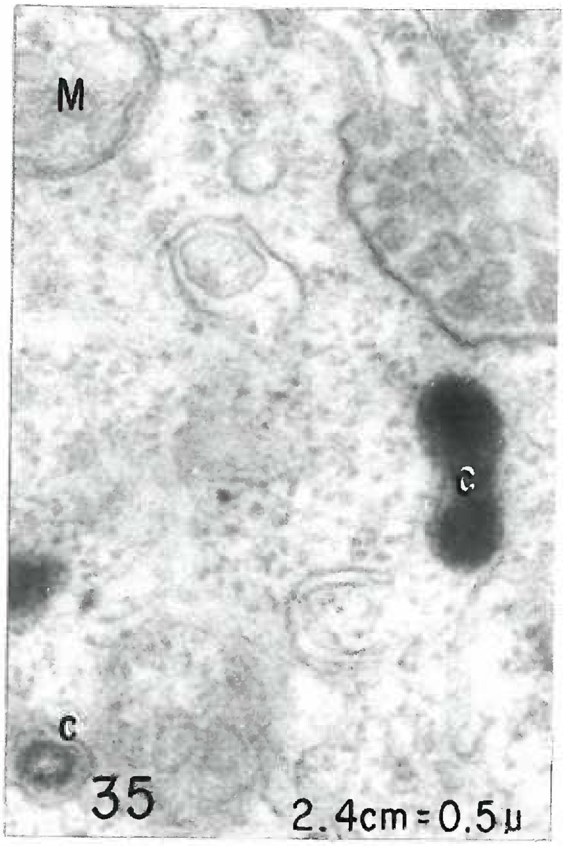

CM
b
a
-ER-
b
NM
34
1.5 cm=0.25 μ

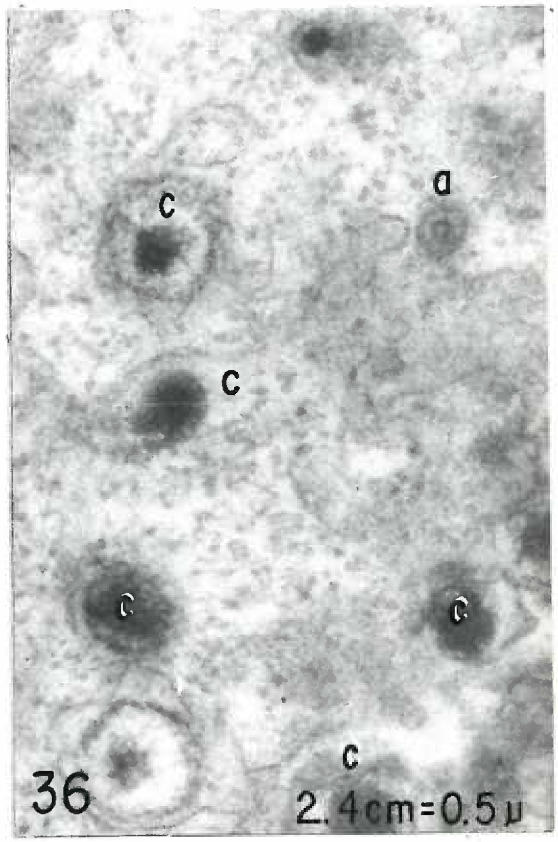
FIGURE 35: An electron micrograph of part of an epithelial cell of an epidermal papilloma. Mitochondrion (M); *c particle* (c). Lead citrate stain. X47,000.

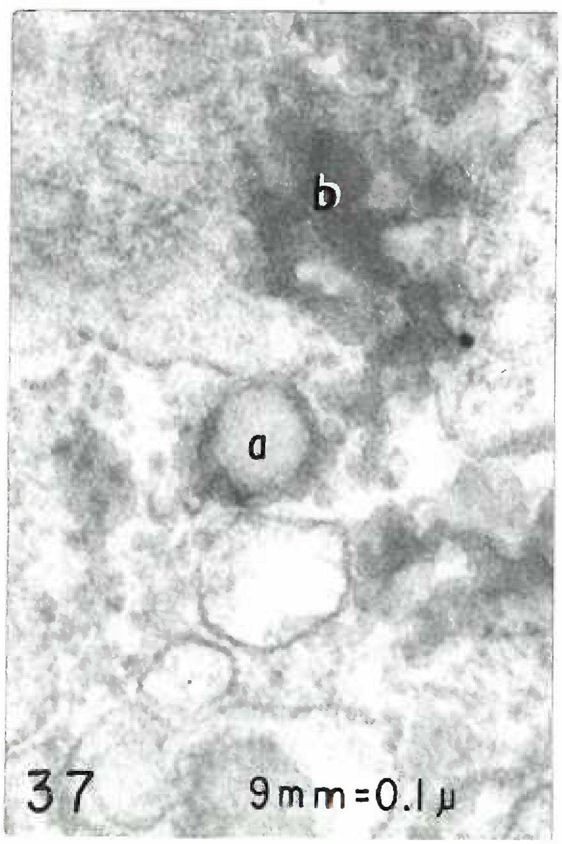
FIGURE 36: An electron micrograph of part of an epithelial cell of an epidermal papilloma. *a particle* (a); *c particle* (c). Lead citrate stain. X47,000.

FIGURE 37: An electron micrograph of part of an epithelial cell of an epidermal papilloma. *a particle* (a); *b particle* (b). Lead citrate and uranyl acetate stains. X90,000.

FIGURE 38: An electron micrograph of part of an epithelial cell of an epidermal papilloma. Mitochondrion (M); *a particle* (a); *b particle* (b); *c particle* (c). Lead citrate stain. X47,000


M
c
c
35
2.4cm=0.5µ


c
a
c
c
c
c
36
2.4cm=0.5µ


b
a
37
9mm=0.1µ

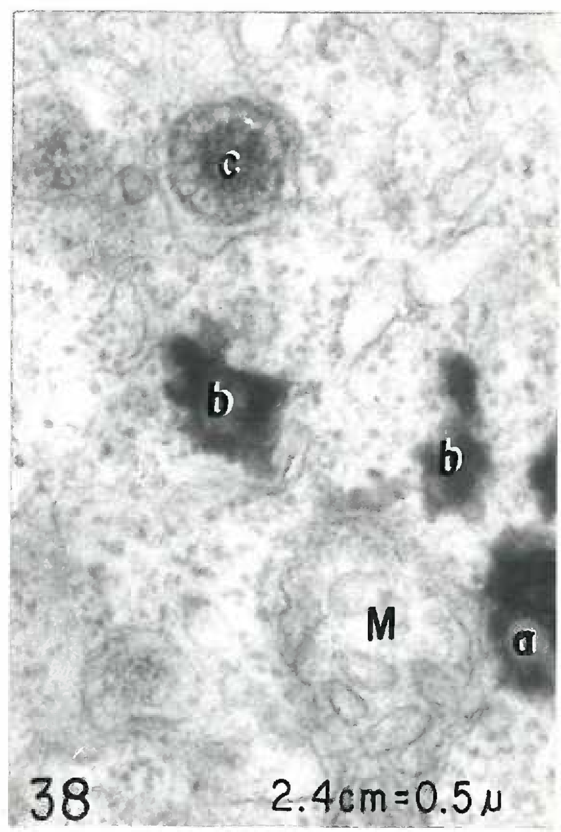
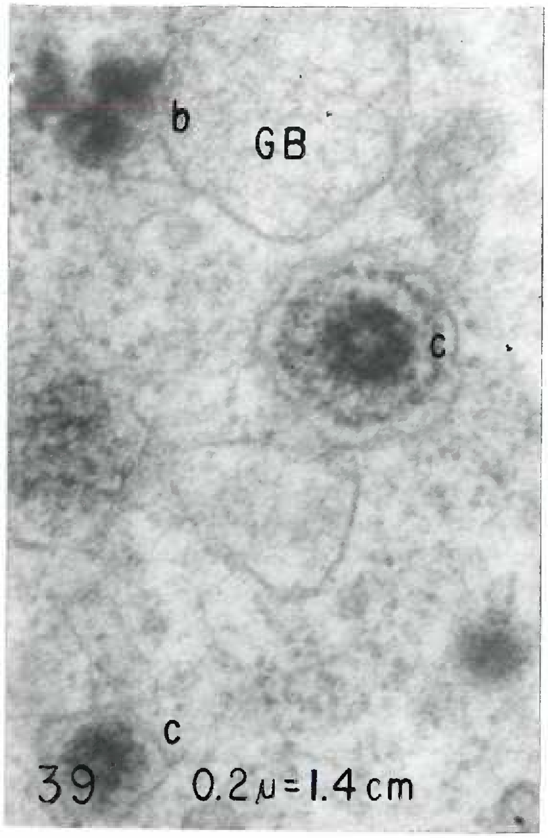
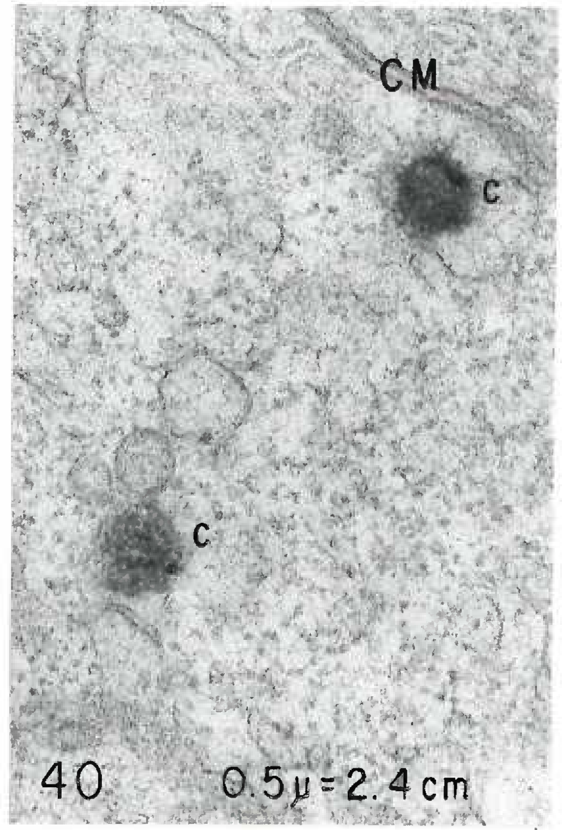

c
b
b
M
a
38
2.4cm=0.5µ

FIGURE 39: An electron micrograph of part of an epithelial cell of an epidermal papilloma. The *b particle* (b) in the upper left is contiguous with a "mitochondrial precursor" (GB) type structure. *c particle* (c). Lead citrate stain. X68,000.

FIGURE 40: An electron micrograph of portions of two adjacent epithelial cells of an epidermal papilloma. Cell membrane (CM); *c particle* (c). Lead citrate stain. X47,000.

FIGURE 41: An electron micrograph of part of an epithelial cell of an epidermal papilloma. *Note* the "mitochondrial precursor" (GB) contiguous with the *b particle* (b) in the lower right. Mitochondria (M); ergastoplasm (ER); *a particle* (a); *c particle* (c). Lead citrate stain. X47,000.


b
GB
c
c
39
0.2 μ = 1.4 cm


CM
c
c
40
0.5 μ = 2.4 cm

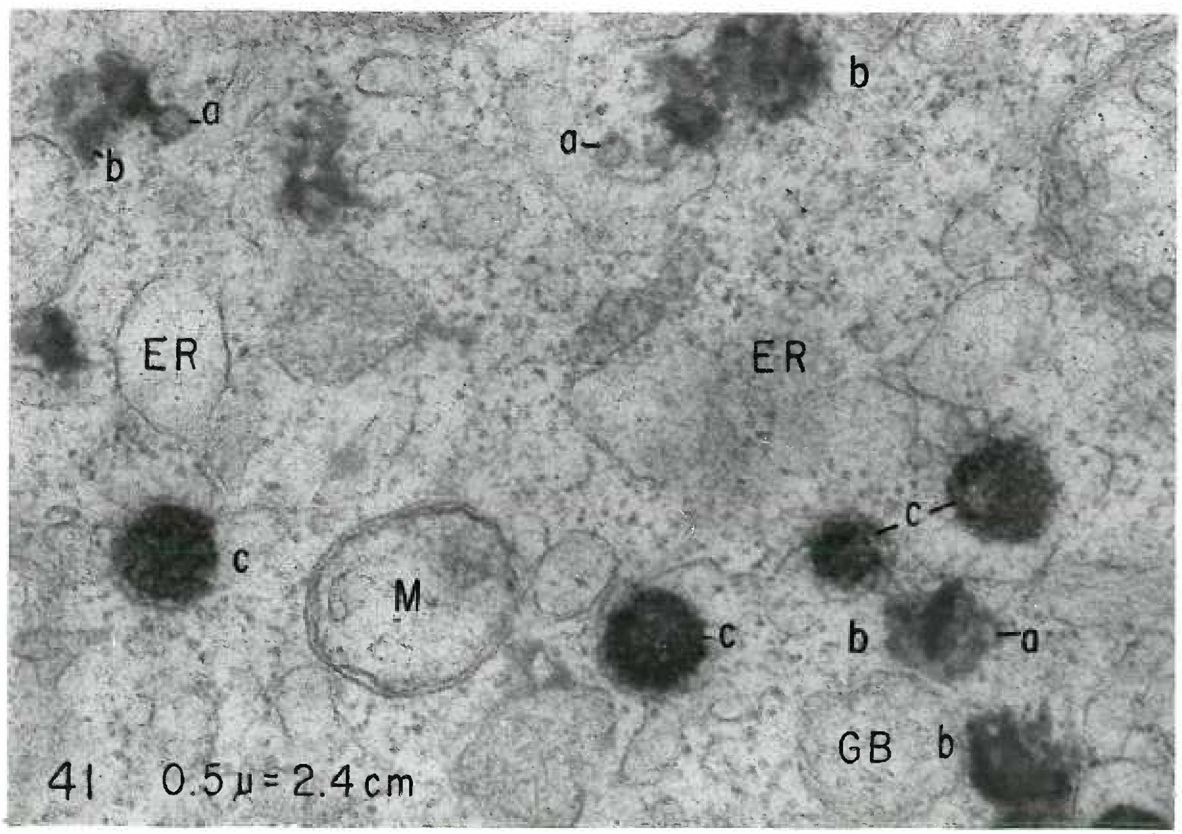
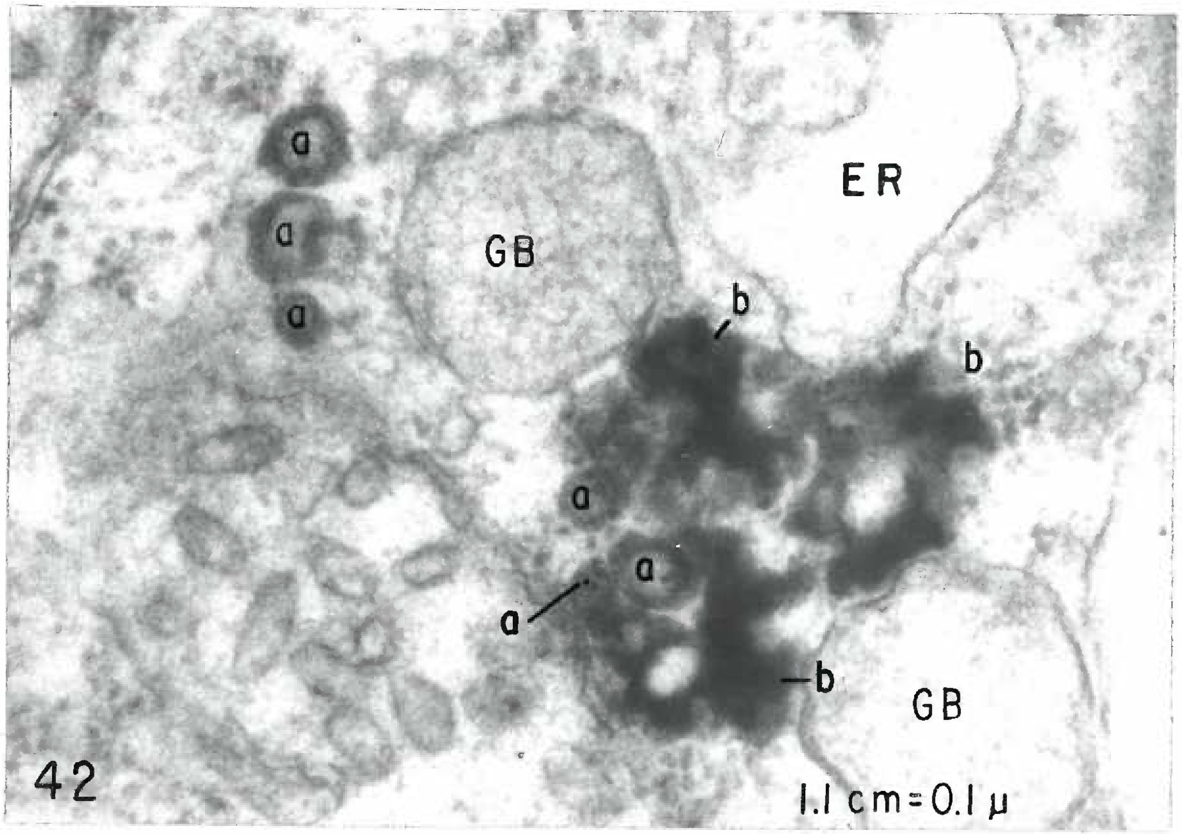
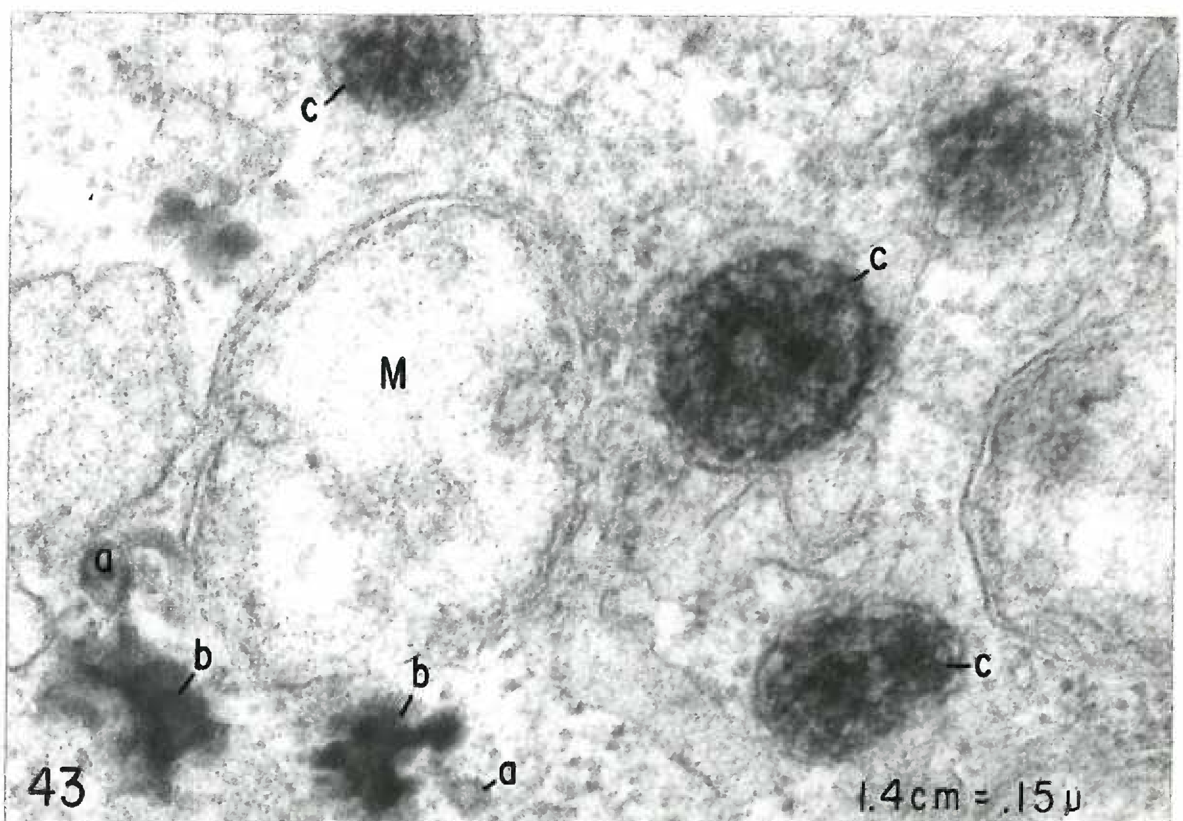

a
b
a
b
ER
ER
c
c
M
c
b
a
GB
b
41
0.5 μ = 2.4 cm

FIGURE 42: An electron micrograph of part of an epithelial cell of an epidermal papilloma. The large *b particle* (b) lies between two mitochondrial precursor-like structures (GB). Mitochondrion (M); ergastoplasm (ER); *a particle* (a). Lead citrate stain. X112,000.

FIGURE 43: An electron micrograph of part of an epithelial cell of an epidermal papilloma. Mitochondrion (M); *a particle* (a); *b particle* (b); *c particle* (c). Lead citrate stain. X90,000.



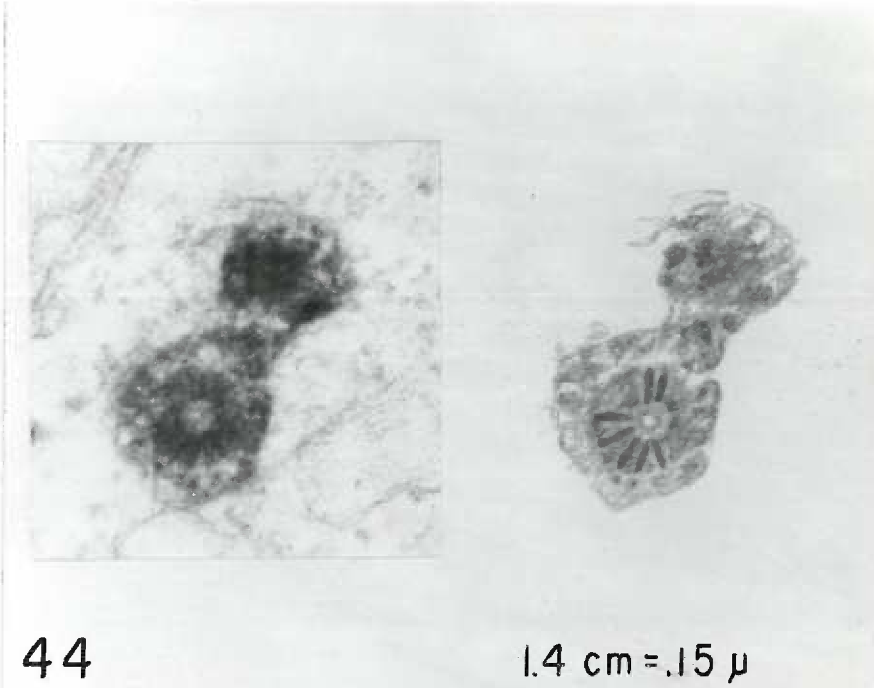
a
a
a
GB
ER
b
b
a
a
a
b
GB
42
1.1 cm=0.1 μ



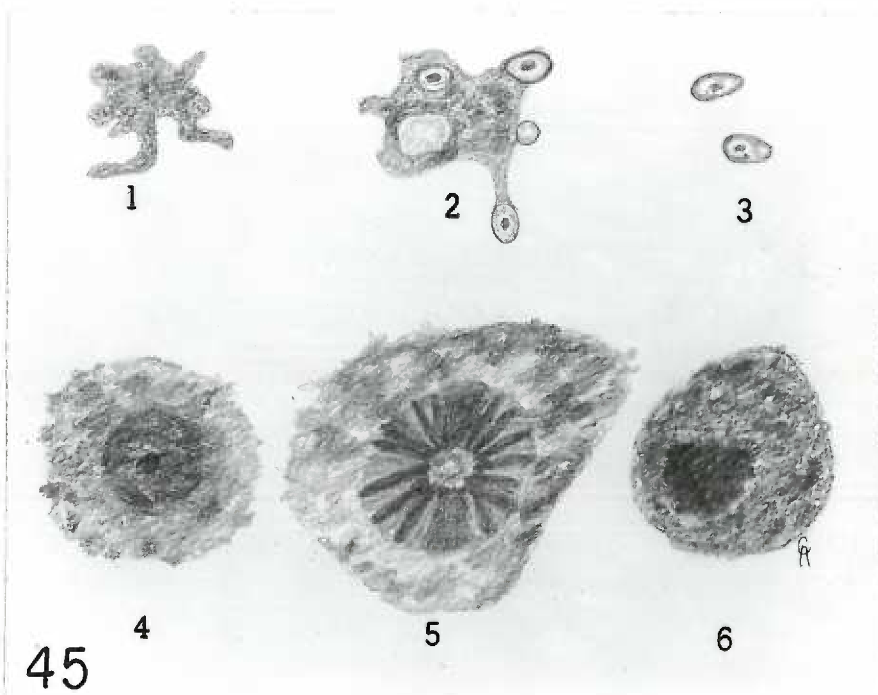
c
c
M
a
b
b
c
a
43
1.4cm=.15μ

FIGURE 44: An electron micrograph (left) of two *c particles* in an epithelial cell of an epidermal papilloma. *Note* suggestions of radially oriented rods (capsomeres?) also represented in the diagram (right). Lead citrate stain. X90,000.

FIGURE 45: Diagramatic representations of the cytoplasmic virus-like particles seen in electron micrographs of epithelial cells of epidermal papillomas of flathead sole: (1) the dense, homogeneous *b particle*, the presumed "viroplasm"; (2) the *b particle* showing observed locations of associated vesicular, virus-like *a particles*; (3) vesicular, virus-like *a particles*; (4, 5, 6) three different morphologic forms of the presumed mature, virus-like *granular body* or *c particle*. The hypothetical schema is that the dense *b particles* are a locus of virus formation and give rise to the vesicular *a particles*, and these subsequently develop into the mature *c particles*.



44



45