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Ultrastructure of Tubular Inclusions in Endothelial Cells of Pituitary Tumors Associated with Acromegaly*

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Summary. Tubular inclusions were present in 13 out of 43 pituitary adenomas of acromegalic patients and in a single chromophobe pituitary adenoma. There were none in 76 other pituitary adenomas with differing endocrinological symptomatology. The arrays were usually located in the perinuclear cistern of capillary endothelial cells. The tubule diameter in osmium fixed material measured 19–26 nm and the light core averaged 6–11 nm. A longitudinal period of about 4.5 nm could be demonstrated with PTA block staining. Fixation with glutaraldehyde and block staining with ethidium bromide as well as permanganate fixation followed by RNAse treatment showed only the core of the tubules consisting of globular subunits. Several histochemical reactions (perchloric acid extraction, methenamine-silver staining, trypsin and DNAse digestion of frozen sections) suggested that the particles consist of a core of DNA coated with protein. No virus multiplication could be detected in cell cultures or in mice inoculated with fresh tumor material. No significant antibody titers against several virus antigens could be demonstrated.

Key words: Pituitary adenoma — Acromegaly — Tubular inclusions — Endothelial cells.

Introduction

Tubular inclusions within the endoplasmic reticulum of endothelial cells were first described by Fresco (1968) in systemic lupus erythematosus. Since then similar looking structures have been found in various human tumors (Hodgkin's disease, osteosarcoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma, Kaposis sarcoma, hepatoblastoma brain tumors, pituitary adenoma), Letterer-Siwe disease, scleroderma, encephalitis, myositis, myopathies, nephritis, nephrosis, mononucleosis infectiosa, congenital rubella syndrome as well as in material of cell culture origin (for review see Kistler and Groscurth, 1973; Lyon et al., 1972; Schaff et al., 1972; Uzman et al., 1971).

Two possible interpretations have been proposed in the literature: The particles may represent an unknown but widely distributed virus (Györkey et al., 1969; 1971; Jenson et al., 1971; Norris et al., 1972; Norton, 1969; Smith and Northrop, 1971) or may be the product of the endoplasmic reticulum responding to certain stimuli (Barringer and Swoveland, 1972; Chandra, 1968; Jenson et al., 1971; Landry and Winkelman, 1972; Schaff et al., 1972, 1973; Uzman et al., 1971).

We have applied a variety of fixation, staining, and digesting techniques in a series of pituitary adenomas in order to get further insight into the nature

* Dedicated to Prof. Dr. G. Töndury on the occasion of his 70th birthday.

of at least one variety of tubular arrays because the conclusions of most papers dealing with this subject have been drawn almost exclusively from the interpretation of osmium or glutaraldehyde-osmium fixed and epoxy embedded material. Our observations as well as the results of the literature mentioned will deal only with human biopsy material and not with cell cultures since secondary changes may have taken place.

Material and Methods

A total of 120 pituitary adenomas were examined with the electron microscope. Fourty-three of these tumors were removed from acromegalic patients. Fifty-eight tumors did not show any clinical signs of endocrine hyperfunction (so called chromophobe adenomas). In addition there were seven oncocytomas, six cases of Forbes-Albright syndrome, three cases of Nelson's syndrome, two cases of Cushing's syndrome and one case of Addisons disease (for review of the detailed ultrastructural findings see Landolt, 1975).

The following methods of tissue fixation, embedding, staining and histochemical examination were applied (for detailed descriptions see Landolt, 1975):

1. Osmium tetroxide fixation, embedding in Durcupan® (Fluka AG, Buchs/SG, Switzerland).
2. Glutaraldehyde-osmium tetroxide fixation, Durcupan® embedding.
3. Glutaraldehyde fixation, Durcupan® embedding, without osmium treatment.
4. Glutaraldehyde fixation, ethidium bromide (Sigma) block staining, Durcupan® embedding.
5. Block staining of osmium fixed material with phosphotungstic acid (PTA), Durcupan® embedding.
6. DNA staining with the potassium permanganate (Luft, 1956) RNAse (Fluka) treatment of Yotsuyanagi and collaborators (1960, 1965).
7. Trypsin-RNAse (Fluka) and trypsin-DNAse (Fluka) digestion of p-formaldehyde fixed frozen sections with secondary osmium fixation and Durcupan® embedding.
8. Perchloric acid extraction of osmium tetroxide fixed and Durcupan® embedded material (Douglas, 1970).
9. Methenamine-silver staining of glutaraldehyde fixed and Durcupan® embedded tissue (Peters and Giese, 1970, 1971).

The biopsy material was homogenized immediately and a 10% suspension in Eagle's MEM with fetal bovine serum was inoculated into tubes with monkey kidney (Cynomolgus) and human lung fibroblast (WI 38) cell cultures. The same material was also injected intracerebrally in weaning mice. Pre-operative sera of the patients were examined for antibodies against different viruses by hemagglutination-inhibition or complement fixation testing using the micro-titer system.

Results

Undulating tubular structures were found in 13 out of 43 adenomas of acromegalic patients (= 30%), in only one chromophobe adenoma, and in none of the remaining cases. They were observed only in endothelial cells and never in epithelial cells, pericytes or fibroblasts. In some tumors up to 50% of the endothelial

Fig. 1. Low power electron micrograph demonstrating aggregation of tubular structures (arrow) in perinuclear cistern of endothelial cell of a pituitary adenoma in a case of acromegaly. Osmium fixation, uranyl acetate staining, ×15,400

Fig. 2. Detail of perinuclear cistern containing interwoven tubules with connections to nuclear membrane (arrowhead) and external membrane of cistern (arrow). Osmium fixation, uranyl acetate and lead citrate staining, ×138,000

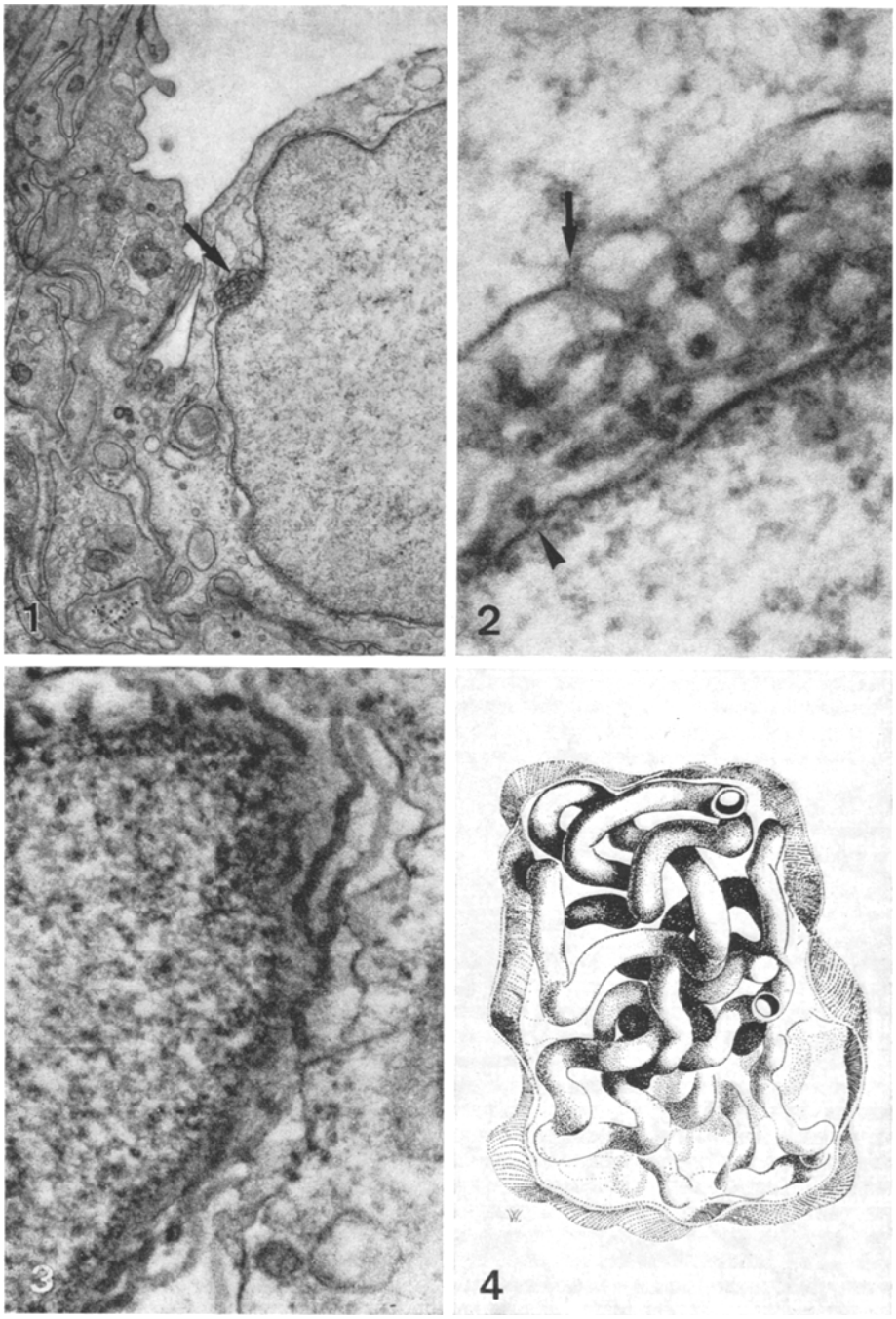

Fig. 3. Perinuclear cistern containing relatively straight tubules which can be followed for a distance of 0.5 μm. Osmium fixation, uranyl acetate and lead citrate staining, ×90,200

Fig. 4. Artist's impression of three dimensional arrangement of tubules as seen in stereomicrographs

cells sectioned through the region of the nucleus were affected. The tubules were usually located in the perinuclear cistern (Figs. 1 and 2). In some instances they could be observed in the dilated cisterns of the rough-surfaced endoplasmic reticulum not in contact with the nucleus. No tubules or similar structures were present in the interior of the nucleus. The tubular units measured approximately 22 nm in outside diameter in osmium fixed material (Table 1). They possessed a light core with a diameter of about 8 nm. This core was best seen in osmium fixed material and was much less distinct in glutaraldehyde-osmium or osmium-PTA fixed material. The core diameter was not significantly altered by the different osmium containing fixation techniques. The tubules were usually twisted and appeared to be branched (Fig. 2). They could be followed only rarely for distances up to 0.5 μm (Fig. 3). No branching could be observed along the straighter sections of the tubules.

Stereoscopic micrographs of "thick" sections (interference color blue), corresponding to a section thickness of 190–240 nm (Pease, 1964) gave further insight

Table 1. Dimensions of the tubular structures in acromegaly after different fixation procedures

Fixation technique	Staining[a]	External diameter of tubule (nm)	Diameter of core (nm)	Thickness of wall (nm) (calculated)	Diameter of unit membrane in other cell organelles (nm)
Osmium	UAc-Pb	22.3 (19.6–26.8)	8.5 (5.6–11.2)	7.0	7.5 (6.1–9.0)
Glutaraldehyde-osmium	UAc-Pb	23.1 (20.6–26.1)	7.9 (6.6–10.8)	7.5	8.5 (7.8–9.4)
Osmium	PTA	23.1 (20.2–24.8)	6.3 (4.5–10.9)	8.4	8.2 (6.8–9.0)
Glutaraldehyde-ethidium bromide	UAc-Pb		12.7 (9.4–18.0)		
Potassium permanganate	RNAse UAc		8.0 (5.8–9.4)		9.0 (7.9–11.2)

[a] UAc = Uranyl acetate; Pb = Lead citrate; PTA = Phosphotungstic acid

Fig. 5. Cross section of tubule in perinuclear cistern demonstrating trilaminar structure of tubule wall (arrow). Osmium fixation, uranyl acetate and lead citrate staining, ×277,000

Fig. 6. Cross striation pattern of tubules (arrow) visible after PTA staining. Occasional cross sections demonstrate hexagonal array (arrowheads) of dark dots which can be enhanced by photographic "symmetrizing" (inset). Osmium fixation, PTA block staining, ×356,000 (inset ×534,000)

Fig. 7. Demonstration of tubular structure by glutaraldehyde-osmium fixation and uranyl acetate and lead citrate staining, ×146,000

Fig. 8. Hazy picture of cisternal inclusion material as observed after glutaraldehyde fixation alone combined with uranyl acetate and lead citrate staining, ×159,000

Fig. 9. Demonstration of electron dense strands instead of tubular structure by glutaraldehyde fixation and ethidium bromide treatment combined with uranyl acetate and lead citrate staining, ×155,000

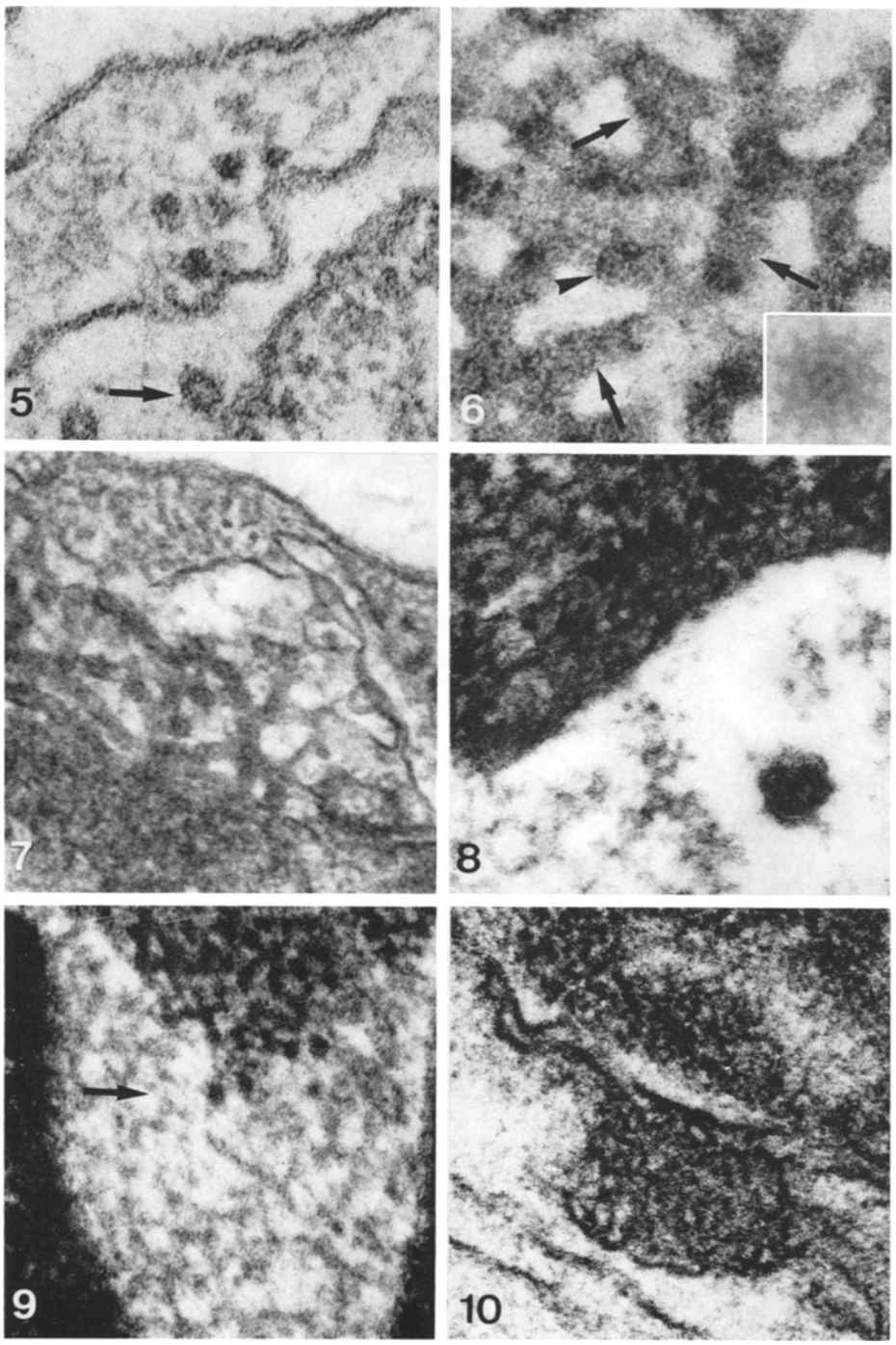

Fig. 10. Accumulation of electron dense strands within perinuclear cistern demonstrated by the permanganate-RNAse method combined with uranyl acetate staining. Ribosomes are not seen. ×113,000

into the three dimensional arrangement of the tubular structures. This technique showed that the apparent branching was caused by the superimposition of two tubules running in different directions. This is easily possible since the normally used sections (interference color silver) are about 3–4 times as thick as the tubules. An artist's impression of the stereomicrographs is shown in Fig. 4. No crystalline patterns were present in the material examined.

The tubules appeared to be continuous with the external and internal membrane of the perinuclear cistern (Fig. 2). The tubular core was therefore related to the nuclear material. The tubular wall demonstrated the unit membrane pattern (Fig. 5). Visualization of the trilaminar pattern was rather difficult because of the marked twisting of the structures. The calculated thickness of the tubular wall coincided with measurements of the unit membrane of other organelles in all fixation techniques using osmium (Table 1). Block staining with PTA demonstrated a definite cross striation of the tubules which could not be seen after osmium or glutaraldehyde-osmium fixation (Fig. 6). The pattern showed a periodicity of 4.0–4.5 nm suggesting a helical structure of the tubule wall. Occasional cross sections demonstrated a hexagonal array of dark and bright dots arranged around a dark central core. This pattern could be enhanced by photographic "symmetrizing" (Fig. 6, inset).

We have eliminated osmium as a fixative in several experiments after realizing that all papers demonstrating tubular structures used this particular technique. A series of experiments compared the tubular structure of osmium (Fig. 2) and glutaraldehyde-osmium fixed tissue (Fig. 7) with material which had never been in contact with osmium tetroxide. Glutaraldehyde alone (Fig. 8) gave an extremely hazy picture. No tubules could be recognized. The combination of glutaraldehyde and ethidium bromide changed the image completely (Fig. 9). The arrays within the perinuclear cistern consisted of electron dense strands with an average diameter of 12.7 nm. They showed a definite globular substructure if viewed in appropriate position (arrow in Fig. 9). No membranous exterior was depicted. The unit membranes of the cells were not visible with this technique. Ribosomes, nuclear material and lysosomes were heavily stained. Fixation with potassium permanganate, incubation in RNAse and staining with aqueous uranyl acetate again showed electron dense strands of globular material with a diameter of 8 nm approximately. Ribosomes were not demonstrated with this technique (Fig. 10).

The comparison of perchloric acid extracted sections with control sections of osmium fixed and Durcupan embedded material (Figs. 11, 12) showed an almost complete destruction of the tubules in the perchloric acid treated material. This effect was not specific for the structures in question as other membranous

Figs. 11 and 12. Control and adjacent perchloric acid extracted sections, both osmium fixed and araldite embedded, demonstrating almost complete destruction of the tubules in the extracted material. Nuclear membrane and Golgi complex remain visible. ×47,000

Fig. 13. Absence of tubular formation in perinuclear cistern after trypsin-DNAse treatment of p-formaldehyde fixed frozen sections obtained from a biopsy containing the structures after routine osmium fixation. Osmium fixation of digested thick sections, uranyl acetate and lead citrate staining, ×38,500

Fig. 14. Frozen section of the same material as in Fig. 13 treated with trypsin-RNAse. Osmium fixation of digested thick sections, uranyl acetate and lead citrate staining, ×38,500

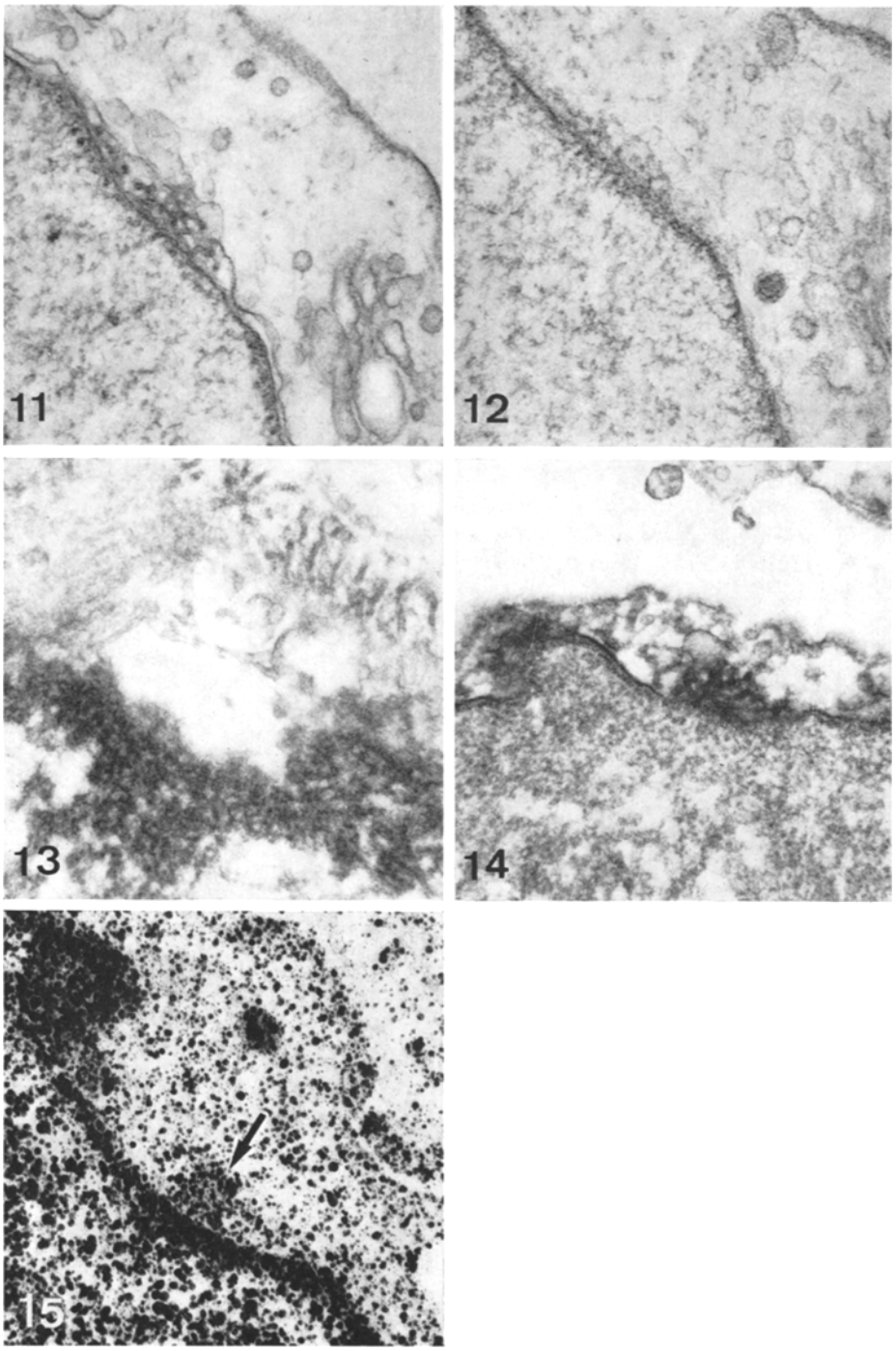

Fig. 15. Silver deposit (arrow) of intermediate density in supposed area of tubular aggregation as demonstrated by methenamine-silver method. ×69,000

organelles (e.g. the Golgi cisterns) were also partially affected. No tubular inclusions could be found in p-formaldehyde fixed and trypsin-DNAse digested material (Fig. 13). The perinuclear cisterns were empty in dilated regions. The trypsin-RNAse treatment left the structures intact (Fig. 14). The methenamine-silver method showed accumulations of silver grains in circumscribed areas of the perinuclear cistern. These grains were less densely arranged than those within the nucleus (Fig. 15).

One patient underwent repair craniotomy for plastic correction of the skull defect three months after initial tumor extirpation. The tumor biopsy had shown numerous tubular arrays in the cisterns of the endothelial cells. A muscle biopsy removed during the second surgical procedure showed the similar type of tubular structures in the endothelial cells of the muscle capillaries. No similar structures could be seen in the muscle fibres or peripheral nerves. Muscle biopsies taken during the initial procedures of seven subsequent cases did not contain tubules.

Tubular structures were present in the tumor biopsies of two out of 12 acromegalic patients (= 17%) who had received a course of radiotherapy prior to surgery and in 11 out of the remaining 31 patients (= 35%) who had not undergone preoperative irradiation. The difference is not statistically significant ($p = 0.44$). Radiotherapy therefore does not seem to influence their presence or absence.

Fourteen patients (4 cases with and 10 cases without tubular structures) showed negative serologic reactions for systemic lupus erythematosus (Waaler-Rose test and antiglobulin consumption test for cell nuclei). The remaining patients were not examined. No significant antibody titers could be observed with different viral antigens (measles, rubella, herpes, mumps). In 5 cases the tumor biopsy material was tested in cell cultures and in mice (observation time 3 months). Only in one case could Echovirus type 11 be detected, and, in this instance no tubular inclusions were found. The significance of this finding is questionable as at that time an outbreak of Echovirus type 11 was affecting patients and hospital staff alike.

Discussion

Pituitary adenomas of acromegalic patients are an excellent source for the study of intracisternal, undulating, tubular arrays in endothelial cells since 30% of the cases examined contained these structures. The formations are quite inconspicuous and the tissue has to be examined thoroughly in order to detect the tubules. This is the reason why they have been described previously only in a single case of thyrotropic adenoma (Curé et al., 1972) even though more than 150 cases of acromegaly with ultrastructural examination of the pituitary adenomas have been published to date (for review see Landolt, 1975). Only systemic lupus erythematosus and dermatomyositis show a higher incidence. This renders the finding a diagnostic aid according to the view of different authors (Grausz et al., 1970; Hashimoto and Thompson, 1970, Landry and Winkelmann, 1970; Prunieras et al., 1970).—The nature of the particles remains unknown in most cases in which they have been described. Their three dimensional structure had not been elucidated before and for this reason they have been called "branched tubules" (Baringer and Swoveland, 1972; Jenson et al., 1971). Stereoscopic

examination has shown that this is not correct. This misinterpretation is due to the fact that the section thickness amounts to three to four times the tubule diameter. Ramifications are rare if they occur at all.

The continuity of the tubules with the membrane of the perinuclear cistern as well as the fact that the tubules show a trilaminar wall in favorable sections has been interpreted as proof of origin from the endoplasmic reticulum (Baringer, 1971; Baringer and Swoveland, 1972; Chandra, 1968; Hashimoto and Thompson, 1970; Jenson et al., 1971: Uzman et al., 1971). The striking coincidence of the dimensions of the unit membrane and of the tubular wall can also be seen in our material after fixation with osmium, glutaraldehyde-osmium, and osmium-PTA (Table 1). In addition to the relationship with the cisternal membrane there are definite contacts with the nuclear membrane which sometimes form funnel-like modifications. The cross striation pattern of the tubule wall, which can be visualized after PTA block staining, showed a period of 4.0–4.5 nm. This coincides with that of measles nucleocapsids in subacute sclerosing leucoencephalitis (Oyanagi et al., 1971; Perrier et al., 1967) and in the paramyxovirus isolated from multiple sclerosis brain tissue (Lewandowski et al., 1975). Occasionally a hexagonal pattern could be seen in cross sections of PTA stained material. However nucleocapsids of myxoviruses (Herdon and Rubinstein, 1968; Jenis et al., 1973: Oyanagi et al., 1971, Toga et al., 1969) and paramyxoviruses (Pincus et al., 1970, Uzman et al., 1971) are considerably thinner than the tubular structures in acromegaly.

The four different methods used for demonstration of nucleic acids and especially DNA have shown positive results (extraction with perchloric acid, ethidium bromide staining, potassium permanganate fixation and RNAse incubation, and the methenamine-silver stain). The conclusion that the tubule core consists of a strand of DNA-containing material is further substantiated by the results of enzymatic digestion of frozen tissue sections. It is known that nucleocapsids of paramyxoviruses are resistant to RNAse treatment in vitro (Duesberg and Robinson, 1965). Pretreatment with trypsin is necessary in order to remove the protein coat of the nucleoprotein core to allow free access of the RNAse or DNAse. This may explain the resistance of the tubular structures in similar experiments performed by Schaff and collaborators (1972, 1973) who omitted the trypsin pretreatment because they thought that ultrathin sectioning was sufficient to expose the tubular cores. We have demonstrated above that the section thickness amounts to three to four times the tubular diameter. Therefore ultrathin sectioning does not expose sufficient amount of core material to allow a visible effect of RNAse or DNAse.

The diameter of the tubule core as shown after combined potassium permanganate treatment coincided with the diameter of the bright center after osmium fixation (Table 1). The tubular core shown by ethidium bromide was considerably thicker. We have to assume that not only the core but also the inner third of the tubule wall is stained by the latter procedure.

The presence of tubular arrays in the endoplasmic reticulum of transplanted human tumors of lymphoid origin and in patients with autoimmune diseases has been related to increased IgG synthesis (Pothier et al., 1973). In acromegaly

an abnormal amount of growth hormone is synthesized by the epithelial tumor cells which do not characteristically show the tubular arrays. There is no reason to postulate protein synthesis in the tubule bearing endothelial cells. Adenomas with active prolactin synthesis (Landolt, 1975) never show these particular structures in spite of the fact that both hormones are globulines.

Serological examination of our cases showed no auto-immune reaction of the lupus erythematosus type that could be responsible for the formation of the tubules. Antibody titer determination in patients sera against several different viruses did not show any significant results. From several adenomas examined only in one case Echovirus type 11 could be isolated. No tubular structures were present in this particular case. The isolation of an adenovirus from a chromophobe adenoma was reported by Cooper (1967). The significance of this remains undetermined.

The question of the possible nature of the DNA containing tubular structures located in the perinuclear cistern of endothelial cells in acromegaly can only be answered by isolation of the etiological agent. This was the case of systemic lupus erythematosus where a self-propagating, cytotoxic agent resembling a myxovirus has been isolated from the blood of patients (Moolden et al., 1973) or in the congenital rubella syndrome where similar arrays have been described (Kistler and Groscurth, 1973). DNA viruses known to cause infection in man and animals generally exhibit a cubical capsid symmetry such as the icosahedral structure of adenoviruses (Horne, 1974). The Ff-group of filamentous bacteriophages and the polyhedrosis virus of insects are the only rod shaped viruses containing DNA (Fraenkel-Conrat, 1974). Based on morphological aspects the tubular structures are quite unlike viral structures.

The presence of a virus in a tumor does not necessarily imply an etiological relationship (Cooper, 1967). The particle may simply be a passenger profiting from a beneficial internal milieu. The structures may also be interpreted as another form of extranuclear or extramitochondrial DNA e.g. the informational DNA for which a control function in protein synthesis during cell differentiation was postulated. Transcription is supposed to occur in the outer nuclear membrane (Bell, 1969). The type of extranuclear DNA described in our paper might also be a product of a reaction of the endothelial cells to certain environmental stimuli or changes in tissue metabolism due to acromegaly.

References

Baringer, J. R.: Tubular aggregates in endoplasmic reticulum in herpes simplex encephalitis. New Engl. J. Med. **285**, 943–945 (1971)

Baringer, J. R., Swoveland, P.: Tubular aggregates in endoplasmic reticulum: evidence against their viral nature. J. Ultrastruct. Res. **41**, 270–276 (1972)

Bell, E.: I-DNA: Its packaging into I-somes and its relation to protein synthesis during differentiation. Nature (Lond.) **224**, 326–328 (1969)

Chandra, S.: Undulating tubules associated with endoplasmic reticulum in pathologic tissues. Lab. Invest. **18**, 422–428 (1968)

Cooper, J. R.: Tumor tissue growth—The growth of tumor tissues from the central nervous system in tissue culture. J. Kans. med. Soc. **68**, 340–343 (1967)

Curé, M., Trouillas, J., Lhéritier, M., Girod, C., Rollet, J.: Inclusions tubulaires dans une tumeur hypophysaire. Nouv. Presse Med. **1**, 2309–2311 (1972)

Douglas, W. H.: Perchloric acid extraction of deoxyribonucleic acid from thin sections of epon-araldite-embedded material. J. Histochem. Cytochem. **18**, 510–514 (1970)

Duesberg, P. H., Robinson, W. S.: Isolation of the nucleic acid of Newcastle disease virus (NDV). Proc. natl. Acad. Sci. (Wash.) **54**, 794–800 (1965)

Fraenkel-Conrat, H.: Descriptive catalogue of viruses. In: Comprehensive virology, vol. 1. New York: Plenum Press 1974

Grausz, H., Earley, L. E., Stephens, B. G., Lee, J. C., Hopper, J.: Diagnostic import of virus-like particles in the glomerular endothelium of patients with systemic lupus erythematosus. New Engl. J. Med. **283**, 506–511 (1970)

Györkey, F., Min, K.-W., Sincovics, J. G., Györkey, P.: Systemic lupus erythematosus and myxovirus. New Engl. J. Med. **280**, 333 (1969)

Györkey, F., Sinkovics, J. G., Györkey, P.: Electron microscopic observations on structures resembling myxovirus in human sarcomas. Cancer (Philad.) **27**, 1449–1454 (1971)

Hashimoto, K., Thompson, D. F.: Discoid lupus erythematosus. Arch. Derm. **101**, 565–577 (1970)

Herndon, R. M., Rubinstein, L. J.: Light and electron microscopy observations on the development of viral particles in the inclusions of Dawson's encephalitis (subacute sclerosing panencephalitis). Neurology (Minneap.) **18**, 8–20 (1968)

Horne, R. W.: Virus structure. In: M. Locke (ed.), Ultrastructure of cells and organisms. New York: Academic Press 1974

Jenis, E. H., Knieser, M. R., Rothouse, P. A., Jensen, G. E., Scott, R. M.: Subacute sclerosing panencephalitis. Immunoultrastructural localization of measles-virus antigen. Arch. Path. (Chic.) **95**, 81–89 (1973)

Jenson, A. B., Spjut, H. J., Smith, M. N., Rapp, F.: Intracellular branched tubular structures in osteosarcoma. An ultrastructural and serological study. Cancer (Philad.) **27**, 1440–1448 (1971)

Kistler, G. S., Groscurth, P.: Intrazisternale Tubuluskomplexe in Zellen menschlicher Keimlinge mit Röteln-Embryopathie. Virchows Arch. Abt. B **14**, 77–82 (1973)

Landolt, A. M.: Ultrastructure of human sella tumors. Acta neurochir. (Wien), Suppl. **22** (1975)

Landry, M., Winkelmann, R. K.: Tubular cytoplasmic inclusion in dermatomyositis. Mayo Clin. Proc. **47**, 479–492 (1972)

Le Pec, J.-B., Yot, P., Paoletti, C.: Interaction du bromhydrate d'ethidium (BET) avec les acides nucléiques. Etude spectrofluorimétrique. C. R. Acad. Sci. (Paris) **259**, 1786–1789 (1964)

Lewandowski, L. J., Walers, D., Koprowski, H.: Characterization of a paramyxovirus isolated from multiple sclerosis brain tissue. In: Mahy, B. W., Bary, R. D. (eds.), Negative strand viruses, p. 203. London: Academic Press 1975

Luft, J. H.: Permanganate—a new fixative for electron microscopy. J. biophys. biochem. Cytol. **2**, 799–801 (1956)

Lyon, G., Griscelli, C., Lebon, P.: Endothelial intracisternal tubular inclusions in a case of chronic encephalitis associated with immunological deficiency. Neuropädiatrie **3**, 459–469 (1972)

Moolden, S. E., Silver, A. M., Mihalyfi, M.: Self-propagating cytotoxic agent in systemic lupus erythematosus. Amer. J. clin. Path. **60**, 123 (1973)

Norris, F. H., Aguilar, M. J., Harmann, C. E.: Virus-like particles in a case of vasculitis with brain tumor. Arch. Neurol. (Chic.) **26**, 212–217 (1972)

Norton, W. L.: Endothelial inclusions in active lesions of systemic lupus erythematosus. J. Lab. clin. Med. **74**, 369–379 (1969)

Oyanagi, S., Rorke, L. B., Katz, M., Koprowski, H.: Histopathology and electron microscopy of three cases of subacute sclerosing panencephalitis (SSPE). Acta neuropath. (Berl.) **18**, 58–73 (1971)

Pease, D. C.: Histological techniques for electron microscopy, 2nd ed. New York: Academic Press 1964

Périer, O., Vanderhaegen, J. J., Pelc, S.: Subacute sclerosing leuco-encephalitis. Electron microscopic findings in two cases with inclusion bodies. Acta neuropath. (Berl.) **8**, 362–380 (1967)

Peters, D., Giese, H.: Detection of DNA in thin sections. In: Proc. VII. Int. Congr. Electron Microscopy, vol. 1, Grenoble 1970

Peters, D., Giese, H.: Elektronenmikroskopischer Nachweis von DNS. Acta histochem. (Jena), Suppl. **10**, 119–125 (1971)

Pincus, T., Blacklow, N. R., Grimley, P. M., Bellanti, J. A.: Glomerual microtubules of systemic lupus erythematosus. Lancet **1970 II**, 1058–1061

Pothier, L., Uzman, B. G., Kasac, M. M., Saito, H., Adams, R. A.: Immunoglobulin synthesis and tubular arrays in the endoplasmic reticulum in transplanted human tumors of lymphoid origin. Lab. Invest. **29**, 607–613 (1973)

Prunieras, M., Grupper, Ch., Durepaire, R., Beltzer-Garelly, E., Regnier, M.: Etude ultrastructurale de la peau dans 42 cas de lupus érythemateux. Presse méd. **78**, 2475–2479 (1970)

Schaff, Z., Barry, D. W., Grimley, P. M.: Cytochemistry of tubuloreticular structures in lymphocytes from patients with systemic lupus erythematosus and in cultured human lymphoid cells. Comparison to a paramyxovirus. Lab. Invest. **29**, 577–586 (1973)

Schaff, Z., Heine, U., Dalton, A. J.: Ultramorphological and ultracytochemical studies of tubuloreticular structures in lymphoid cells. Cancer Res. **32**, 2696–2706 (1972)

Smith, R. D., Northrop, R. L.: Paramyxovirus-like structures in the nephrotic syndrome. Amer. J. clin. Path. **56**, 97–103 (1971)

Toga, M., Berard, M., Tripier, M. F., Cesarini, J. P., Choux, R.: Etude ultrastructurale de quatre cas de leuco-éncéphalite sclérosante subaigue. Acta neuropath. (Berl.) **14**, 1–13 (1969)

Uzman, B. G., Saito, H., Kasac, M.: Tubular arrays in the endoplasmic reticulum in human tumor cells. Lab. Invest. **24**, 492–498 (1971)

Yotsuyanagi, M.: Mise en évidence au microscope électronique des chromosomes de la levure par une coloration spécifique. C. R. Acad. Sci. (Paris) **250**, 1522–1524 (1960)

Yotsuyanagi, M., Guerrier, C.: Mise en évidence par des techniques cytochimiques et la microscopie électronique de l'acide désoxyribonucléique dans les mitochondries et les proplastes d'Allium cepa. C. R. Acad. Sci. (Paris) **260**, 2344–2347 (1965)

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