Notes on the Electron Microscopy of Tissue Sections

II. Neoplastic Tissue*

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In the study of tissue sections with the electron microscope hundreds of micrographs have impressed us with some aspects characteristic of the over-all picture of cellular structure. We have observed the cells in our sections to exhibit such distinctive traits that we feel justified in listing some of these as indicative of normal tissue and some as pointing to neoplastic phenomena, regardless of the anatomical location of the tissue.

The electron micrographs of the majority of our sections of normal tissue portray an unmistakably consistent picture in the appearance of some of their cellular constituents. The membranes, either the outer or nuclear envelopes, are reproduced as firm, solid, clear-cut lines or boundaries. The nuclei are well formed, being round or oval in shape and their contents seem to be of a rather uniform, finely granular texture, while their nucleoli appear to be much denser in structure. The cytoplasm usually shows a coarser granular texture frequently with a fine fibrillar structure as a background consisting of delicate and often almost indistinct fibrils. Occasionally some round empty spaces having the appearance of "vacuoles"¹ appear in the connective tissue but only to a very moderate extent.

In contrast to such electron microscopic characteristics of normal cell structure, our sections of neoplastic cells seem to exhibit a somewhat aberrant picture. Greenstein emphasizes that tumors "tend chemically to resemble each other more than they do normal tissues or than normal tissues resemble each other" (5). It is not unreasonable to expect then that tumor cells would exhibit during their life cycle over-all characteristics in their structure generic to cancer that differ from those of normal cells. We did not wish to restrict ourselves to animal tumors induced by close inbreeding or the use of carcinogens whose limitations, determined by the present narrow state of knowledge, may constitute a very definite barrier in gaining a generic understanding of neoplastic phenomena. Such induced tumors may not be fully representative of human tumors arising from conditions unknown at the present time and so we have selected a major part of our tissue material from human origin. We believe that the hundreds of electron micrographs of neoplastic tissue which we have taken and studied in comparison with normal tissue sections may also justify us to list some common characteristics that impress us as specific to them, regardless of their location or source. We have selected the micrographs presented here from our large collection of electron micrographs of cancerous tissue sections in the hope that they may be best suited in the limited space available to present the subject and portray some of the aberrations that occur without and within the cells during cancerous development.

DESCRIPTION OF FIGURES

The material of our human tumor sections whose micrographs are presented here was selected by the pathological laboratories of the hospitals as well as by us and great care was taken not to include in our sections areas that displayed signs of necrosis. A list of the tumors as well as extracts from the reports of the hospitals on the tumors whose sections are presented here will be found in the Appendix.

In order to obtain a mental picture of different zones of development within the tumors, a method was adopted by which sections following progressively a diametrical path through the tumor were taken. By this method our sections generally start in the normal tissue adjacent to the tumor and progress through the apparent edge of the tumor proper, as probably its most active area, to the tumor center as generally the most indurated area. The micrographs presented here were obtained in this way and an attempt is made to present different

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¹ The word "vacuoles" is used throughout the paper in a descriptive sense without connecting it with the routine definition.
areas of development within the neoplastic growth particularly by a series of intestinal tumor sections.

**DISCUSSION**

We are convinced and have been able to observe that the indication of morphological changes of tissue elements is often intensified by the electron microscope. Slight changes from normal that may appear questionable as seen under the light microscope are in many cases indicated more clearly and more distinctly by the higher resolution of the electron microscope. We want to add to this sentence that it would not be unreasonable to expect that some histological characteristics connected with neoplasms would also be more clearly revealed.

Our experience has been limited to tumors of the intestine, skin, and breast, so far, but we believe that the consistent observations we have made in the study of hundreds of micrographs reveal sufficient generic characteristics of neoplastic tissue in contrast to normal tissue that an attempt can be made to catalogue and classify them. We are not aiming at an extended description of the morphological and histological detail revealed in our micrographs but rather are attempting to describe some of the generic manifestations by which our tumor sections seem to differ from sections of normal tissue of the same anatomical location. Thus, we have attempted to compare electron micrographs of sections of malignant epithelial tumors of the intestine to sections of the epithelial structure of normal intestine, or sections of malignant epithelioma to sections of normal skin. Unfortunately, due to the peculiar structure of breast tissue we have not been able so far to obtain satisfactory sections of normal human breast tissue though we encountered no difficulty in sectioning and portraying parts of malignant breast tumors. In consequence, our figures of breast carcinoma cannot be compared directly to the host tissue but we believe that notwithstanding they are important by their generic characteristics which are very similar to those portrayed in our electron micrographs of cancers of the skin and of intestine.

We are impressed by the consistency of a pattern that seems definitely to express a difference between normal and neoplastic tissue although we are fully aware that before these differences can be accepted as truly specific to cancer much more electron microscopic research must be done to ascertain that similar characteristics do not exist in some other types of non-cancerous tissue which we have not had the opportunity as yet to study. In this light we are presenting and reporting the observations we have made so far merely as notes on our work, which we intend to follow with additional data.

Our work is much too limited to give a clear electron microscopic description of neoplastic tissue, yet, as stated before, our electron micrographs of cancerous tissue impress us as exhibiting some definite characteristics which appear to be materially different from those of normal tissue. We do not know how many active neoplastic cells are portrayed in our micrographs since nobody as yet has been able to point out the specific difference between a malignant and a normal cell, nor do we know how many of the cells shown are normal cells that exhibit their reaction to nearby cancerous infiltration, yet all our cancerous sections seem, with surprising consistency, to follow a certain general trend. In cataloguing our observations we could sum it up by saying that signs of aberration in malignant tissue often begin with the appearance of "vacuoles" in the intercellular spaces of affected areas. It is emphasized here that the word "vacuole" is merely used in a descriptive sense without in any way connecting it with the routine definition of this term. It seems then, as aberration increases, that these vacuoles appear more numerous and also of larger size and are observed not only in connective tissue but also in the cytoplasm of some of the cells. It must be said here that vacuoles have been observed by us in our electron micrographs of normal tissue sections, yet they never seem to appear as numerous or as large. Their appearance in cancerous sections seems to be of a different order of activity. It is a possibility that they indicate a weakening or dissolving of tissue since one of the characteristics of tumor growth is its capacity to invade and break down adjacent normal tissue. It may be due, on the other hand, to lack of development of cells and supporting tissue, caused by too rapid a rate of growth. They may be the beginning of necrosis, though we have tried strictly to exclude necrotic portions of tumor. A phenomenon more important and generic than the appearance of large numbers of vacuoles in the cytoplasm seems to be a breaking or disappearing of the nuclear membranes. It is accompanied by a splitting or breaking of the nuclei into a number of fragments appearing as dense irregular masses. In great contrast to this appear the round or oval shapes of normal nuclei enveloped by clearly portrayed membranes and revealing a finely structured interior. This is the most characteristic observation that our electron micrographs of neoplastic tissue have enabled us to make since this phenomenon was persistently portrayed no matter what cancerous tissue we have sectioned.
It should be added here that we also obtained the impression of the disappearance of nuclear membranes when we approached this problem from another angle, which concerned itself with the segregation of nuclei. By this work we were able easily to isolate normal cell nuclei in high purity and sufficient quantities, while we have not fully succeeded in duplicating this for nuclei of tumors. Most of their membranes seem to be destroyed or too fragile to stand up under the mechanical strain which this operation entails.

Another and often simultaneous phenomenon seems to be a similar breaking or disappearing of the outer cellular membranes, accompanied frequently by the appearance of fine fibrils of submicroscopic diameters. Often these fibrils, apparently coming from the extracellular tissue, seem to follow in their growth the outline of the disappearing membrane of the cell so that they frequently adopt the shape of a fibrillar shell. Often a complete filling of the cellular interior is revealed, with networks of fibrils replacing most or all of the cell and its surroundings. The presence of these fibrils in large numbers may explain in some parts the induration that so frequently occurs in tumors. Frequently the irregular dense masses of nuclear fragments are embedded in or encased by the network of these fibrils.

The diameter of fibrils as we have observed them is of the order of 100 A. in their first appearance of single fibrils. They seem to increase rapidly in number and dimensions and often the union of a number of single fibrils into ribbons or strands is observed. The fibrils themselves are constructed of regularly spaced segments and it should be noted here that judged by the dimensions and spacing of their segments they resemble the collagen fibrils described by Schmitt and collaborators (8). It is possible that the fibrils in our electron micrographs may be identical with the fine fibrils discussed by Howes (6) and it is interesting to note that Howes closes his paper by saying "It should be possible to investigate with the electron microscope the changes in the collagenous fibres and the reticulin that occur in spontaneous tumors."

In respect to this observation of an apparently progressing fibrillar growth it is interesting to reflect that, in all probability, the preponderant protein of these structures is collagen. The amino acid composition of collagen or rather gelatin, into which it converts so easily, is characterized by some rather exceptional proportions. One of them is the almost complete absence of tryptophane and another is the high percentage of arginine occurring in the order of an approximate ratio of 1 to 4 to 8 for histidine, lysine and arginine respectively (2), while normal muscle tissue has an approximate ratio of 1 to 4 to 3, and organs like liver, kidney and stomach, a ratio of 1 to 3 to 3 of these acids (1). Our many analyses of human and animal neoplastic tissue by microbiological assay (9) have very consistently disclosed a tryptophane level of half or less than that of normal muscle or organ tissue and have further disclosed a lysine-arginine ratio with arginine considerably in excess. Unfortunately, in human neoplasms not all of the tissue cell area consists of malignant cells (7) and the material we have analyzed must therefore be considered to be diluted or extended with normal tissue in different and varying degrees, so that our analytical results could only be expected to reflect a trend. Nevertheless the results, or the trend expressed by them, are sufficiently distinct to be interpreted as a support for the phenomenon observed in our electron micrographs that fibrillar replacement plays an important role in tumor development.

We were disappointed that no mitotic figures were found or portrayed in our electron micrographs since they are characteristic signs of cancer if investigated with the light microscope. A simple explanation, however, would present itself in the reflection that our sections for the electron microscope are of the average thickness of 1/10–3/10 of a micron while sections for the light microscope are 5 to 10 microns thick. A mitotic cell sliced to the latter thickness and in a favorable plane cannot fail to portray many of the mitotic bodies existing in it at that particular phase. However, the same cell sliced to the minute thickness of 1/10 of 1 micron, even if sliced in a favorable plane, could not portray whole mitotic bodies but only thin cross-sections of them, perhaps showing themselves as dots here and there that would be quite difficult to interpret. It is true that in many of our electron micrographs we have observed dots and lines here and there which we suspected to be sections of mitotic bodies but so far we have not been able clearly to demonstrate them as such. Also it must be reasoned that the fixations as used by us may happen to possess no selective absorption or affinity to chromosomes or mitotic bodies in general and that these thereby largely escape being portrayed by the photographic plate.

SUMMARY

High-speed microtome slicing of tissue into sections approximately 1/50 as thick as presently used

* To be published in a later paper.
sections for the light microscope, as well as new technics of fixation required by the high resolution of the electron microscope have been developed and employed for the investigation of neoplastic tissue. Some observations in particular are presented by our electron micrographs which we believe are not consistently revealed by the light microscope since often dimensions are involved below that instrument's threshold of resolution.

Signs of aberration observed in our electron micrographs of neoplastic tissue sections often seem to begin with the appearance of large numbers of vacuoles in intercellular tissue and cells. A characteristic aberration seems to be the breaking or disappearing of nuclear membranes and subsequent shrinking or breaking of the nuclear mass into dense fragments. A similar breaking of the outer cellular membranes frequently goes hand in hand, accompanied by the appearance of fibrils replacing them and often filling the cell interior.

In contrast, normal cells investigated by the same technics portray few vacuoles and solid, clear-cut boundaries for membranes. Their nuclei are well formed, round or oval shaped, and possess fine granular texture.

Every precaution has been taken to use only portions of tumors believed to be viable and we are convinced that some of the phenomena described are signs of neoplasia.

ACKNOWLEDGMENT

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APPENDIX

List of Tumors Used for Micrographs

<table>
<thead>
<tr>
<th>Fig.</th>
<th>Tumor Description</th>
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<tbody>
<tr>
<td>1</td>
<td>T-25</td>
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<td>T-9</td>
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Fig. 1 presents an electron micrograph of a cross section of tissue of human intestine at 6,700× magnification, prepared with Grey's fixation. Two goblet cells and a number of other epithelial cells are shown. This section originates from a part of the tissue that by gross examination is beyond the outer edge of the tumor.

REFERENCES

DESCRIPTION OF FIGURE 2

Fig. 2 is an electron micrograph of a section of the same human intestinal carcinoma as in the previous figure, at 6,700X magnification and prepared with Grey's fixation. Widespread appearance of vacuoles is strongly indicated within and without the cells, together with strongly represented fibrous structure. Some cells (a and b) appear to be foreign to this type of tissue and may be cancer cells. These cells differ from normal cells of the colon in the lack of uniformity in their over-all structure and in the presence of many vacuoles and fibrillar elements. Their membranes are not clear cut and particularly the nuclear membranes seem to be ragged and indistinct in most cases. Substantial evidence that vacuolization of the cytoplasm was present before fixation is the indentation of the nucleus in cell (a) Fig. 2.
DESCRIPTION OF FIGURE 3

Fig. 3 is an electron micrograph of the same tissue at 6,700× magnification and prepared with Grey's fixation. It seems to reveal greater differences from normal cells than the previous pictures, by showing still wider spread of vacuoles and fibrillar network. Three cells (a, b and c) are particularly shown whose cytoplasms are filled with vacuoles, whose cellular membranes as well as nuclear membranes exhibit ragged structure, and which seem to show distortion, possibly by pressure. The nucleus of the cell (b) at the left side and the cell (c) on top show severe spottiness.
DESCRIPTION OF FIGURE 4

Fig. 4a is an electron micrograph of a section of a human breast carcinoma prepared with 10 per cent neutral formalin in Tyrode's solution at 13,500 X magnification. It portrays a cancerous cell whose outer membrane has disappeared and has been replaced by a fibrillar envelope, part of which is clearly indicated. Fibrils have replaced cytoplasmic structure to a very large extent though some cytoplasmic bodies and granules are still present. Fibrils have bridged over and attached themselves to the nucleus which however seems to be still fairly intact though too dense to show interior structure. Fig. 4b of 2 stereoscopic electron micrographs of the same section should be advantageously viewed with a stereoscope to observe the three dimensional arrangement of the fibrils.
DESCRIPTION OF FIGURE 5

Fig. 5 is an electron micrograph of a section of a mammary mouse tumor at 10,000× magnification prepared by gradual fixation with osmic acid and picric acid. It appears to present a disintegrating cell surrounded by dense fibrous tissue. Both cellular and nuclear membranes have disappeared almost completely. The nuclear outline has become very ragged and the nucleus seems to be in the process of separating into clumps of dense masses. Occasional bodies resembling mitochondria and other granular material are still present.

*See Part I of this paper.
DESCRIPTION OF FIGURE 6

Fig. 6 is an electron micrograph of a section of a human carcinoma of the breast at 10,000× magnification. It represents in its main part a degenerated cell whose outer membrane has been almost entirely replaced by a fibrillar envelope strong enough to preserve the shape of the cell and parts of its contents. Large vacuoles seem to appear throughout the cell. Some opaque granular particles, perhaps mitochondria, are still visible as well as dense broken-up masses of fragments.
Fig. 6
DESCRIPTION OF FIGURE 7

Fig. 7 is an electron micrograph of a section of human mammary adenocarcinoma at 16,500 × magnification, prepared by gradual fixation with osmic and picric acids. The figure portrays what we believe to be 2 adjacent cells whose original enveloping membranes have been replaced by a network of extremely fine fibrils, thinner than in the previous figures. Fibrillar structure seems to be advancing from all sides into the cytoplasm, of which little is left except well preserved particles which may be mitochondria. The nucleus of the cell in the lower half of the picture has lost its membrane and its contents have been separated into clumps of heavy masses, while the nucleus of the upper cell seems to have disintegrated or have been outside the plane of the section, just as many sections of an egg could be made without striking its yolk.
DESCRIPTION OF FIGURE 8

Fig. 8 is a montage of 5 electron micrographs of a section of a squamous cell epithelioma at 4,000X magnification and prepared with Bouin's fixing solution. The successful fixation of this section by Bouin's fluid proved to be an exception to the usual unsatisfactory results when using standard fixatives. This figure also shows a group of cells resembling cells of the stratum Malpighii. Nuclear degeneration is indicated in a number of cells (a, b and c) together with loss of nuclear membranes. In contrast, the two cells (d and e) at the extreme left of the micrograph seem to present a more normal appearance. The bridges between most of the cells seem to be well preserved and the tonofibrils in the cytoplasm of the cells are demonstrated.
DESCRIPTION OF FIGURE 9

Fig. 9 is an electron micrograph of a section of the same human epithelioma as Fig. 8 at 9,000× magnification, prepared with Bouin’s fixation. The portrayed cell appears to be abnormal in the irregular formation of the nucleus and lack of a normal membrane.
Fig. 9
DESCRIPTION OF FIGURE 10

Fig. 10 is an electron micrograph of a section of stratum Malpighii of the same human epithelioma as in Figs. 8 and 9. It is taken at 7,000× magnification and prepared with Grey's fixation. At least 4 cells can be recognized showing different stages of advanced nuclear disintegration. Fibrillar structures have replaced most of the cytoplasm of the cells in the upper half of the micrograph and their nuclei have broken up into a number of separate clumps. The intercellular bridges are gone, to a great extent, and cellular membranes are only partly preserved. The two cells in the lower part of the figure appear to be of normal development. The figure may represent the difference between disintegration of diseased cells and normal nuclear shrinkage of skin cells.
DESCRIPTION OF FIGURE 11

Fig. 11 is a section of connective tissue of human carcinoma of the breast at 30,000× magnification. It has been prepared by gradual fixation with osmic and picric acids and has been intensified by chromium shadowing. It portrays at this magnification some of the fibrils that we have observed in many of the preceding pictures at lower magnification. Some of these fibrils are in single strands which appear to be the primary units, while others have joined to form strands of 2 or 3, producing thereby a flat band or ribbon effect. The beaded structure of the fibrils can be clearly observed whether they are single or in formations of double or triple strands. The diameter of single fibrils is of the order of 400–500 Å. The ends of the fibrils are broad or slightly rounded and cannot be observed in tipped or pointed form. It is difficult to decide whether the bulging effect observed on some fibrils is a natural phenomenon or is due to a possible reaction of the protein of the fibrils with the fixing agents (3). The repeat distance of the segments seems to vary from 450 to 750 Å, which variation may be due to stretching or to a perspective effect. Submicroscopic spherical bodies which can be observed in this figure have already been reported by us (4).  

4 A second report on submicroscopic spherical bodies will appear concurrently with this in "Experimental Medicine and Surgery."
Fig. 11
Notes on the Electron Microscopy of Tissue Sections II. Neoplastic Tissue


_Cancer Res_ 1948;8:549-573.

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