Abnormal Intranuclear and Cytoplasmic Formations Associated with a Chemically Induced, Transplantable Chicken Sarcoma

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PLATES 55 TO 64

(Received for publication, July 15, 1958)

ABSTRACT

Thirty GRCH/15 tumors (a 1, 2, 5, 6-dibenzanthracene-induced chicken sarcoma) were examined in the light and the electron microscope.

Associated with the sarcoma were two types of abnormal intranuclear lesions, one in the form of a vacuole, the other as an aggregate containing glycogen. In the electron microscope, one type of lesion observed showed an organized microfibrillar structure.

Abnormal cytoplasmic formations occurred as massed clusters of thread-like or tubular material, which gave rise to small bodies with concentric shell structure; similar bodies were found associated with vacuoles.

INTRODUCTION

The GRCH/15 chicken tumor was experimentally induced by Peacock (36) with 1,2,5,6-dibenzanthracene; it has since been transmitted by cellular transplantation in the pectoral muscle of young chickens. A faster growing form (30 to 60 days evolution as compared with 80 to 150 days) was obtained by Carr (9) at the 32nd passage and has subsequently been maintained at this Institute.

This fast growing form, used in the present study, is a fibrosarcoma of firm consistency containing spindle cells and large round cells with greatly enlarged, round, or oval nuclei. Some intercellular collagen is generally found. The tumor frequently metastasizes and is often accompanied by a predominantly myeloid or erythromyeloid leukemic reaction (11); the tumor tissue may be invaded by myeloid elements. Contamination with a leukemia virus from infected stock cannot be excluded in these cases.

In conjunction with studies of various other types of cancer cells, an electron microscope investigation of this tumor was undertaken to ascertain if there were any characteristic cytological features associated with it.

Materials and Methods

Tumors of 30 chickens of the white Leghorn and Sussex strain were examined. The tumor-bearing animal was killed and portions of the tumor, spleen, and bone marrow were immediately dissected out and fixed. The material to be described was obtained from the peripheral, actively growing region of the tumor. For electron microscopy, small pieces of the tumor were excised, fixed for 1 hour in a 2 per cent solution of buffered osmium tetroxide of pH 7.3 (34), embedded in butyl methacrylate, and polymerized with 2 per cent luperco at 45°C. Sections obtained with a Porter-Blum microtome were examined in a Siemens Elmiskop I.

Histochemical tests were carried out on smears, paraffin sections (fixed in alcohol Bouin, Carnoy, or Zenker), and sections of methacrylate-embedded material. For the methacrylate sections, standard staining techniques (hematoxylin and eosin, Feulgen, Giemsa, toluidine blue, periodic acid–Schiff (29), and carmine-Best) were used slightly modified as recommended in references 23, 21, 32. Phase contrast was used on unstained sections.

Since the limited sampling available by ultrathin sectioning for electron microscopy may falsely suggest the absence of lesions in some tumors, the additional use of thick sections (both of methacrylate and of paraffin-embedded tissue) has proved to be particularly important in the present study.

RESULTS

The tumor was found to show nuclear alterations (Figs. 1 to 13) and some unusual formations in the cytoplasm (Figs. 14 to 21). No similar structural modifications have so far been observed in any other material studied in this laboratory, including the GRCH/16 sarcoma.

Light and electron microscopical results are described separately and their features compared subsequently. "Lesion" will be used as a collective term for abnormal features in the nucleus, rather than "inclusion" which is now so frequently used in association with virus infections.

Light Microscopical Study

Cytochemical Results.—Two kinds of nuclear lesions were distinguishable: one that gave a negative reaction with PAS and appeared as a vacuole, and another that stained intensely red. Both lesions were well defined; affected nuclei were observed which contained one or other of them (Text-fig. 1(*1* to *3*) and (*4* to *5*)), or both together; if together in the same nucleus, they could occur well separated (Text-fig. 1(*6*)), in close touch with one another (Text-fig. 1(*7*)), or with the staining aggregate actually inside the vacuolar formation (Text-fig. 1(*8*, *9*, *12*)).

Vacuoles of various sizes have been found in many nuclei, ranging from one or several small ones to an enormous vacuole which practically occupies the entire nucleus (Text-fig. 1(*4*, *5*, and *10*); compare also Figs. 1 and 2).

The aggregates of PAS-positive material may also vary greatly in size and number. More than one formation may be found per nucleus (Text-fig. 1(*2*, *3*)), which may range from barely perceptible to large deposits (Text-fig. 1(*11*)), culminating in a compact, jagged, or round aggregate which almost fills the nucleus. It is only in very thin sections that these large, apparently solid masses (Fig. 3 *c*) may be shown to be not homogeneous but rather to consist of intertwined thread-like components (Figs. 3 *a* and 3 *b*), their widths ranging from 200 to 400 mμ.

The *PAS-positive formation* is assumed to contain glycogen, since the simple test of extracting glycogen by salivary digestion, done on smears and paraffin sections, caused loss of stainability by PAS. Similar results were obtained when carmine-Best was used instead of PAS.

The Feulgen reaction showed certain abnormal masses of material similar in size and distribution to the PAS-positive bodies as in Fig. 4, and some intravacuolar formations as in Text-fig. 1(*8*, *9*, and *11*). This would indicate that the nuclear aggregates also contain some DNA if one assumes that the specificity of the Feulgen reaction is not impaired under the existing experimental conditions.

The *vacuolar formations* proved to be Feulgen-negative (Figs. 1 and 2); it would appear that there is a redistribution but no significant loss of DNA in such nuclei (25).

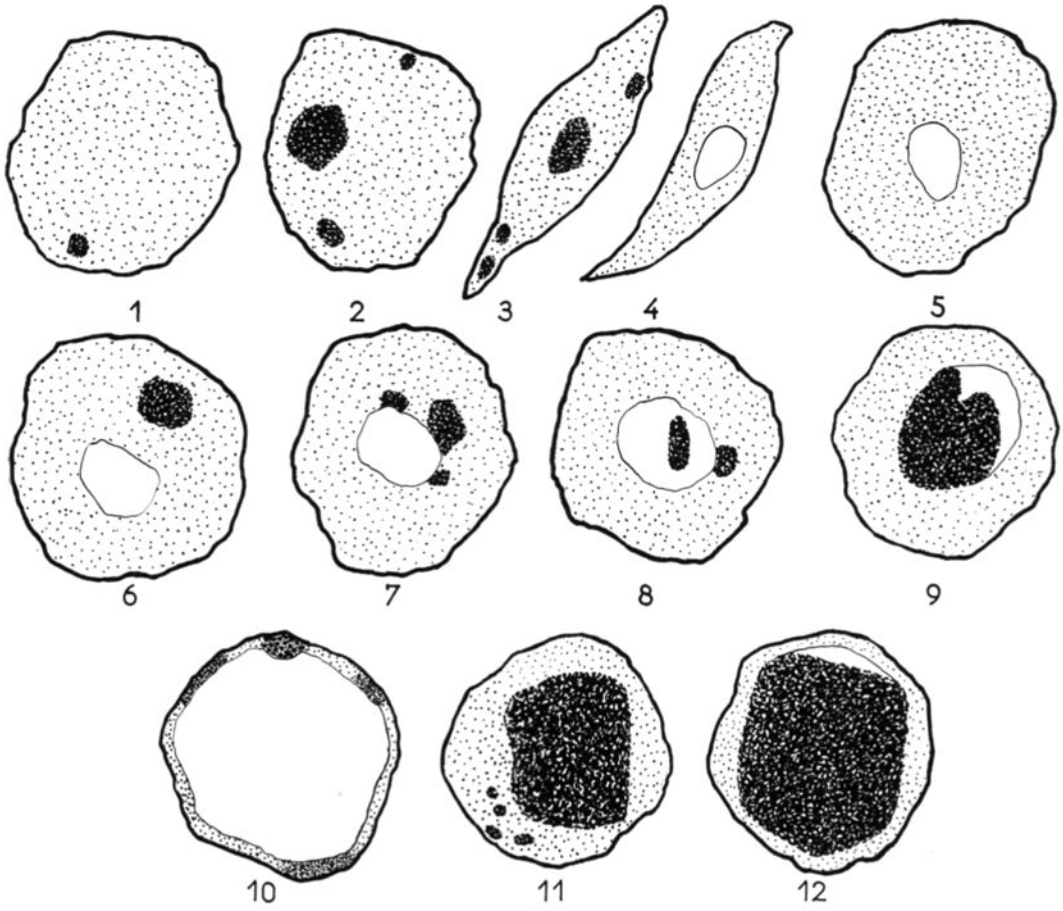
The PAS-positive components seemed to be morphologically independent from the nucleoli in the nuclei examined. The infrequent appearance of small PAS-positive granules in contact with the nucleolus was only observed when the nucleus already contained a very large positive lesion. Where only a simple, small granule was found per nucleus, which may be supposed to represent an earlier stage in lesion formation, the granules and the nucleolus were well separated.

Phase Contrast Microscopy.—Nucleoli, and both vacuolar and dense intranuclear formations could be differentiated in unstained preparations. The spatial relationships between them, described earlier, were confirmed. Vacuoles are not always completely lacking in contrast; some gradation in contrast through the lesion may be distinguishable, implying some variation in the density of its content.

The Bearing of Tumor Age on Nuclear Lesions.—Out of a total of 30 tumors, 28 showed a positive PAS reaction as well as vacuoles. The two negative PAS reactions were obtained with samples containing many myofibrils; *i.e.*, in a region in which the tumor was invading muscle tissue. Such regions, which presumably represent the young, actively growing tumor cells, contained only very occasional nuclear lesions of the vacuolar type.

No correlation was found between the age of the chicken which ranged from 1 to 40 days at the time of the tumor graft, and the appearance of nuclear lesions. Cellular changes associated with the age of the tumor do, however, appear to permit a broad qualitative classification. A table summarizes the findings based on approximately four samples per tumor examined.

A low incidence of nuclear lesions was observed in very young tumors, and remained low up to the age of 25 days; in older tumors, cells with nuclear lesions became more widespread and the lesions themselves more pronounced (they became more



TEXT-FIG. 1. Abnormal intranuclear formations.
White: vacuolar portion; densely dotted: PAS-positive region.
1. Nucleus with single PAS-positive aggregate.
2 and *3.* Several aggregates per nucleus.
4 and *5.* Vacuole in nucleus of round and spindle shape respectively.
6. Vacuole and aggregate in same nucleus (separate).
7. Aggregates at edge of vacuole.
8. Aggregates at edge and inside vacuole.
9. Large aggregate inside vacuole.
10. Vacuole, occupying nearly entire nucleus.
11. Large aggregate, and some very small aggregates.
12. Large aggregate occupying most of nucleus; small remnant of former vacuole still recognizable.

extensive or several would be found per nucleus). It may be of interest to note here that in tissue culture (from a 10-day-old tumor) (18) in a state of active growth, a number of small vacuolar lesions and only some very rare PAS-positive lesions were found in nuclei.

The significance of such lesions and their variation with tumor age are obscure.

Electron Microscopical Study

Low power survey micrographs (as Fig. 5) showed considerable polymorphism of cell type and cytoplasmic characteristics. The tumor tissue was formed by round cells and elongated spindle cells, with some collagen. The occurrence of striking unusual formations was noted, both in nuclei

TABLE I

Age of tumor	Nucleus				Comments
	Vacuolar lesions		PAS-positive aggregate		
	Size of lesion	Frequency of occurrence	Size of lesion	Frequency of occurrence	
days					
12–20	Small	++	Small	+	Nuclear lesions found localized in groups of cells
21–25	Small Medium	+++ ++	Small	++	
26–30	Mostly medium	+++	Small Medium Large	+ +++ +	
31–35	Mostly medium	++	Various, mostly large Aggregates within vacuolar lesions	+++ ++	
36–58	Large	+	Medium	Increase continuing	
			Large	++++	
			Aggregates within vacuolar lesions.	+++	Cells with intranuclear PAS-positive aggregates found increasingly widely distributed with advancing tumor age

+, Rare; ++, occasional; +++, frequent; ++++, very frequent.
Small, $< 2\ \mu$.
Medium, 2 to 4 μ.
Large, $> 4\ \mu$.

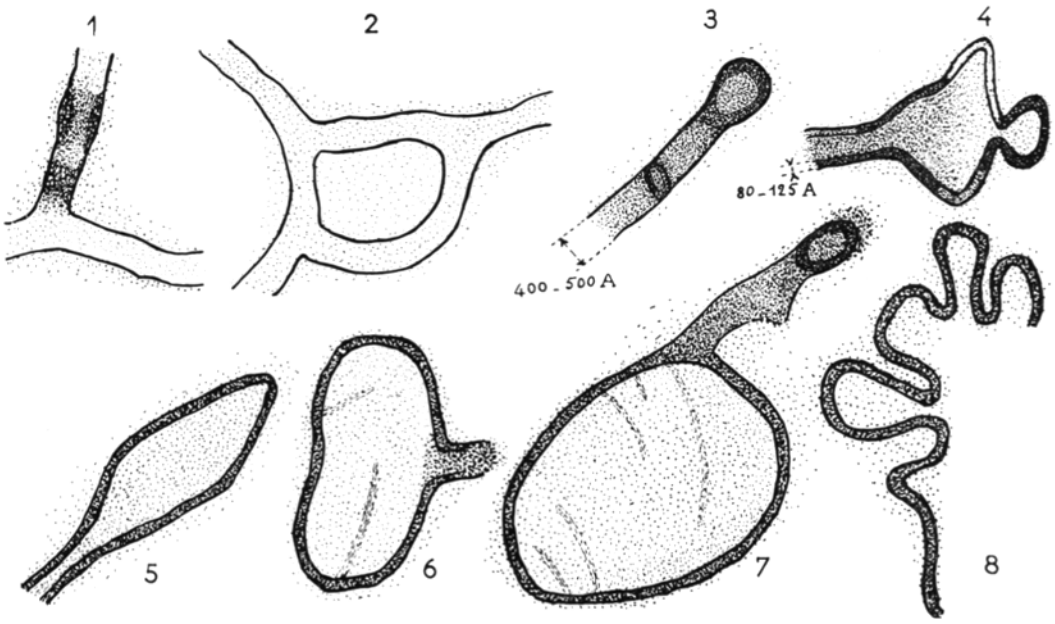
and in cytoplasm of tumor cells. Nuclear and cytoplasmic features will be described separately.

The Nucleus.—Two kinds of nuclear alterations were encountered. Intranuclear *lesions of type 1* (Figs. 5 and 6) occurred as well defined regions of lowered density and somewhat flaky appearance. A loose network of some partially interconnected material could sometimes be recognized at higher magnification (Figs. 7 to 9). The most regular fibrillar constituents had a width of 250 to 350 A, though values below 200 and above 500 have been encountered. Granules, similar in size and relative density to those normally present in nuclei, were often found associated with the surface of the fibrillar components. The apparently empty regions between the fibrils may correspond to an additional component of the lesion, such as an amorphous substance lacking in contrast under the given experimental conditions, or removed in the course of specimen preparation.

The lesions appeared in some instances fairly compact, in others diffuse. No limiting membrane was recognized separating the lesion from the rest of the nucleus. Chromatin tended to be pushed towards the nuclear periphery (Fig. 9); occasionally, some fibrillar structures could be traced from the chromatin into the lesion.

Type 1 lesions varied in size, ranging from being just large enough to permit identification to almost filling the entire nuclear cross-section. Nuclei of spindle cells more often showed intermediate or small lesions (Fig. 8) whereas the large lesions, extending over more than half the cross-section of the nucleus, appeared to be confined to enlarged round or oval nuclei (Fig. 6). Such major lesions were frequently accompanied by vacuolation of the cytoplasm (*cf.* Fig. 6).

Another kind of intranuclear formation, to be called *type 2 lesion*, occurred as one or more aggregates of closely packed, regular filaments



TEXT-FIG. 2. Abnormal cytoplasmic formations.

1. and *2.* Tubular components, showing local increase in density, and some branching.

3. Differentiation and budding along tubule.

4. Tubule, showing double structure at periphery, and some local swelling.

5. Tubule, with local modifications.

6. and *7.* Structures with interior of low density, and protruding portion resembling tubule-like components above.

8. Typical thread-like formation with double structure.

(Fig. 13). Such filaments had a diameter of about 170 A; transverse sections revealed in some cases a light core surrounded by a denser zone. A belt of low density usually separated the lesion from the rest of the nucleoplasm (Fig. 10). Any resemblance of the lesion to nucleoli was superficial (Fig. 11). Although transverse sections of filaments at low power might appear like granules (Fig. 12), these were considerably larger than nucleolar granules (Fig. 10).

The nucleolus occurred as a rather opaque structure or in the form of "nucleolonema" (7, 13), with normal substructures (37). Masses of considerable density were occasionally observed inside nucleoli. No consistent topographical relationship between the nucleolus and either type of lesion could be established.

Lesions were often small (Fig. 11), but could occupy a large portion of the nucleus (Fig. 10). Their size and number per nucleus, and their frequency of occurrence in the tissue increased with tumor age.

The Cytoplasm.—Considerable variations in ultrastructure were observed in different cells. Some tendency towards dedifferentiation was noted. The *Golgi zones* were small and accompanied by only small vacuolation; often, several of these zones occurred in one cell section. Lamellar *ergastoplasm* was frequently little developed, and usually scant in cells with type 1 lesions. The degree of organization varied even locally within one cell, and lamellar ergastoplasm often appeared only in close association with mitochondria. *"Annulate lamellae"* (43, 39, 46) or *"cisternae fenestratae"* (35, 41) were observed comparatively frequently in this tumor in groups of from 4 to 12 (Fig. 10), usually in cells with a type 2 lesion. Such lamellae appeared to have some connection with the ergastoplasm. *Lipide deposits* were generally found to be scanty except in some presumably old cells. *Mitochondria* were plentiful, and most of them had numerous closely spaced cristae. Certain mitochondrial modifications tended, however, to accompany the abnormal cytoplasmic formations previously mentioned, and will be described together with them.

A striking phenomenon associated with the tumor was the appearance in the cytoplasm of a large number of cells of dense, rather opaque formations (Figs. 14 and 16). Such clusters could form several foci within one cell section, and could occupy large areas (Fig. 14). They might occur anywhere within the cytoplasm (Figs. 14 to 19), although frequently located near the nucleus.

Structural details of such formations (as in Figs. 15 to 19) are schematically illustrated by Text-fig. 2. Components which appeared as tubules in cross-section (Fig. 17), sometimes showed some branching (Text-fig. 2(*1* and *2*)); the extent of their visible interconnection depended on the plane and thickness of the section. Usually over a short distance, but sometimes for even up to 2 μ, they would show no visible differentiation, had a fairly uniform width of 450 $\pm$ 50 A, and appeared of medium density. The characteristically opaque appearance of the clusters is due to very dense structural modifications along tubular components (Fig. 17), appearing as a peripheral and sometimes transversal thickening (Text-fig. 2(*1*) and Figs. 16, 17). An opaque double membrane from 80 to 120 A in width, with constituent membranes of less than 40 A (Figs. 17 and 18) would become distinguishable at the periphery of tubules (Text-fig. 2(*4, 5*)) or at their budding ends (Text-fig. 2(*3*)), where small structures consisting of concentric shells appeared (Figs. 18 and 19). The more regular structures had a diameter of 650 A, and were frequently found separate from the tubules (Figs. 16 and 17). Although the possibility that such structures represent cross-sectioned tubules cannot be excluded, the general appearance and the variation in size of such regular forms suggested rather that they corresponded to complex spherical bodies, observed at different levels of sectioning. Quite frequently, single very opaque thread-like formations consisting of a double membrane were recognized (Fig. 15 and Text-fig. 2(*8*)); these sometimes extended over considerable distances even in a thin section, one having been traced for 5 μ, and could be interpreted as representing corrugated lamellae.

Although the majority of cells containing dense cytoplasmic clusters had apparently normal nuclei, small type 2 lesions were also observed sometimes. Only on very rare occasions were pronounced nuclear lesions of either type found together with such cytoplasmic formations.

In tumors after the age of 30 days, rather similar clusters of small bodies could be found within or close to vacuoles throughout the cytoplasm (Figs. 20, 21). The round bodies had a diameter of 400 to 500 A, compared with the regular forms of 600 to 700 A found in the cytoplasmic clusters described above; they were surrounded by a single or double membrane. Whereas the intracytoplasmic clusters showed a preponderance of differentiating tubular components, the vacuoles were found to contain predominantly spherical and some irregular, concentrically layered structures. Despite some differences in size and location, it is suggested that these formations may be linked developmentally, and may be present either as clusters or in vacuoles.

Cytoplasmic clusters were accompanied by numerous mitochondria some of which showed certain modifications (Figs. 17 and 18). Protuberances of modified mitochondria resembled tubular and thread-like formations in their neighbourhood, but such aspects were rarely found. Other mitochondria were swollen in their vicinity. Large numbers of osmiophilic granules (45, 26) have been encountered in some mitochondria, frequently noted near vacuoles containing clusters of small bodies (Fig. 20).

A single, very old tumor was examined at the age of 235 days (compared with the usual maximal 60 days), and found to contain a particular kind of mitochondrial abnormality. Usually, oval or round mitochondria in the GRCH/15 sarcoma were less than 1 μ, and often below 0.5 μ (Figs. 8, 20); in this particular tumor, many cells contained unusually large mitochondria, up to 3.5 μ in diameter, which did not appear swollen but had a matrix of normal density and large numbers of cristae, mostly long and interbranched. Other big mitochondria (1.5 to 2 μ) contained opaque granular formations (Fig. 22) which, at higher magnification (Fig. 23) were seen to consist of dense rodlets about 200 A wide surrounded by some matrix of low density. Cells with such mitochondria did not show the nuclear or cytoplasmic lesions described earlier; the nuclei were found to contain numerous opaque granules of various sizes.

DISCUSSION

A correlation between the cytochemical and ultrastructural features of the nuclear lesions depends on establishing a correct correspondence between light and electron microscopical observations.

Whereas the light microscope showed both

vacuolar formations and PAS-positive aggregates in the nucleus, the electron microscope revealed loose fibrillar or flaky regions of comparatively low electron density (type 1 lesion), and closely packed fibrillar aggregates of apparently high density (type 2 lesion).

Attempts at direct comparison of the same lesions in adjacent sections for light and electron microscopy have met with difficulties, and no unequivocal results have been obtained so far. A less direct correlation is therefore proposed, based on observations from similar areas. For the following reasons it is, then, suggested that type 2 lesions (Figs. 10 and 13) correspond to the PAS-positive aggregates (Fig. 3), and the type 1 lesions (Figs. 9 and 11) to the vacuoles:—

1. The compact, clearly delineated type 2 lesions correspond more closely to the well defined glycogen deposits, with respect to size, occurrence, and variation with tumor age.

2. Bundles of closely aligned microfibrils, such as constitute type 2 lesions would logically account for the thread-like formations revealed by PAS (Figs. 3 *a* and 3 *b*) and Feulgen staining (Fig. 4).

3. A type 2 lesion is very closely packed, and thus appears more dense than the background nucleoplasm. It does not seem logical to suppose that a similarly fixed section viewed under the light microscope should appear as a vacuole; *i.e.*, less dense than the background.

4. Tumors with a particularly high incidence of major type 1 lesions showed a correspondingly large number of nuclei with large (PAS-negative) vacuoles in the light microscope.

The cytochemical identification of glycogen by amylase digestion, in conjunction with stains such as PAS, has been widely adopted. Hale (20), in reviewing the extensive literature on the subject, maintains, however, that "... on the general use of enzymes, there is always an element of doubt in the conclusion to be reached when enzymatic digestion is used to identify glycogen." The suggestion that glycogen is responsible for the strong PAS-positive reaction of the intranuclear aggregates in the GRCH/15 tumor is therefore put forward with similar reservations.

Normally occurring cytoplasmic glycogen has mostly been studied with the electron microscope in liver (16), where it appeared as somewhat diffuse areas of low electron density, thus resembling the type 1 lesions of the sarcoma rather than the dense aggregates of lesion 2.

However, another formation presumed to be glycogen but which is morphologically different from that found in liver cells (16) has now been found in the cytoplasm of cells from turtle atrium (17). Intracellular glycogen is known to occur in different forms, with different degrees of solubility.

Meyer (30) suggests the existence of a number of naturally occurring glycogens with different solubilities. One may conclude that the form of glycogen aggregation will depend on the particular way in which it is linked with other components (*e.g.* it may occur as a glycogen-protein complex (2, 38, 47)), or on its degree of polymerization (30, 31).

In view of the above, one may indeed expect a variety of morphological forms to be revealed by the electron microscope; particularly nuclear glycogen, as found in the GRCH/15 tumor, may differ from glycogen found in cytoplasm.

Glycogen, present in the *cytoplasm* of many normal cells (*e.g.* embryonic tissues, muscle, cartilage, liver, etc.), is also found in numerous tumors (15, 14, 8).

The classical example of *nuclear glycogen* is found in diabetic liver (27, 1, 19, 44) in which an inverse relationship exists between the glycogen content of nucleus and cytoplasm (44). Though to a much lesser extent, nuclear glycogen was also detected in liver in other diseases associated with a variety of metabolic disturbances (Graves's disease, Hodgkin's disease, certain anemias, etc. (10)). Intranuclear liver glycogen has, however, been found to occur normally in metamorphosing tadpoles (22). Glycogen deposits and vacuoles occurred in the liver cells of both thioacetamide-treated rats and, spontaneously, in some mice (24). Recently, a chemically induced mouse hepatoma was found to contain intranuclear glycogen (42). No electron microscope study of this case has hitherto been reported.

Nothing is known of the significance of intranuclear glycogen for the GRCH/15 sarcoma.

The presence of mitochondria in the vicinity of cytoplasmic clusters might suggest a possible relationship between them, whereas no connection with the Golgi apparatus was indicated. Some mitochondria seemed to have undergone a loss of internal organization, sometimes to a point at which their distinction from vacuoles became difficult. Some protuberances from structures suggestive of mitochondria after considerable internal breakdown showed a resemblance to tubular cluster components. Occasionally, a group of swollen mitochondria was found near a cluster. It

might be supposed that these cytoplasmic clusters have deteriorating effects on some mitochondria. A connection between the formation of cytoplasmic clusters and mitochondria seems likely but it cannot be considered established.

The outside mitochondria found in only one GRCH/15 tumor are of some interest. Very large mitochondria have been reported elsewhere in the literature. Giant spheroids in the adrenal gland (3) up to 3 μ in diameter, and nearly filled with tubules were interpreted as representing highly specialized mitochondria. Even larger mitochondria of about 5 μ have been seen in solid, apparently actively growing metastases of the Ehrlich ascites tumor (46). It would be interesting to know whether such hypertrophic mitochondria might be a symptom of degeneration or regression of the tumor.

The results show that the cytoplasmic and nuclear anomalies do not usually coincide although some cytoplasmic clusters have been found together with small nuclear lesions of type 2. It is not clear whether any connection exists between nuclear and cytoplasmic formations.

In spite of many biological tests, the GRCH/15 has never yielded a tumor-inducing virus. Nevertheless, one could argue that a virus was present, though "masked" and not detectable by biological methods. It was thought possible that electron microscope studies might throw some light on this problem by revealing particulate matter. The lesions reported in this paper scarcely resemble any aspects of cellular modification induced by known viruses. Furthermore, no comparable formations have ever been observed in electron microscope studies of several chicken neoplasms known to be induced by viruses (Rous sarcoma (6, 33, 12); Murray-Begg endothelioma (40); Fujinami myxosarcoma (28); erythroblastosis (4, 5)). Very occasionally, the presence of a few virus-like particles morphologically identical with those found in such tumors and leukemias has also been noticed in the GRCH/15 sarcoma but they are extremely rare and therefore thought to be related to a contaminating virus that has nothing to do with the cancer process itself. The other bodies which, despite some polymorphism, might have possible viral significance would be the dense particles observed among the clusters in the cytoplasm, and the spherical bodies inside certain cytoplasmic vacuoles. Both types occur in large numbers and show similar substructure (*i.e.* a core of low density and a double outer shell). However, it would be, at the present moment, unwise to attach too much importance to them.

In speculating upon the significance of the intranuclear and cytoplasmic formations it is important to remember that they were found in a tumor which had been serially transplanted in fowl over a long period, since its original induction by a carcinogenic agent. It is clear that they cannot be attributed directly to the toxic effects of carcinogens. They may, however, be the outcome of genetic changes in the cells induced by the carcinogen or occurring spontaneously during tumor transmission, such changes giving the cells a selective advantage and at the same time resulting in an altered metabolism producing the features observed. Cytoplasmic formations much more than the intranuclear formations are to a certain extent reminiscent of viral developmental changes, and it might be envisaged that such a genetic change could give rise to an intrinsic virus which remains in a latent form. (Nevertheless, as previously stated, if viral in nature they could be extraneous.) At this time, both the nuclear and cytoplasmic formations are of unknown significance, but it is of interest for further studies that they are a constant feature of this particular tumor line.

The author wishes to thank Professor Ch. Oberling for much encouragement, and for making available the facilities of the Institute; Dr. W. Bernhard for suggesting the problem, and for his help, criticism and many stimulating discussions; Dr. I. Chouroulinkov, from whom the tumor was obtained, and Dr. R. du Boistesselin for advice on the histochemical part of the work. Grateful acknowledgment is made to Mme. Andrée Pissier and M. Sylvain Halpern for technical assistance.

The author is greatly indebted to the Mutuelle de l'Education Nationale for financial support.

CONCLUSION

1. The GRCH/15 chicken sarcoma (chemically induced by 1,2,5,6-dibenzanthracene and subsequently transplanted by grafting) was studied in thin section in the electron microscope. Thirty tumors were examined.

2. Abnormal formations were consistently observed in some cells, either in the nucleus or in the cytoplasm.

3. Two kinds of intranuclear formations could be distinguished:

(*a*) formations of amorphous character. The comparatively sparse content in the form of a

network with some filamentous and granular components was of only low density.

(*b*) well defined aggregates, consisting of closely packed and intertwined fibrils, about 170 A thick. Both formations may be found in the same nucleus, either separately or in contact with each other.

4. In the light microscope, the intranuclear formations appeared as aggregates and vacuoles. Such intranuclear PAS-positive aggregates were observed in 28 out of 30 tumors, their ages ranging from 12 to 58 days.

5. Standard tests (PAS, carmine-Best, with saliva controls) suggested that glycogen was present in these aggregates.

6. Size and frequency of occurrence of such aggregates proved to be progressive, increasing with the age of the tumor.

7. Unusual cytoplasmic formations have also been observed:

(*a*) Clusters with individual components in the form of broad tubular structures, about 450 A in diameter, within which a dense double membrane may frequently be observed. Such regions are very frequent, may occur in one or several parts of a cell, and are not confined to a particular location.

(*b*) Numerous small spherical and elongated particles, frequently with a dense double structure, found in cytoplasmic vacuoles.

8. A very old tumor showed marked mitochondrial abnormalities and no abnormal nuclear lesions or cytoplasmic formations. The mitochondria were grossly hypotrophic, and many showed localized dense material of granular appearance.

Bibliography

1. Askanazy, M., and Huebschmann, P., *Zentr. allg. Path.*, 1907, **18,** 647.
2. Aubel, E., Reich, W. S., and Lang, F. M., *Compt. rend. Acad. sc.*, 1938, **206,** 777.
3. Belt, W. D., and Pease, D. C., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 369.
4. Benedetti, E. L., Bernhard, W., and Oberling, Ch., *Compt. rend. Acad. sc.*, 1956, **242,** 2891.
5. Bernhard, W., Bonar, R. A., Beard, D., and Beard, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1958, **97,** 48.
6. Bernhard, W., Oberling, Ch., and Vigier, P., *Bull. Cancer*, 1956, **43,** 9.
7. Borysko, E., and Bang, F. B., 9th Congress, Electron Microscope Society of America, November, 1951.
8. Brown, J. W., *Anat. Rec.*, 1954, **118,** 368.
9. Carr, J. G., *Brit. J. Cancer*, 1953, **7,** 131.
10. Cazal, P., and Mirouze, J., *Presse Méd.*, 1951, **59,** 571.
11. Chouroulinkov, I., Guerin, M., Oberling, Ch., and Vigier, P., *Bull. Cancer*, 1957, **44,** 135.
12. Epstein, M. A., *Brit. J. Cancer*, 1957, **11,** 268.
13. Estable, C., and Sotelo, J. R., *Ann. Inst. Cien. Biol., Montevideo*, 1950, **1,** 105.
14. Euler, H. V., and Skarzynski, B., Biochemie der Tumoren, Stuttgart, Ferdinand Enke, 1942.
15. Ewing, J., Neoplastic Disease. A Treatise on Tumors, Philadelphia, W. B. Saunders Co., 1942.
16. Fawcett, D. W., *J. Nat. Cancer Inst.*, 1955, **15,** suppl., 1475.
17. Fawcett, D. W., and Selby, C. C., *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 63.
18. Febvre, H. L., 1957, personal communication.
19. von Gierke, E., Stoerungen des Stoffwechsels, *in* Aschoff, I., Handbuch der allgemeinen pathologischen Anatomie, Jena, Gustav Fischer, 8th edition, 1936, IV, B, 326.
20. Hale, A. J., *Internat. Rev. Cytol.*, 1957, **6,** 194.
21. de Harven, E., *Compt. rend. Soc. biol.*, 1956, **150,** 63.
22. Himes, M. H., Pollister, A. W., and Moore, B. C., *J. Histochem. and Cytochem.*, 1956, **4,** 433.
23. Houck, C. E., and Dempsey, E. W., *Stain Technol.*, 1954, **29,** 207.
24. Kleinfeld, R., and Koulish, S., *Anat. Rec.*, 1957, **128,** 433.
25. Leuchtenberger, C., 1957, personal communication.
26. Low, F. N., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 337.
27. Lubarsch, O., *Virchows Arch. Path. Anat.*, 1906, **183,** 188.
28. Mannweiler, K. L., and Bernhard, W., *Bull. Cancer*, 1958, **45,** 223.
29. McManus, J. F. A., *Stain Technol.*, 1958, **23,** 99.
30. Meyer, K. H., *Advances Protein Chem.*, 1945, **2,** 249.
31. Meyer, K. H., *Advances Enzymol.*, 1943, **3,** 109.
32. Moses, M. J., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 397.
33. Oberling, Ch., Bernhard, W., and Vigier, P., *Nature*, 1957, **160,** 386.
34. Palade, G. E., *J. Exp. Med.*, 1952, **95,** 285.
35. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 567.
36. Peacock, P. R., *Cancer Research*, 1946, **6,** 311.
37. Porter, K. R., *J. Histochem. and Cytochem.*, 1954, **2,** 346.
38. von Przylecki, St. J., and Majmin, R., *Biochem. Z.*, 1934, **271,** 168.
39. Rebhun, L. I., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 93.

40. Rouiller, Ch., Haguenau, F., Golde, A., and Lacour, F., *Bull. Cancer*, 1956, **43,** 10.
41. Schulz, H., *Oncologia*, 1957, **10,** 307.
42. Shelton, E., *J. Nat. Cancer Inst.*, 1955, **16,** 107.
43. Swift, H., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 415.
44. Warren, S., and Lecompte, P. M., The Pathology of Diabetes Mellitus, Philadelphia, Lea and Febiger, 1952.
45. Weiss, J. M., *J. Exp. Med.*, 1955, **102,** 783.
46. Wessel, W., and Bernhard, W., *Z. Krebsforsch.*, 1957, **62,** 140.
47. Willstätter, R., and Rohdewald, M., *Z. Physiol. Chem.*, 1934, **225,** 103.

Explanation of Plates

Plate 55

Fig. 1. Feulgen-stained, methacrylate-embedded section, after fixation with osmic acid. Arrows point to vacuolar (Feulgen-negative) lesions. Note nucleus (arrow near center) which has two separate vacuolar lesions. × 800.

Fig. 2. Feulgen preparation as above. Note large vacuoles (arrows), which may fill almost entire nucleus (right arrow). ×1600.

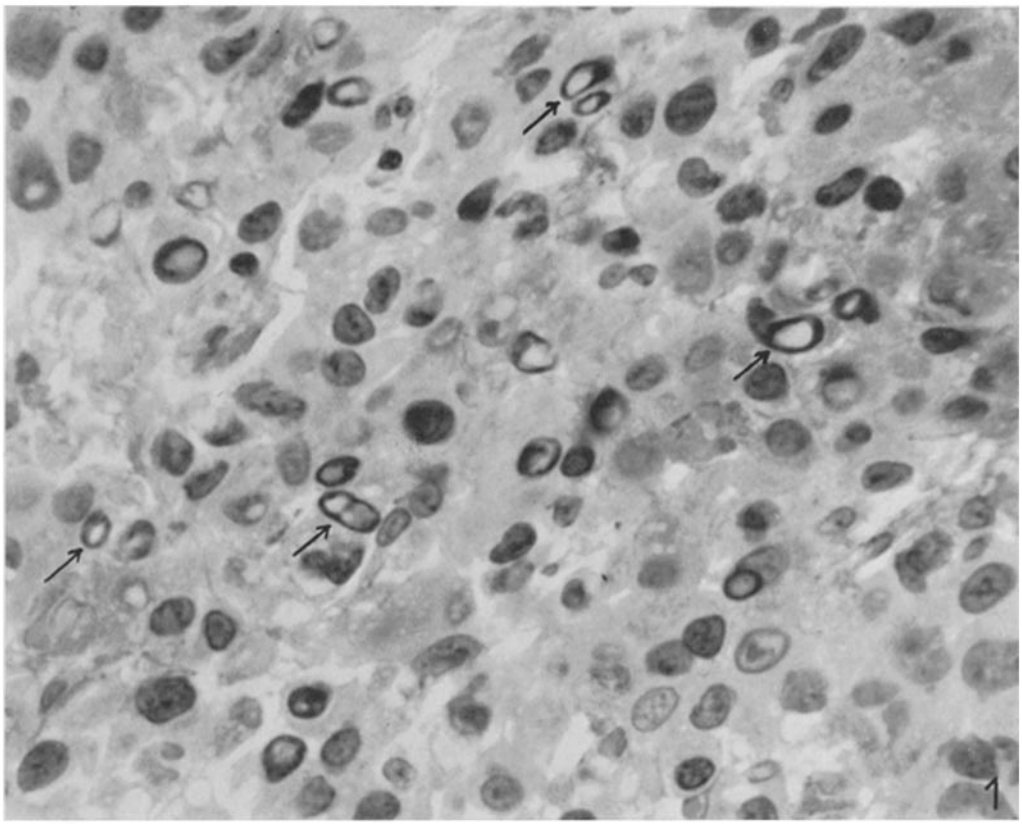
Fig. 3. Periodic acid–Schiff on methacrylate section, after fixation with osmic acid. × 800.

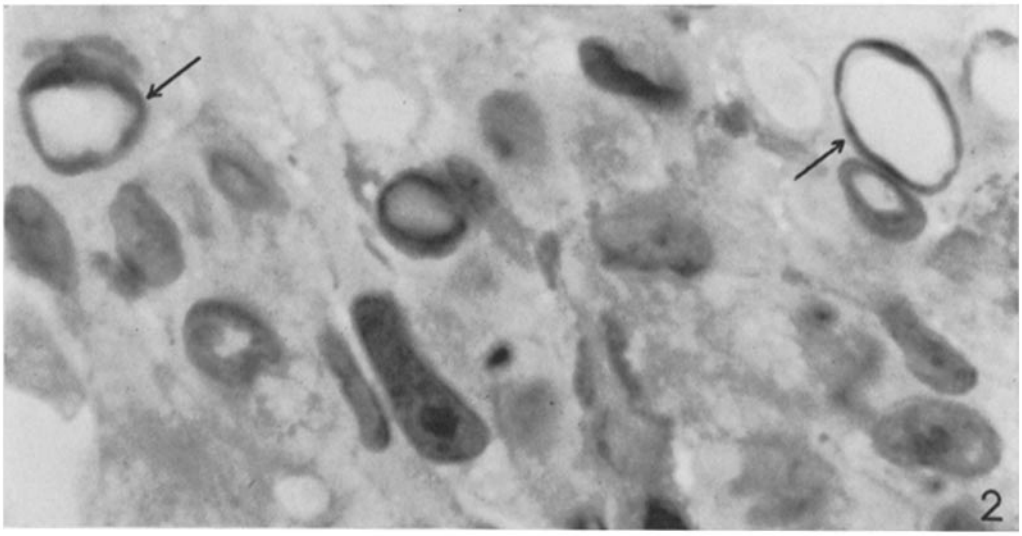
3 *a*. Nucleus with large PAS-postive aggregate. Strands may be seen (arrow) which link aggregate and chromatin rim. A separate small aggregate is located within the chromatin portion (bottom), and connected with the main aggregate by strands.

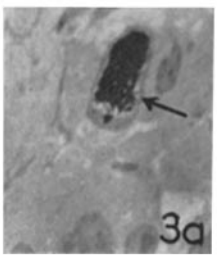
3 *b*. Round nucleus, containing intertwined thread-like formation. Note the comparatively narrow chromatin rim.

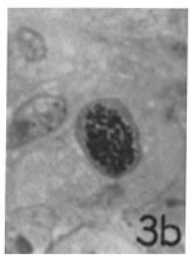
3 *c*. Nucleus with large aggregate and adjacent vacuolar portion. Note that the aggregate appears uniformly opaque owing to the greater thickness of this section.

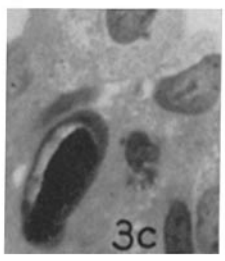
Fig. 4. Feulgen-stained preparation, as for Figs. 1 and 2. A positive portion projects from the chromatin rim into the negative region of the nucleus. Note the thread-like heterogeneities recognizable in the positive central portion. ×2000.

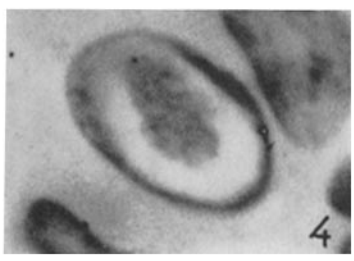








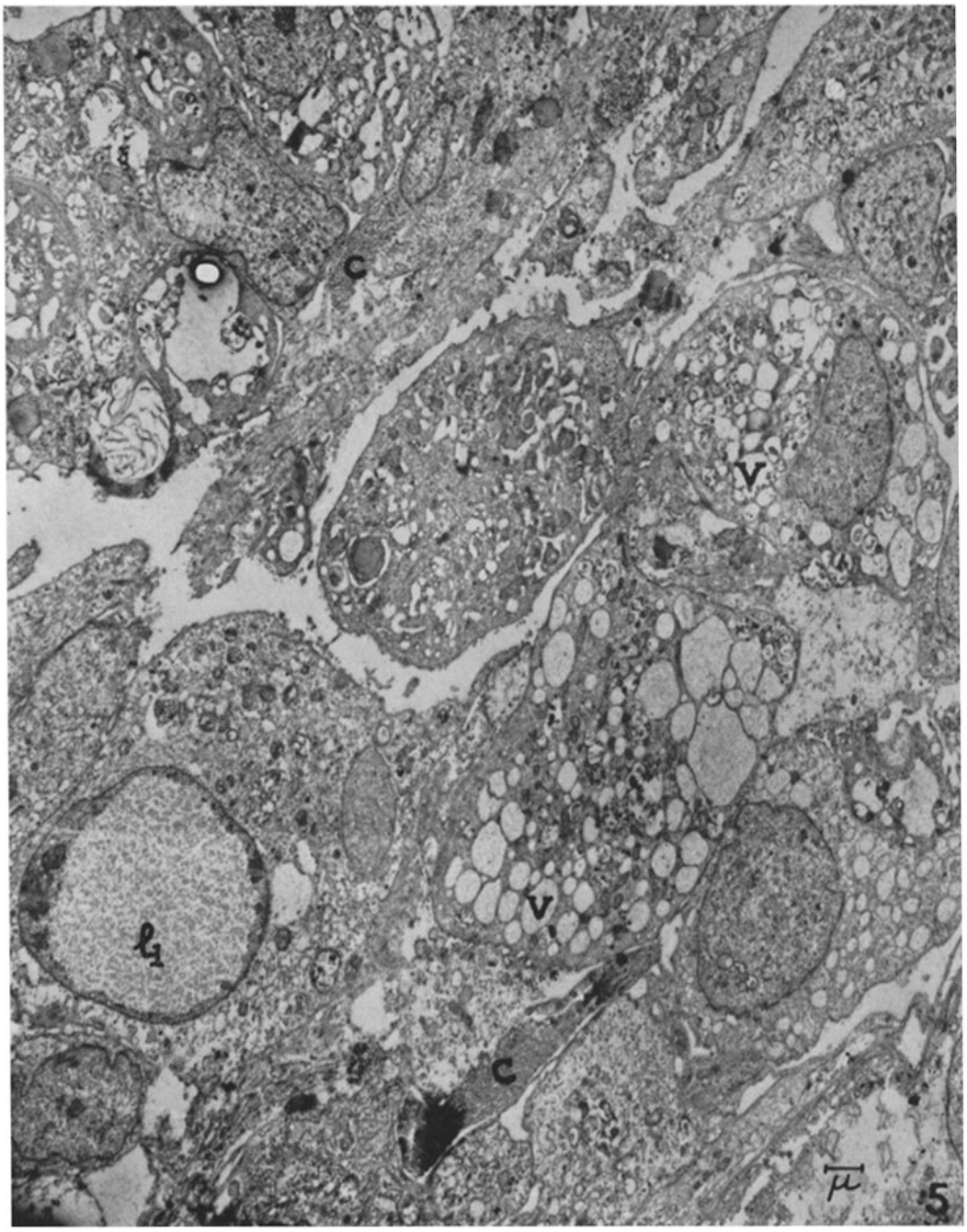




(Friedlaender Binggeli: Intranuclear and cytoplasmic formations)

PLATE 56

FIG. 5. Low power electron micrograph of a tumor area, with one round nucleus containing a large type 1 lesion (l_1), some cells showing some vacuolation of the cytoplasm (v), and intercellular collagen (c). $\times 5000$.

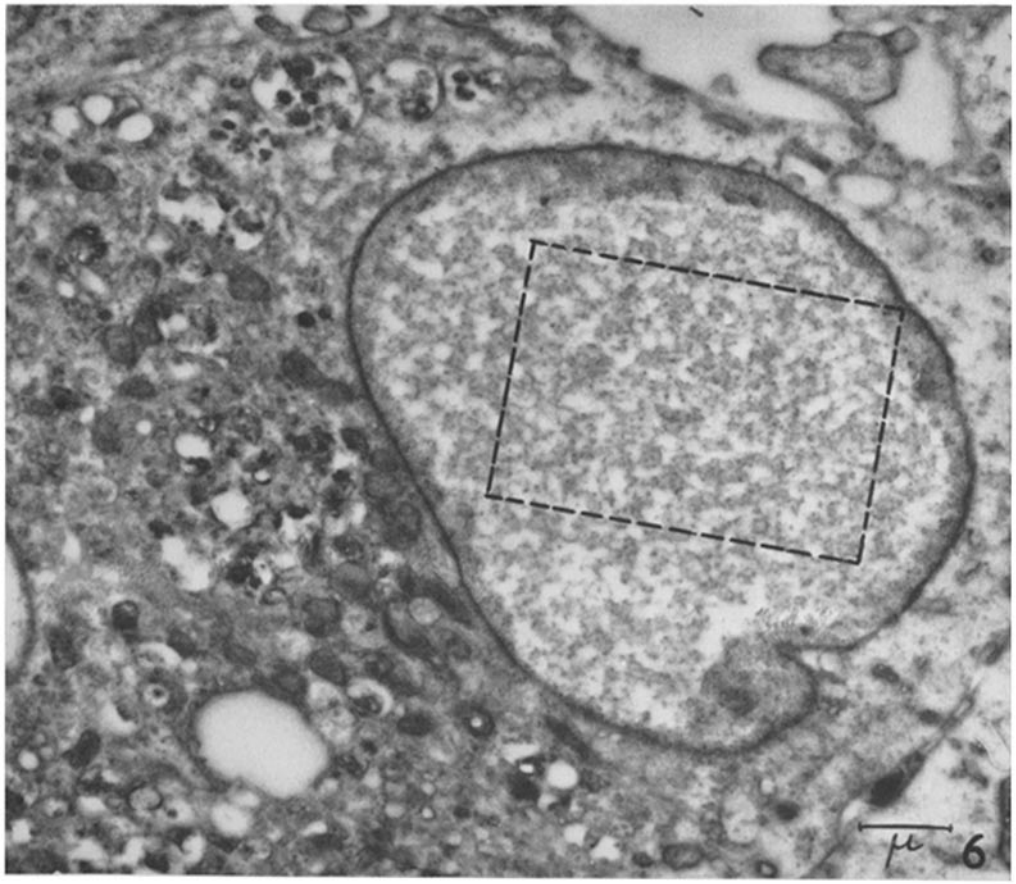


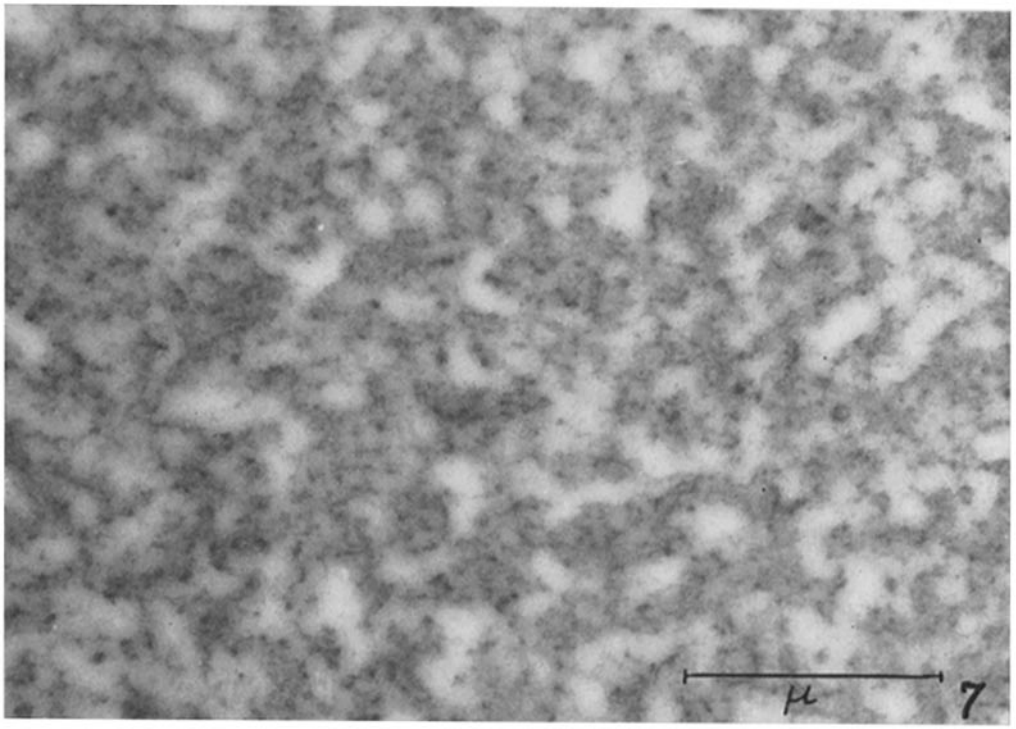
(Friedlaender Binggeli: Intranuclear and cytoplasmic formations)

PLATE 57

FIG. 6. Nucleus with large type 1 lesion, leaving only a narrow rim of chromatin near the nuclear membrane. The cytoplasm appears somewhat vacuolated. ×12,000.

FIG. 7. Portion of nuclear lesion, corresponding to encircled region in Fig. 6, at higher magnification. ×34,000.



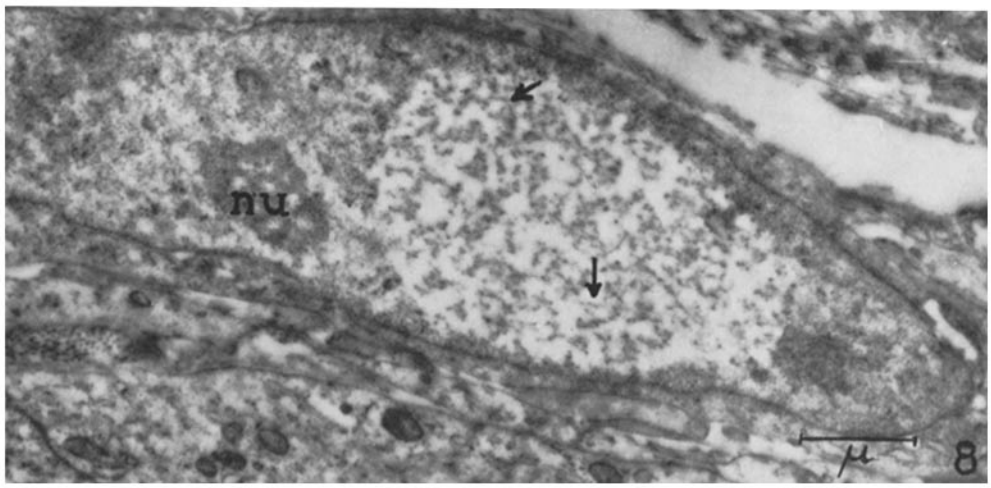


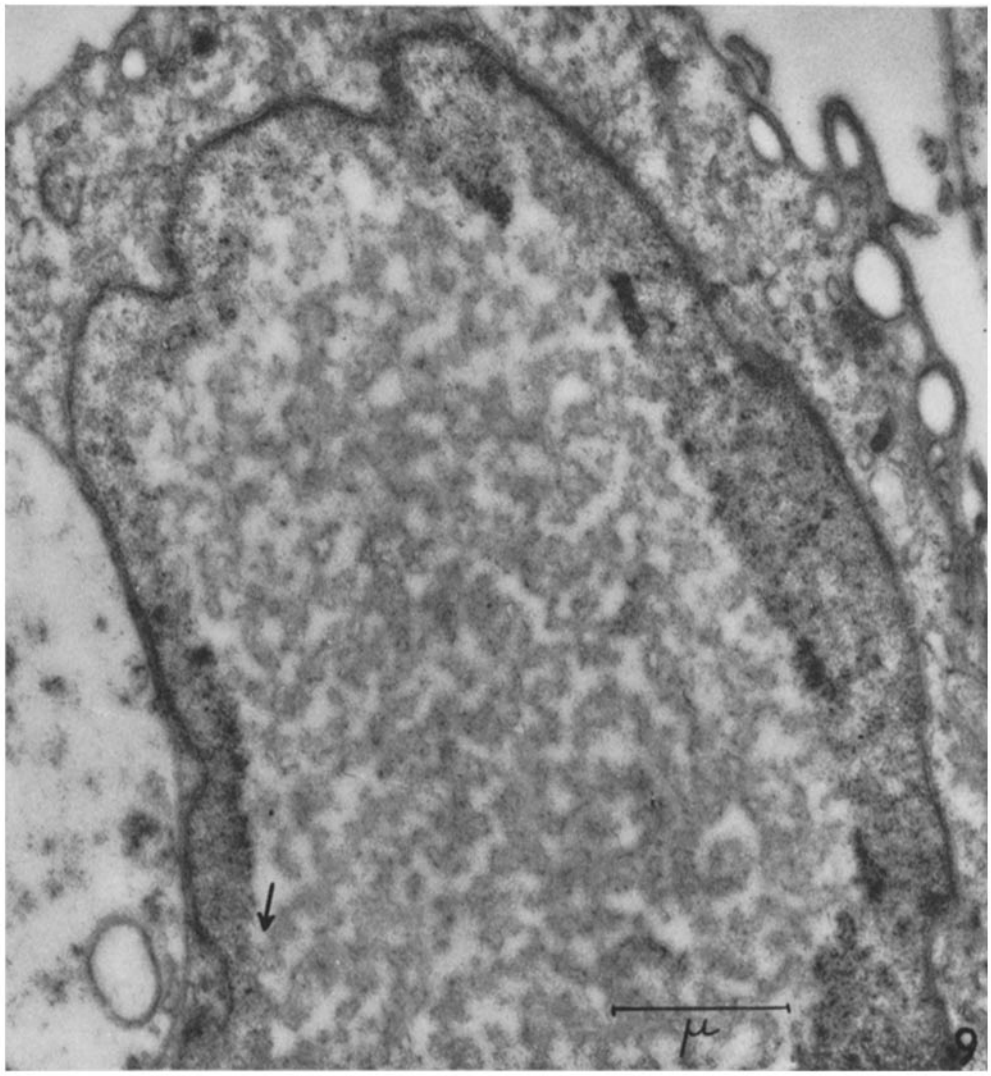
(Friedlaender Binggeli: Intranuclear and cytoplasmic formations)

PLATE 58

FIG. 8. Spindle cell, with medium-sized nuclear lesion of type 1; some components of fibrillar appearance are indicated by an arrow. Note nucleolus (*nu*), at some distance from the lesion. ×15,000.

FIG. 9. Nuclear lesion (type 1). Note continuity of some of the internal components and the chromatin rim (as at arrow). ×23,000.

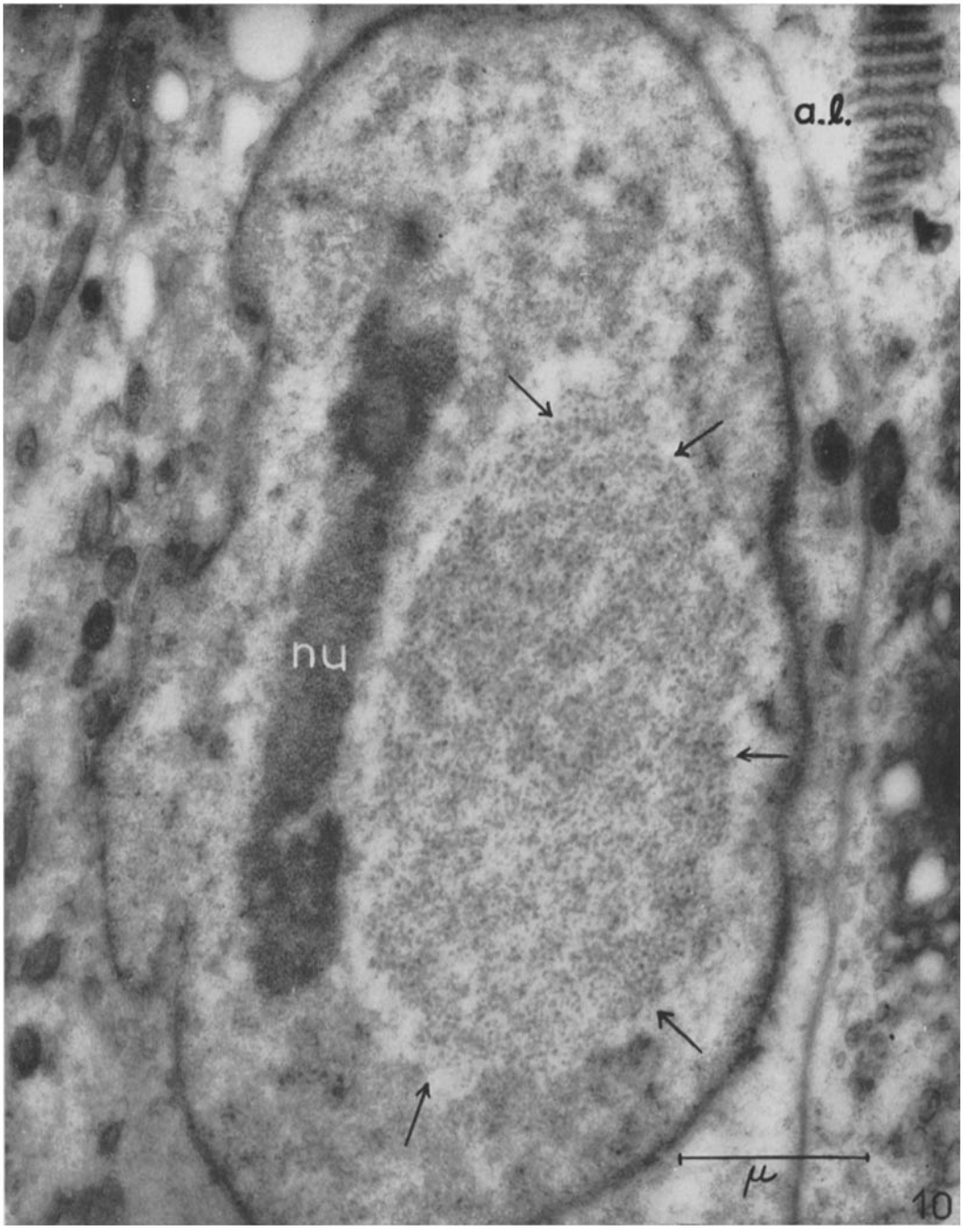




(Friedlaender Binggeli: Intranuclear and cytoplasmic formations)

PLATE 59

FIG. 10. Nucleus with large type 2 lesion (see arrows). Note the difference between the granular appearance of the nucleolus (*nu*), and the much coarser apparent granularity of the lesion. A group of 11 annulate lamellae (*a.l.*) are visible in the right top corner. Note the transverse fibrillar connections which continue into the cytoplasm. ×28,000.



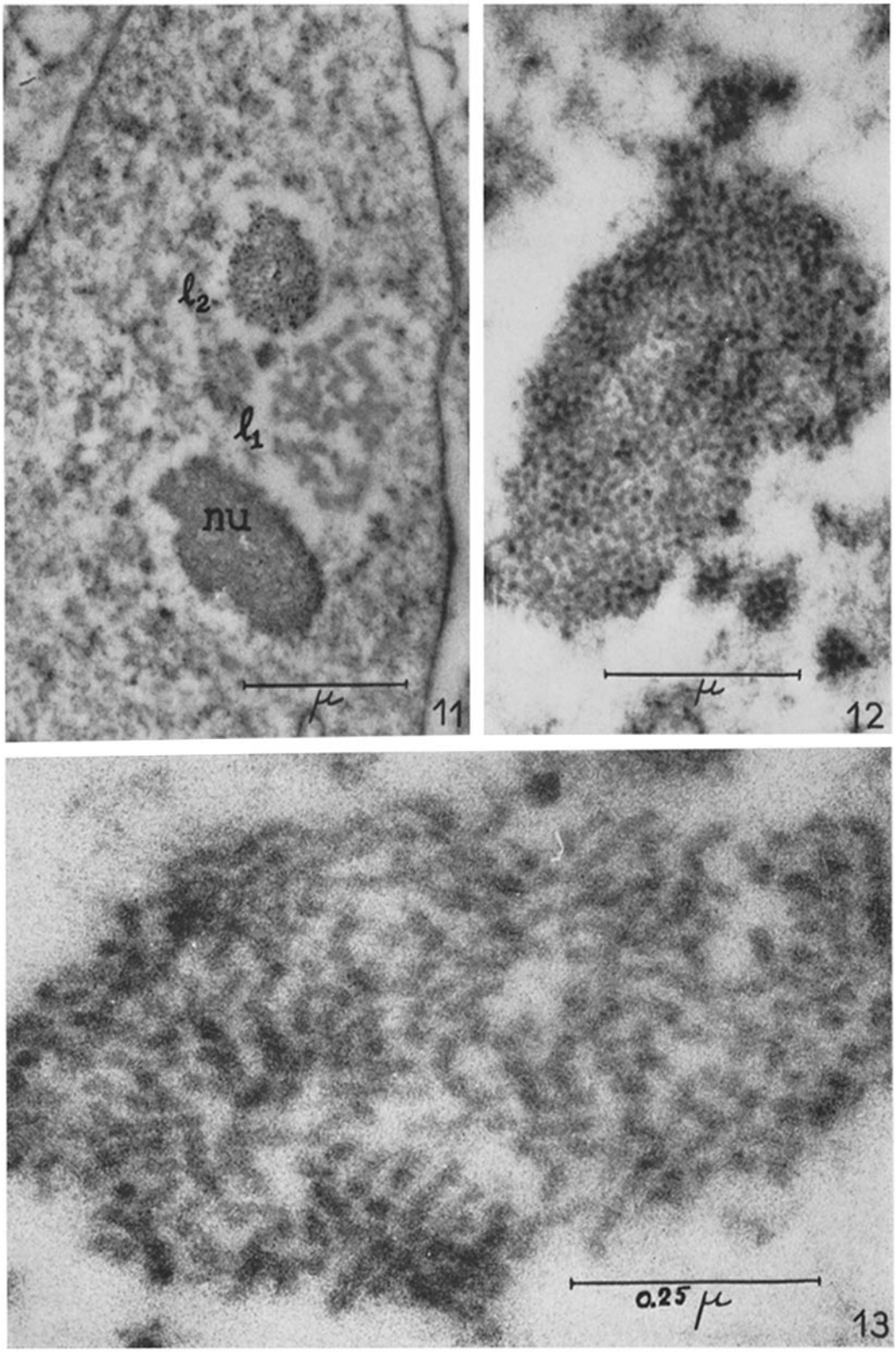
(Friedlaender Binggeli: Intranuclear and cytoplasmic formations)

PLATE 60

FIG. 11. Nucleus with nucleolus (*nu*) and two small lesions l_1 and l_2, corresponding to abnormal formations of type 1 and type 2, respectively. Note differences in density and organization between the two lesions. ×26,000.

FIG. 12. Nuclear lesion (type 2). Note close packing of components, and their granular appearance in cross-section. The lesion is surrounded by a zone of low electron density. ×61,000.

FIG. 13. A type 2 lesion at higher magnification. Note the fibrillar aspect of the constituent structures. ×155,000.



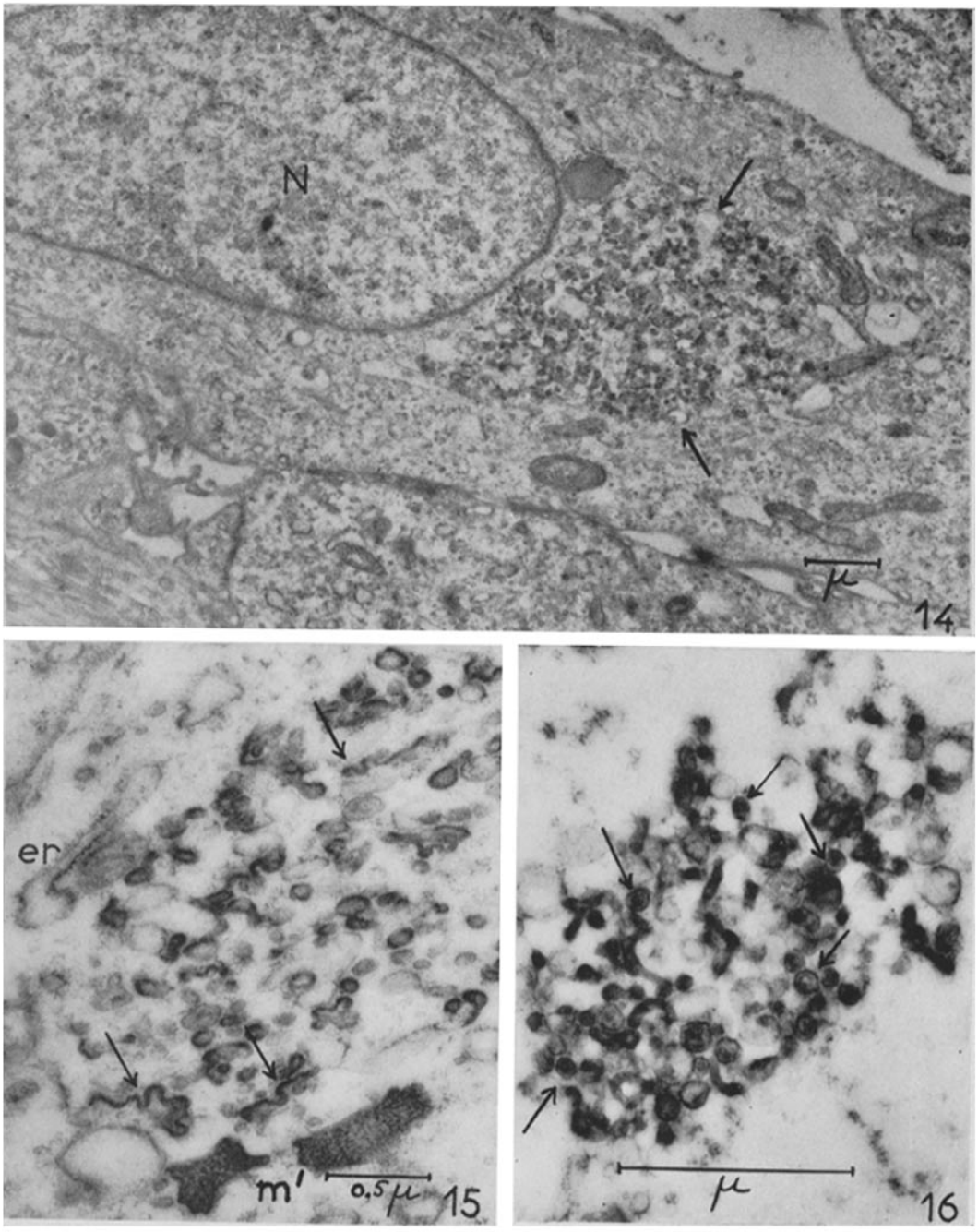
(Friedlaender Binggeli: Intranuclear and cytoplasmic formations)

PLATE 61

FIG. 14. Low power electron micrograph showing nucleus (*N*) and abnormal cytoplasmic formation, with its limits indicated by arrows. ×12,000.

FIG. 15. Higher power micrograph of abnormal cytoplasmic formation. Note the folded opaque threads at arrows. *m'* represents a structure of the type often found near such formations (modified mitochondrion?). Some ergastoplasm (*er*) is visible. ×30,000.

FIG. 16. Abnormal cytoplasmic formation in a compact cluster, with some small dense bodies (at arrows). × 34,000.



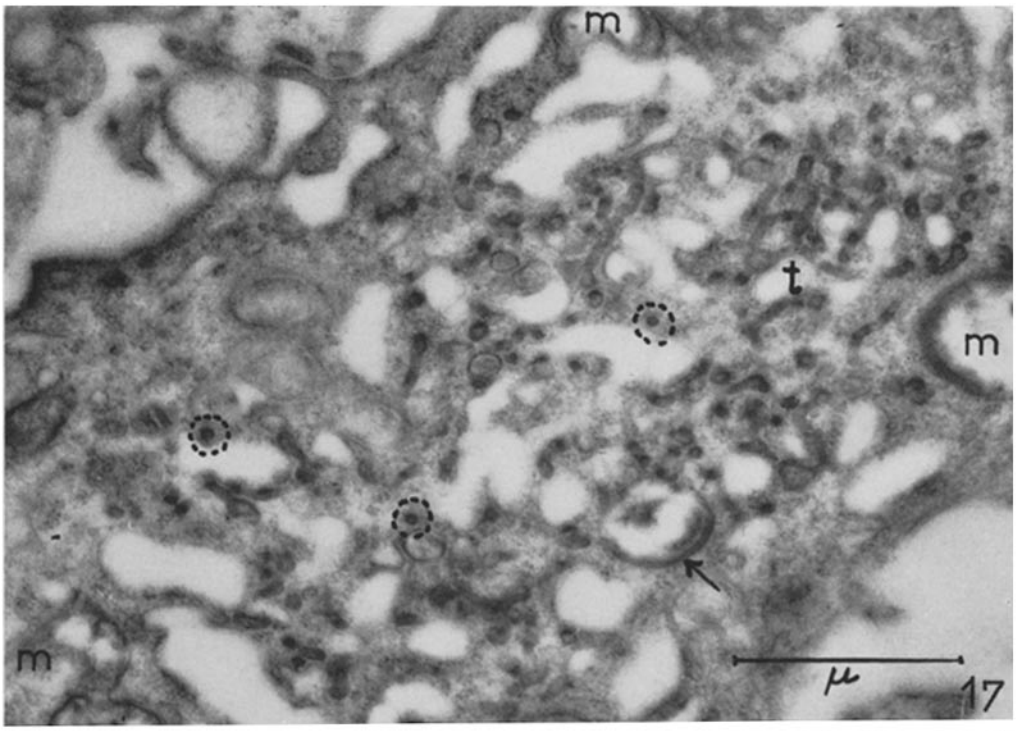
(Friedlaender Binggeli: Intranuclear and cytoplasmic formations)

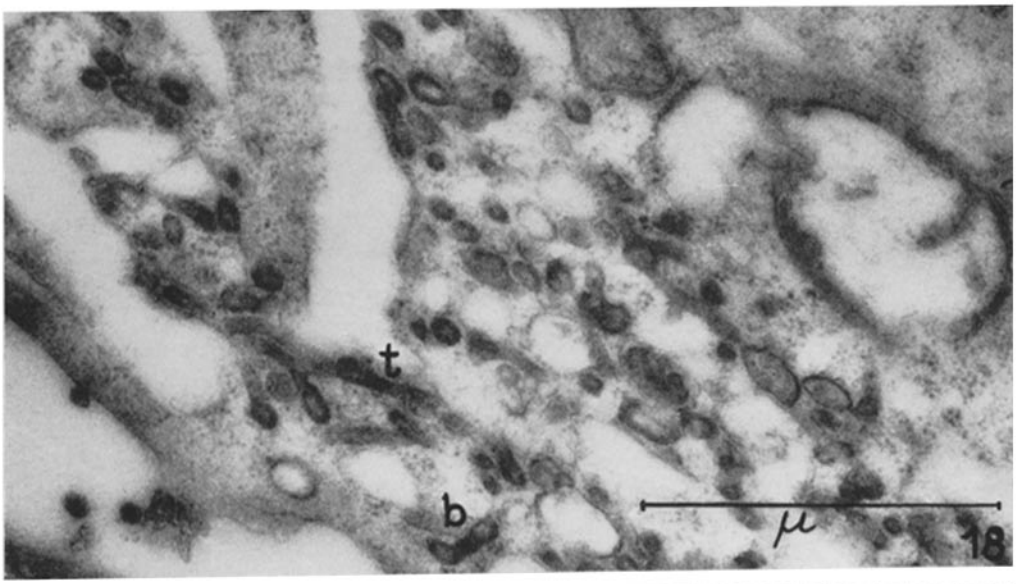
PLATE 62

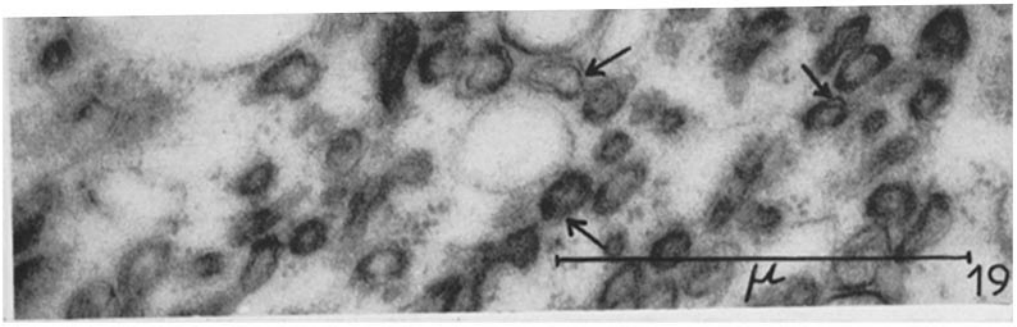
FIG. 17. Fairly thick section of abnormal cytoplasmic formations, in a more vacuolated portion of cytoplasm. Numerous tubular structures are visible at (*t*); dotted lines encircle some individual round structures. Mitochondria show localized swelling as at *m*, sometimes with broadening of boundary (see arrow). ×30,000.

FIG. 18. Abnormal cytoplasmic formations, showing tubular structures (*t*) and budding (*b*). × 48,000.

FIG. 19. Abnormal cytoplasmic formations. Note the two concentric membranes (arrows) of the elements indicated. ×54,000.





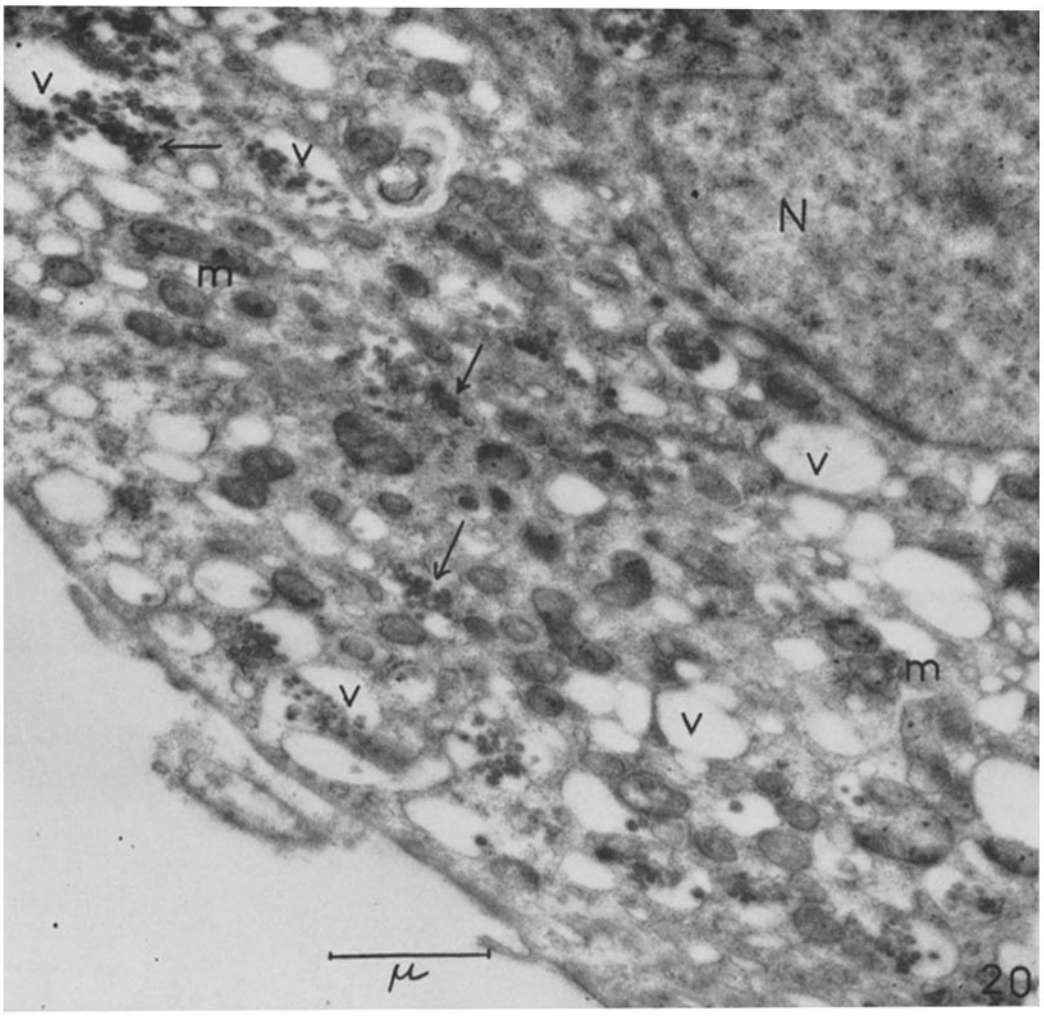


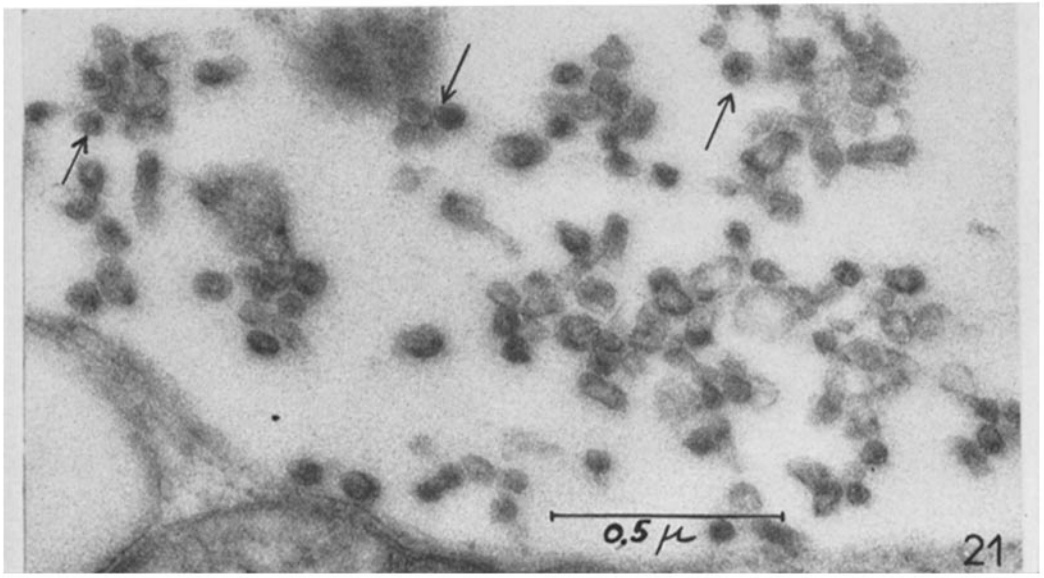
(Friedlaender Binggeli: Intranuclear and cytoplasmic formations)

PLATE 63

FIG. 20. Cell with numerous clusters of particles (some indicated by arrows) in the cytoplasm itself and in some of the vacuoles (*v*). Such a group is also found near the nucleus (*N*) at the top left. Note mitochondria (*m*) which contain many dense granules. ×21,000.

FIG. 21. Close-up of a vacuole. Arrows point to some of the more regular round structures. ×61,000.



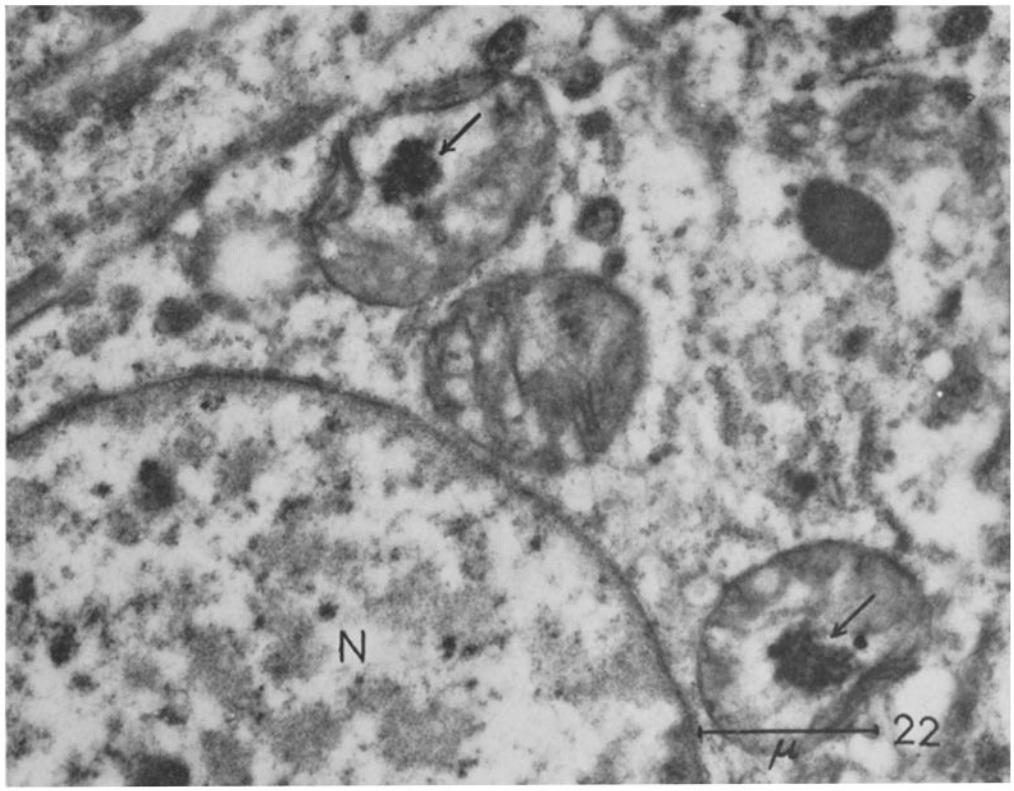


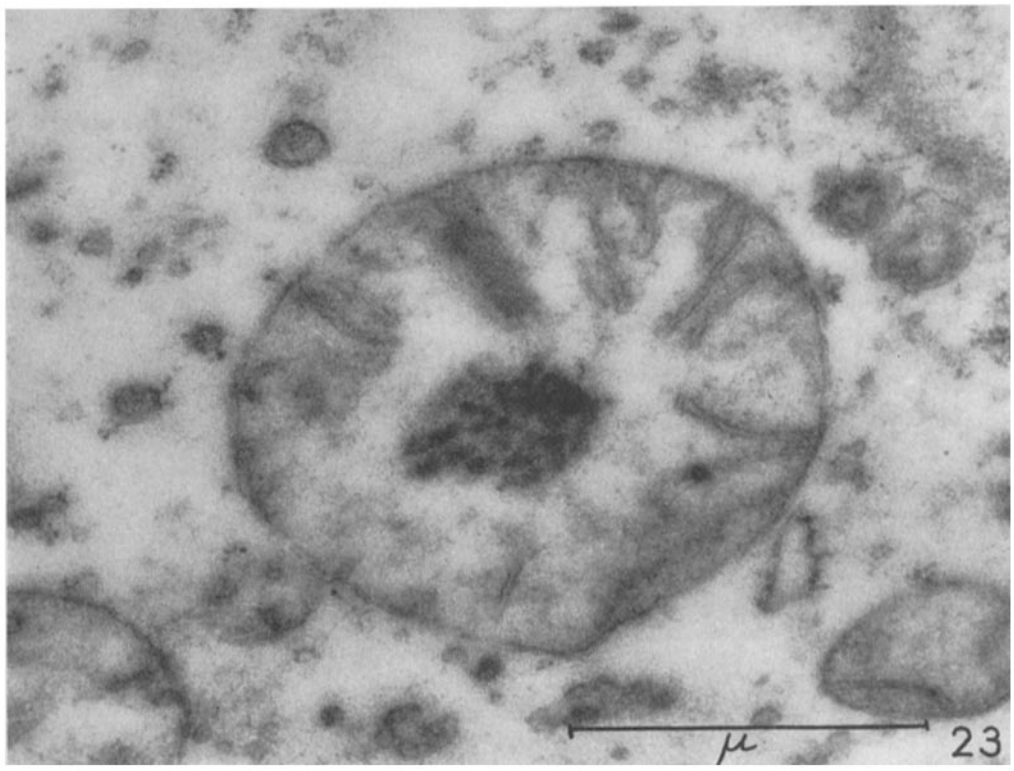
(Friedlaender Binggeli: Intranuclear and cytoplasmic formations)

PLATE 64

FIG. 22. Cells from an exceptionally old (235-day) tumor. Note the large modified mitochondria, two of which near the nucleus (*N*) contain dense heterogeneous formations (arrows). ×23,000.

FIG. 23. Close-up of a similar large mitochondrion of modified structure. Note the normal-sized mitochondrion on the right, bottom, for comparison. ×48,000.





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