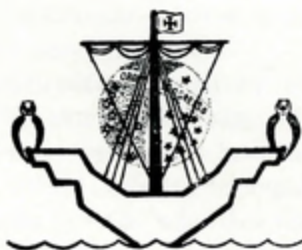


M.^a DE LOURDES BORGES and J. F. DAVID-FERREIRA

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METEL L. HEALTHY AND INFECTED WITH POTATO VIRUS X
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COMPARATIVE STUDY OF CELL STRUCTURE IN *DATURA METEL* L. HEALTHY AND INFECTED WITH POTATO VIRUS X OR POTATO VIRUS Y (*)

by

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According to BRANDES and WETTER (1959) and BRANDES (1964) potato virus X and potato virus Y are elongated plant viruses belonging to different groups. The initial criterion used in their distinction was the average length of the particles. The two viruses are also serologically unrelated, have different thermal inactivation points, present different concentrations in the host and no cross protection. In their distinction are not included the different cytological alterations caused in the host cells.

The ultrastructural modifications caused by potato virus Y have been studied some years ago by FERREIRA and BORGES (1958) using *Datura Metel* as host. Subsequently two short references have been made in the subject, by MATSUI and YAMAGUCHI (1966), referring to unpublished data of KIKUMOTO and MATSUI and by EDWARDSON (1966b) who, describing cytoplasmic inclusions caused by rod-shaped viruses, presents an electron micrograph of *Nicotiana Tabacum* L. cells infected with potato virus Y.

The alterations caused by potato virus X have been studied by BORGES and FERREIRA (1959) in *Datura Metel* cells and by KIKUMOTO and MATSUI (1961) using *Datura Stramonium* as host.

As far as we know there are no comparative studies on the ultrastructural alterations caused by potato virus X and potato virus Y in single infections in the same host in similar environmental conditions. The purpose of this paper is to compare normal *Datura Metel* L. cells with cells infected with

(*) Dedicated to Prof. Flávio Resende.

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potato virus X or potato virus Y in order to observe the alterations caused by each one of these viruses and investigate the cellular organelles more affected by their replication.

MATERIAL AND METHODS

Plants of *Datura Metel* L. kept in an insect-proof glasshouse were sap inoculated on the same day with potato virus X (PVX) or potato virus Y (PVY) and others were left as controls. The plants of the three groups were kept in the same environmental conditions. The strain XP-11 of potato virus X used in the present work is the most common in Portugal (BORGES, 1962). It causes in *Datura Metel* L. a mild mottle and it is the same referred to in a previous paper (BORGES & FERREIRA, 1959). The potato virus Y used was the strain YP-N the only one of the «necrotic type» found in this country. The symptoms it produced are: vein chlorosis and chlorotic vein banding, chiefly near the leaf tip. Pieces of leaves of 0.5×2 to 3 mm were cut in the chlorotic zone near the veins, or in the green zones, between the secondary veins of the potato virus Y infected plants and in similar regions, in plants infected with potato virus X, and in healthy ones.

Fixations have been made always at 9 to 10 am.

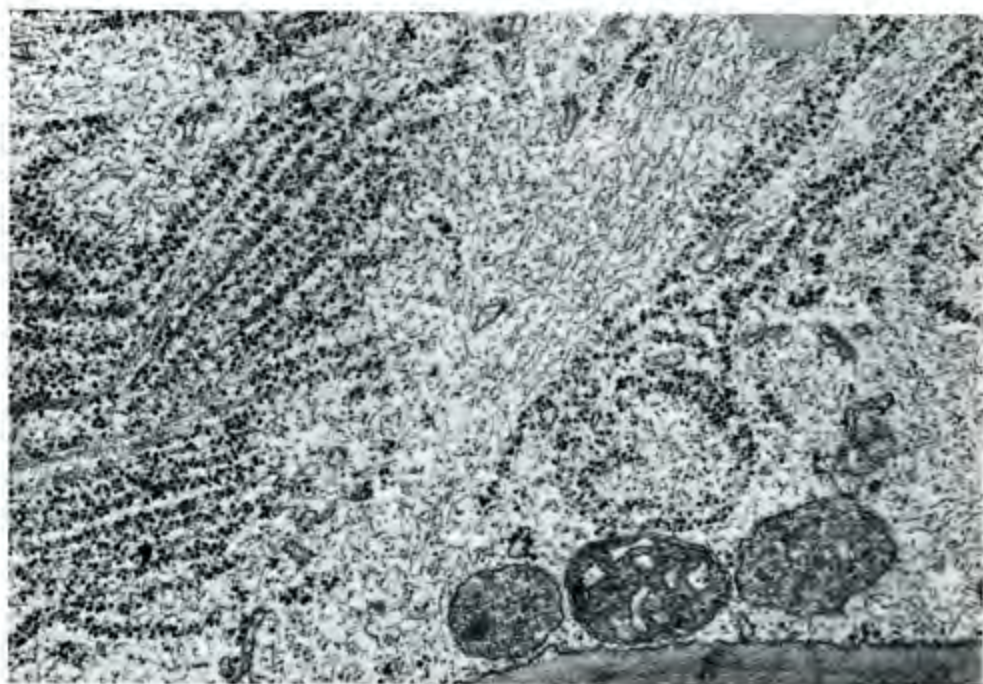
The pieces of leaves were fixed 1 hour in 3 % glutaraldehyde phosphate buffered at pH 7.3 (Millonig). They were washed in the same buffer and immersed in 2 % osmium tetroxide phosphate buffered at pH 7.3 (Millonig). After fixation the tissues were dehydrated in increasing concentration of ethanol and embedded in Epon 812 mixtures (LUFT, 1961). Thin sections were cut on a LKB ultratome and picked up on carbon coated grids. After double staining with uranyl acetate (saturated solution in 50 per cent alcohol) and lead citrate (REYNOLDS, 1963) they were examined in a Siemens Elmiskop 1A at 80 kv.

RESULTS AND DISCUSSION

In cells of plants infected with PVX the most striking aspect is the presence of large cytoplasmic areas characterized by unusual accumulations of ribosomes (Fig. 1). In these areas the ground substance is crowded with ribosomes and filaments of variable length and 40 to 50 Å in diameter (Figs. 2 and 3). Neither the control cells nor the cells infected with PVY presented such ribosome-filament rich areas.



Fig. 1 — Section of *Datura Metel* leaf cell infected with PVX. In the cytoplasm the ground substance is crowded with ribosomes and filaments. These areas are interpreted as virus assembly sites. $\times 21\,000$.



Figs. 2 and 3 — Sections of *Datura Metel* leaf cell infected with PVX. High magnifications of the ribosome-filament rich areas. The ribosomes are in some places attached to the filaments forming unusual long chains. $\times 35\,000$ and $\times 50\,000$.

Frequently the ribosomes are attached to the filaments forming unusual long chains. This aspect of the cells infected with PVX has been recently described by DAVID-FERREIRA and BORGES (1968) and interpreted as virus assembly sites. Owing to the role of ribosomes in protein synthesis it is not surprising to find such big accumulation of ribosomes in the cells infected with PVX. As this virus attains a high concentration in the host, it requires a very active protein synthesis for the replication.

The increase of ribosomes and polysomes is not an unusual feature in virus infected cells. It has been reported among others by HAMRE *et al.* (1967) who describes an increase of ribosomes 3 hours after infection of animal cells with a new RNA virus isolated from the human respiratory tract and their accumulation 6 to 12 hours later. Also SHIKATA and MARAMOROSCH (1967) signaled the presence of polysomes closely associated with virus assembly sites in plants and leafhoppers (*Agallia constricta*) infected by a wound tumor virus.

An aspect similar to the one we described has been reported by KOLEHMAINEN *et al.* (1965) in mesophyll cells from tobacco leaves infected with tobacco mosaic virus.

An observation characteristic of the cells infected with PVY is shown in Figs. 4 and 5. In these cells mitochondria are frequently observed surrounded by filaments of indeterminate length and 90 to 100 Å in diameter. In some sections, as the one shown in Fig. 6, it is possible to observe that the filaments are parallel to the long axes of mitochondria.

Images of associated mitochondria and filaments were never observed in controls or PVX infected cells of the same host. They cannot be regarded as an artifact and the constancy of their observation in cells infected by PVY is interpreted as connected with the presence or replication of this virus.

To our knowledge images of the type we just mentioned have never been signaled in normal or plant infected cells. Their significance is open to many questions.

Are the filaments surrounding the mitochondria virus particles or a stage in their formation? If in some way these filaments are related with virus, what is not surprising regarding their morphology and diameter, do they represent an initial step of virus infection or a stage in the virus replication? Why are they so closely associated with the mitochondria?

Is their mitochondrial association an expression of special energy requirements?

At the moment all these questions are valid but the techniques used are insufficient for an interpretation.

Another cellular alteration occurring after infection with PVY is the presence in the cytoplasm of the infected cells of dense bands, rings and

rosettes or pinwheels (Figs. 4 to 6). Some of these formations have been previously observed in connection with another strain of the same virus by FERREIRA and BORGES (1958) as well as by MATSUI and YAMAGUCHI (1964) in *Datura Stramonium* infected with tobacco etch virus. Lately similar formations have been reported by several authors in other virus infections (Table I).

TABLE 1 — Viruses causing cytoplasmic inclusions similar to those induced by PVY

	Virus	Host	Reference
750 m μ	Bean yellow mosaic	<i>Vicia Faba</i> L.	WEINTRAUB et al. 1966
		<i>Lupinus luteus</i> L.	EDWARDSON, 1966 b
	Bean common mosaic	<i>Phaseolus vulgaris</i> L.	EDWARDSON, 1966 b
	Lettuce mosaic	<i>Lactuca sativa</i> L.	EDWARDSON, 1966 b
	Sugar cane mosaic	<i>Zea Mays</i> L.	SANTOS, 1962; EDWARDSON, 1966 b
730 m μ	Turnip mosaic	<i>Brassica rapa</i> L.	HAYASHI et al. 1965
		<i>Petunia hybrida</i> Vilm.	HAYASHI et al. 1965
	Potato virus Y	<i>Datura Metel</i> L.	FERREIRA et al. 1958
		<i>Nicotiana Tabacum</i> L.	EDWARDSON, 1966 b
		<i>Capsicum annuum</i> L.	RUBIO-HUERTOS, 1966
702 m μ	Tobacco etch	<i>Datura Stramonium</i> L.	MATSUI et al. 1964
	Tobacco severe etch	<i>Nicotiana Tabacum</i> L.	RUBIO-HUERTOS et al. 1964
	Watermelon mosaic	<i>Citrullus vulgaris</i> S.	PURCIFULL et al. 1967
	Wheat streak	<i>Triticum durum</i> Desf.	LEE, 1965

RUBIO-HUERTOS *et al.* (1966) described the presence of rings, rosettes and plates in cells of *Capsicum annuum* infected with an unidentified virus, probably a strain of potato virus Y. From the examination of serial sections RUBIO-HUERTOS (1966) and EDWARDSON (1966a) interpreted those formations as tangential or longitudinal sections of more or less coiled laminated aggregates of virus particles with a common axis.

EDWARDSON (1966b) claims that these cylindrical inclusions are typical of viruses belonging to potato virus Y group, the group 5 of BRANDES (1964). In fact they are described in relation to several viruses all but one (wheat streak mosaic virus) belonging to group 5 (Table I).

The wheat streak mosaic virus which has an average length of 702 m μ has been tentatively included by BRANDES (1964) in group 4. It would be important to study the serology of this virus in order to reconsider its



Figs. 4 and 5—Sections of *Datura Metel* leaf cells infected with PVY. In the cytoplasm numerous rings, rosettes and plates characteristic of cells infected with PVY and interpreted as aggregates of virus particles. The mitochondria (*m*) are surrounded by filaments visible in transverse and oblique sections $\times 35\,000$.



Fig. 6 — Section of *Datura Metel* leaf cell infected with PVY. In the cytoplasm longitudinal sections of laminated aggregates. Some of the filaments surrounding the mitochondria are parallel to their long axes. A dense cytoplasmic body (*l*) is visible in this section, $\times 30\,000$.

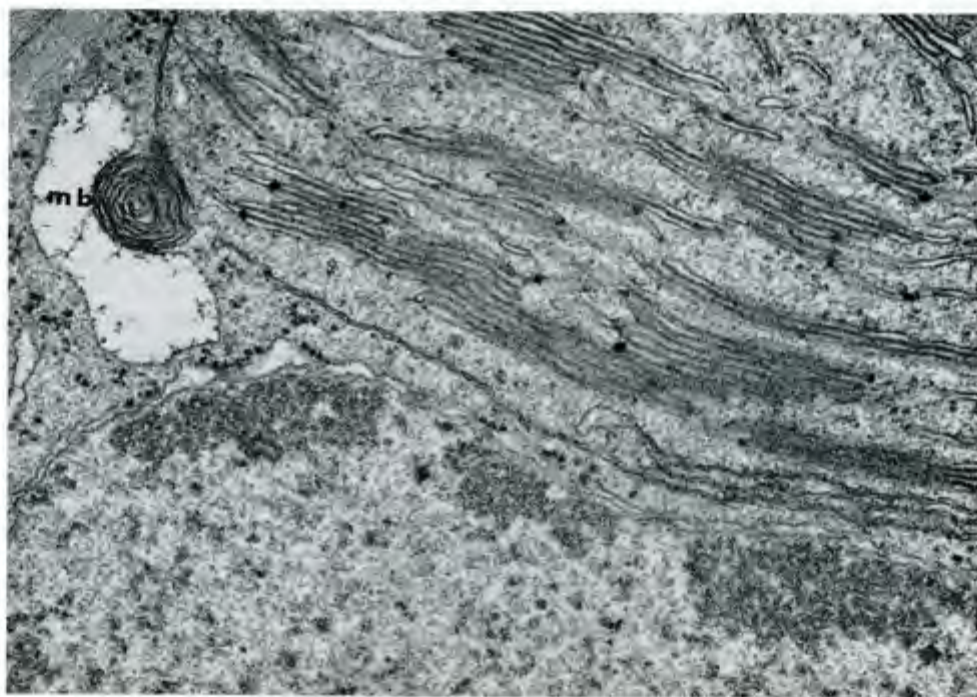


Fig. 7 — Section of *Datura Metel* leaf cell infected with PVX. Myelin body (*m b*) associated with the chloroplast membrane, $\times 52\,500$.

classification. It would also be interesting to know the behaviour in the host cells of rye grass mosaic virus, considered to have the same average length as wheat streak mosaic virus. Perhaps these two viruses are more related to group 5 of BRANDES than with those of group 4 where they are now included.

The *chloroplasts* are cell organelles frequently reported as affected by viruses. The symptoms due to PVX in *Datura Metel* are a mild mottle (BORGES, 1962). The green observed is not far from the normal colour and a great alteration of the plastids is not to be expected. In the plants infected by the PVY, the leaves maintain their normal colour except near the leaf tips where some veins are chlorotic and show a chlorotic vein banding (BORGES, 1964). In the present work we observed in both virus infections some ultrastructural alterations in the plastids of chlorotic areas. A severe degeneration was never seen. The changes consisted in: a decrease of the grana layers, fragmentation of the stromatic lamellae, and an unusual big number of lipid droplets. Occasionally the limiting membrane of the chloroplasts presented some disruptions. In these points myelin-like figures were frequently located (Fig. 7). No virus particles have been observed inside the plastids.

Alterations in the chloroplast morphology are signaled by different authors as a consequence of virus infection. CHALCROFT *et al.* (1966) in an electron microscopic study on Chinese cabbage infected by turnip yellow mosaic virus, described in the chlorotic zones a decrease of the grana and a small number of lamellae. In the green zones of the leaf there were no apparent alterations. This work has confirmed some previous light microscope observations by RESENDE-PINTO *et al.* (1952).

Fragmentation of the stromatic lamellae is referred to by GEROLA *et al.* (1965) in chloroplasts of *Nicotiana glutinosa* leaves infected with a strain of cucumber mosaic virus. LEE (1965) shows a complete desintegration of lamellae and a great number of lipid droplets in wheat plants infected with wheat streak mosaic virus.

These changes are similar to some seen in our material. But in all the cases we studied they seem to be less pronounced. We cannot exclude the possibility that some of these alterations are related to the specimen preparation.

The *Golgi apparatus* was observed in the normal as well as in the virus infected cells of *Datura Metel*. Usually with five cisternae (Fig. 13) surrounded by many vacuoles and vesicles it appears slightly enlarged in PVX infected cells.

A hypertrophy of the dictyosomes has been described by RUBIO-HUERTOS (1965) in *Vicia Faba* L. cells infected by Petunia ringspot virus. The same

author however has not observed similar aspects in *Vicia Faba* infected by other virus nor in *Nicotiana Tabacum* infected by tobacco severe etch virus.

The slight hypertrophy of the Golgi we noted in some cells infected with PVX is not comparable to the one described by RUBIO-HUERTOS (1965).

The presence of *dense cytoplasmic bodies* were frequently observed in preparations from controls and plants infected with PVX or PVY. In thin sections they are represented by circular or elliptical profiles 0,7 to 1 μ in diameter. They are bounded by a *single membrane* and their matrix is denser than the cytoplasmic ground substance. In some of them a dense crystal is present in the matrix. The crystal is formed by a ordered pattern of closely spaced, parallel, dense lines of about 40 Å (Figs. 8 and 9).

Often an endoplasmic reticulum cisterna is seen in the vicinity of the surrounding membrane of these bodies. We have not been able to substantiate any relationship between the number of dense bodies and virus infection. Apparently they are identical to the crystal containing bodies described by THORNTON *et al.* (1964) in *Avena sativa* coleoptile cells and in *Phycomyces blakesleeana* sporangiophores. CRONSHAW (1964) also described crystal containing bodies in several species in different experimental conditions. Both authors signaled the resemblance of the crystal containing bodies with the *lysosomes* of the animal cells.

Spherical or elongated cytoplasmic corpuscles from 0,2 to 1 μ in diameter, bounded by a single membrane and associated with the endoplasmic reticulum have also been described by MOLLENHAUER *et al.* (1966) in plant tissues fixed with potassium permanganate, osmium tetroxyde and glutaraldehyde. He compares these organelles with the *microbodies* of the animal cells. The crystal containing bodies we observed in our preparations are also morphologically more similar to the microbodies, which also have a crystal, than to the lysosomes which generally have a homogeneous matrix.

We can assume that the crystal containing bodies in plant cells belong to the same group of organelles as lysosomes and microbodies in animal cells. The nature of the enzymes they contain could only be known after their isolation and biochemical study.

The *cell wall* and the *nucleus* are also cell organelles where no apparent changes due to the viruses have been seen.

Some plasmodesmata have been observed in the cell wall in longitudinal (Fig. 10) and in transversal sections (Fig. 11). In the cells with a mild plasmolysis we observed images where the connections between plasmodesmata and plasmalemma are evident. In the micrograph of Fig. 10 an endoplasmic reticulum cisterna is seen closely related to the plasmodesmata. In our micrographs we never observed the continuity of endoplasmic reticulum of

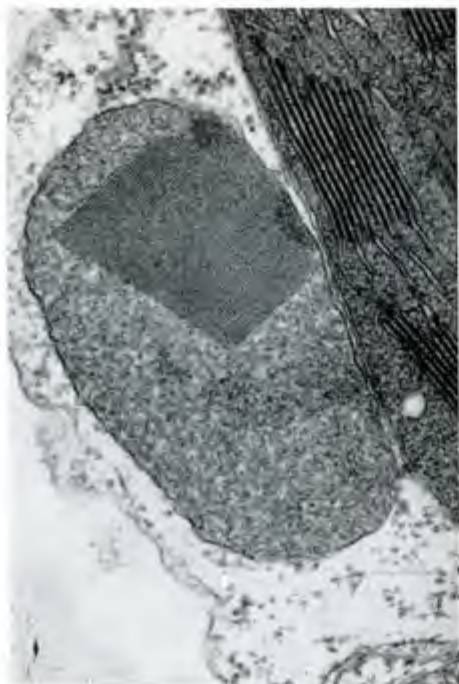


Fig. 8 — Dense cytoplasmic body with crystal in the cytoplasm of *Datura Metel* leaf cell infected with PVY. $\times 52\,500$.

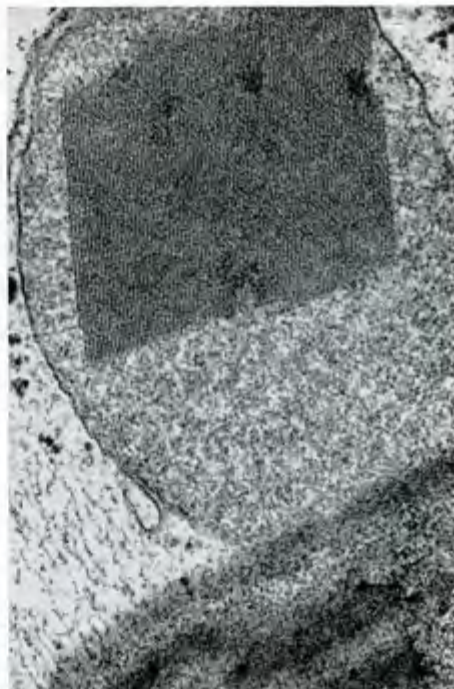


Fig. 9 — Dense cytoplasmic body with crystal in the cytoplasm of *Datura Metel* leaf cell infected with PVX. $\times 52\,500$.



Fig. 10 — Longitudinal section of plasmodesmata in cell wall of *Datura Metel* leaf cell. $\times 35\,000$.



Fig. 11 — Transversal section of plasmodesmata in *Datura Metel* leaf cell. $\times 35\,000$.

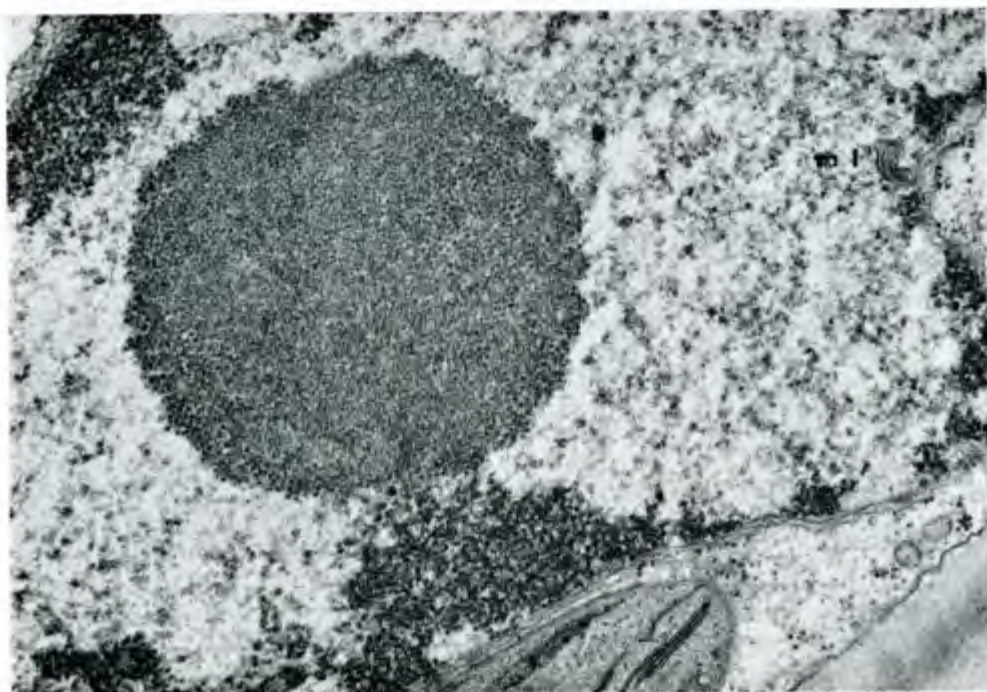


Fig. 12—Section of *Datura Metel* leaf cell. Aspect of the nucleus and nucleolus. Myelin figure (ml) associated with the nuclear membrane. $\times 35\ 000$.



Fig. 13—*Datura Metel* leaf cell infected with PVY. The section is tangential to the nuclear (N) surface and several nuclear pores are visible, Golgi apparatus (G). Associated to the mitochondria many filaments. $\times 30\ 000$.

neighbor cells which LOPEZ-SAEZ *et al.* (1966) described in plasmodesmata of *Phalaris Canariensis* cells.

Also O'BRIEN *et al.* (1966) from electron micrographs showing longitudinal and transversal sections in plasmodesmatas of *Avena sativa* coleoptiles concluded that «the endoplasmic reticulum does not seem to be continuous through them».



Fig. 14—Section of *Datura Metel* leaf cell infected with PVX. Virus inclusion body, $\times 35\,000$.

We never observed particles near or in the plasmodesmata as have been clearly demonstrated by ESAU *et al.* (1967) with beet yellows virus in beets.

In the nucleus no virus particles or other formations which could be related to the presence of virus have been observed. The nucleus show the normal structure with a limiting double membrane (Fig. 12) where pores are seen when the sections are tangential to its surface (Fig. 13). The nucleolus, usually big, presents a normal structure. Its granular component is sometimes seen dispersed in the surrounding nucleoplasm.

CONCLUSIONS

From the comparative study of cell structure in *Datura Metel* L. healthy and infected by potato virus Y or potato virus X the following conclusions have been made:

- 1 — The nucleus, Golgi apparatus, crystal containing bodies (cytosomes) and cell wall show no appreciable changes due to the presence of these viruses.
- 2 — In the virus infected cells most of the chloroplasts show a normal structure. However in some cases a reduced number of grana lamellae, discontinuity in stroma lamellae and an unusual number of lipid droplets was observed. The signaled alterations are common to both viruses.
- 3 — The morphology of mitochondria are sometimes irregular in the virus infected cells. It is not possible to correlate these variations with the presence of the viruses.
- 4 — The mitochondria of cells from plants infected with PVY are frequently surrounded by filaments 90 to 100 Å in diameter. It is possible that these filaments represent virus particles. The significance of their association with the mitochondria is discussed. It will be interesting to learn if other viruses, belonging to group 5 of BRANDES, cause similar changes in their hosts.
- 5 — In the *Datura Metel* cells infected with PVX we have observed cytoplasmic areas crowded with ribosomes and filaments 40 to 50 Å in diameter. These zones are supposed to be virus assembly sites.
- 6 — Some of the cell alterations caused by PVX and PVY should be considered together with other characteristics in the classification of these viruses. In PVX infected cells, the virus are dispersed in the cytoplasm or accumulated in inclusions protruding into the central cell vacuole (Fig. 14). In the PVY infected cells the particles can be found also dispersed or forming laminated aggregates.

Note added in proof — After this article had been presented for publication, a note was published by B. D. Harrison and I. M. Roberts in J. Gen. Virology (3, 121, 1968) describing a close association between tobacco rattle virus and mitochondria in infected cells of *Nicotiana clevelandii*. The observation described by these authors is comparable to the one we reported in *Datura Metel* leaf cells infected with potato virus Y.

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