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Cytoplasmic Tubular Arrays in Latent Chronic Glomerulonephritis

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Summary. Sub-microscopic studies on renal tissue, obtained by percutaneous biopsy from a patient with *Latent Chronic Glomerulonephritis*, have revealed the presence of round bodies in the cytoplasm of endothelial cells of the glomeruli. The bodies generally occur as aggregates in an apparent association with the endoplasmic reticulum. Along with the round forms, other profiles are observed also. It is believed that they represent one of the many images of a system of undulating tubules.

By virtue of the fact that these bodies occur irrespective of the presence of virus particles in the tissue under study, it is concluded that they are not viral in nature.

Zusammenfassung. Im Nierenbiopsiematerial von einem Patienten mit latenter chronischer Glomerulonephritis konnten submikroskopisch rundliche Körper im Cytoplasma der glomerulären Endothelzellen nachgewiesen werden. Die Körper erscheinen generell als Aggregate in direkter Verbindung mit dem endoplasmatischen Reticulum. Neben rundlichen Körpern lassen sich auch noch weitere Strukturformen beobachten. Es wird angenommen, daß die Cytoplasmakörper eines der vielen Erscheinungsformen tubulärer Struktursysteme repräsentieren. Aus der Tatsache, daß diese Körper in Geweben ohne Nachweis von Viruspartikeln in Erscheinung treten, wird der Schluß gezogen, daß sie nicht virusbedingt sind.

Introduction

Recently, distinctive crystalline, reticular or tubular arrays have been reported in the cytoplasm of endothelial and other cells in a variety of conditions. Among the circumstances in which such structures have been found are normal human cells (Chandra, 1968), normal monkey tissue (Ishikawa, 1963; Sebuwufu, 1968; Finegold, 1967; de Martino *et al.*, 1969; Battifora and Markowitz, 1969; Rosen and Tisher, 1968), kidney tissue in Systemic Lupus Erythematosus (de Martino *et al.*, 1969; Grausz *et al.*, 1970; Györkey *et al.*, 1969; Norton, 1969; Hurd *et al.*, 1969; Kawano *et al.*, 1969; Sinkovics *et al.*, 1969; Pincus *et al.*, 1970; Haas and Yunis, 1970; Datsis, 1972), lipoid nephrosis (de Martino *et al.*, 1969; Duffy, 1969), syphilitic nephrosis (Datsis, 1972), and other renal disease (Battifora and Markowitz, 1969; Norton, 1969; Hurd *et al.*, 1969; Shearn and Stephens, 1970); sarcomas (Chandra, 1968; Moore and Chandra, 1968; Lombard *et al.*, 1967; Munroe *et al.*, 1964; Bucciarelli *et al.*, 1967), lymphomas (Chandra, 1968; Kakuk *et al.*, 1968), leukemias (Recher *et al.*, 1968; Siegal *et al.*, 1968; Uzman *et al.*, 1968), a variety of viral infections, and in experimental nephrotoxicity studies (Datsis, 1972). The particles have been regarded by many as viral or virus-like in nature.

The purpose of the present communication is to report the occurence of cytoplasmic tubular structures in a kidney biopsy from a patient having clinically

proven *latent chronic glomerulonephritis.* Though the appearances of the particles vary in the several conditions, their presence by and large within the endoplasmic reticulum as well as their basic structural similarities, profoundly suggest that these diverse observations may all concern a single phenomenon whose widespread occurence has not been biologically appreciated.

Case Report

A 14-year old male child was first admitted to KSH in 7/1968 for generalized convulsions of a few hours duration. On the day after admission, he was noted to have gross hematuria and questionable oedema. He had *Impetigo Contagiosum* some weeks earlier. The culture at the time of admission failed to show Group A β-hemolytic Streptococci. The ASO titer was 100 Todd Units. His blood pressure at the time of admission was 140/90. Percutaneous renal biopsy at that time was compatible with the diagnosis of *Acute Glomerulonephritis.* Urinalysis became normal approximately 3 to 4 months later and had remained so until the second admission in June 1970. At the time of a visit prior to the second admission, the patient was noted to be moderately hypertensive (140/100), and to have +1 proteinuria. Urinalysis following admission showed a specific gravity of 1.018 and was negative for albuminuria.

Methods

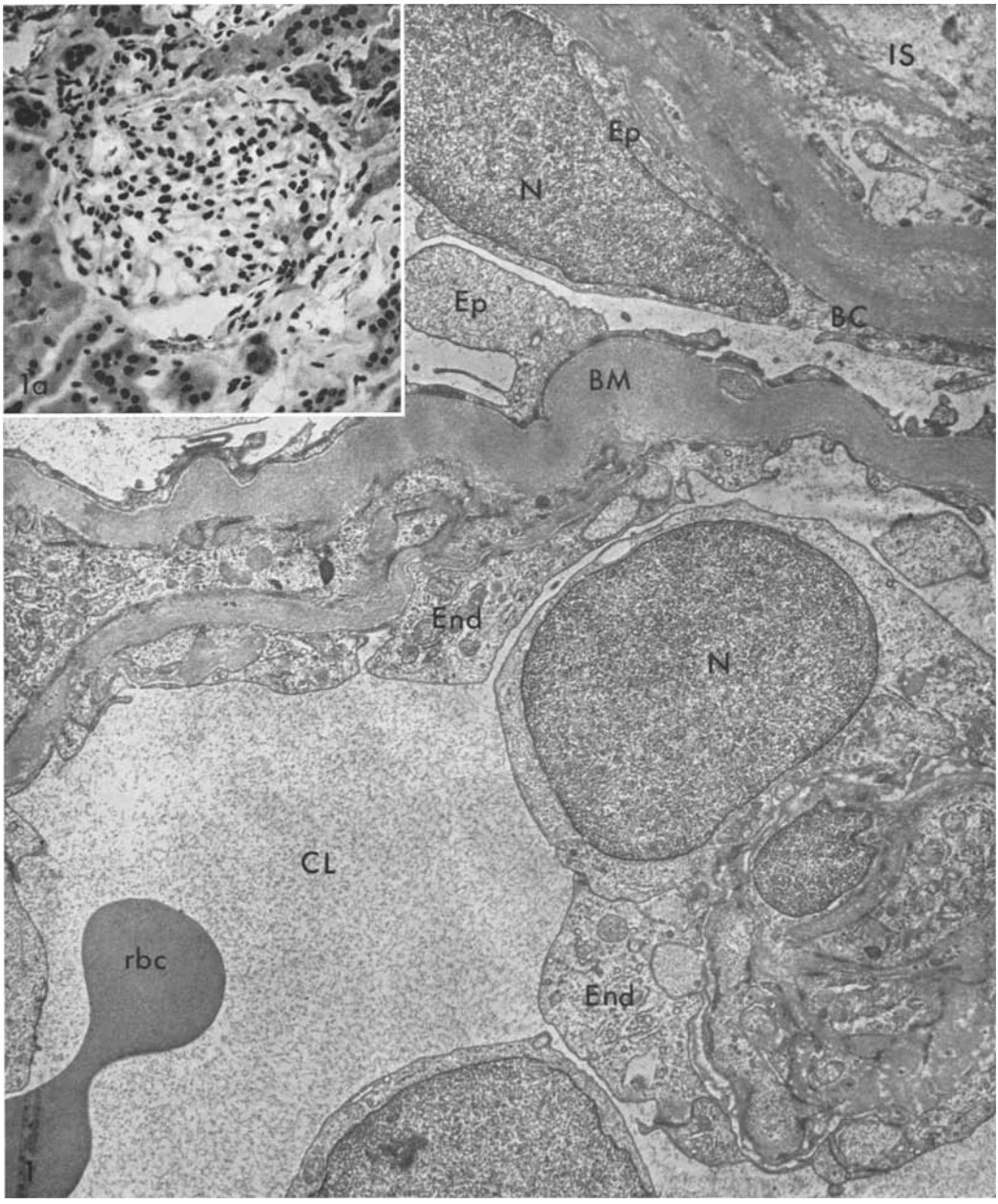
Diagnosis based on light microscopy observations was performed on tissues obtained by percutaneous renal biopsy according to the method advocated by Kark and Muehrcke (1954). The tissue was fixed in Zenker's fixative for 1 hour, and embedded in paraffin; sections cut at a thickness of 6 to 8 μ were stained with Hematoxylin and Eosin and Periodic Acid Schiff (PAS).

For ultrastructural studies, tissue obtained as described above, was fixed for 1 hour in 1% s-collidine buffered osmium tetroxide. Thin sections were stained with uranyl acetate (Watson, 1958), and lead hydroxide (Karnovsky, 1961), prior to the examination under the electron microscope.

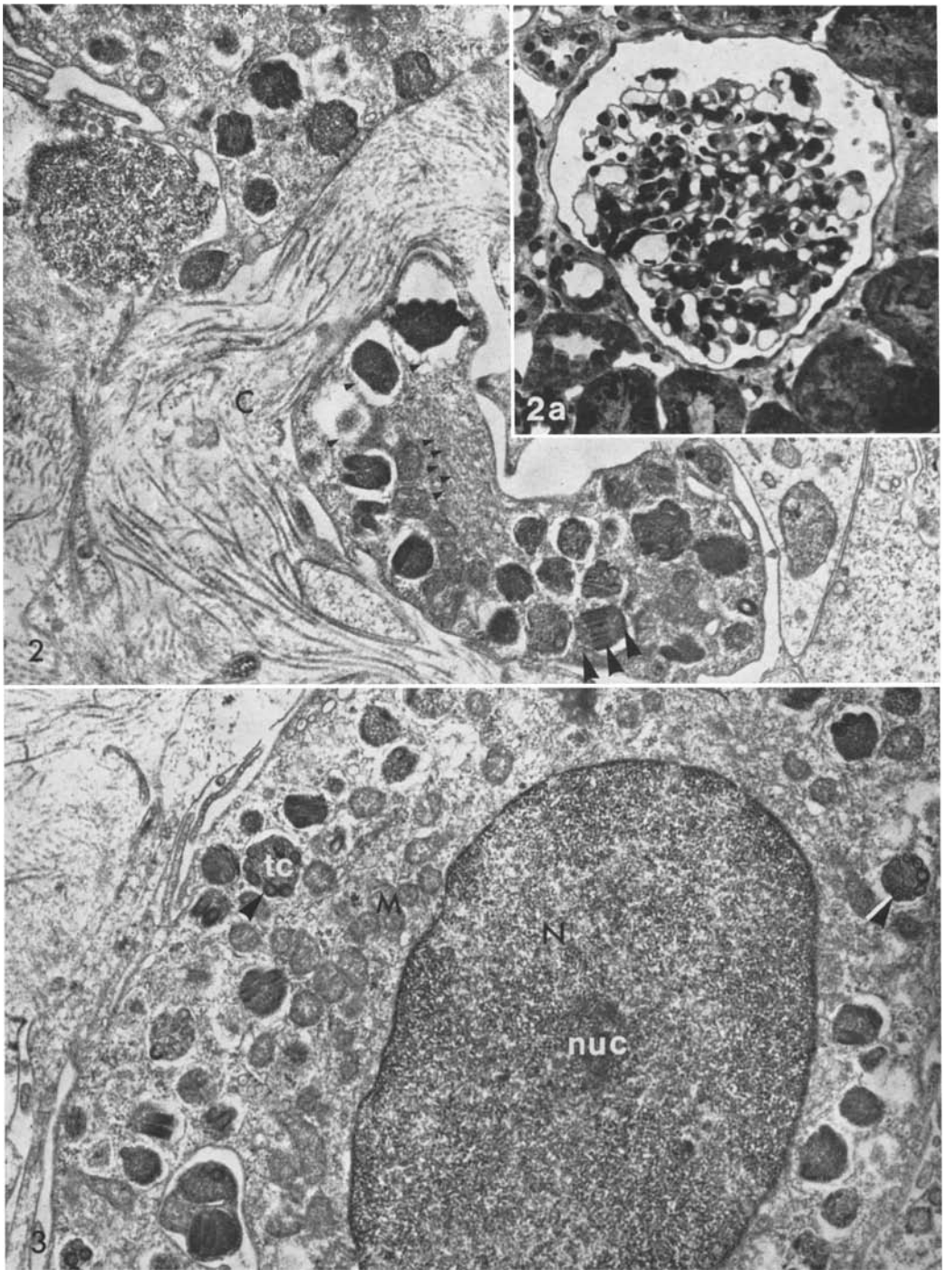
Results

The appearance of the glomeruli varied from mild hypercellularity to relatively normal size and cellularity. Those glomeruli involved, exhibited some mild focal intercapillary cellular hyperplasia, without resulting in severe lobular distortion (Fig. 1a). The hypercellularity was related to focal mesangial cell proliferation and small clusters of polymorphonuclear leukocytes in the capillary lumena (Fig. 2a). Areas of folding and condensation of the glomerular basement membrane were present with some mild narrowing of the glomerular capillary lumena. There were no diffuse basement membrane alterations in the glomerulus, and there were *no* basement membrane "humps" which distort the epithelial cell surface. The glomerular epithelial cell displayed conspicuous foot processes and there were only occasional areas bridging Bowman's space. No fibroepithelial crescents or synechiae were encountered (Fig. 1).

Fig. 1. Electron micrograph illustrating portion of the glomerulus depicted in Fig. 1a. The epithelial cell foot processes cover the basement membrane but, except for being less dense, than normal in some areas, are not remarkable. The basement membranes (*BM*) are intact, and the endothelial layer covering the membrane internally is slightly swollen (*End*). An erythrocyte (*rbc*) is seen in the lumen of the capillary (*CL*). Three nuclei (*N*) of mature endothelial



cells are noted. Fixation in 1% s-collidine buffered O_sO_4; stained with uranyl acetate and lead hydroxide. × 16500 (reduced to 5/7) Fig. 1a. Light microscopic appearence of a glomerulus from the patient, two years after onset of disease. Glomerulus shows mild hypercellularity, generally located in the lobular stalks. The capillaries are widely patent. Toluidine Blue. × 250 (reduced to 5/7). *BC* Bowman's Capsule, *BS* Basal Lamina, *C* Collagen, *CL* Capillary Lumen, *End* Endothelium, *Ep* Epithelium, *erg* ergastoplasm, *M* Mitochondrion, *N* Nucleus, *nuc* nucleolus, *tc* tubular complex



Figs. 2 and 3

Large amorphous masses were present in the majority of the endothelial cells examined. These masses consisted of fibrogranular material within which were found denser structures of various sizes (Figs. 2—4). The amorphous fibrogranular elements varied in size and form from the larger spheres mentioned, to smaller clusters delimited by a single, smooth membrane, most likely derived from the smooth surfaced endoplasmic reticulum. A clear zone or "halo" intermitted between the dense masses and the boundary smooth membrane. Fine strands, closely resembling microtubules extending into a clear zone of cellular cytoplasm were encountered in numerous sections (Fig. 2).

Fig. 3 represents a section from one of the numerous endothelial cells examined. The area contains numerous round, electron dense, compact structures of a fibrogranular appearance. When viewed at a higher magnification, each granular body appeared containing aggregates of small round bodies, some of which appear to be hollow, round, smooth membrane-bound structures with electron opaque cores (Fig. 4). However, profiles of other bodies occurring within the same round, dense, fibrogranular body, indicate that they may not be spherical but rather tubular. The tubular nature of these bodies is revealed most clearly in fortuitous longitudinal sections. This is seen in Fig. 3 and 4, in which, in addition to round bodies, images resulting from greater obliquity of planes of section are observed. Occasionally the plane of section is such as to produce images of greater complexity. In addition to the structures described above, there appear to be circular profiles bound by two or more membranes, thus giving the appearance of five-unit membranes.

A careful examination of Fig. 4, suggests that the prifiles are formed by two or more undulating tubules lying within the thickness of the section, such that the crest of one lies opposite the trough of the other. At different planes of section, these structures displayed a rather rectangular structure suggestive of developing cilia or replicating centrioles.

Discussion

The present report adds another observation to the growing list of reports of tubular or crystalline arrays within endoplasmic reticulum in a variety of conditions. Observations of particles of this type and/or of somewhat modified structure have been made in several states:

Fig. 2. Survey electron micrograph illustrating part of the cytoplasm of an endothelial cell from the glomerulus in Figure 2a. Large dense, amorphous masses containing tubular and/or discrete round denser structures are present. (Large arraows). Fine strands, closely resembling cytoplasmic microtubules extending into the clear zone of cellular cytoplasm, are seen. Fixation as in Fig. 1. $\times$ 11200 (reduced to 7/9). Fig. 2a. Light microscopic appearence of another glomerulus from the same patient. Glomerulus shows a moderate degree of intercapillary hypercellularity related to focal mesangial proliferation, accompanied by small clusters of polymorphonuclears in the capillary lumina. Toluidine Blue. $\times$ 250 (reduced to 7/9)

Fig. 3. Electron micrograph indicating a portion of the cytoplasm of an endothelial cell from the glomerulus in Fig. 1a. Dense, tubular complexes (*tc*), comprise a major portion of the ultrastructural picture of the fibrogranular round cytoplasmic masses. Fixation as in Fig. 1. $\times$ 11200 (reduced to 7/9)

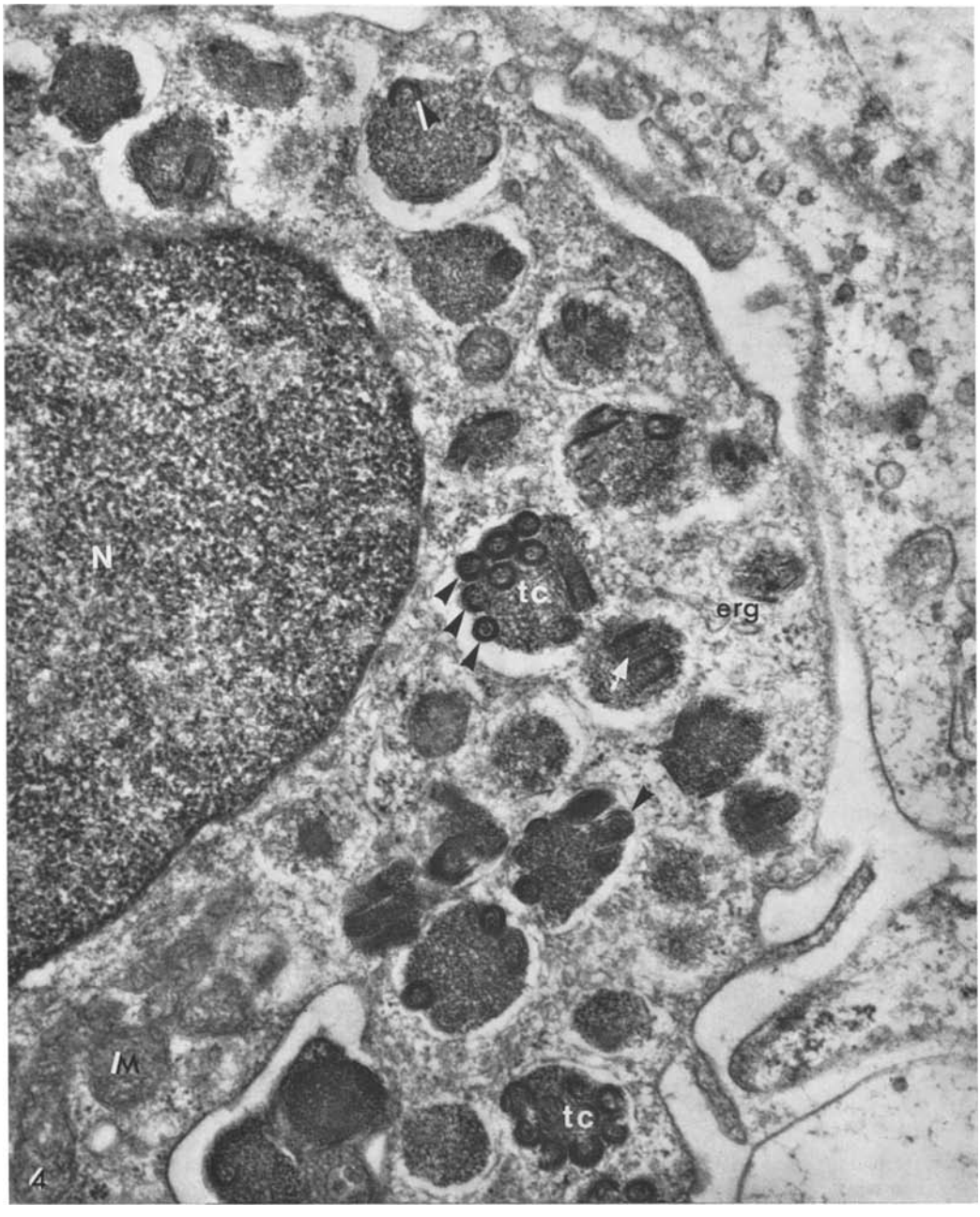


Fig. 4. High magnification electron micrograph from an endothelial cell showing the undulating, tubular nature of the fibrogranular, electron — dense, round masses. The tubular nature of these bodies is clearly visible in fortuitous longitudinal sections. A clear zone or "halo", situated between the dense masses and the boundary smooth membrane is seen. Fixation as in Fig. 1. $\times$ 32600 (reduced to 5/7)

Normal human tissue-culture cells (Chandra, 1968), and normal monkey tissues (Ishikawa, 1963; Sebuwufu, 1968; Finegold, 1967; de Martino *et al.*, 1969; Battifora and Rabinowitz, 1969; Rosen and Tisher, 1968); kidney biopsy tissue from patients with Lupus Nephritis (de Martino *et al.*, 1969; Grausz *et al.*, 1970; Györkey *et al.*, 1969; Norton, 1969; Hurd *et al.*, 1969; Kawano *et al.*, 1969; Sinkovics *et al.*, 1969; Pincus *et al.*, 1970; Haas and Yunis, 1970; Datsis, 1972), or less frequently in other renal diseases (de Martino *et al.*, 1969; Battifora and Markowitz, 1969; Norton, 1969; Hurd *et al.*, 1969; Duffy, 1969; Datsis, 1972); neoplastic conditions, including sarcomas (Chandra, 1968; Moore and Chandra, 1968; Lombard *et al.*, 1967); Rous-sarcoma virus infection induced tumors (Munroe *et al.*, 1964; Bucciarelli *et al.*, 1967); lymphomas (Kakuk *et al.*, 1968), including Burkitt's lymphoma (Chandra, 1968), and leukemias (Chandra, 1968; Recher *et al.*, 1969; Siegal *et al.*, 1968; Uzman *et al.*, 1972); infections including Semlikis Forest virus infection (Pincus *et al.*, 1970); Aleutian-mink disease (Tsai *et al.*, 1969); Foot-and-mouth disease Breese and Graves, 1966); St. Louis encephalitis (Murphy *et al.*, 1968); Murray Valley encephalitis (Fishie and Rehacek, 1968); Wesselbron-virus infection (Parker and Stannard, 1967); distemper (Blinzinger and Deutschländer, 1969); Rubella (Kim and Boatman, 1967); equine viral arteritis (Estes and Cheville, 1970); Poliomyelitis (Simon *et al.*, 1970; Blinzinger *et al.*, 1969; Koestner *et al.*, 1966; Kanamitsu *et al.*, 1967); Coxsackie myocarditis (Haas and Yunis, 1970); canine hepatitis (Givan and Jézéquel, 1969); mononucleosis (Uzman *et al.*, 1972; Moses *et al.*, 1968); staphylococcal enterotoxemia (Finegold, 1967); scleroderma (Norton, 1969); Dego's disease (Nishida and Howard, 1968); Krabbé's leucodystrophy (Haas and Yunis, 1970); and the Chediak-Higashi syndrome (Douglas *et al.*, 1969). Similar undulating tubules were recently found occurring in experimental nephrotoxicity studies (Datsis, 1972).

The finding of the material in normal tissue has been confined to the observations of the aggregate in cultured human cells from normal persons (Chandra, 1968), and to a series of observations in monkeys (Ishikawa, 1963; Sebuwufu, 1968; Finegold 1967; de Martino *et al.*, 1969; Battifora and Markowitz, 1969; Rosen and Tisher, 1968), describing the aggregate within the endoplasmic reticulum of the endothelial cells, in retina, lung, liver and kidneys. Although it is difficult to establish the frequency with which these aggregates may be found in normal tissues, most studies indicate that the structures are found much more often in disease. The morphologic character of the aggregates has differed in the variety of circumstances in which they have been found. In normal tissues, neoplastic conditions and virus-infected tissues, they have usually appeared as a rather tightly packed array of particles that have only occasionally been recognized as tubular in nature (Chandra, 1968). In contrast, in most of the material from patients with Lupus Nephritis and other renal disease, the appearance is that of loosely arrayed tubules (Datsis, 1972). A number of features, however, are common to both types of arrays: (a) their occurence related to and more commonly within the endoplasmic reticulum, and (b) their localization to endothelial cells or less frequently to macrophages. The common features have suggested that the several observations may all concern the same structures, and that variations in appearence of the structures may be related to the packing density of the aggregates.

Great strides have been made in unifying the many and various morphologic types of tubular or crystalline in pattern arrays found within the cells in the above mentioned conditions on the basis of their intimate relationship to viral infections. But to limit consideration of these apparent tubular structures to the general concept of intracellular viral inclusions, simply because they have been documented in diseases of a viral origin, would not be the most constructive approach.

There would, then, be a tendency to dismiss further investigation of the biological importance of this cytoplasmic reactivity. While there are apparent points of similarity between these tubular and/or crystalline arrays and the viral particles as well as the normally occurring centrioles and cilia, to permit this general conceptualization to deter specific consideration of these intracellular tubular structures would only obstruct rather that extend clarification of the basic cytoplasmic tubular nature of this cytoplasmic change.

The several reports concerning the appearance of apparent tubular structures in kidneys of patients with clinically proven Lupus Erythematosus have generated much interest in the possible viral etiology, since those particles bear some resemblance to myxoviruses. Although several investigators did not find similar particles in normal human renal tissue, the particles were found in biopsies from patients with other renal diseases. The particle size of 20 to 25 nm given in most of these reports is larger than that for the expected diameter of the nucleocapsid of the myxovirus or paramyxovirus group (Davis *et al.*, 1967). In addition, localization of the aggregate within the endoplasmic reticulum has been seen quite frequently in biopsy material from Lupus Erythematosus cases. Structural data obtained from studies of 57 cases of human renal biopsy material, profoundly indicate the direct relation of these tubular structures to the endoplasmic reticulum as physical connections between these two subcellular structures have been documented. This has not been a feature of myxovirus or of paramyxovirus infections, in which the virus is found either within the nuclei or free in the cytoplasm. The centriolar or ciliary origin of these structures, on the other hand, viz., the possible induced replication of the cellular centrioles or even the formation of new cilia, is improbable. A typical centriole is, by definition, a cylinder about 200 mμ in outside diameter and 300 to 500 mμ long. In its wall are nine evenly spaced fibrils running the length of the centriole; each fibril further appears, in cross section, as a band of three microtubules so closely apposed that they seem to share common walls. The band of three is inclined at an angle of 30 to 40° from a tangent to the circumference of the cylinder. A fine filamentous, granular or amorphous material forming a wall matrix embedding the fibrils and extending to embrace the cylinder in a sleeve of variable extent and density was seen in the tubular structures in biopsy material under consideration; but this is as far as similarity goes. The usual 9 + 2 pattern of the typical centriole was in no instance encountered in our material. Though variations in the appearance of centrioles and/or cilia have been reported in the literature (Phyllips, 1966), the characteristic cartwheel structure consisting of a central cylindrical hub and nine delicate slightly swirled spokes extending toward the peripheral fibrils, was in no instance seen.

The biological existence of systems of undulating tubules having organized or unorganized orientations was recently reported by Chandra (1968). In that study, substantial proof for the existence of mutually perpendicular systems of undulating tubules forming a sign of plus, was the most often encountered. Since many other orientations of the tubules are possible (Datsis, 1972c), various images can be observed in sub-microscopic studies. It is apparent that the most frequently observed images would be the round bodies or profiles. Aggregates of round profiles were observed in tumors induced by Rous-sarcoma virus (Bucciarelli *et al.*,

1967; Munroe *et al.*, 1964), in the Sticker sarcoma of the dog (Lombard *et al.*, 1967), and in cell cultures infected with rubella virus. The evidence indicates that they represent images of systems of undulating tubules by certain planes of section. In an organized state, the tubules could produce images exhibiting a crystalline or tubular pattern. The round bodies, cannot, therefore, be conceived as viral in nature.

Examples of highly organized three-dimentional tubular structures are found predominately in two non-mammalian tissues. In the clear cells of the dentritic organ of some marine catfish, a "well-developed endoplasmic reticulum in the form of a more or less tubular network" occurs, the significance of which is not clear (Van Lennep and Lanzing, 1967). In the luminous cells of the polynoid worm *Elytra*, Bassot (1966), described circular profiles similar to those described in the present study. Bassot believes that the tubules are "a specialized form of endoplasmic reticulum".

With regard to the mammalian tissues, Sisson and Fahrenbach (1967), describe crystalline structures formed by a system of straight tubules. The different images resulting from a system of undulating tubules in pathological conditions have been described by several investigators, but no correlation between the various images have been shown to exist. It is suggested that the several observations may all concern the same structures and that variations in appearance of the structures may be related to the packing density of the aggregates. Furthermore, the evidence presented, coupled with the unique appearance of the aggregates and their distribution within the endoplasmic reticulum of endothelial cells, suggest that the particles, though described in a variety of conditions, may represent a common cellular response to a variety of pathologic processes.

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