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The Structure and Development of a Genetic Tumour of the Pea

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With 12 Figures

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Summary

The development of the neoplasm of the pea pod has been examined by electron microscopy. The subsidiary cells of the stomata are the site of neoplastic development, and the earliest signs of tumour growth are the accumulation of lipid material in these cells and a decrease in the degree of their vacuolation. Mitosis in these cells gives rise to a population of neoplastic cells similar in structure to normal parenchyma. The only structural abnormality which persists in mature neoplastic tissue is the degeneration of the plastids. The chloroplasts of the original subsidiary cells undergo a continuous series of degenerative changes involving loss of photosynthetic function. The mode of development of the neoplasm and the ultrastructural changes associated with it are discussed in relation to other plant tumours.

1. Introduction

The morphology of a genetic neoplasm of the pea pod has been described by DODDS and MATTHEWS (1966) and SNOAD and MATTHEWS (1969). It consists of discrete outgrowths of colourless callus tissue from the stomata of the maturing pod, and it only appears under conditions of greenhouse growth or low light intensity (SNOAD and MATTHEWS 1969). In favourable growth circumstances it may eventually cover the majority of the surface of the pod. The neoplasm appears to develop as a result of mitoses in the subsidiary cells of the stomata, and its development is under the control of a single dominant gene (NUTTALL and LYALL 1964). This suggests that it may present an ideal model for the study of a primary chemical or structural event controlling development.

Several types of neoplastic and hyperplastic growth in plants have been the subject of electron microscope study (AMES 1972, GEE, SUN and DWYER 1967, GEROLA and BASSI 1966, LIPETZ 1970, MANOCHA 1970). Most authors, comparing the cells within the mature tumourous tissue with parenchymal cells

of the host plant have concluded that there are few major differences in ultrastructure between the two. The organelles most frequently showing some difference are the endoplasmic reticulum and the plastids. LIPETZ (1967, 1968, 1970) has claimed to demonstrate a quantitative difference in the amounts of endoplasmic reticulum present in cells of crown galls and auxin-induced neoplasia, and this observation has been restated by MANOCHA (1970). AMES (1972), describing genetic tumours of tobacco, suggests that they arise from a population of meristematic cells and that it is these cells which show the increased amount of endoplasmic reticulum. Unfortunately this is „not evident" from the published figures. The behaviour of plastids is likewise not consistently documented. AMES (1972) implies that the meristematic population within the tumour contains proplastids which differentiate into chloroplasts with tumour development, but other authors describe simple degenerative processes leading to destruction of internal lamellae of the chloroplasts of the originating tissue and eventual loss of plastids (GEE *et al.* 1967, GEROLA and BASSI 1966).

The work to be described here is a study of the development of the pea pod neoplasm. Since the site of its origin is known (SNOAD and MATTHEWS 1969) it represents an excellent system in which to study the primary events giving rise to neoplastic growth.

2. Materials and Methods

Young pods showing evidence of neoplastic development were removed from plants of pea "Chemin Long" Np (SNOAD and MATTHEWS 1969) growing in a greenhouse. Small pieces of the pod were fixed in 5% glutaraldehyde in 0.02 M phosphate buffer containing sucrose (BURGESS 1970). After post-fixation in veronal buffered 1% osmic acid, specimens were dehydrated in an alcohol series and embedded in Araldite. Sections were cut on an LKB Ultrotome III and examined in an AEI EM 6B.

3. Results

The structural development of the neoplasm at the light microscope level has been described by SNOAD and MATTHEWS (1969) and will not be considered further here. Fig. 1 shows a section through a closed stomatal

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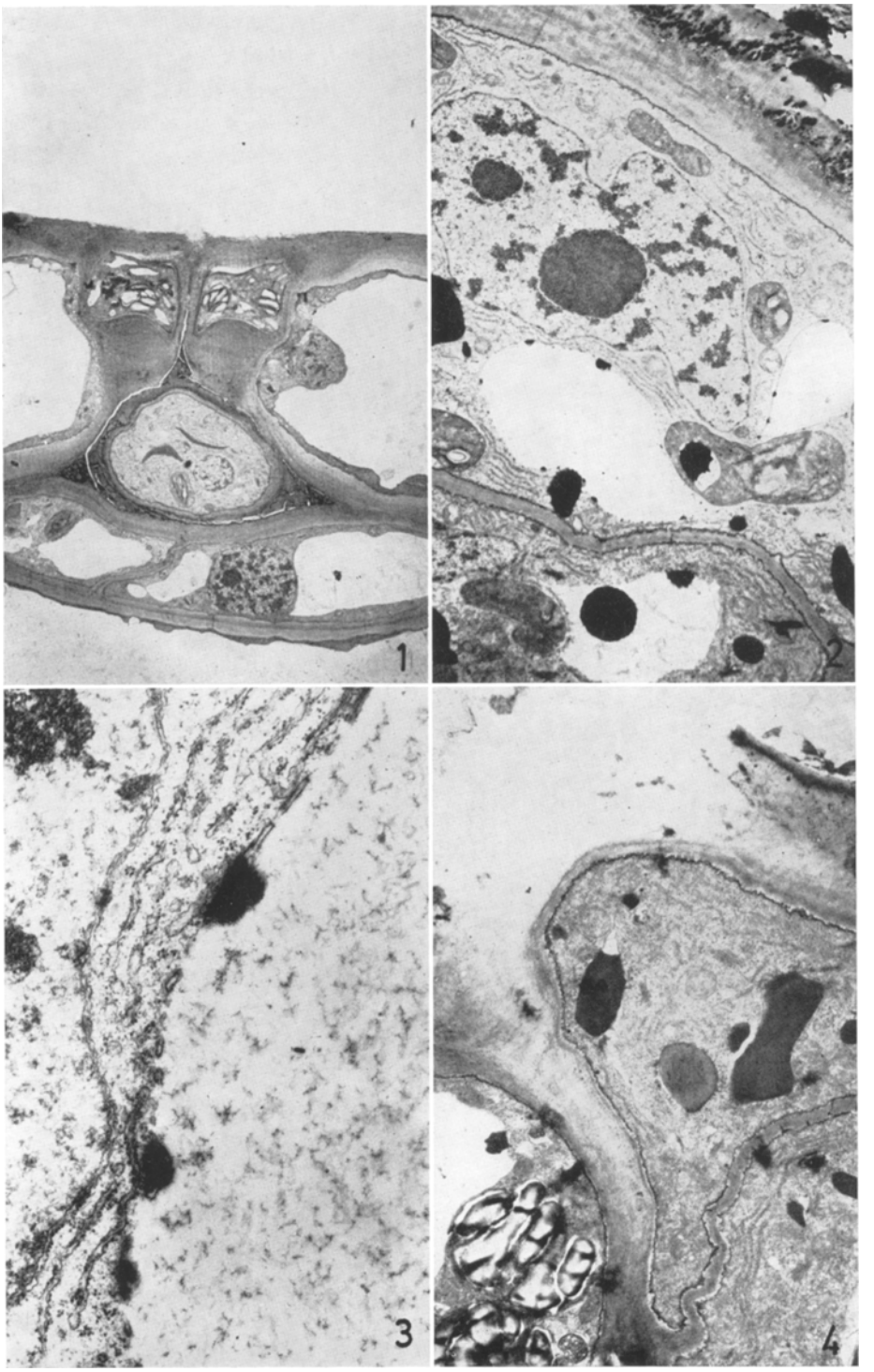
Fig. 1. Section through a closed stomatal complex in the pea pod. The subsidiary cells are smaller and less vacuolate than ordinary epidermal cells. ×1,800

Fig. 2. Subsidiary cell at an early stage of neoplastic growth. Osmiophilic material is present within the cytoplasm and along the tonoplast. The larger of the two vacuoles shows a fragmented tonoplast. The tonoplast around the smaller vacuole is intact. ×4,000

Fig. 3. Part of a similar cell to that of Fig. 2. The tonoplast is vesiculated. One of the osmiophilic droplets is apparently enclosed in a membrane, possibly the tonoplast. ×24,000

Fig. 4. Section through a young neoplasm. A neoplastic cell has burst through the old epidermis next to the guard cells. The starch filled plastids of the guard cell are visible. ×6,700



Figs. 1–4

complex of the pea pod before neoplastic growth has begun. The guard cells are characterised by a dense cytoplasm and plastids containing large accumulations of starch. The subsidiary cells are highly vacuolate with small nuclei of circular section. The plastids of the subsidiary cells will be described below (Fig. 6). The epidermal subsidiary cells are distinguishable from other epidermal cells not associated with stomata only by their slightly smaller size and lesser degree of vacuolation, not by any differentiation of cytoplasmic organelles. Plasmodesmata appear to interconnect the subsidiary cells and are seen fairly frequently; between subsidiary and guard cells no plasmodesmata have been seen.

Two characteristic changes occur in the ultrastructure of the subsidiary cell at the onset of neoplastic growth. Large osmiophilic droplets appear within the cell and the vacuole fragments into several smaller pieces (Fig. 2). In certain cells the tonoplast appears to be associated with small osmiophilic particles and may fragment into very electron dense vesicles (Figs. 2 and 3). The large dense droplets may occur either in the cytoplasm or within the vacuolar space, and do not appear to be membrane bound. At this stage of neoplastic growth the cell nucleus takes on a more ameboid appearance and the regular outline of the plastids is also lost. The young neoplasm grows by division of these modified cells, causing a local swelling of the pod which eventually develops into a visible pustule of white callus tissue (SNOAD and MATTHEWS 1969). Sections of young neoplasia show that the original epidermal wall may split as a result of the growth of the neoplastic tissue (Fig. 4).

A group of cells within a young neoplasm is shown in Fig. 5. The cells contain large osmiophilic droplets and small disperse vacuoles. The cells are separated by thin walls containing plasmodesmata, and in general have the appearance of a typical meristematic tissue. Mitotic activity is quite low as judged by the frequency of mitotic figures within sectioned material, and no abnormalities associated with mitotic stages have been observed.

The growth of the neoplasm is accompanied by a series of changes in the morphology of the plastids. Fig. 6 shows a chloroplast within the peripheral cytoplasm of a subsidiary cell prior to neoplastic development. Such chloro-

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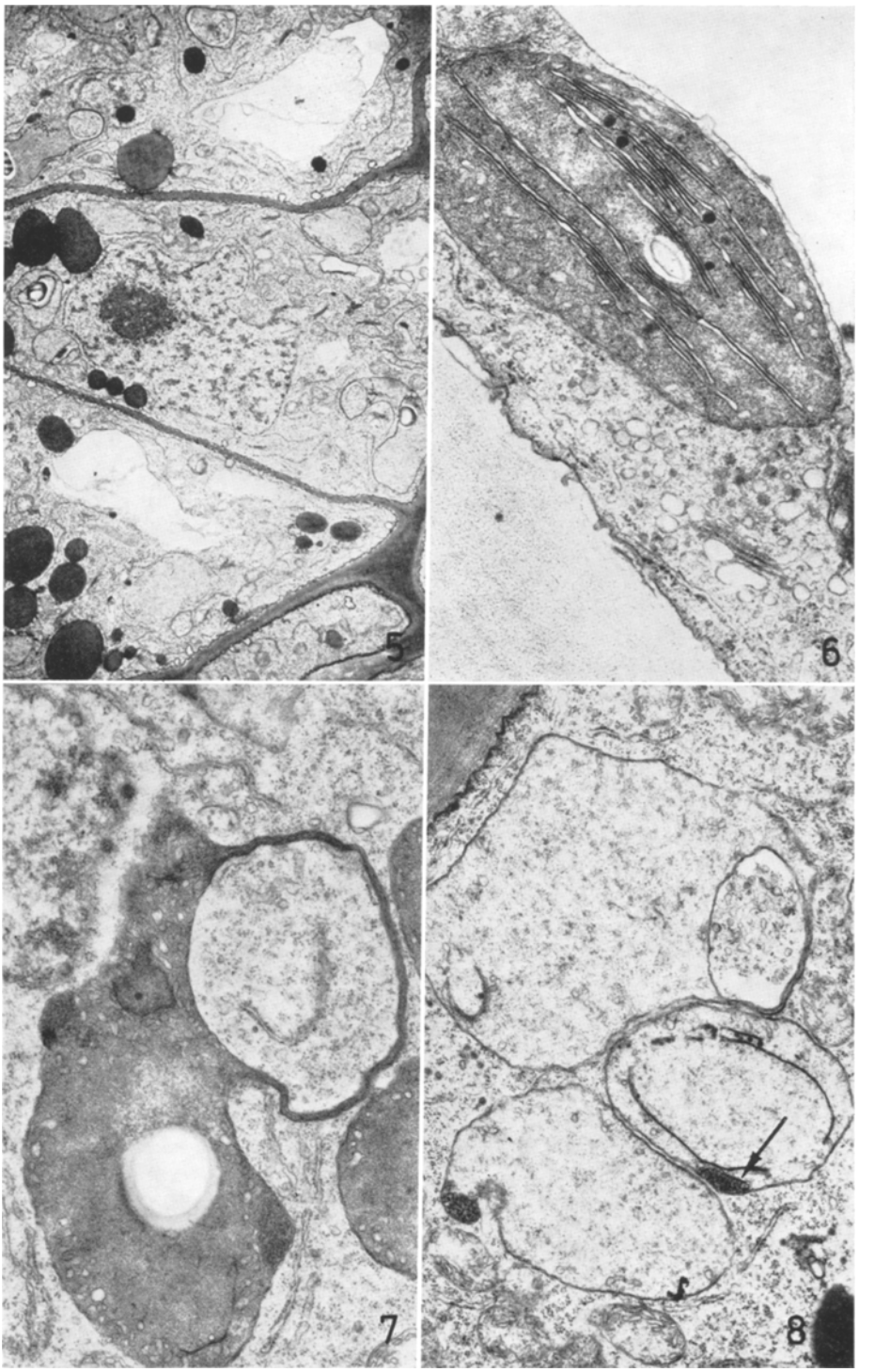
Fig. 5. Section through the meristematic-like cells of a young neoplasm. Lipid droplets in the cytoplasm and a reduced degree of vacuolation characterise the cells. ×3,700

Fig. 6. Part of the peripheral cytoplasm of a subsidiary cell. The plastid is of ellipsoidal section and contains lamellar systems and starch. ×25,000

Fig. 7. A plastid from a developing neoplastic cell. The ameboid shape is characteristic. Two regions containing staining interconvoluted tubules are visible in the section. ×19,000

Fig. 8. A group of plastids within a maturing neoplastic cell. Very few membranes remain and starch is absent. Two of the plastids contain regions of staining tubules, one associated with a single internal plastid membrane (arrow). ×16,000



Figs. 5–8

plasts are typically ellipsoidal in section and contain organised grana and stroma lamellae, as well as more reticulate membranes. There are usually small amounts of starch present, and a few osmiophilic droplets amongst the stroma lamellae. This type of chloroplast structure is common to the epidermal cells also, and is morphologically intermediate between the starch- and reticulum-containing plastids of the guard cells (*cf.*, PALLAS and MOLLENHAUER 1972) and the lamellate chloroplasts of the mesophyll. As the neoplasm begins to develop, the plastids first assume a more irregular outline in section (Fig. 2) and frequently seem to engulf large cytoplasmic organelles such as lipid droplets and mitochondria. The ameboid shape sometimes involves quite extensive and tenuous projections into the cytoplasm (Fig. 7). In larger neoplasia, degeneration of much of the plastid structure occurs. The plastids enlarge and tend to have a more spherical appearance (Fig. 8). The intensity of staining of the stroma is much reduced, and the internal lamellar systems either disappaer entirely or exist as single membranes (Fig. 8) or disorganised clumps of beaded vesicular appearance (Fig. 9). There is little or no starch in such plastids, and presumably they contain no visible pigments since the mature neoplasm is itself white in appearance.

One unusual and persistent feature of these degenerated plastids is the presence just inside the plastid envelope of a region containing stained tubules (Figs. 8 and 10). The tubules are sometimes associated with a single internal membrane, and appear to be interconvoluted. They have a diameter of about 20 nm. They often occupy or cause a small extension of the plastid envelope (Figs. 7, 8, 10). Occasionally two such groups are seen within a single plastid section (Fig. 7). They are not exclusively confined to totally degenerate plastids within the mature neoplastic cell, (*cf.*, Fig. 6), but they have not been observed in normal chloroplasts of the epidermis, subsidiary cells, or guard cells.

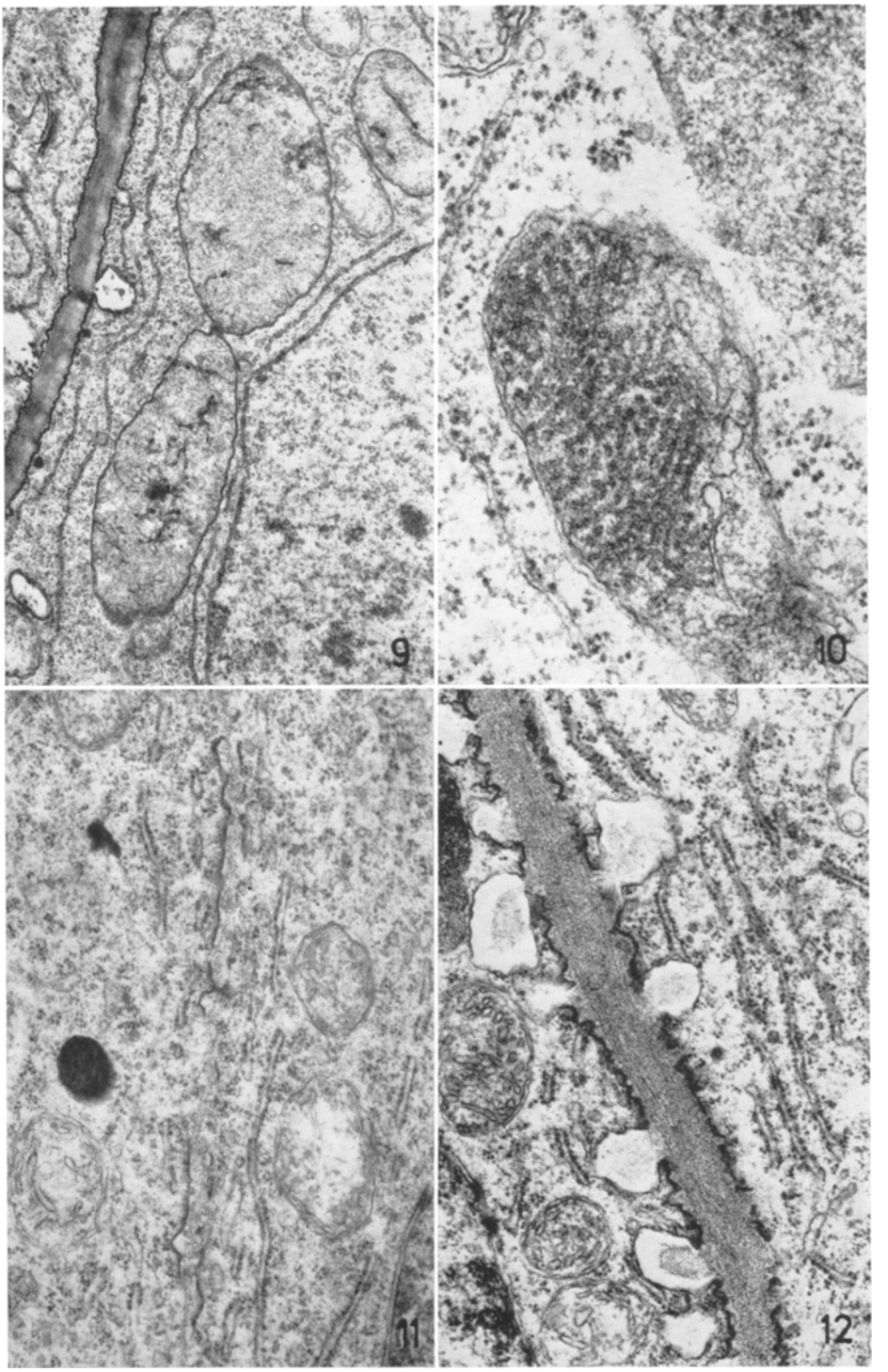
It is very noticeable that the plastids within a single cell are closely synchronised in the stage of their morphological appearance. Conversely adjacent cells within the developing neoplasm may contain plastids populations which are quite different in their structure.

Fig. 9. Part of the cytoplasm of a maturing neoplastic cell. The plastid shows an outline suggestive of a fission. A few wall ingrowths are also visible. ×11,000

Fig. 10. The region within a degenerating plastid which contains staining tubules. Fragments of membranes bound this region within the plastid. ×62,000

Fig. 11. Part of a recently formed cell plate in the dividing region of the neoplasm. Although a continuous plate of fibrillar material is visible, the plasmalemma shows random discontinuities. ×25,000

Fig. 12. The appearance of the wall between two neoplastic cells. Ingrowths containing fibrillar material are randomly distributed on both sides of the wall, as are small discontinuities in the plasmalemma. ×26,000



Figs. 9–12

Other cytoplasmic organelles appear to be of normal structure in both developing and mature neoplasia. The mature neoplasm consists of highly vacuolate cells separated by thin walls, similar to the general description of other neoplastic tissues. The large osmiophilic droplets characteristic of early neoplastic development do not persist in the older tissue. Two minor peculiarities concerning the plasmalemma appear in both developing and mature neoplasia. The plasmalemma itself shows many small discontinuities (Figs. 11 and 12). This effect is particularly striking at the time of cell plate formation (Fig. 11), but is also seen in older walls. In addition, walls separating neoplastic cells frequently show the presence of numerous extensions into the cytoplasm, which range to 100 nm in diameter (Fig. 12). These extensions usually contain staining fibrillar material. There is no evidence from the appearance of cytoplasmic organelles however that they represent sites of rapid incorporation of vesicular material from the cytoplasm.

Examination of normal, non-neoplastic pods reveals none of the foregoing ultrastructural features ascribed to neoplastic development. Stomatal fine structure is similar to that described by Figs. 1 and 6.

4. Discussion

The problem of neoplastic growth has received some attention from electron microscopists, but the majority of published reports consist of a comparison of the structure of the mature tumourous growth with some corresponding tissue of the originating plant. In general little difference emerges between such cells. The neoplasm of the pea pod presents an opportunity to examine the course of events which occur as a particular small group of cells become transformed from their stable differentiated state in the normal plant into being the originating cells of a proliferating callus growth.

The first changes to be observed at the electron microscope level in the subsidiary cells of the pod stomata may be considered as representing „de-differentiation" towards the meristematic state. The fragmentation of the vacuole, and the enlargement and apparent activation of the nucleus exemplify this. The appearance of large quantities of osmiophilic material within the cells at this time is quite characteristic, and it is possible that this is in some way related to the dispersal of the vacuole. The tonoplast membrane is associated with small droplets of osmiophilic material and sometimes appears to fragment into vesicles, some of which contain the osmiophilic material. It is difficult to understand how fragmentation of the tonoplast could occur *in vivo* without resulting in the large-scale release of vacuolar contents into the cytoplasm, and cell death. One possible explanation is that the vesiculation of the membrane is entirely an artefact of handling or preparation of the specimen; however, intact tonoplast membranes may be

found in the same cell with vesiculated ones (Fig. 2), and furthermore, the vesiculated appearance of the membrane is limited to those cells in which dispersal of the vacuole is probably taking place. Alternatively the accumulation of lipid droplets at the tonoplast may result from a real transformation in the membrane which allows it to condense, and the vacuole to diminish in size. The same changes in the properties of the membrane might render it locally unstable to the fixation procedure adopted.

The plastids of the subsidiary cells, in contrast to the findings of KAUFMAN *et al.* (1970) and SRIVASTAVA and SINGH (1972), are quite differentiated and contain starch. This differentiation is not reversed during the formation of the population of meristematic-like cells, but rather it appears that neoplastic growth is accompanied by a continuous series of degenerative changes in the chloroplast. These changes, finally resulting in large organelles of simple structure which presumably lack photosynthetic capability, take several mitotic generation times to be completed. It is thus unlikely that a malfunction of the chloroplasts acts as a nutritional or hormonal trigger for neoplastic growth.

The ultrastructural changes associated with the plastids follow a different course from other examples of tumour development. In genetic tumours of tobacco, the plastids of the mature tumour are similar to normal cortical chloroplasts, and arise from "proplastids" within a tumourous meristem (AMES 1972). In tumours induced by maize rough dwarf virus, there is breakdown of the plastid envelope and destruction of the chloroplasts (GEROLA and BASSI 1966). In crown gall tissue there is either no change in the plastids (LIPETZ 1970) or they are reduced in size and number following initial changes to grana structure (GEE *et al.* 1967). The plastids within pea pod neoplasm show a degeneration in structural complexity, but this is a synchronized change within any particular cell, suggesting that it is under central genetic control. Furthermore, despite considerable modification and simplification of structure, the plastids appear to retain their ability for replication, as is shown both by their maintained numbers and the frequent appearance of possible division figures. This situation appears to be intermediate between that of the tobacco genetic tumours and the virus- and bacteria-induced neoplasia. The loss of normal developmental controls which results in the growth of the neoplasm extends to a basic aspect of the functioning of the chloroplast as a photosynthetic unit, but apparently not to its survival as a self-replicating organelle within the cytoplasm. The tubular structures observed in degenerating plastids appear similar to those described as a normal component of guard cell plastids in two species by ALLAWAY and SETTERFIELD (1972) and PALLAS and MOLLENHAUER (1972). No satisfactory explanation of their presence has been given, and their resemblance to cytoplasmic microtubules seems slight (ALLAWAY and SETTERFIELD 1972).

The significance of the minor changes in ultrastructure associated with the plasmalemma is difficult to assess. As has been discussed above with regard to the appearance of fragmented vesicles at the boundary of the vacuole, discontinuities in cell membranes usually represent nothing more than faulty fixation procedure. Conceivably however a real change has occurred in the plasmalemma which results in local instability to fixative. The ingrowths of material from the wall could similarly represent plasmolysis during fixation; certainly they are never extensive enough to warrant their interpretation as a specialised differentiation of the wall (JONES and NORTHCOTE 1972). A satisfactory explanation of these ultrastructural changes may have to wait until the biochemical nature of the changes accompanying neoplastic growth can be ascertained.

The growth of the tumour can thus be simplified into three different phases; an initial "de-differentiation" of the subsidiary cells, equipping them for mitosis, a cell division phase, and a re-differentiation phase into the mature neoplasm. Superimposed on these is the continuous series of changes observed in the plastids. Growth of the neoplasm is dependent upon certain environmental conditions, of which quality of illumination is the most critical. It is hoped that work in progress on the effects of environmental conditions on neoplastic growth will enable the relationships between the phases of its development to be more clearly elucidated.

References

ALLAWAY, W. G., and G. SETTERFIELD, 1972: Ultrastructural observations on guard cells of *Vicia faba* and *Allium porrum*. Canad. J. Bot. **50**, 1404—1413.

AMES, I. H., 1972: The fine structure of genetic tumour cells. Amer. J. Bot. **59**, 341—345.

BURGESS, J., 1970: Microtubules and cell division in the microspore of *Dactylorchis fuschii*. Protoplasma **69**, 153—264.

DODDS, K. S., and P. MATTHEWS, 1966: Neoplastic pod in the pea. J. Hered. **57**, 83—85.

GEE, M. M., C. N. SUN, and J. D. DWYER, 1967: An electron microscope study of sunflower crown gall tumour. Protoplasma **64**, 195—200.

GEROLA, F. M., and M. BASSI, 1966: An electron microscopy study of leaf vein tumours from maize plants experimentally infected with maize rough dwarf virus. Caryologia **19**, 13—40.

JONES, M. G. K., and D. H. NORTHCOTE, 1972: Nematode induced syncytium—a multinucleate transfer cell. J. Cell Sci. **10**, 789—809.

KAUFMAN, P. B., L. B. PETERING, C. S. YOCUM, and D. BAIC, 1970: Ultrastructural studies on stomata development in internodes of *Avena sativa*. Amer. J. Bot. **57**, 33—49.

LIPETZ, J., 1967: Fine structure studies of plant tumour cells. J. Cell Biol. **35**, 82 A.

— 1968: Changes in the endoplasmic reticulum of plant tumour cells. J. Cell Biol. **39**, 81 A—82 A.

— 1970: The fine structure of plant tumours. I. Comparison of crown gall and hyperplastic cells. Protoplasma **70**, 207—216.

MANOCHA, M. S., 1970: Fine structure of sunflower crown gall tissue. Canad. J. Bot. **48**, 1455—1458.

NUTTALL, V. W., and L. H. LYALL, 1964: Inheritance of neoplastic pod in the pea. J. Hered. **55**, 184—186.

PALLAS, J. E., and H. H. MOLLENHAUER, 1972: Physiological implications of *Vicia faba* and *Nicotiana tabacum* guard-cell ultrastructure. Amer. J. Bot. **59**, 504—514.

SNOAD, B., and P. MATTHEWS, 1969: Neoplasms of the pea pod. In: Chromosomes Today. Volume 2 (C. D. DARLINGTON and K. R. LEWIS, eds.), pp. 126—131. Edinburgh: Oliver and Boyd Ltd.

SRIVASTAVA, L. M., and A. P. SINGH, 1972: Stomatal structure in corn leaves. J. Ultrastruct. Res. **39**, 345—363.

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