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AN INVESTIGATION OF THE ETIOLOGY OF BOWEN'S DISEASE

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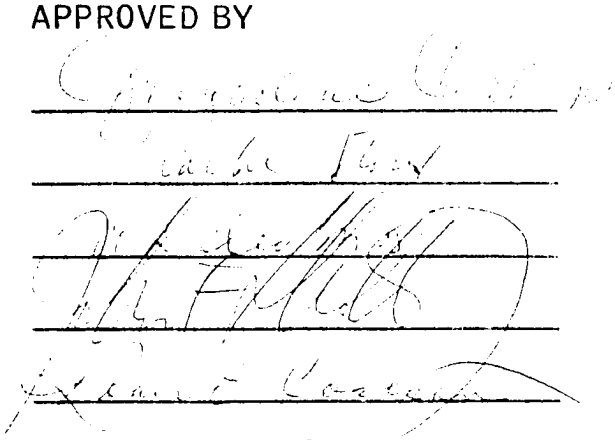

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AN INVESTIGATION OF THE ETIOLOGY OF BOWEN'S DISEASE

CHAPTER I

INTRODUCTION

Bowen's disease, a squamous cell carcinoma in situ, was originally described in 1912 by J. T. Bowen as a precancerous dermatosis. This in situ carcinoma is generally restricted to the trunk region of Caucasians and makes its appearance during the fifth and sixth decades of life. This epidermal lesion is slow growing and appears grossly as a dull red, scaly plaque of 2-3 cm in diameter.

Bowen's disease has been of interest to many investigators because of a relatively long period of quiescence. The lesion may remain as a carcinoma in situ for as long as five years before invading the underlying dermis (Bowen, 1920; Lever, 1967). Several investigators have also noted the high incidence of internal neoplasms which either precedes or follows the appearance of the epidermal lesion (Graham and Helwig, 1959; Hugo and Conway, 1967). These studies showed a nine fold increase of internal malignancy when compared with expected values of a control group. Graham and Helwig (1959) were the first to notice the association of Bowen's lesions and systemic cancer. They reported a 25 per cent incidence of this association and listed the types of internal tumors in the following order of

frequency: respiratory system, gastrointestinal tract, genitourinary organs, reticulo-endothelial system, endocrine system, breast and eye.

This incidence of multiple tumors prompted a search for the etiology of Bowen's disease. Early studies indicated that the lesion sometimes accompanied high arsenic intake (Anderson, 1932). Later, investigators found that arsenic was preferentially accumulated in Bowen's lesions, and that the values obtained were 82 per cent higher than those of control skin and 40 per cent higher than arsenic levels in other types of skin disorders (Graham, et al., 1961; Fergusson, et al., 1960).

In 1963, Yeh reported that a lesion histologically similar to Bowen's was a frequent finding among the Chinese population on Taiwan. Subsequent investigations revealed that well water in these villages contained large quantities of arsenic. However, it should be emphasized that Bowen's lesions were not always correlated with an arsenic background, in fact, the majority of cases available for this study had no history of arsenic exposure or intake.

Since arsenic exposure has been an inconsistent finding in Bowen's disease, other etiologic agents should be considered. Moreover, the high incidence of systemic involvement is suggestive of an infectious process, and is compatible with a virus etiology.

The theory of a viral origin for tumors was suggested first by Borrel in 1907. He noted that some virus infections induced hyperplasia in various tissues. Experimental evidence for the viral etiology of animal tumors was introduced by Ellerman and Bang in 1908 with the transmission of avian leukosis. Later, Rous (1910; 1911) implicated viruses in various avian neoplasms. The significance

of these investigations escaped the leading oncologists of the day, and consequently did little to stimulate work in this area. A long period of inactivity was ended in the 1930's by Shope's work in the isolation of viruses from papilloma and other neoplasms in rabbits (Shope, 1932). Bittner (1936) and Lucké (1938) showed the cell-free transmission of murine mammary carcinoma and frog renal carcinoma respectively. At the beginning of the 1950's, Gross (1951) demonstrated the viral etiology of "spontaneous" leukosis in mice and observed that the virus was transmitted vertically to each generation by infection of the ova and/or sperm. His later experiments (Gross, 1953, 1955, 1958) showed that some mouse strains were free of the infectious agent, while other genetic strains were compromised. Continued experiments utilizing serial transfer in newborn mice resulted in the production of the highly virulent "Gross passage A" virus. This virus strain induced leukemia in as many as 90 per cent of injected young mice.

The advent of useful electron microscopy in the 1950's provided a new research tool which greatly stimulated research activity involving animal tumor models. Subsequent advances in viral oncology are well described in a comprehensive review article by Shope in 1966.

In the late fifties and sixties, many investigators turned their attention from animals to the study of human tumors of suspected viral etiology, and since that time many types of human neoplasias have been studied ultrastructurally, virologically and immunologically (Dmochowski, 1964; Smith, et al., 1964; Porter, et al., 1964; Dmochowski, et al., 1965; Epstein, et al., 1965; Zeve, et al., 1966; Dmochowski, 1967; Feller and Copra, 1968; Hinz, et al., 1968;

Maruyama, et al., 1968; Raucher, 1968; Seman and Seman, 1968; Stewart, et al., 1969; Morton, et al., 1969).

Among the agents associated with human tumors, the best known virus is the Epstein-Barr virus (Epstein, et al., 1964) which was isolated from an African patient with Burkitt's lymphoma. This virus has been shown to have a DNA core and has been placed in the herpes group. However, the most promising candidate for carcinogenesis in humans is probably the C-type RNA virus. Recently, several electron microscopic studies have reported this type of virus in human tumors. One of these studies (Morton, et al., 1969) reported that extracts of osteosarcoma containing C-type particles induced malignant transformation in human tissue culture cells. However electron microscopic examination of the transformed culture cells failed to show virus particles of the C-type. The same group reported that subcultures of a liposarcoma contained virus-like particles similar to the C-type murine virus described by Bernhard in 1960, but again, when the original tumor was examined electron microscopically, no viruses were seen. The only original tumor studied by this group which revealed virus-like particles was a chondrosarcoma. This tumor contained virus-like particles that budded from the endoplasmic reticulum in the tumor cells, and were similar to the "A" particles in a human reticulum cell sarcoma as described by Seman and Seman in 1968.

A spectacular report dealing with human tumorogenesis was the growth of feline leukemia virus in human embryo lung cells (Jarrett, et al., 1969). This agent, a C-type RNA virus, was demonstrated by electron microscopy and uridine labeling experiments showed that it proliferated readily in cultured human cells.

It has been shown that a serological relationship exists between feline and human leukemias. It has been reported previously by Geering that an antigenic relationship existed among the viruses which produced leukemia in rats, mice and cats (Geering, et al., 1968).

Although the information regarding the role of viruses in the etiology of many animal tumors is unequivocal, no malignant human tumor has yet proven to be directly attributable to a virus. However, the major works cited above would prompt one to investigate further the possible etiologic role of infectious agents in human tumor oncology.

The work to be reported on the following pages is the product of an investigation which was originally undertaken to study only the histochemical and morphological alterations in a carcinoma in situ, Bowen's disease. However, the repeated visualization of virus-like particles in association with these lesions prompted an expansion of the experimental protocol to include the investigation of an etiologic role of these virus-like particles in this disease entity.

CHAPTER II

MATERIALS AND METHODS

Clinical Material

Clinically characteristic Bowen's lesions of 2 to 3 centimenters in diameter were removed from patients under local anesthesia. The specimens were weighed (wet tissue weight approximately 0.2 gm) and divided with a scapel into three equal parts: for light microscopy, for electron microscopy and for determination of biological activity.

Histochemistry

Light microscopy. Several histochemical methods were applied in an attempt to characterize some of the cytochemical alterations that occur in the development of the Bowen's lesions. These histochemical methods are listed under the following general catagories:

Polysaccharides

Periodic Acid-Schiff - with and without diastase digestion (Barka, 1963)

Astrablau - (Kelly, 1962)

Hale's Colloidal Iron - (Barka, 1963)

Alcian Blue - (Barka, 1963)

Lipids

Sudan B - (Barka, 1963)

Nucleic Acid

Feulgen - with and without desoxyribonuclease digestion (Barka, 1963)

Enzymes

Acid phosphatase - (Barka, 1963)

Electron microscopy. Methods for the ultrastructural demonstration of two hydrolytic enzymes were utilized to study the involvement of the lysosomes in the morphological alterations. The methods selected were for the localization of acid phosphatase and desoxyribonuclease. The following method is our modified version of Barka and Anderson's modification of the Gomori acid phosphatase method.

Wet glutaraldehyde fixed tissues were sectioned to 20-40 microns on an S and F tissue chopper (University of Southern California, Development and Research Laboratory). The sections were incubated for 20 minutes at 37°C in the following substrate:

10 ml of 0.1M tris-maleate buffer

20 ml water with .125 per cent sodium beta-glycerophosphate

20 ml 0.1 per cent lead nitrate

This substrate was adjusted to pH 5.0.

The method for demonstrating desoxyribonuclease was that of Vorbrodt

(1961), and the tissue was prepared for incubation in the manner described above.

Morphologic Studies

Light microscopy. The tissue for light microscopy was fixed in 10 per cent neutral buffered formalin, embedded in tissuemat, sectioned at 5-7 microns, and examined histologically to assure that the specimens were true Bowen's lesions (Lever, 1967).

Electron microscopy. The tissue for electron microscopy was fixed in 2 per cent glutaraldehyde (Sabatini, et al., 1963), buffered to pH 7.2 with 0.1M phosphate buffer (Barka and Anderson, 1963) with 5 per cent sucrose. After two hours of fixation, the specimens were placed in 0.1M phosphate buffer with 5 per cent sucrose, pH 7.2, and stored for subsequent examination, pending confirmation of the histological diagnosis. Storage in the buffer for periods up to one week produced no apparent damage. The specimens were then post-fixed with 2 per cent osmium tetroxide, in 0.1M phosphate buffer, pH 7.2 with 5 per cent sucrose. After two hours the specimen was dehydrated in graded alcohols and embedded in Cargille's epoxy resin (Araldite 6005 obtained from R. P. Cargille's Laboratories, Inc, N. J.).

Tissue culture cells taken for electron microscopy were treated in the manner described above, except that a low speed centrifugation (600-800 X g) was performed between each step, in order that all cells might be pelleted. Virus-like particles from these cultures were centrifuged at 60,000 X g in a Beckman L-2 ultracentrifuge using a SW 25.1 swinging bucket head. The pellets obtained were fixed and embedded in the manner described above.

After polymerization of the resin for 24 hours at 60°C the blocks were thin sectioned and stained with acetic acid-uranyl acetate and lead citrate (Reynolds, 1963). Micrographs were taken at various magnifications (3,000 to 100,000 x) on an Hitachi HU-11B electron microscope.

Tissue Culture Methods

Established tissue culture lines of HeLa Chessen were maintained at 37°C. The cells were grown in Eagle's Minimum Essential Medium (MEM) without glutamine, enriched with 10 per cent heat denatured fetal calf serum, 1 per cent glutamine and 1 per cent penicillin-streptomycin mixture (5,000 units per ml). (All tissue culture media and supplements were obtained from Grand Island Biological Company, New York).

Biological Assays

Tissue Culture Experiments

The portion of tissue from the Bowen's lesion allotted for biological assays was immediately frozen with liquid nitrogen and maintained at -70°C. Later, the tissue was quickly thawed and cut in half. The first half was sliced in small segments and placed directly in 20 ml of medium on monolayers of HeLa cells (5×10^6 cells) in milk dilution bottles. The remaining half was ground to an emulsion by a mortar and pestle, diluted to 10 ml with sterile saline, sonicated, and filtered through a 0.2 micron Millipore filter. The resulting cell free filtrate was layered over a monolayer of HeLa cells (5×10^6 cells) and incubated at 37°C for two hours. Following the incubation, the fluid was decanted and replaced with

enriched MEM. The cultures were then gassed with 5 per cent CO_2 and air mixture and placed in an incubator at 37°C. Control, normal skin was processed in an identical manner as described above.

Following the earliest discernible cytopathic effect, serial transmission was accomplished by collection of the tissue culture cells and medium. These cells and medium were lightly sonicated and filtered through a 0.2 micron Millipore filter. The filtrate was then applied to a new monolayer of HeLa cells for two hours at 37°C. After two hours, the filtrate was removed and fresh medium added and again incubated at 37°C. Each subsequent passage was performed in an identical manner, until termination of the experiment.

Egg Experiments

Ten day embryonated hen eggs were injected with 0.2 ml of the cell free filtrate from the human tumor. It was injected into either the amniotic sac fluid or the chorio-allantoic cavity fluid. The injection sites in the shells were sealed with dental wax and the eggs incubated at 37°C for 72 hours to 10 days. After the fluids were drawn off, the serosa (chorio-allantoic) and amniotic sacs were removed by cutting off the top of the egg and dropping the embryo from the shell. Pieces of both membranes were prepared for electron microscopy by the methods described in the morphological studies section. The remaining membranes and fluids were frozen and stored for further tissue culture studies.

Immunology

Cells from supernatent from the first passage in the tissue culture was

emulsified with Freud's adjuvant (Difco Laboratories, Detroit, Michigan) in a 1:1 ratio. This emulsion was then injected into young, white, New Zealand rabbits. The total injection volume was 3 ml, with each rabbit receiving 0.25 ml in each foot directly in the footpads, and the remaining 2 ml was injected into the flanks, 1.0 ml in each side. Ten days later each rabbit received a 2 ml booster of the same emulsion, 1.0 ml in each flank. Forty-five days after the second injection, the rabbits received a third and last injection of 2 ml of emulsion, 1.0 ml in each flank.

Ten days following the last injection, the rabbits were bled by cardiac puncture, each rabbit contributing 50 ml. The fresh blood was allowed to clot at room temperature, and the serum withdrawn. The serum was divided into 3 ml aliquots and stored in sterile glass vials at $-70^{\circ}C$.

Plasma was obtained from patients with Bowen's disease and other types of cancer, as well as normal volunteers. Plasma samples were number coded at the time of withdrawal, to insure objectivity during subsequent experiments. All number coded plasma samples were assayed for the Bowen's virus antigen by immunodiffusion against the hyperimmune rabbit antiserum using the Ouchterlony technique (Ouchterlony, 1948). In this immunodiffusion procedure, an agar medium (1.0 per cent Special Agar-Noble, Difco Laboratories, Detroit, Michigan) allows the diffusion of macromolecules at rates which correspond to their respective molecular weight and steric configuration. At the site of junction of two or more macromolecules which are diffusing from opposite sites, an opaque line is formed. The opaque line is due to the coupling of antibody with an antigen and subsequent precipitation

of that complex.

Prior to the preparation of the double diffusion plates, the rabbit antiserum was absorbed against human cells to remove the nonspecific antibodies. Patient plasma and the absorbed hyperimmune rabbit serum then were loaded into circular wells, 3 mm in diameter, on an agar plate of 3-4 mm in thickness. The circular wells were cut with a cork borer with an inter-well distance of 10 mm from lip to lip. After loading, the plate was placed in a moist chamber, and stored at room temperature. The plates were scanned for the opaque percipitin lines at 24, 48 and 72 hours, and photographed at the first appearance of a reaction.

CHAPTER III

RESULTS

Light Microscopic Morphology

The plaques of Bowen's disease appeared as intra-epidermal squamous lesions. The over-all epidermal field was marked by dysplasia in which the normal organization and polarity of the stratified epithelium was lost. Individual sites were marked by hyperkeratotic surfaces, parakeratosis and marked acanthosis. The most notable change in individual cells was the propensity for malignant dyskeratosis, or disarranged keratinization, seen as premature individual cell keratinization in the basal and spinous layers. This type of keratinization contrasted with normal keratinization in that the normal process occurred in the stratum granulosum and stratum corneum and utilized keratohyaline formation. The cells within the thickened epidermis were often seen in mitosis, many of which were abnormal, e.g., multipolar. Occasional cells were noted to have as many as six nuclei of variable size. The rete ridges were sometimes thickened, rounded and appeared blunted due to the narrowing of dermal papillae. The contiguous dermis frequently contained a mononuclear infiltrate consisting of plasma cells, lymphocytes and macrophages.

Histochemistry

Light microscopy. The periodic acid-Schiff method demonstrated increased amounts of carbohydrates in a granular form, and when coupled with diastase digestion, the results indicated an increased glycogen content as compared to normal skin. The Feulgen reaction did not demonstrate desoxyribose in unusual locations or configurations.

The other histochemical methods (Astrablau, Alcian Blue, Hale's Colloidal Iron and Sudan B) utilized for the study of Bowen's disease were essentially consistent with the findings in normal skin. Consequently, these methods did not contribute to the understanding of the disease process.

Electron microscopy. Localization of the hydrolytic enzyme or enzymes known as acid phosphatase indicated the presence of large granules of the lysosome family in all the epidermal layers (Plate XVIII, Figure 18). The localization also indicated that dyskeratosis was intimately involved with the hydrolytic enzyme system since the dyskeratotic cells showed diffuse enzyme localization (Plate XVIII, Figure 19).

Desoxyribonuclease was also localized to structures of lysosomal appearance, however no definitive interpretation could be made due to the large amounts of nonspecific reaction product.

Electron Microscopic Morphology

The most dramatic ultrastructural alteration was the finding of numerous breaks in the continuity of the basement membrane (Plate I, Figure 1; Plate II,

Figure 2; Plate III, Figure 3; Plate IV, Figure 4; Plate V, Figure 5; Plate VI, Figure 6). Villous processes from basal cells projected through the basement membrane (Plate II, Figure 2) and appeared to be followed by a diapedesis-like action (Plate III, Figure 3; Plate IV, Figure 4; Plate V, Figure 5). The downward migration of keratinocytes resulted in the destruction of large segments of basement membrane (Plate VI, Figure 6), and occasionally the penetrating cell processes were found in direct contact with fibroblast-like cells in the adjacent dermis (Plate V, Figure 5). Conversely, infiltrating dermal cells of the mononuclear types were seen also to penetrate the basement membrane and migrate into the epidermis (Plate VII, Figure 7).

The surfaces or plasma membranes of the basal and spinous cells were very irregular and thrown up in villous projections (Plate I, Figure 1; Plate VI, Figure 6; Plate VIII, Figure 8). These cellular projections protruded into widened spaces between cells and sometimes made contact with the projections from an adjacent cell. Some of these connections appeared to function as rudimentary desmosomes (Plate VII, Figure 7). Normal skin was always noted to have narrow intercellular spaces, and large numbers of well-defined desmosomes (Plate IX, Figure 9).

The Golgi apparatus of squamous cells in Bowen's disease was found to be very well-developed and some sections showed as many as four distinct perinuclear zones in a single cell (Plate X, Figure 10). In normal cells, the Golgi zones are both uncommon and poorly developed (Plate IX, Figure 9). Mitochondria were present in increased numbers and frequently exhibited extreme size variation and pleomorphism (Plate XI, Figure 11). Normal epidermal cells contained rela-

tively few mitochondria, and these were usually small and oval-shaped (Plate IX, Figure 9). Accumulations of beta glycogen were observed in the basal and lower spinous layers as membrane bound localizations in a perinuclear position (Plate XII, Figure 12). The quantity of melanin was markedly reduced within the affected epidermis (Plate I, Figure 1), and although dendritic processes containing melanin were present between cells (Plate VIII, Figure 8), the majority of keratinocytes contained no melanosomes. However, those keratinocytes which did contain melanin accumulated large amounts of the pigment granules in lysosome-like structures (Plate XIII, Figure 13).

Most nuclei varied in size and shape, and occasionally multinucleated giant cells with closely packed nuclei were present (Plate XX, Figure 20). The nuclear outlines were moderately irregular with distinct bilameller nuclear membranes (Plate X, Figure 10; Plate XIV, Figure 14; Plate XV, Figure 15; Plate XVI, Figure 16). Nucleolar size was markedly increased, and when measured by the method of Nix, et al., (1965) the average nucleolus was 60 per cent larger than controls. The enlarged nucleolus consisted of dense strands which branched to form a reticulated nucleolonema (Plate X, Figure 10). This reticulate appearance may be seen in normal epidermal cells but is usually less prominent (Plate IX, Figure 9).

The degree of premature keratinization differed greatly from cell to cell, and often there was only a displacement and aggregation of tonofilaments in a perinuclear position (Plate XIV, Figure 14). However, in many cells, there were discrete cytoplasmic foci of mature keratin plugs which were sometimes lined by

membranes and joined by desmosomes (Plate XIV, Figure 14 and Plate XV, Figure 15). Occasionally whole dyskeratotic cells were observed within the cytoplasm of other keratinocytes (Plate XVI, Figure 16; Plate XVII, Figure 17 and Plate XIX, Figure 20). These "engulfed" dyskeratotic cells appeared to be condensed and often exhibited a strong acid phosphatase activity (Plate XVIII, Figure 19).

The giant cells which were described at the light microscopic level were found to be of two types, the first type was obviously a mononuclear keratinocyte which had phagocytosed one or more dyskeratotic cells as described above (Plate XIX, Figure 20). The second type of giant cell appeared to be a true multinucleated cell having as many as six nuclei and abundant cytoplasm (Plate XX, Figure 21).

In six of seven lesions of Bowen's disease, a virus-like cytoplasmic particle, measuring approximately 800 to 1100 A in diameter, was demonstrated in extracellular spaces of the basal and spinous layers (Plate XXI, Figure 22). These virus-like particles appeared to form by a budding process, either from the plasma membrane (Plate XXII, Figures 23, 24, 25, 26) or from a plasma membrane-like surface within the cytoplasm. Some particles also appeared to originate from tubular structures adjacent to the plasma membrane. The earliest morphological indication of this budding sequence was an increase in the electron density and in the number of laminations of the plasma membrane. This altered area began to evaginate as the membrane increased in complexity and density. As the final structure became more evident, the plasma membrane pinched off behind the emerging particle, which was then extruded into the intercellular space. The postulated developmental sequence is depicted in Plate XXII, Figures 23, 24, 25, 26.

In each of the lesions studied, the predominant particle was extracellular, showing an outer shell consisting of alternating light and dense layers (Plate XXIII, Figure 27). The less dense nucleoid area, which appeared to be continuous with the innermost membrane, contained two or more small, electron-dense particles measuring approximately 100 A (Plate XXII, Figure 26; Plate XXIII, Figures 27, 29, 30).

During the course of this investigation, no particles of the type described have been found in 44 samples of normal skin or 12 samples of other epidermal diseases studied as controls. Furthermore, no virus-like particles were found in the one case of Bowen's disease in which there was a history of arsenic intake (see discussion).

Tissue Culture

HeLa cells were inoculated with cell-free extracts of the original tumor or with small pieces (1 mm^3) of frozen tumor. After an infection became evident, the HeLa cell monolayer and medium were sonicated, filtered and serially transferred to a new monolayer of tissue culture cells.

During the first and second cell-free passages, the HeLa cells became very granular, and after five days many (90 per cent) detached from the substrate. As each passage was completed, these gross cytopathic effects became less apparent until at the fifth passage the cells became only slightly granular and remained tightly fixed to the glass. Although the gross cellular effects decreased after each passage, the numbers and morphology of the particles apparently remained the same. Due to the large number of specimens involved for electron microscopic study, the

number of passages (five) was arbitrarily selected. Pellets of particles from the media of tissue cultures were examined by thin sectioning techniques and were found to contain many particles similar to the types described in the human tumor.

Cell specimens taken for electron microscopy from each passage were found to contain particles similar to those described above in the human primary tumor (Plate XXIV, Figures 31, 32). Occasional particles with sac-type membranes and eccentrically located nucleoids were observed clinging to the outer surface of the plasma membrane of the cell. Two extracts from normal skin did not produce the cellular effects, and no virus-like particles were observed in any cell cultures incubated with the normal skin extracts.

Chick Embryo

Inoculation of 10 day embryonated hen eggs was accomplished by a cell-free extract of the original tumor, or by cell-free supernatents from infected HeLa cells. Samples of membranes from the inoculated eggs were taken at various intervals and examined electron microscopically.

Ultrastructural examination revealed virus-like particles (800-1000 A in diameter) in the embryonic serosa (chorio-allantoic) membrane of infected eggs. Some particles were seen within the cytoplasm and budding from villous projections (Plate XXV, Figures 35, 36), while other particles were found free in extracellular space adjacent to the basal cells (Plate XXVI, Figures 39, 40). These particles were similar in size and morphology to particles found in HeLa cells infected with the cell-free filtrate and to those found in human tumors (Plate XXV, Figures 33, 34, 35, 36; Plate XXVI, Figures 37, 38, 39, 40). The extracts from normal

skin did not produce particles on the amniotic membrane or serosa of any eggs examined.

Immunology

Rabbit antiserum made against the virus-like particles obtained from tissue culture, cross-reacted with a plasma antigen in patients with Bowen's disease. This antigen-antibody reaction precipitated in agar using the Ouchterlony gel diffusion technique.

Plate XXVII, Figure 41 is a reaction of the antiserum with: (1) plasma from a Bowen's patient obtained prior to surgery, (2) plasma from the same patient obtained three months post-operative and (3) plasma from a normal control. A precipitin reaction occurred to the pre-operative plasma but not to the post-operative or control plasma. An additional series of 33 cancer and 51 non-cancer patients was assayed for antigen content.

The technique showed positive reactions on 27 of the 33 cancer patients and negative reactions on 46 of the 51 non-cancer patients. Of the five cancer patients whose plasma did not react, one was a treated Bowen's disease patient, two had acute lymphoblastic leukemia in complete remission and the remaining two patients were negative even though their disease was not arrested in any way.

In the normal group, five individuals had positive reactions, one of those patients had a five year post-operative history of a lung tumor, another patient was hospitalized for myocardial ischemia, and a third patient had a hemolytic anemia of unknown etiology. Positive reactions were obtained with the fourth and fifth patients in several trials even though a complete clinical work-up revealed no evidence of neoplasia or other diseases.

CHAPTER IV

DISCUSSION

Bowen's disease is classified as a squamous carcinoma *in situ*, which implies that the process is limited to the epithelial layer and is contained by the basement membrane. The ultrastructural discontinuity of the basement membrane in the observed cases of Bowen's disease is in contrast to the intact basement membrane found in cervical carcinoma *in situ*. However, the basement membrane damage in Bowen's disease was strikingly similar to that observed in invasive cancer of the cervix (Luibel, *et al.*, 1960). Dobson and Griffin (1962) also found basement membrane dissolution prior to invasion of experimental epidermal tumors. Ordinarily Bowen's disease is not invasive for long periods of time. This study casts doubt upon the hypothesis that the basement membrane is important in restraining the invasion of carcinoma into connective tissue.

Ultrastructurally, the dyskeratosis seen in Bowen's disease may be considered to be of two types, primary and secondary. Primary dyskeratosis occurs entirely within one cell and usually results from central displacement, aggregation and condensation of tonofilaments. These same phenomena are also seen in benign dyskeratosis (Wilgram and Weinstock, 1966). One feature of primary dyskeratosis not reported in benign disorders is interference by the dyskeratotic material with

cell division. The tonofilaments are usually separated from the mitotic apparatus by a clear zone (Olsen, et al., 1969), however in the dyskeratosis of Bowen's disease the tonofilaments appear in this area and become entangled with the spindles of the mitotic apparatus. It is unlikely that normal division can proceed to completion in such cells, and it is probably that some giant cells (Plate XX, Figure 21) are formed in this manner. Although nuclear material was observed to separate, cytoplasmic division was not seen in these cells.

Dyskeratotic plugs in various stages of disintegration were seen in many keratinocytes, these plugs were frequently lined by unit membranes and connected to the keratinocyte by desmosomes. This suggested that the dyskeratosis seen in some cells probably occurred through phagocytosis by one keratinocyte of either fully or partially keratinized material from another keratinocyte. This type of dyskeratosis may be considered to be secondary and has not been reported with benign dyskeratosis.

The acid phosphatase activity observed in dyskeratotic keratinization was closely analogous to acid hydrolase activity in normal superficial epidermis prior to normal keratinization. These observations on acid phosphatase activity suggested a role for hydrolytic enzymes in the degradation of cellular organelles prior to keratinization. The importance of a disturbed desmosomal-tonofilament relationship in abnormal keratinization has been emphasized by Caufield and Wilgram (1963) in Darier's and in Hailey-Hailey disease. They reported that condensation and retraction of tonofilaments occurred following their separation from the desmosomes and resulted in dyskeratosis. Braun-Falco and Vogell (1965)

in an opposing view have shown that apparently normal keratinization may occur in areas with abnormal desmosomal-tonofilament complexes.

The abundance of endoplasmic reticulum and Golgi zones in the presence of large numbers of abnormal mitochondria suggested very active metabolism and synthesis of cell constituents and may be related to nucleolar changes. Increased nucleolar size has been observed in a variety of tumors. This finding is not specific for cancer, however, as Nix, et al., (1965) found nuclear enlargement following ultraviolet light exposure of normal skin.

The above discussion of Bowen's disease was concerned only with the morphological characteristics of the lesions, but the occurrence of virus-like particles in six out of seven clearly defined Bowen's lesions was an unexpected finding. The only Bowen's lesion in which virus-like particles were not seen occurred in a patient who had ingested considerable amounts of arsenic, a known etiological agent in skin cancer. However, the high percentage of non-arsenical cases that contained virus-like particles suggests more than a casual relationship.

Although many biological agents have been reported in association with human neoplasms (Meesen and Schultz, 1957; Sykes, et al., 1962; Dalton, et al., 1964; Dmochowski, 1964; Lunger, et al., 1964; Porter, et al., 1964; Bryan, et al., 1965; Dmochowski, 1965a, 1965b; Dmochowski, et al., 1965; Zeve, et al., 1966; Dmochowski, et al., 1967; Harris, 1967; Sabin, 1967; Dalton, et al., 1968; Feller and Chopra, 1968; Rauscher, 1968; Stewart, et al., 1969; Nordquist, et al., 1970), no virus has been demonstrated to be the causative agent in malignant human tumors, with the possible exception of

Burkitt's lymphoma. A review of Burkitt's lymphoma by Bell in 1967 revealed that 14 different biological agents had been isolated from these tumors. The existence of multiple agents in a single tumor type indicate the hazards involved in premature conclusions.

In 1967, Huebner suggested criteria for characterization of suspected particles and proof of their identity, by which a suspected biological agent could be unequivocally defined. These criteria are in the form of questions: is this virus a true virus, can the virus be recovered by other laboratories, did the virus originate in the tissue under study, and is the virus the cause of the disease? However, human tumor viruses are not as readily studied as animal tumor viruses, and only the human papilloma virus has a proven etiological role in solid tumors of humans and has been confirmed by Huebner's criteria.

The morphology of the virus-like particles in Bowen's disease is quite similar to the virus-like particles reported by Dalton, et al., in 1968. This group described a thick-walled particle containing several subparticles in the nucleoid in sections of plasma pellets from a patient with chronic lymphocytic leukemia. These workers also showed a budding sequence in the formation of C-type particles in murine leukemia induced by the Rauscher virus. This budding sequence of a murine C-type virus is remarkably similar to that reported above in Bowen's disease (Plate XXII, Figures 23, 24, 25, 26).

The ultrastructure of the Bowen's particles is similar to the structure of a new virus group, the arenoviruses (Rowe, et al., 1970). The arenoviruses are characterized by a spherical particle which measures 1100-1300 A in diameter.

The particles are bound by a unit membrane envelope and the unstructured interior contains a variable number of electron dense particles.

The viral nature of the particles observed in Bowen's disease was assessed by: (1) distinct virus-like morphology of the particles in human tissue, (2) the appearance of similar particles in tissue culture, after inoculation with either cell-free filtrates or whole cells, (3) the observation of particles of similar size and morphology on the serosa of infected eggs, and (4) the absence of growth of any contaminating forms such as PPLO, bacteria, and yeast. Furthermore, no viruses of this type have been observed in normal skin (44 cases studied) or other epidermal lesions (12 cases studied).

Treatment of tissue culture cells with tumor extracts produced extensive cellular changes, or cytopathic effects (CPE). The apparent decrease of CPE following each passage is of considerable interest. This marked loss of CPE could be due to attenuation of the particle, leading to loss of virulence, or to a cytotoxic substance which was diluted out by the five subsequent medium changes. Although CPE was reduced after each passage, the number of virus-like particles was apparently the same after five passages.

The precipitin reaction obtained with hyperimmune rabbit antiserum to the virus-like particles grown in tissue cultures indicated that an antigen in the infected tissue cultures was common to patients with Bowen's disease and to patients with other types of neoplasia. Although the antiserum was considered to be relatively specific, the cross-reactions with plasma antigens of other cancer patients was perplexing. Originally it was assumed that the antiserum would be

specific for an antigen which could be used for typing the virus and group identification. However, the cross-reactions indicated that the same (or a closely similar) antigen was present in other neoplastic diseases. Three possibilities are proposed to explain these unexpected immunological findings: (1) the antiserum is not specific for a viral antigen but for an enzyme or a metabolic product of the tissue culture cells (which are also human tumor cells), (2) the antiserum is specific for a group of cross-reacting tumor cell membrane antigens which could be available in many types of tumors. In this case, a seemingly specific reaction could occur to many different antigens if the antibody was a "loose fit" type and the membrane antigens were closely similar or (3) the antiserum is truly specific, and the reactions showed many different human tumor types with a single etiologic agent.

The ultrastructural, virological and immunological evidence indicates that the virus particles in Bowen's disease could have a functional role in the disease process. As discussed above, the morphology of the virus is consistent with the known structure of other viruses, and the biological activity of the particle has been established in chick embryo and human tissue culture cells. Although virus particles were seen in six cases, the case with an arsenic history did not contain similar structures. This could indicate that pathological lesions that are grossly and histologically similar may be the result of two quite different agents. Since it is known that arsenic has a role in skin cancers, it is not unrealistic to assume that a similar lesion could be due to a virus.

CHAPTER V

SUMMARY AND CONCLUSIONS

The principle finding in this study on the ultrastructure, virology and immunology of Bowen's disease are: (1) frequent breaks in the subjacent basement membrane, with microinvasion of the dermis by malignant epidermal keratinocytes, (2) large numbers of dyskeratotic cells of the secondary or malignant dyskeratotic type, (3) acid phosphatase activity in lysosomes and diffuse activity in dyskeratotic areas, (4) abnormal amounts of cellular metabolic products, e.g., glycogen, (5) abnormal size and amount of cytoplasmic organelles, e.g., Golgi apparatus, endoplasmic reticulum and mitochondria, (6) decreased numbers of desmosomes and formation of rudimentary desmosomes between villous processes, (7) virus-like particles budding from the plasma membrane and free in the extracellular space of the original tumor, (8) production of similar virus-like particles in HeLa cells and chick embryo membranes after inoculation with cell-free extracts of Bowen's tumors and (9) the presence of a new antigen in patients with Bowen's disease and the observation of a similar antigen in patients with other types of neoplasia.

The morphological observations reported herewithin are consistent with malignant cells that are programmed for rapid synthesis of structural components

and equally rapid mitosis. It is not unrealistic to suggest that this sequence of events could be triggered by a virus. The frequency of cases containing viruses (85.9 per cent) indicates that a causal relationship must be considered, the added observations of a new antigen in the host and in tissue cultures strongly support this hypothesis.

REFERENCES

Anderson, N. P. 1932 Bowen's precancerous dermatosis and multiple benign superficial epithelioma: evidence of arsenic as an etiologic agent. Arch. Dermat. Syph., 26:1052.

Barka, T. and Anderson, P. J. 1963 Histochemistry: Theory, Practice and Bibliography. Harper and Row, Inc., New York.

Bell, T. M. 1967 Viruses associated with Burkitt's tumor. Progr. Med. Virol., 9:1.

Bernhard, W. 1960 The detection and study of tumor viruses with the electron microscope. Cancer Res., 20:712.

Bittner, J. J. 1936 Some possible effects of nursing on the mammary gland tumor incidence in mice. Science, 84:162.

Borrell, A. 1907 Le probleme du cancer. Bull. Inst. Pasteur, 5:497.

Bowen, J. T. 1912 Precancerous dermatoses: a study of two cases of chronic atypical epithelial proliferation. J. Cutan. Dis., 30:241.

Bowen, J. T. 1920 Precancerous dermatoses: the further course of two cases previously described. Arch. Dermat. Syph., 1:23.

Braun-Falco, O. and Vogell, W. 1965 Electron microscopic investigation of dynamics of acantholysis in Pemphigus Vulgaris. Arch. Klin. Exptl. Dermatol., 223:328.

Bryan, W. R., Moloney, J. B., O'Connor, T. E., Fink, M. A. and Dalton, A. J. 1965 Viral etiology of leukemia. Ann. Internal Med., 62:376.

Caulfield, J. B. and Wilgram, G. F. 1963 An electron microscope study of dyskeratosis and acantholysis in Darier's disease. J. Invest. Dermatol., 41:57.

Dalton, A. J., Maloney, J. B., Porter, G. H., Frei, E. and Mitchell, E. 1964 Studies on murine and human leukemia. Trans. Assoc. Am. Physicians, 77:52.

Dalton, A. J., Rowe, W. P., Mitchell, E. Z. and Pugh, W. E. 1968 Detection of virus particles in leukemia - lymphoma by electron microscopy. In: Proceedings of the International Conference on Leukemia - Lymphoma. Lea and Febiger, Philadelphia.

Dmochowski, L. 1964 Viruses as related to cancer. Proc. Natl. Cancer Conf., 5:57.

Dmochowski, L. 1965a Viruses and their relationship to animal and human tumors. Texas Rept. Biol. Med., 23:541.

Dmochowski, L. 1965b Electron microscopic observations of leukemia in animals and man. Cancer Res., 25:1654.

Dmochowski, L., Taylor, H. G., Grey, C. E., Dreyer, D. A., Sykes, J. A., Langford, P. L., Roger, T., Schullenberger, C. C. and Howe, D. D. 1965 Viruses and mycoplasma (PPLO) in human leukemia. Cancer, 18:1345.

Dmochowski, L., Grey, C. E., Langford, P. L., Williams, W. C., Sykes, J. A., Young, E. L. and Migliore, P. J. 1967a Viral factors in mammary tumorogenesis. In: Carcinogenesis: A Broad Critique. Williams and Wilkins Co., Baltimore.

Dmochowski, L., Dreyer, D. A., Grey, C. E., Hales, R., Langford, P. L., Pipes, F., Recher, L., Seman, G., Shively, J. A., Schullenberger, C. C., Sinkovics, J. G., Taylor, H. G., Tessmer, C. F., and Yumoto, T. 1967b Studies on the submicroscopic morphology of structures resembling mycoplasma and virus particles in mice and men. Ann. N. Y. Acad. Sci., 143:578.

Dobson, R. L. and Griffin, M. 1962 Histochemistry of cutaneous carcinogenesis. J. Invest. Dermat., 39:597.

Ellerman, V. and Bang, O. 1908 Experimentelle leukamie bei huhnern. Zbl. Bakt., 46:595.

Epstein, M. A., Achong, B. G. and Barr, Y. M. 1964 Virus particles in cultured lymphoblasts from Burkitt's lymphoma. Lancet, I:702.

Epstein, M. A., Henle, G., Achong, B. G. and Barr, Y. M. 1965 Morphological and biological studies on a virus in cultured lymphoblasts from Burkitt's lymphoma. J. Exp. Med., 121:761.

Feller, W. F. and Chopra, H. C. 1968 A small virus-like particle observed in human breast cancer by means of electron microscopy. J. Natl. Cancer Inst., 40:1359.

Fergusson, A. G., Dewar, W. A. and Smith, H. 1960 Arsenic values in various skin diseases. Arch. Dermat. Syph., 81:931.

Geering, G., Hardy, W. D., Jr., Old, L. J. and DeHarven, E. 1968 Shared group-specific antigen of murine and feline leukemia viruses. Virology, 36:678.

Graham, J. H. and Helwig, E. B. 1959 Bowen's disease and its relationship to systemic cancer. Arch. Dermat. Syph., 80:133.

Graham, J. H., Mazzanti, G. R. and Helwig, E. B. 1961 Chemistry of Bowen's disease: relationship to arsenic. J. Invest. Dermat., 37:317.

Gross, L. A. 1951 Spontaneous leukemia developing in C3H mice following inoculation in infancy with AK - leukemia extracts. Proc. Soc. Exp. Biol. Med., 76:27.

Gross, L. A. 1953 Presence of a leukemia agent in normal testis and ovaries of young mice of AK line. Acta Haemat., 10:18.

Gross, L. A. 1955 Mouse leukemia: eggborne viruses disease (with a note on mouse salivary gland carcinoma). Acta Haemat., 13:12.

Gross, L. A. 1958 High susceptability of 1 to 14 day old C3H mice to "passage A" leukemic filtrates. Proc. Soc. Exp. Biol. Med., 97:300.

Harris, R. J. C. 1967 A virus etiology for Burkitt's tumor. Intern. J. Cancer, 2:559.

Hinz, R. W., Bowles, C. A., Conner, G. H., Mitchell, J. R. and Anderson, G. R., 1968 Characterization of a Herpes - like virus recovered from Burkitt lymphoma cells (P3-J) and propagated in dog thymus cells. I. An electron microscopic study. J. Natl. Cancer Inst., 40:477.

Huebner, R. J. 1967 In vitro methods for detection and assay of leukemia virus. In: Carcinogenesis: A Broad Critique. The Williams and Wilkins Co., Baltimore.

Hugo, H. E. and Conway, H. 1967 Bowen's disease: its malignant potential and relationship to systemic cancer. Plastic Reconstruc. Surg., 39: 190.

Jarrett, O., Laird, H. M. and Hay, D. 1969 Growth of feline leukaemia virus in human cells. Nature, 224:1208.

Kelly, J. W. 1962 Histochemistry and Cytochemistry Notes. Published by the Dept. of Pathology, University of Oklahoma School of Medicine.

Lever, W. F. 1967 Tumors of the surface epidermis. In: Histopathology of the Skin. J. B. Lippincott Co., Philadelphia.

Lucké, B. 1938 Carcinoma in the Leopard frog: its probable causation by a virus. J. Exp. Med., 68:457.

Luibel, F. L., Sanders, E. and Ashworth, C. T. 1960 An electron microscopic study of carcinoma in situ and invasive carcinoma of the cervix uteri. Cancer Res., 20:357.

Lunger, P. D., Lucas, J. C., Jr., and Shipkey, F. H. 1964 The ultra morphology of milk fractions from normal and breast cancer patients. Cancer, 17:549.

Maruyama, K., Dmochowski, L., Romero, J. J. and Swearingen, G. R. 1968 A virus containing buffy-coat cell line (MA-1) derived from a patient with chronic thyroiditis and thyroid carcinoma. Med. Res. Annals, 61:388.

Meesen, H. and Schultz, H. 1957 Electronmikroskopischer nachweis des virus in kehlkopfpapillom des menschen. Klin. Wchnschr., 35:771.

Morton, D. L., Malmgren, R. A., Hall, W. T. and Schidlovsky, G. 1969 Immunologic and Virus studies with human sarcoma. Surgery, 66: 152.

Nix, T. E., Jr., Nordquist, R. E., Scott, J. R. and Everett, M. A. 1965 An ultrastructural study of nucleolar enlargement following ultraviolet irradiation of human epidermis. J. Invest. Dermat., 45:114.

Nordquist, R. E., Olson, R. L., Everett, M. A. and Condit, P. T. 1970 Virus-like particles in Bowen's disease. Cancer Res., 30:288.

Olson, R. L., Nordquist, R. E. and Everett, M. A. 1969 Mitosis in human epidermis - an ultrastructural study. Dermatologica, 138:268.

Ouchterlony, O. 1948 In vitro method for testing the toxic-producing capacity of diptheria bacteria. Acta Path. Microbiol. Scand., 25:186.

Porter, G. H., iii, Dalton, A. J., Moloney, J. B. and Mitchell, E.Z. 1964

Association of electron dense particles with human acute leukemia. J. Nat. Cancer Inst., 33:547.

Rauscher, F. J., Jr. 1968 The search for etiologic agents in human leukemia and lymphoma. In: Phenary Session Papers, XII Congress International Society of Hematology, New York.

Reynolds, E. S. 1963 The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol., 17:208.

Rowe, W. P., Murphy, F. A., Bergold, G. H., Casals, J., Hotchin, J., Johnson, K. M., Lehmann-Brube, F., Mims, C. A., Traub, E. and Webb, P. A. 1970 Arenoviruses: proposed name for a newly described virus group. Virology, 5:651.

Rous, P. 1910 A transmissible avian neoplasm (sarcoma of the common fowl). J. Exptl. Med., 12:696.

Rous, P. 1911 Transmission of a malignant new growth by means of a cell-free filtrate. J. Am. Med. Assoc., 56:198.

Sabatini, D. D., Bensch, K. and Barnett, R. J. 1963 Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity. J. Cell Biol., 17:19.

Sabin, A. B. 1967 Search for viral etiology of human leukemia and lymphomas. Past efforts and future perspective. In: Carcinogenesis: A Broad Critique. The Williams and Wilkins Co., Baltimore.

Seman, G. and Seman, D. 1968 Electron microscopic search for virus particles in patients with leukemia and lymphoma. Cancer, 22:1033.

Shope, R. E. 1932 A transmissible tumor-like condition in rabbits. J. Exp. Med., 56:803.

Shope, R. E. 1966 Evolutionary episodes in the concept of viral oncogenesis. Perspect. Biol. Med., 9:258.

Smith, K. O. Benyesh-Melnick, M., and Fernbach, D. J. 1964 Studies on human leukemia. II. Structure and quantitation of myxovirus-like particles associated with human leukemia. J. Nat. Cancer Inst., 33: 557.

Stewart, S. E., Mitchell, E. Z., Whang, J. J., Dunlop, W. R., Ben, T., and Nomura, S. 1969 Viruses in human tumors. I. Hodgkins' disease. J. Nat. Cancer Inst., 43:1.

Sykes, J. A., Dmochowski, L., Schullenberger, C. C. and Howe, C. D. 1962 Tissue culture studies on human leukemia and malignant lymphoma. Cancer Res., 22:21.

Vorbrodt, A. 1961 Histochemical studies on the intracellular localization of acid deoxyribonuclease. J. Histochem. Cytochem., 9:647.

Wilgram, G. F., Caulfied, J. B., and Madgic, E. B. 1964 A possible role of the desmosome in the process of keratinization. In: The Epidermis. Academic Press, New York.

Wilgram, G. F. and Weinstock, A. 1966 Advances in genetic dermatology. Arch. Derm., 94:456.

Yeh, S. 1963 Relative incidence of skin cancer in Chinese in Taiwan: with special reference to arsenical cancer. In: Conference on Biology of Cutaneous Cancer. National Cancer Monograph #10, United States Dept. of H. E. W., Bethesda, Maryland.

Zeve, V. H., Lucas, L. S., and Manaker, R. A. 1966 Continuous cell culture from a patient with chronic myelogenous leukemia. II. Detection of a Herpes-like virus by electron microscopy. J. Nat. Cancer Inst., 37: 761.

APPENDIX

FIGURE LEGEND

The figures shown are micrographs selected from a series of more than 2000 plates. The original micrographs were taken between 3000 and 100,000 times and enlarged to the desired magnification.

List of Abbreviations

AO	after operation
AS	antiserum
BM	basement membrane
BO	before operation
D	dermis
DC	dermal cell
De	desmosome
DKC	dyskeratotic cell
E	epidermis
ER	endoplasmic reticulum
FK	focal keratinization
G	Golgi zone
Gly	glycogen
IP	invading process

L	lysosomes
M	mitochondria
MC	mononuclear cell
MD	melanin containing dendrite
Me	melanin
N	nucleus
No	nucleolus
RDe	rudimentary desmosome
T	tonofibrils

PLATE I

Figure 1. Dermal-epidermal junction showing invasive projections (IP) into the dermis. The basal and spinous cell nuclei (N) are convoluted and thickened, some of which contain large nucleoli (No). Note the widened intercellular spaces and lack of normal desmosomes. 10,000x.

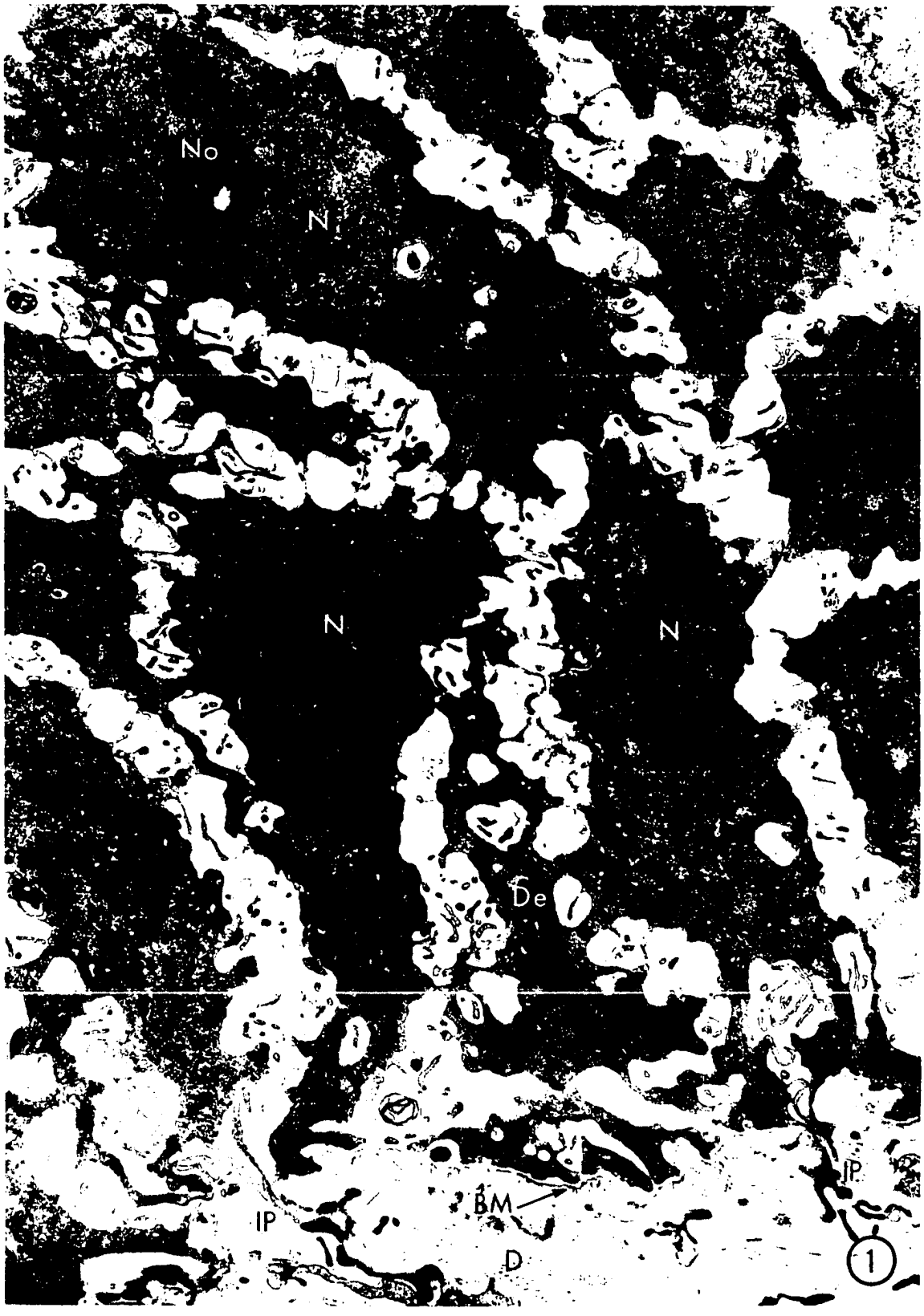

No
N
N
N
De
IP
BM
D
IP
1

PLATE II

Figure 2. Dermal-epidermal junction exhibiting an epidermal villous process (IP) penetrating the basement membrane (BM). 80,000x.


BM
IP
D
BM
2

PLATE III

Figure 3. Dermal-epidermal junction showing a basal cell process which has penetrated the basement membrane and invaded the adjacent dermis. 80,000x.


B
N
D
IP
B
3

PLATE IV

Figure 4. Dermal-epidermal junction demonstrating dermal invasion by two adjacent cell processes. 22,500x.


MC
BM
BM

PLATE V

Figure 5. Dermal-epidermal junction exhibiting basement membrane penetration and dermal invasion by three separate cells. Note that the invading process (IP) of one keratinocyte is in direct contact with a dermal cell. 22,500x.


De
BM
BM
IP
D
5

PLATE VI

Figure 6. Dermal-epidermal junction at a location of complete dissolution of the basement membrane. The basement membrane (BM) is evident in only one small area. Large numbers of mononuclear cells are present and are assumed to be part of the inflammatory process. 10,000x.

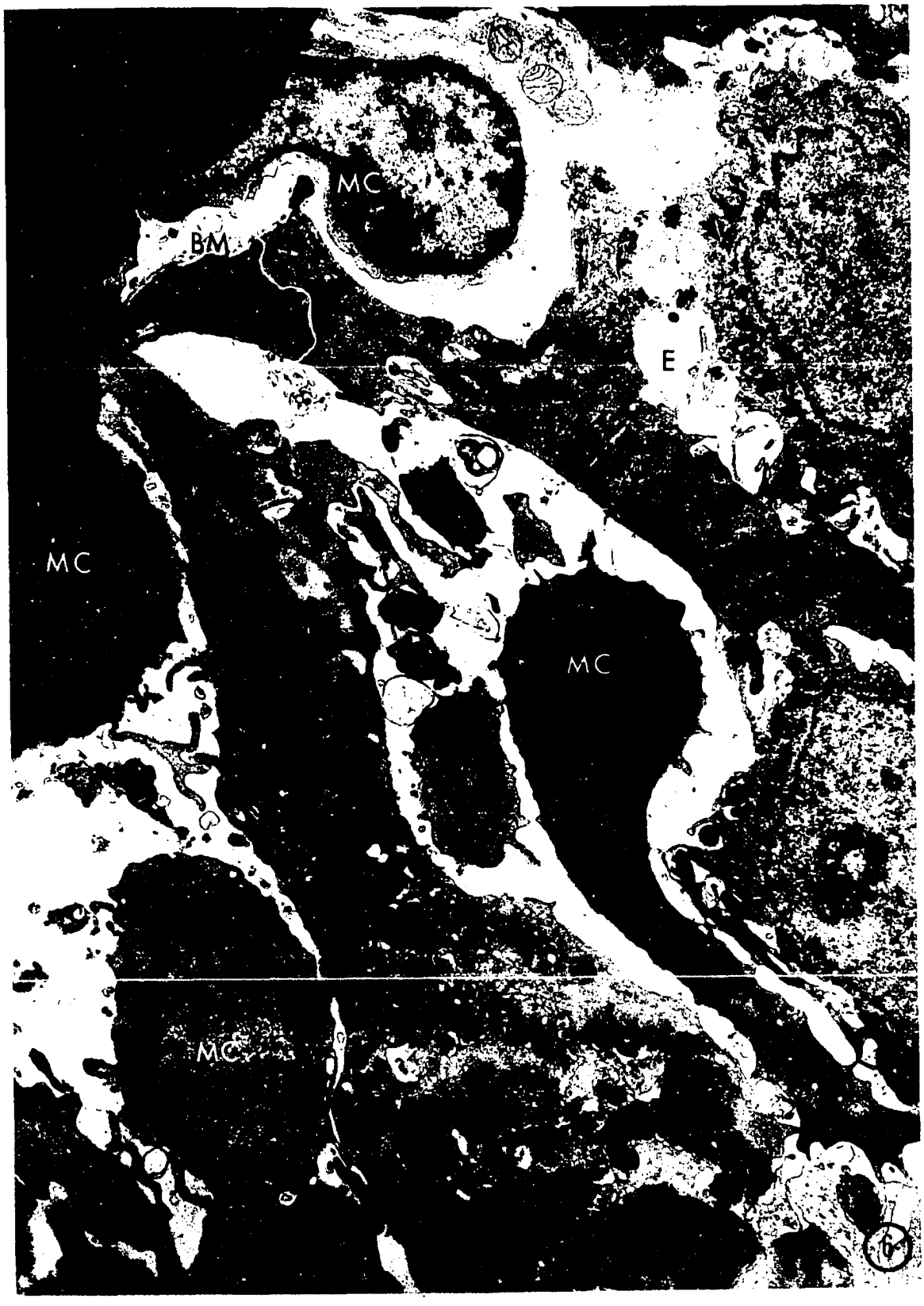

MC
BM
E
MC
MC
MC

PLATE VII

Figure 7. Dermal-epidermal junction showing a monocyte (MC) invading the epidermis through a break in the basement membrane. 17,600x.

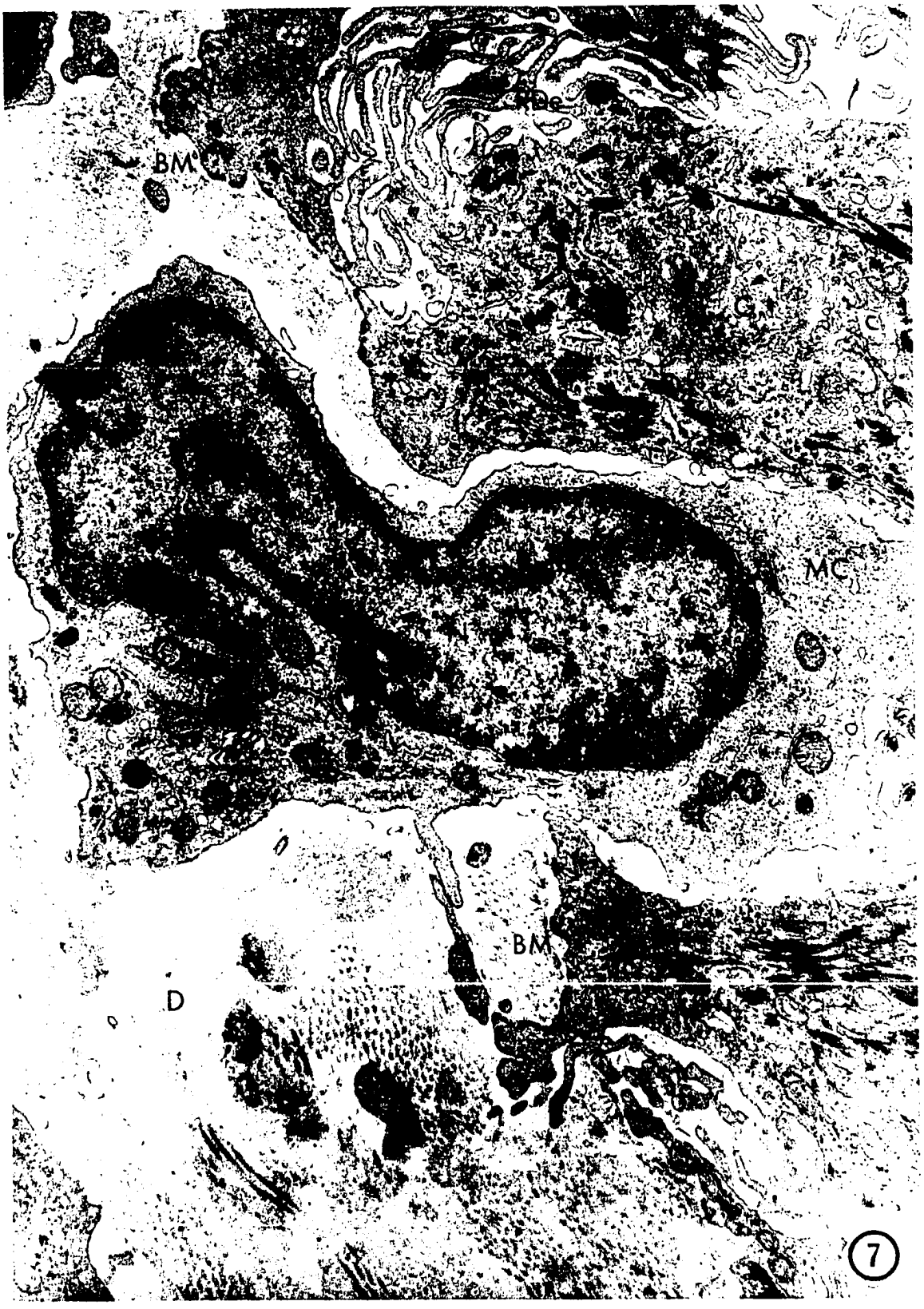

BM
De
MC
BM
D
7

PLATE VIII

Figure 8. Basal and lower spinous layers demonstrating the lack of melanin, widened intercellular spaces and loss of mature desmosomes. Melanin is present in a dendrite adjacent to the basement membrane. Note the melanosomes are single and are not in lysosome-like structures. 14,000x.


N
No
N
N
N
M
MD
RDe
M
BM
D

PLATE IX

Figure 9. Spinous cell from normal epidermis demonstrating normal numbers of mitochondria and other organelles. Tonofilaments are arranged in a peripheral halo and are separated from the nucleus by a clear zone. The nuclear membrane is smooth and thin and the nuclear contents are finely dispersed. Although the nucleolus is present, the nucleolonema is not distinct. Note the narrow intercellular spaces and moderate numbers of desmosomes. 14,000x.


De
No
T
M
9

PLATE X

Figure 10. Spinous cells showing large numbers of mitochondria (M) and many Golgi zones (G), and a few short, unorganized tonofilaments. 14,000x.

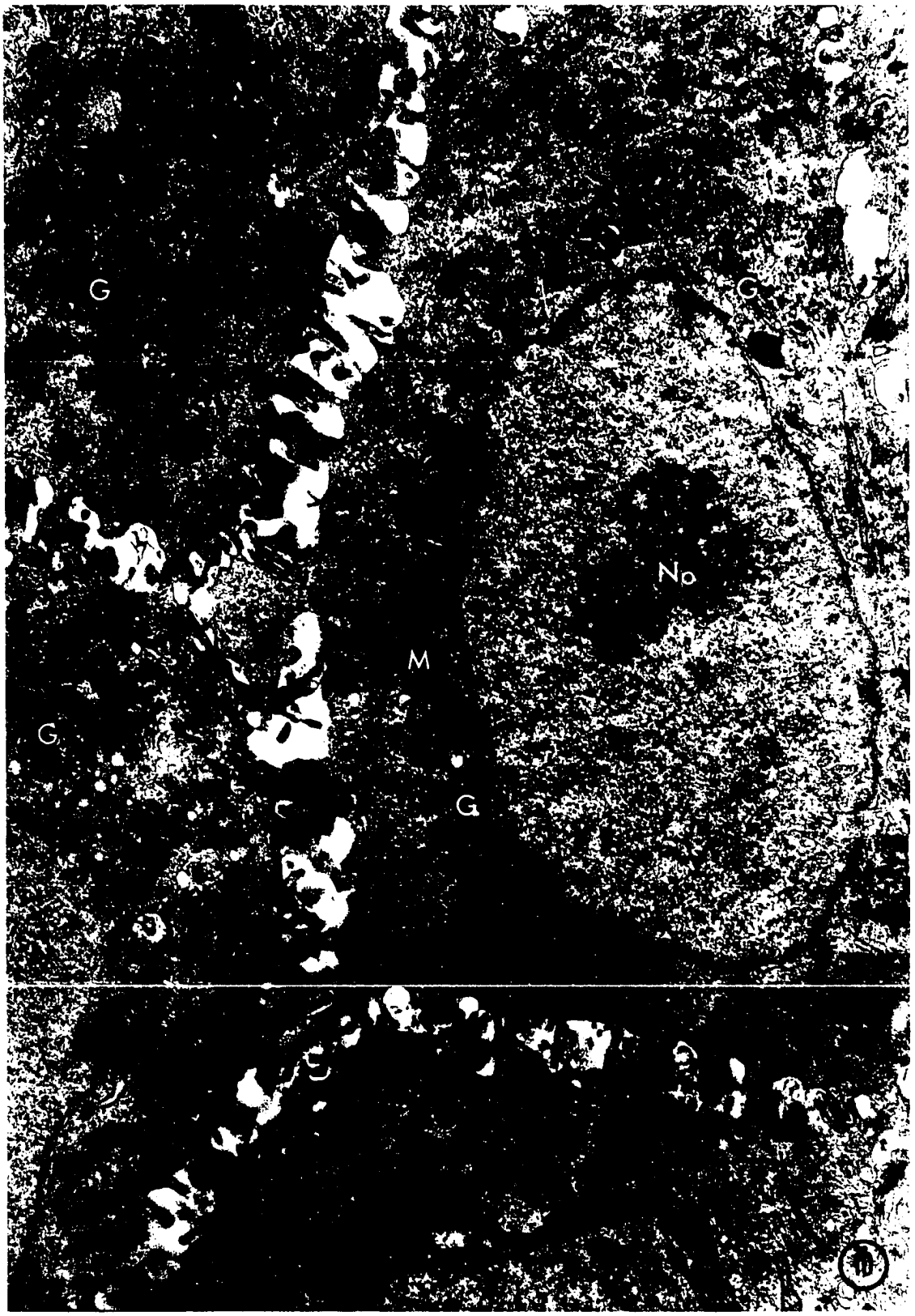

G
G
No
M
G
G

PLATE XI

Figure 11. Spinous cell exhibiting large, pleomorphic mitochondria (M) and condensed tonofilaments. 38,000x.

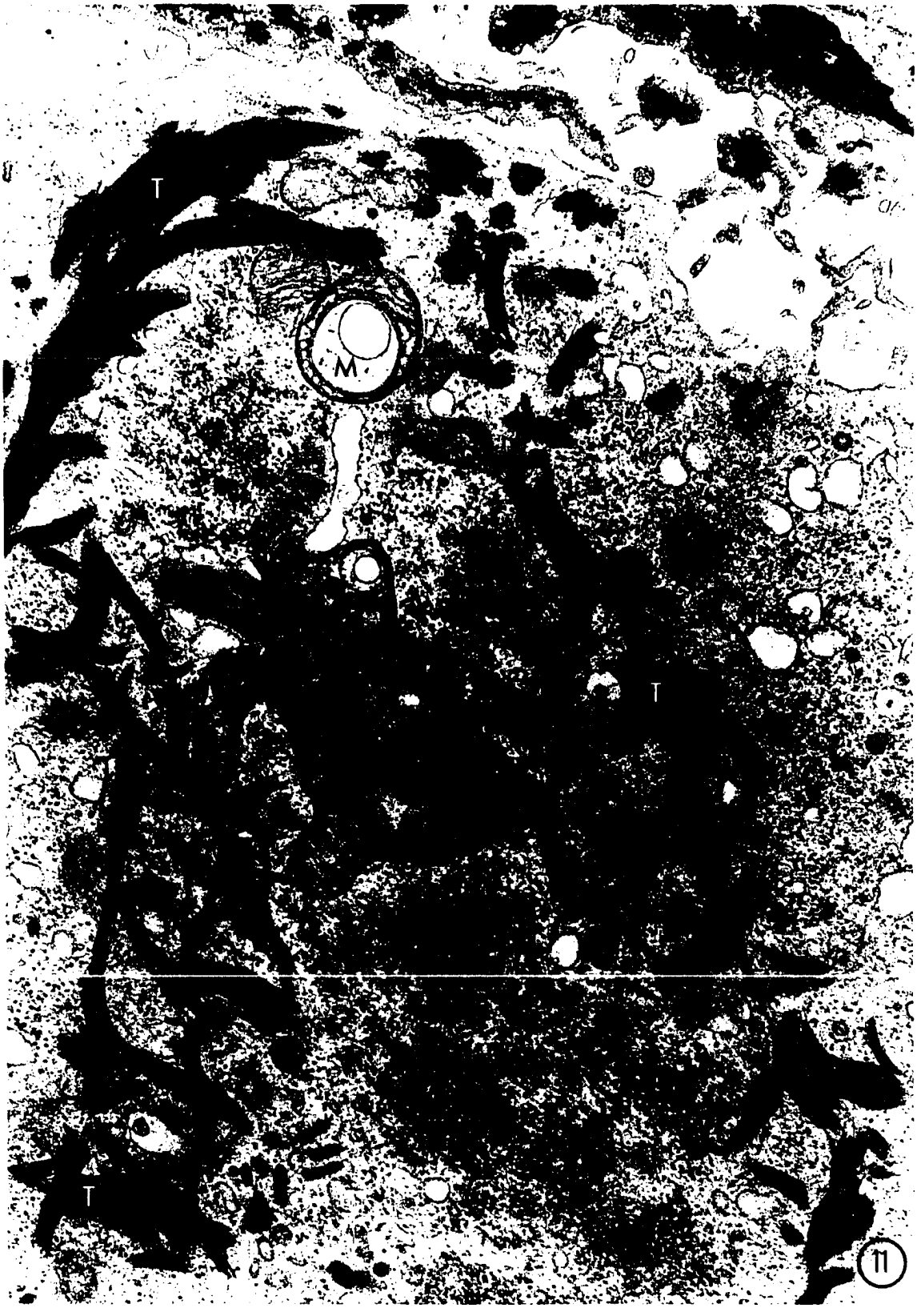

T
M
T
T
11

PLATE XII

Figure 12. Spinous cell containing accumulations of beta glycogen (Gly) in membrane bound bodies adjacent to the nucleus (N). 71,000x.

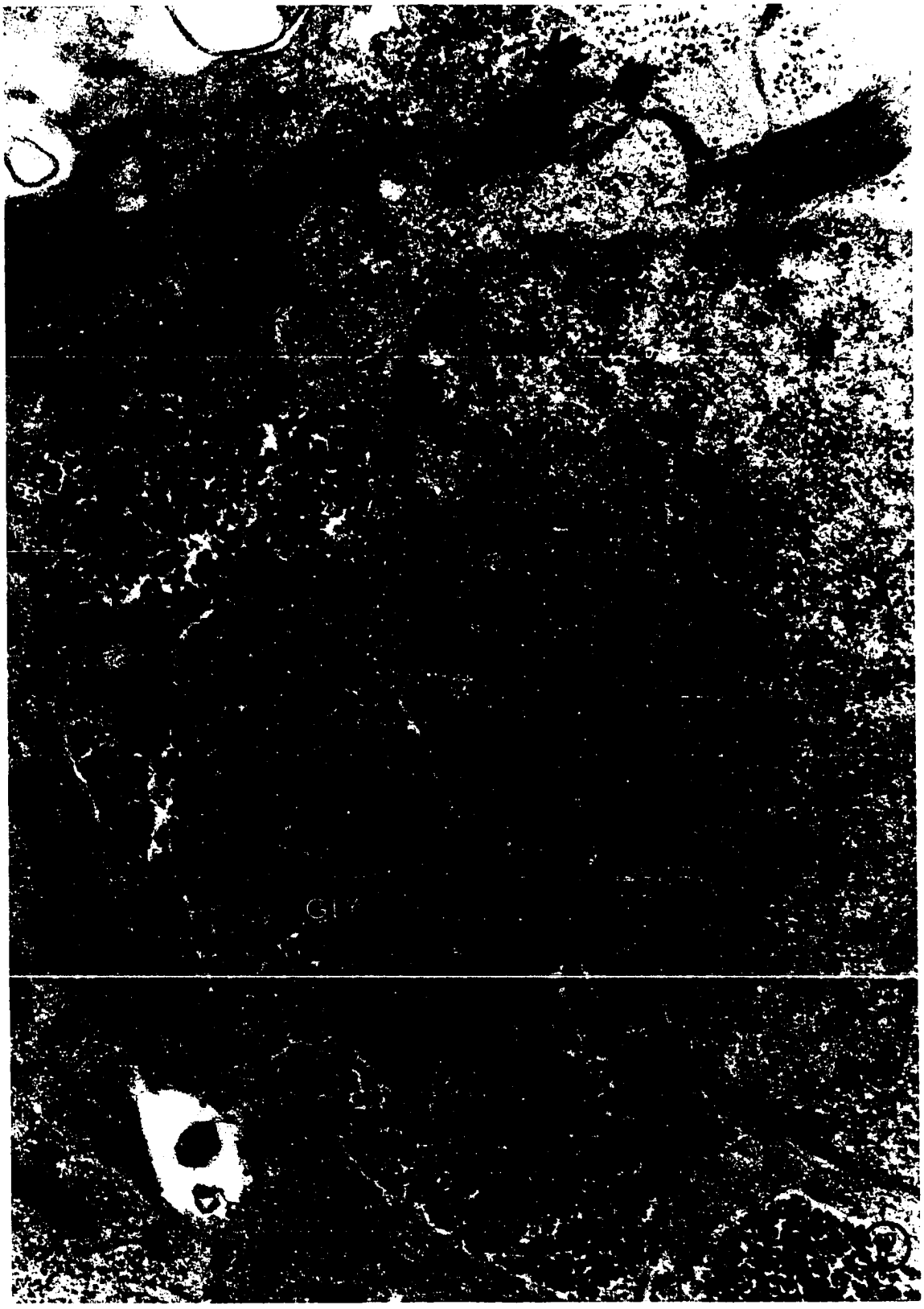


PLATE XIII

Figure 13. Keratinocyte in the spinous layer which contains many lysosome-like structures (L), some of these membrane bound bodies have accumulations of melanosomes (ME). 27,000x.


Me
L
Me
L
L
N
13

PLATE XIV

Figure 14. Spinous keratinocyte exhibiting the two types of keratinization, one of which is a phagocytized dyskeratotic cell (DKC), the other, a focal keratinization (FK) of the keratinocyte cytoplasm. 13,500x.

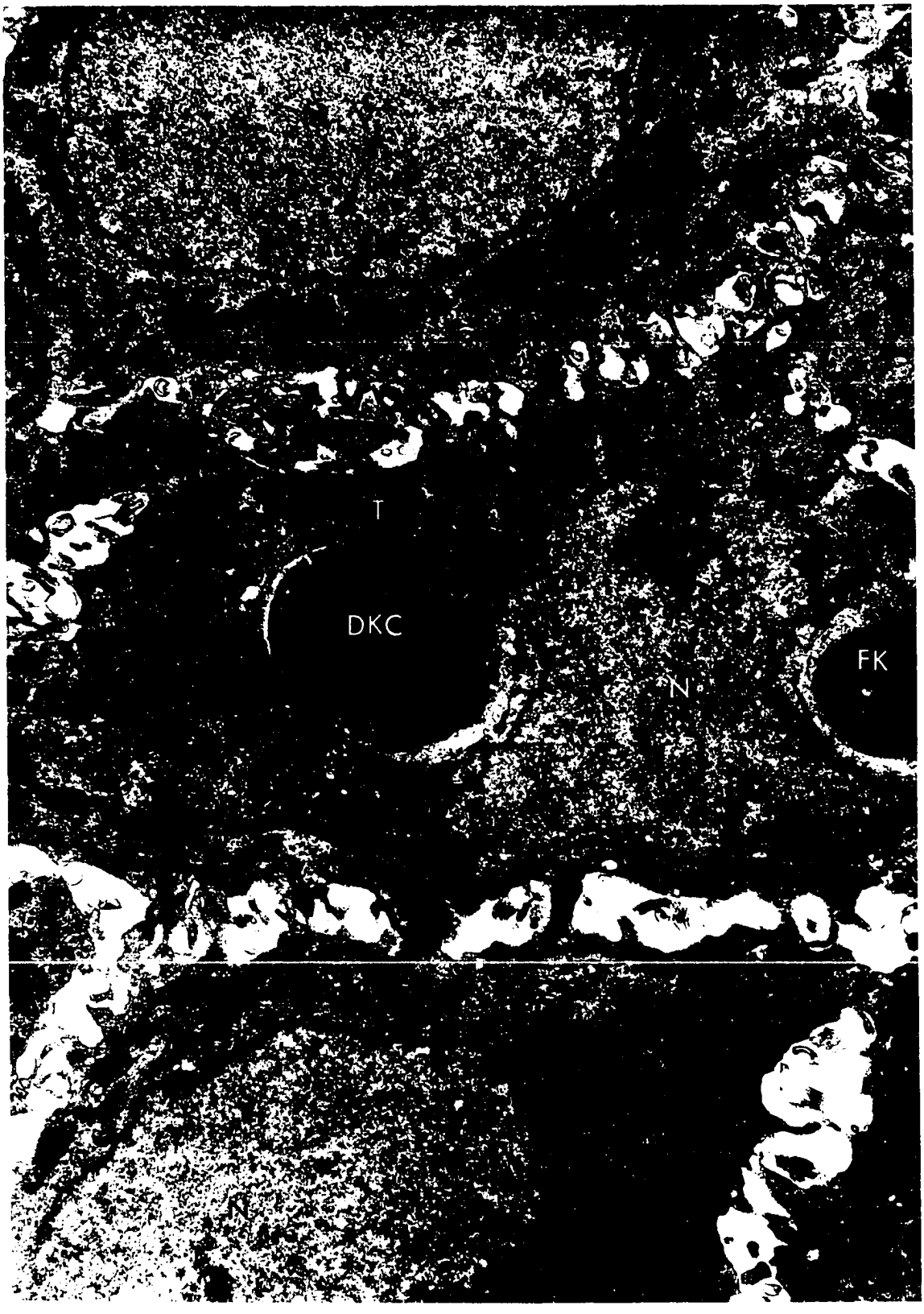

T
DKC
N
FK

PLATE XV

Figure 15. Spinous keratinocyte which contains a discrete area of keratinization (FK) in the cytoplasm. 14,000x.


ER
FK
15

PLATE XVI

Figure 16. Spinous keratinocyte, the cytoplasm of which contains a phagocytized dyskeratotic cell. Note the considerable difference in the density of the two cells, the dyskeratotic cell seems greatly condensed although the nucleus is still apparent. 12,000x.

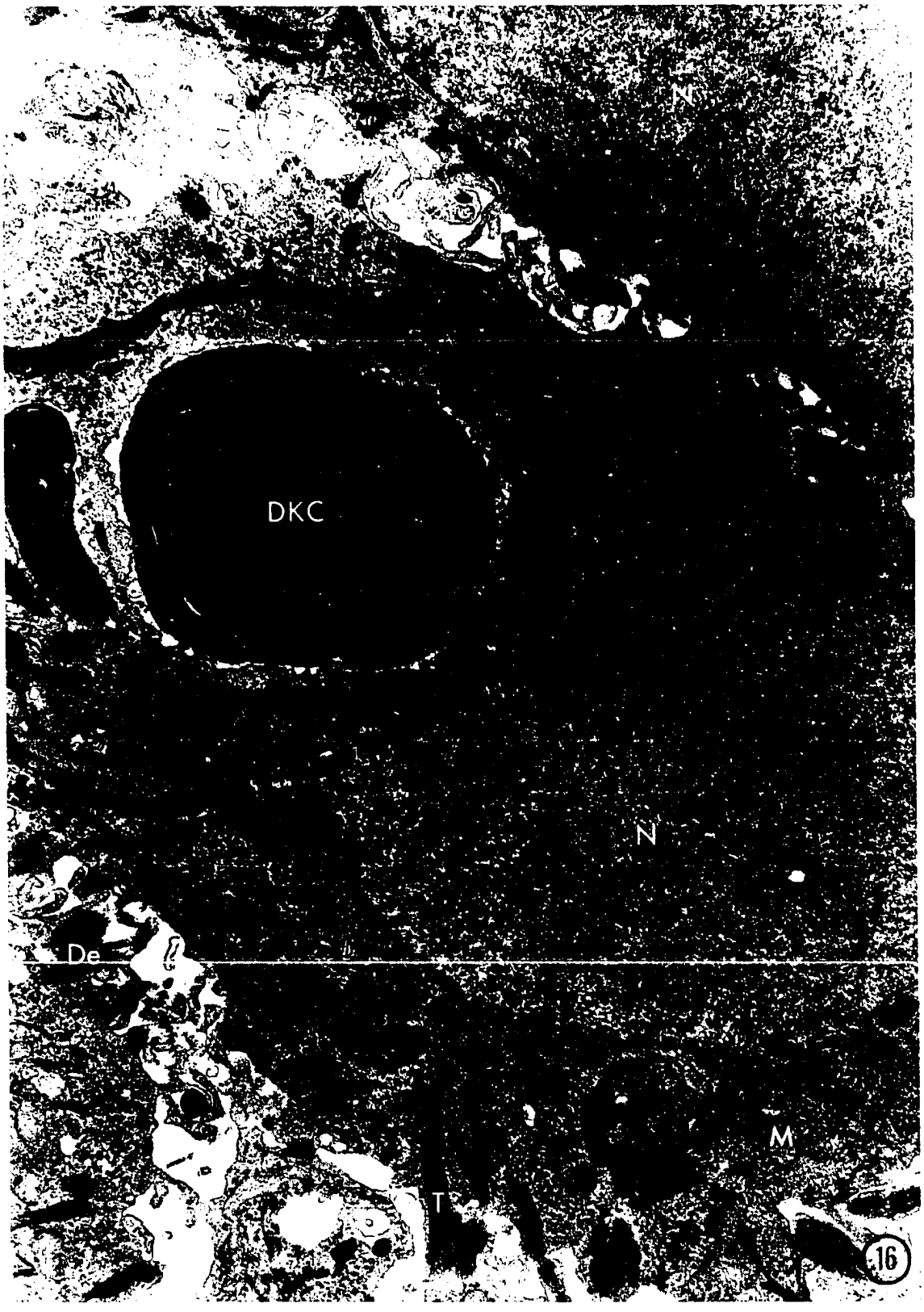

M
DKC
N
De
M
T
16

PLATE XVII

Figure 17. Spinous keratinocyte which contains a dyskeratotic cell and even though a condensation has occurred, the nucleus and cytoplasmic organelles are still discernible. 30,000x.

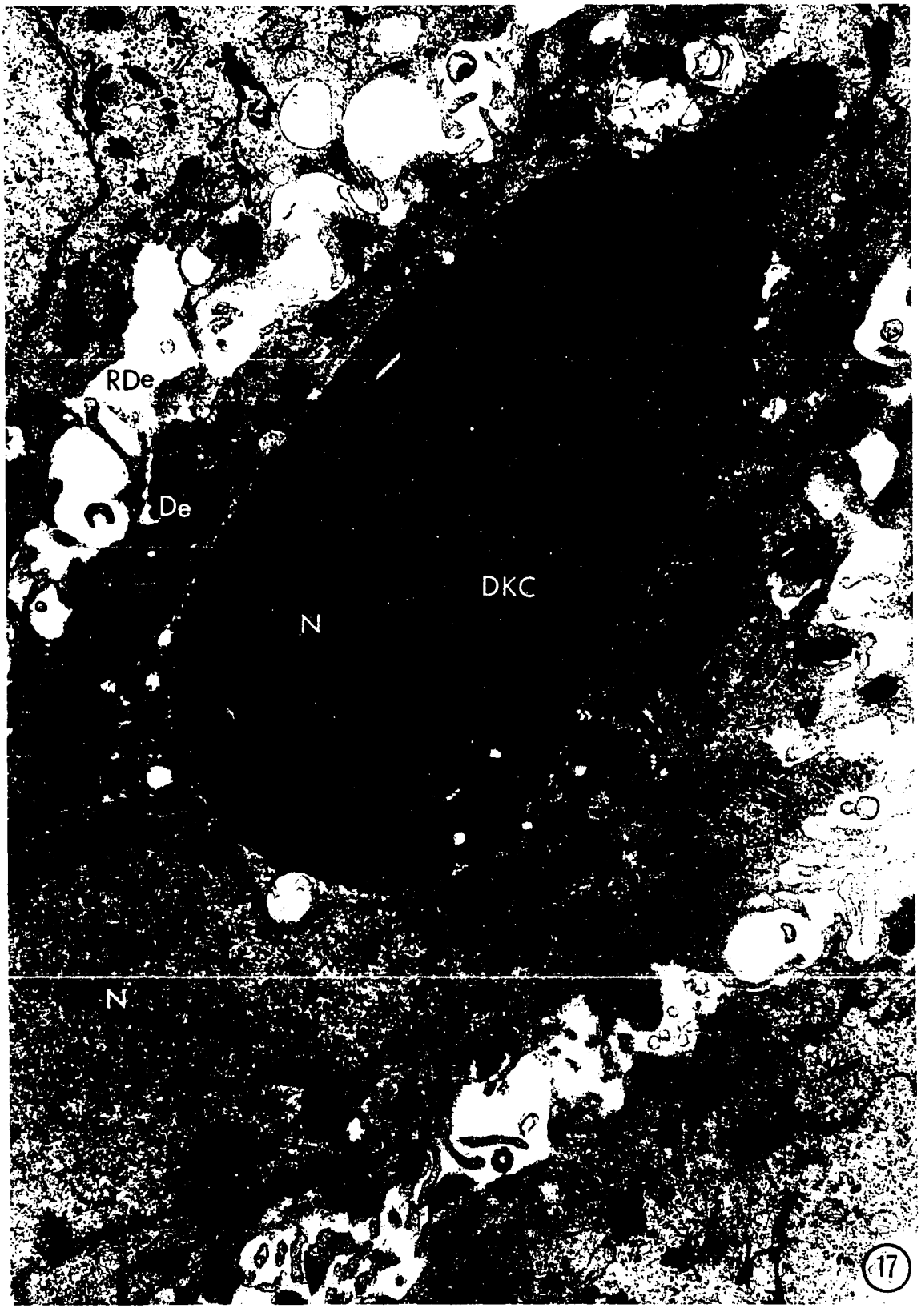
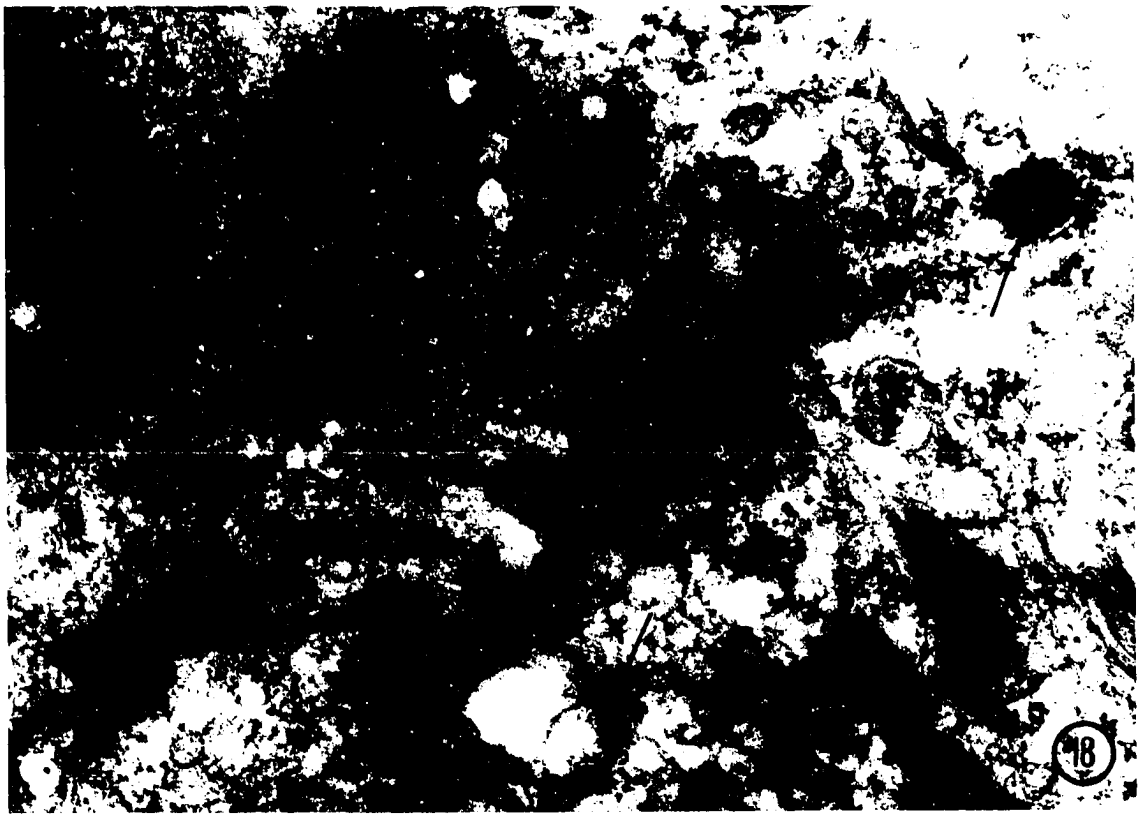

RDe
De
DKC
N
N
17

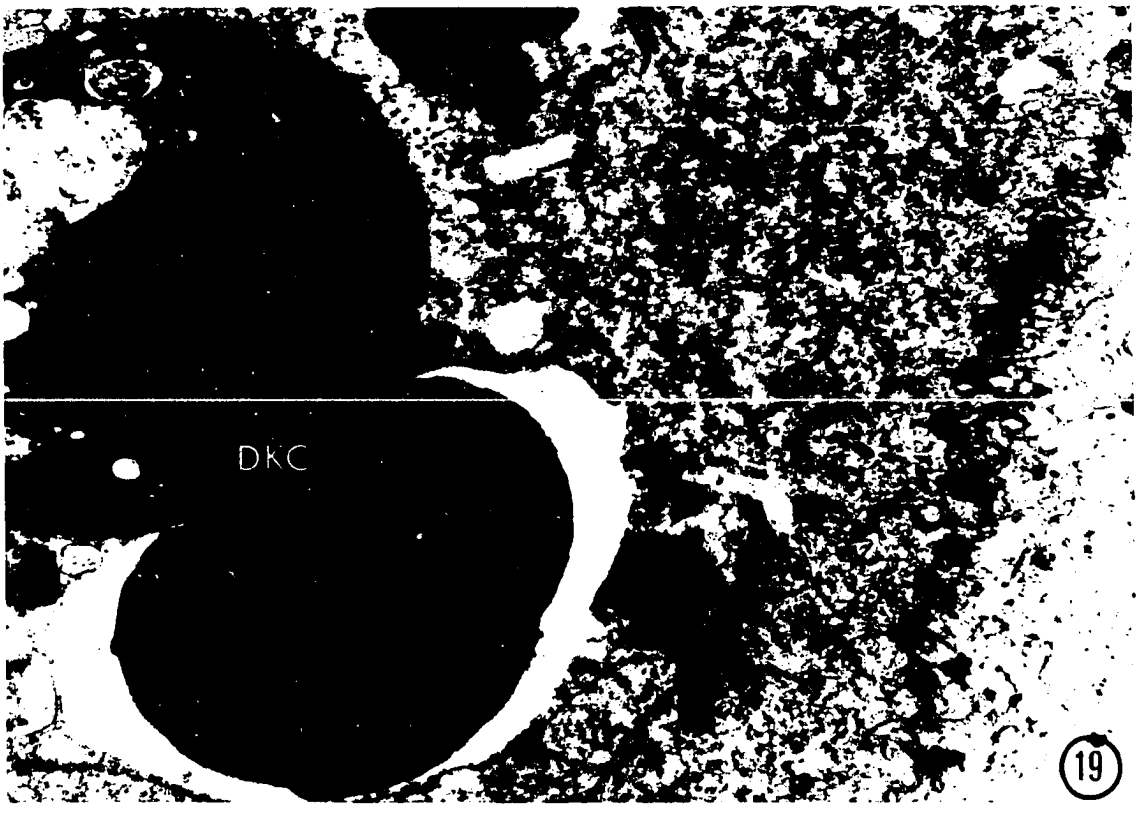
PLATE XVIII

Figure 18. Acid phosphatase reaction, the product of the reaction is localized to discrete membrane bound bodies indicated by arrows. 63,000x.

Figure 19. Acid phosphatase reaction, a dyskeratotic cell is present in the cytoplasm of another cell, the reaction product can be seen diffusely distributed on the dyskeratotic cell. 37,000x.



18



19

PLATE XIX

Figure 20. Spinous keratinocyte which contains a newly phagocytized cell in which all organelles are still present and the nucleus and nucleolus are intact. 13,500x.

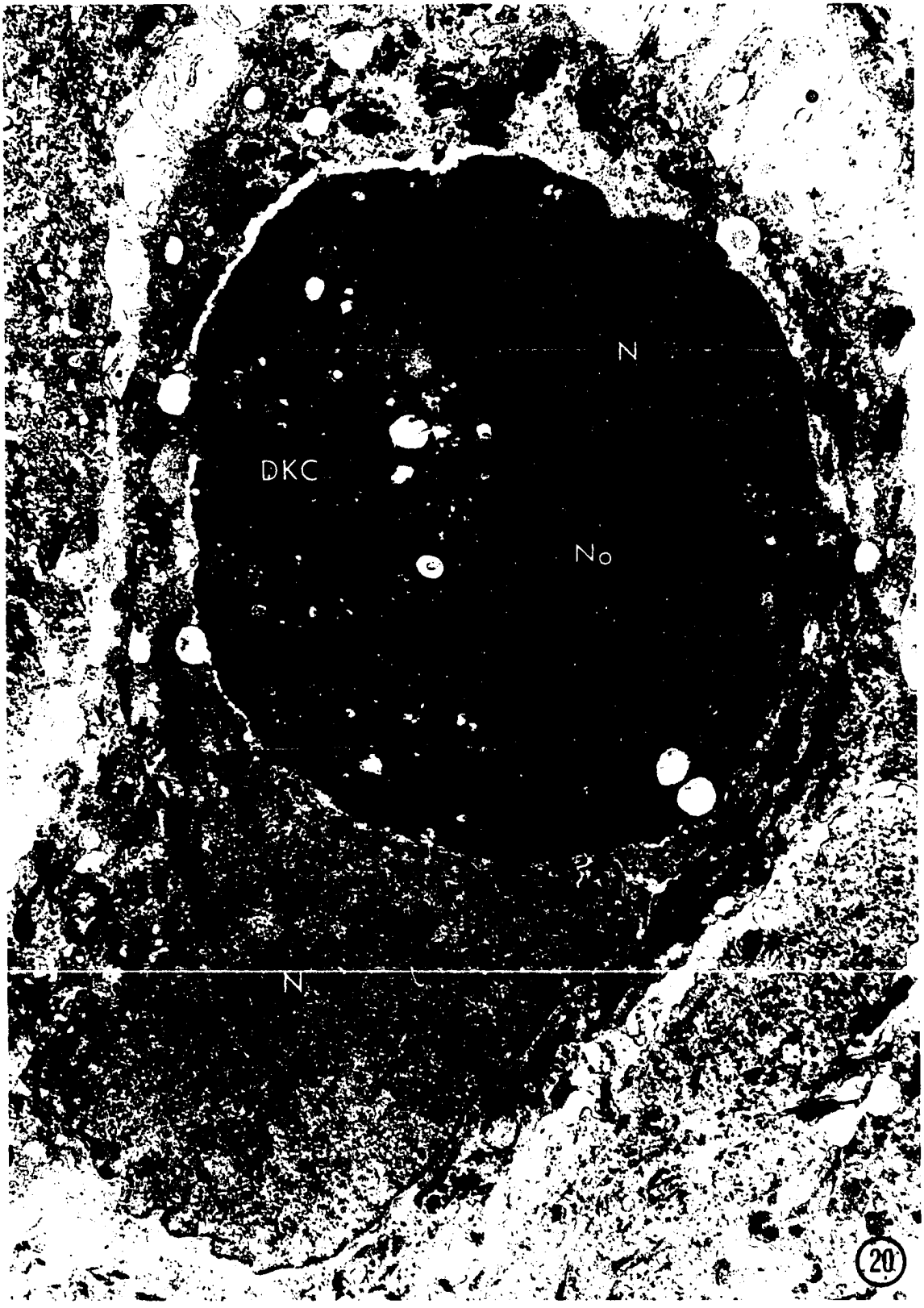

N
DKC
No
N
20

PLATE XX

Figure 21. Giant cell with four individual nuclei, three of which contain large nucleoli. The tonofilaments are aggregated and are no longer individual. 13,500x.

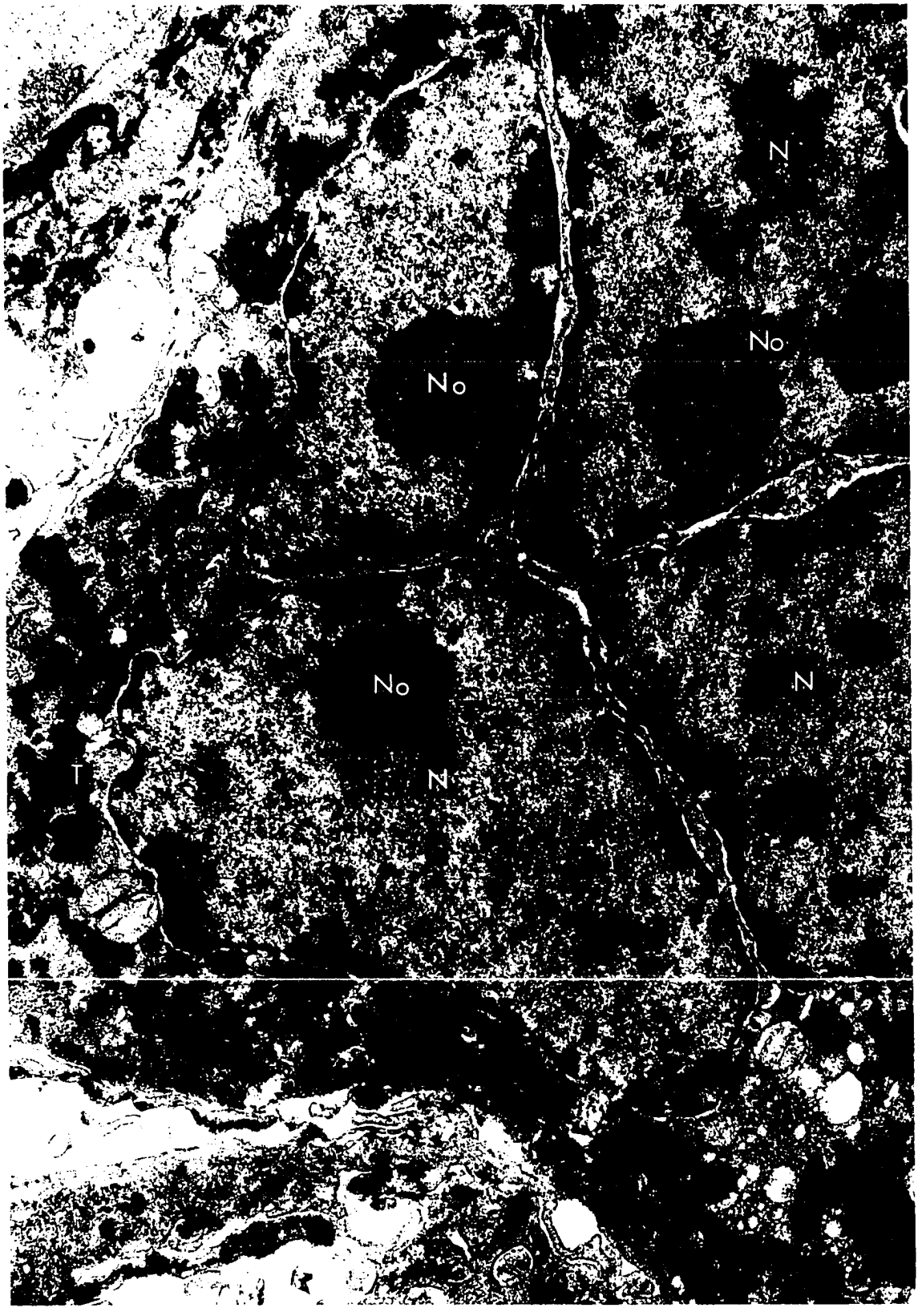

N
No
No
No
N
N
T

PLATE XXI

Figure 22. Spinous layer showing the frequency of extracellular virus particles. The virus particles are located in the dark circles. 10,000x.

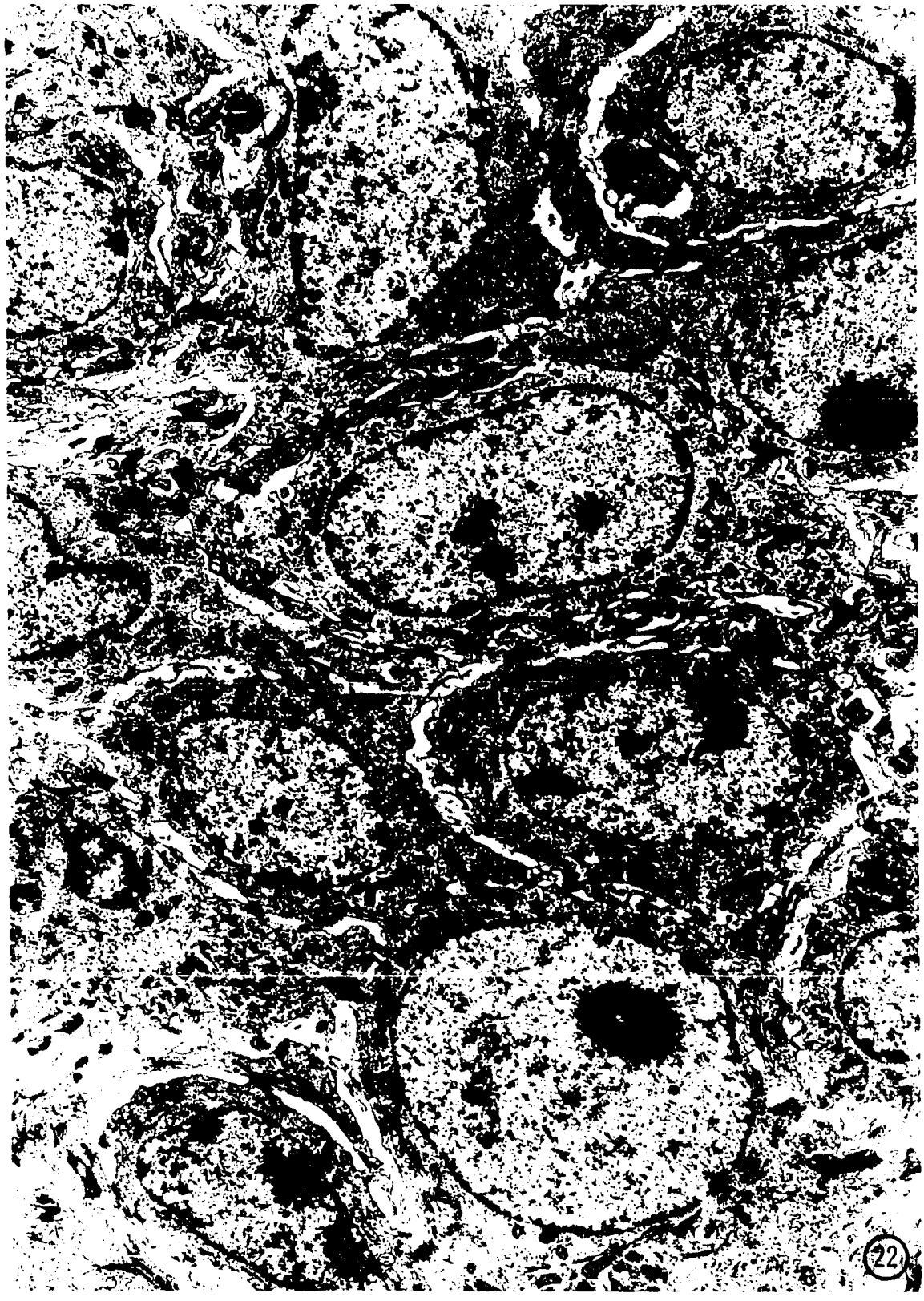
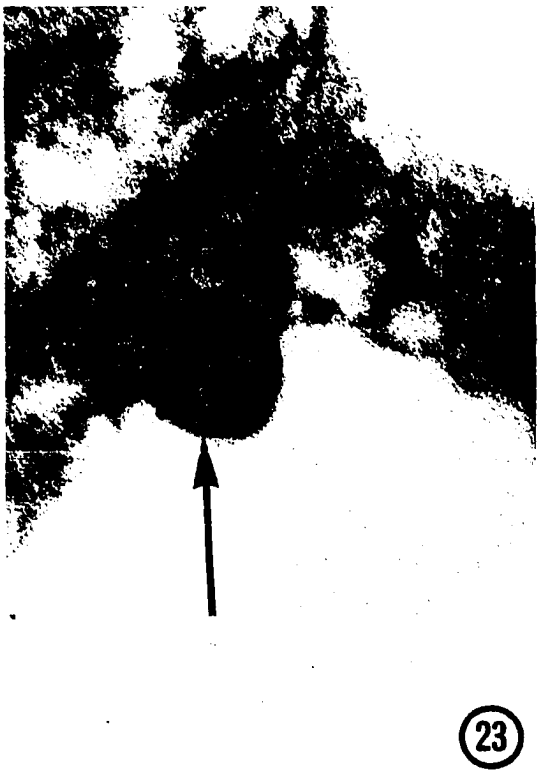

22

PLATE XXII

Figures 27-30. A possible method for the formation of the virus-like particles is shown as a sequence of events beginning with an increase in density of the plasma membrane followed by evagination and the subsequent pinching off and extrusion of the mature particle. 220,000x.



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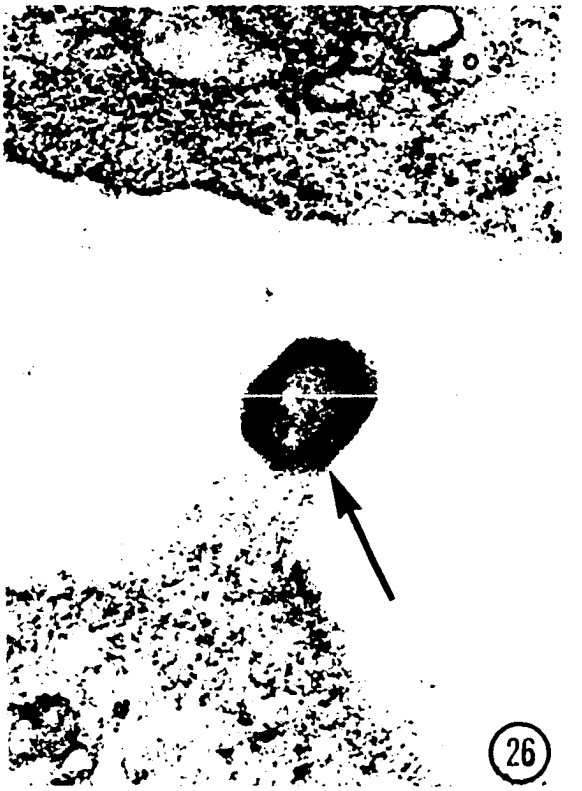

26

PLATE XXIII

Figures 27-30. Extracellular virus particles from four different patients with Bowen's disease. The arrows indicate dense subparticles in the nucleoid. Figure 27, 550,000x; Figures 28-30, 220,000x.

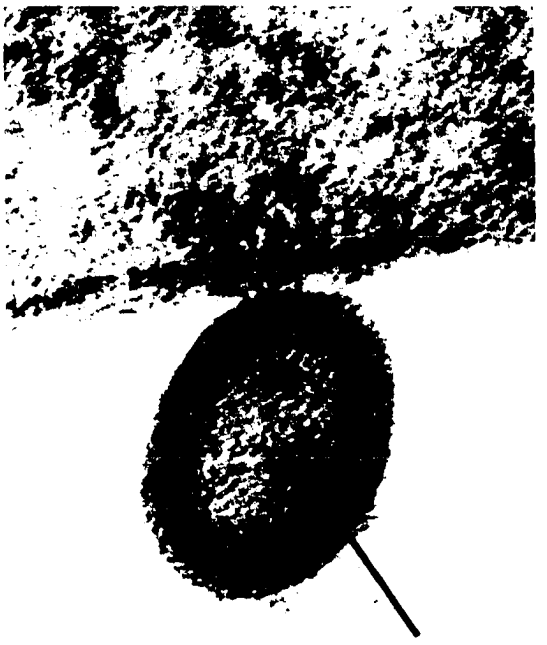

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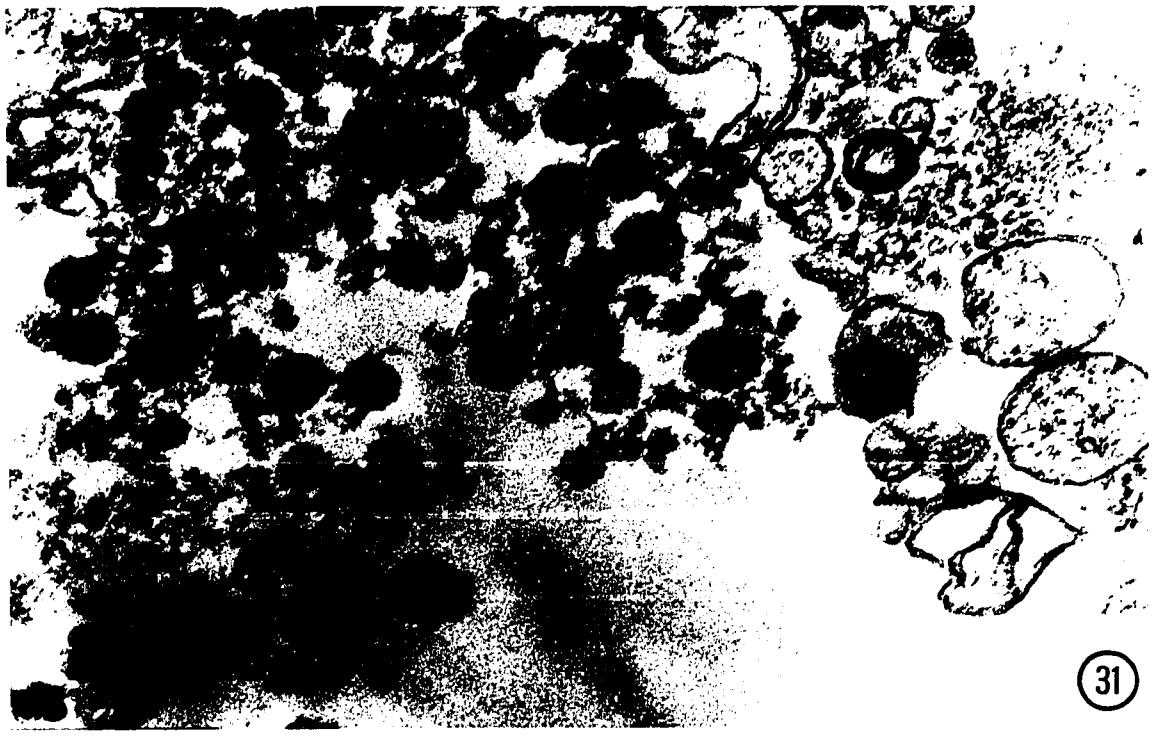
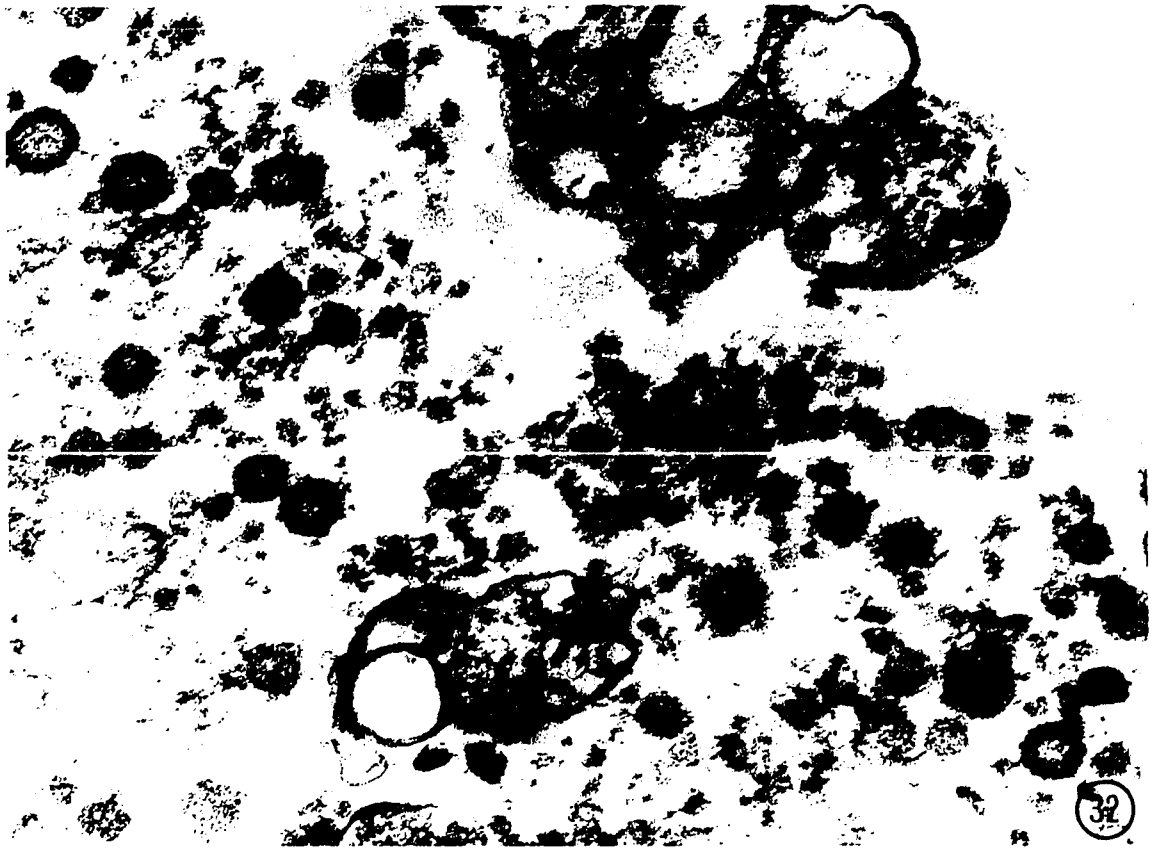
PLATE XXIV

Figures 31 and 32. Thin sections of pellets obtained by centrifugation of infected tissue culture medium. Figure 31 is a sample from the first cell-free passage in HeLa cells. Figure 32 is a collection from the fifth cell-free passage in tissue culture. 220,000x.



31



32

PLATE XXV

Figures 33-36. Figures 33 and 34 depict budding particles from human tumors. Figures 35 and 36 depict budding particles from the serosa of infected eggs, note the similarity of the virus coats in the human (Figure 34) and in the infected egg membranes (Figure 36). 220,000x.


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PLATE XXVI

Figures 37-40. Figures 37 and 38 depict extracellular particles from human tumors. Figures 39 and 40 depict extracellular particles from the serosa of infected eggs. Note the characteristic coats on the human tumor particle (Figure 38) and the particle from infected egg membrane (Figure 40). 300,000x.

37

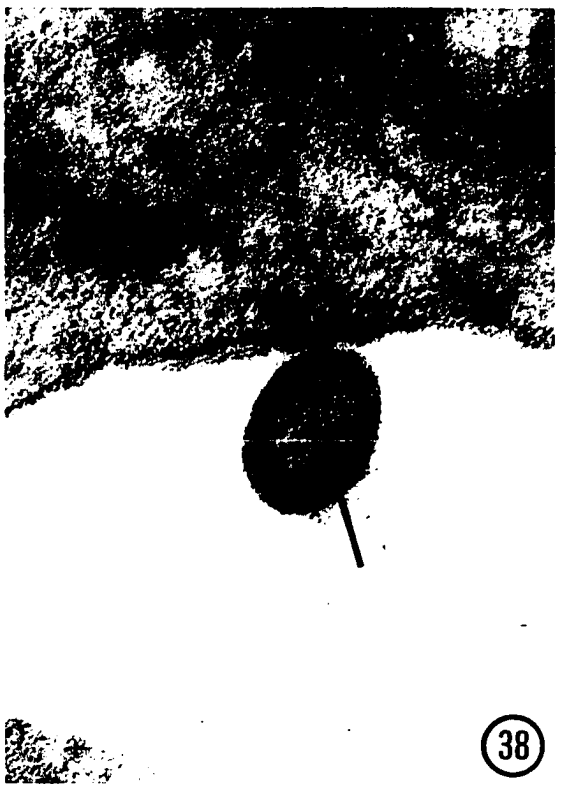

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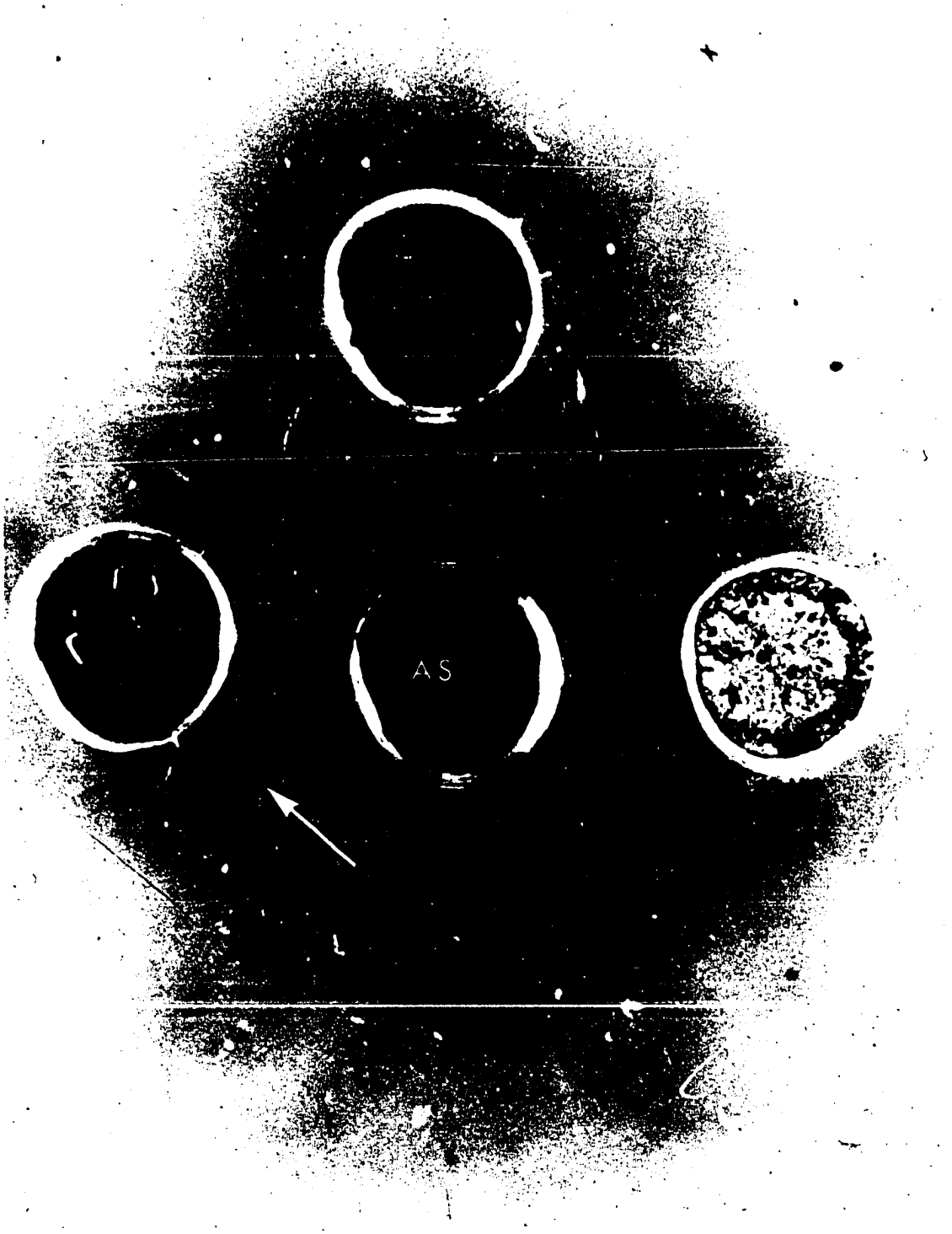
PLATE XXVII

Figure 41. Ouchterlony immunodiffusion plate showing a precipitin line between hyperimmune rabbit antiserum (AS) and the plasma of a patient with an active Bowen's lesion (BO). Note the absence of a line in front of the well which contains plasma from the same patient, three months after surgical removal of the lesion (AO). Also note that the control plasma from a normal volunteer does not form a line with the antiserum. 10x.



(41)