THE DEVELOPMENT OF AMOEBOID INCLUSION BODIES OF TOBACCO MOSAIC VIRUS

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(Plates 1-2)

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Summary

Fixatives that preserve the stromatic structure of normal plastids, show "amoeboid" inclusion bodies also to have a stromatic structure of a coarser and less regular type.

Plastids may be found with distortion of the stroma and an accompanying staining reaction like that given by virus inclusions.

A series of stages may be found between normal plastids and amoeboid inclusion bodies. The inclusion bodies appear to consist of one or more than one distorted and aggregated plastids.

The possibilities of staining methods for the study of virus-host cell relations is discussed.

I. Introduction

During work on the fixation and staining of viruses in infected plant tissues (Bald 1948*a*) some evidence was obtained that amoeboid inclusion bodies may develop from plastids. The only type of plant virus inclusion body whose development has previously been studied in detail, is the granular inclusion of aucuba mosaic of tomato (Sheffield 1931). This virus is a strain of ordinary tobacco mosaic. The granular inclusions are formed by aggregation of visible granules presumably developing in the infected cells, and carried passively in the streaming cytoplasm. The granular inclusions and the crystalline inclusions of tobacco mosaic were later shown (Sheffield 1939) to contain virus in an infective form. The inclusions were dissected out of infected cells, and were used to produce infections by inoculation to healthy plants. This work was repeated with the amorphous inclusion bodies of severe etch virus (Sheffield 1941). Thus some typical inclusion bodies contain a high concentration of virus, and material derived from infected cells (Bawden 1943).

The structure of plastids as it appears after fixation in mixtures that preserve their stromatic character has been described in another paper (Bald 1948*b*). Apart from the stroma, the main features of the fixation image are (i) a region not covered by the stroma, that has been called, for convenience, the stromatic gap, (ii) the starch grains embedded in cytoplasmic material inside the stroma, and (iii) outside the stroma, probably a semi-permeable membrane. A small refractile granule is often situated on the edge of the stromatic gap. These three features of the fixation image remain to be confirmed from living material, or material fixed by other methods.

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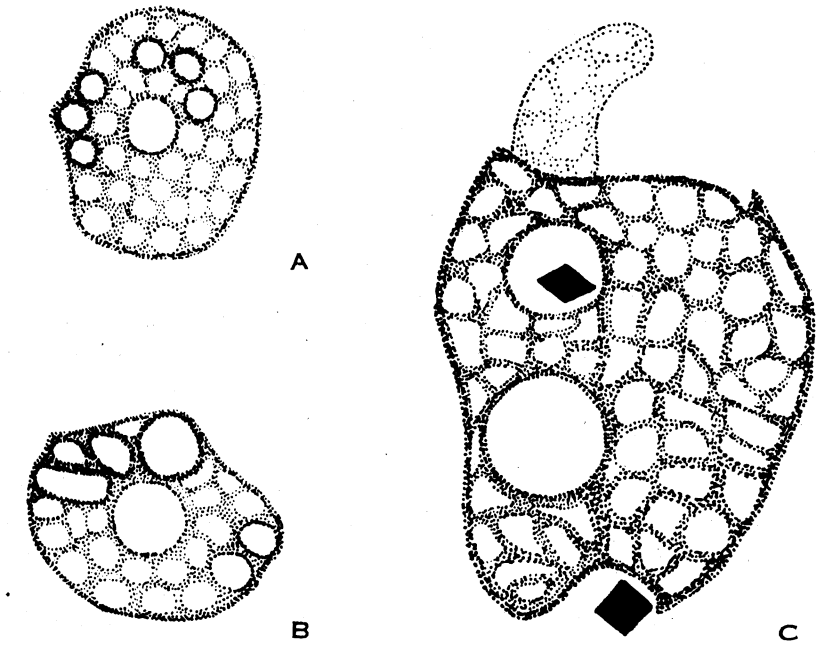
II. Abnormal Plastids and Amoeboid Inclusions

Plastids in epidermal cells of infected leaves, hardly distinguishable from normal plastids, are occasionally found to stain purple or blue with Giemsa, the colour characteristic of virus inclusions. It is more common, however, to find plastids in which the first accompaniment of this staining reaction is an enlarged stromatic gap, or some emphasis or distortion of the stromatic pattern (Figs. 1A, 1B). Apparently before this stage of development is reached, the starch has been converted to sugars, because only in rare instances has the haemotoxylin-iodine staining schedule (Bald 1948*a*) revealed even the remnants of starch grains in distorted plastids. Sometimes at this or a somewhat later stage a "cuboidal body" is seen in the centre of what is probably the enlarged stromatic gap, and occasionally a refractile granule on the edge of the gap. The body enlarges as



Figs. 1A and 1B.—Two abnormal plastids, sketched from camera lucida drawings and enlarged. Plastid (A) illustrates deeper staining and slight distension of 6 pores in the stroma. Plastid (B) illustrates deeper staining, definite enlargement, and distortion of stromatic structure. *c.* x 4500.

Fig. 1C.—An amoeboid inclusion body, rather odd in shape but otherwise typical. Two cuboidal bodies are associated with it. The protrusion above probably represents a distorted leucoplast; the main portion of the inclusion is probably composed of three or four misshapen and aggregated plastids. The original stromatic structure is distorted but preserved. *c.* x 3600.

the stromatic gap and some or all of the stromatal pores enlarge, and it begins to assume its mature form. If it is of a size that would suggest it arises from a small leucoplast in an epidermal cell, only the vacuole developing from the gap may be prominent; if it is of a size that would suggest it arises from a large chloroplast it is more likely to have the form of a multivacuolate network (Fig.

1C and Plate 1, Fig. 1). According to the fixation and staining schedule adopted, the body now stains with Giemsa as a network the same colour as the crystalline inclusion, or half-way between this colour and that taken by the normal plastid. The intensity of colouration may vary to a degree that suggests variation in the concentration of virus.

The distorted plastids or small amoeboid bodies apparently tend to aggregate (Plate 1) and round off to form larger amoeboid bodies. In leaves of tobacco plants about 10 days after inoculation with mosaic, these aggregations are often present in every stage of development. Probably, plastids that remain apparently normal in infected cells for some time also can go through the same cycle, and develop into new amoeboid bodies; for stages intermediate between plastids and fully formed amoeboid bodies have been found in plants infected for a considerable time. Unless new plastids develop from existing primordia or mitochondria, this might contribute to an exhaustion of the chlorophyll and premature yellowing of the leaf.

In the fully developed amoeboid inclusion it is common to find a number of cuboidal bodies, or the spherical forms of them (Fig. 1 and Plate 1) in the centre of the larger vacuoles. This has been observed by a number of workers (Kunkel 1924; Rawlins and Johnson 1925; Goldstein 1926; Holmes 1928; Clinch 1932; Woods 1933). The frequency with which it occurs is often higher than the frequency with which plastids and bodies are associated in normal cells under conditions of bright daylight (Bald 1948*b*).

Following is an observation made several times on epidermal strips from plants on which symptoms had just appeared. Inclusion bodies were just beginning to form and the little material in the cytoplasm that gave the virus staining reaction was mainly still in diffuse form. In control material from healthy plants there was only rare association between plastids and spherical or cuboidal bodies. In infected strips there was more frequent association. In some cells of infected epidermal strips, particularly along the veins where virus was evident, nearly every plastid had around the stromatic gap, or in patches which showed very clearly on the grey-green plastids, material staining red with acid fuchsin like that in the granule of the cuboidal body (Plate 2). It is possible that the frequent association of plastids and cuboidal bodies had some association with virus multiplication, and was connected also with the red-staining masses in the plastids. Alternatively, the protein fractions of the plastids may have dissociated more easily in infected tissues than in healthy, aggregated within the plastids and stained with aniline-fuchsin (Dufrenoy, Stamatinis, and Sarejanni 1929).

III. Discussion

These are preliminary observations, but they have been often repeated on material sampled over a period of more than one year. Some of the fixation and staining methods employed (Bald 1948*b*) both preserve the stromatic character of plastids, and preserve and facilitate the staining of virus. They are therefore more likely to reveal such a relationship between plastids and amoeboid inclusion bodies, if it exists, than established fixatives.

These results suggest many interesting speculations about virus multiplication and virus-cell relationships. The fixing and staining techniques described offer contributory methods for establishing or disproving them. Some evaluation of what these methods will and will not do therefore seems desirable.

The uses and limitations of the staining technique depend fundamentally on the manner and sites of virus multiplication. If viruses multiply by division of previous virus particles floating at random in the cell, there is little point in a detailed cytological study by these special methods of virus-cell relationships. Two other alternatives exist; that multiplication occurs by some such process as division, but mostly at certain points where suitable concentrations of metabolites and suitable energy conditions exist; or that, in order to reproduce, the virus particles become part of the structure of the cell, e.g. join with cell constituents to become one of the phospholipin-ribonucleoprotein complexes that are sometimes represented (Davidson 1945) as the characteristic self-perpetuating units of living matter. The latter alternative best explains the varied and complicated phenomena of immunity and interaction between related viruses (Price 1940; Bawden 1943; Bawden and Kassanis 1945).

According to this view of virus multiplication, virus particles, as distinct from unformed elements or precursors of particles, would exist in an infected cell (*a*) in active form at relatively limited loci, (*b*) passively concentrated around the multiplication loci, (*c*) dispersed throughout the cell in an inert form, or (*d*) as masses of virus particles aggregated into more or less clearly defined inclusion bodies. The position of these bodies in the cell might bear little relation to the loci of multiplication.

Classes (*c*) and (*d*) would intergrade; also aggregates might include other elements than virus particles. Inclusion bodies might vary in composition from almost pure virus to the products of abnormal metabolism, containing relatively little virus.

If the loci of multiplication were inside organs of the plant cell, inclusions might consist of these organs in a degenerate condition containing virus in both forms (*a*) and (*b*). If the organs had become completely degenerate and their loci of virus multiplication were no longer active, the virus might be in form (*b*) only.

Although they are in the highest degree speculative, these assumptions are not unreasonable, and they put reasonable bounds on what results may be expected from staining techniques. Some of these are:

(1) It is possible that the small amounts of active virus at the multiplication loci would never be revealed by staining.

(2) Possibly, also, only viruses that multiply sufficiently in host cells to produce a great excess of virus particles above the number needed to fill active centres of multiplication are likely to be revealed by selective staining.

(3) It does not follow that the location of virus in high concentration automatically discovers the centres of multiplication. Virus particles probably circulate freely in the microscopically structureless portions of the cell cytoplasm

(Sheffield 1931), and must pass from cell to cell (Uppal 1934). It is possible even that virus particles may pass from cells where they developed to other cells before they aggregate into definite cell inclusions. However, the presence of legume virus inclusions in such *formed* elements as the nucleoli (McWhorter 1941), if these inclusions are finally proved to contain virus, might suggest that the virus multiplies in the nucleoli or at their borders. If confirmation is found for the hypothesis that the amoeboid inclusions of tobacco mosaic develop from plastids (see above), the presence of virus in these inclusions might suggest that virus multiplies in the plastids. *A priori,* there seems less chance of virus particles passing through a membrane or phase boundary and of being concentrated passively in an organelle in which they were not formed, than of virus particles concentrating in an organelle where they are being formed.

(4) It seems that the most likely organs for the multiplication of virus would be those, normally containing ribonucleic acid (Davidson 1945), in or near which high concentrations of virus often appear in infected cells. Great care would be needed in judging whether these concentrations of virus had not lodged at such points after circulation around the cell.

(5) In tissues beyond the meristematic stage, the organs of the cell that normally contain ribonucleic acid are the microsomes (if the microsomes in plant cells are analogous to the microsomes of animal cells), the mitochondria, the plastids, and the nucleoli. It would be interesting to discover if the so-called "cuboidal bodies" also contain ribonucleic acid. Special attention might be paid to the condition of these organs in healthy and diseased tissues.

IV. Acknowledgments

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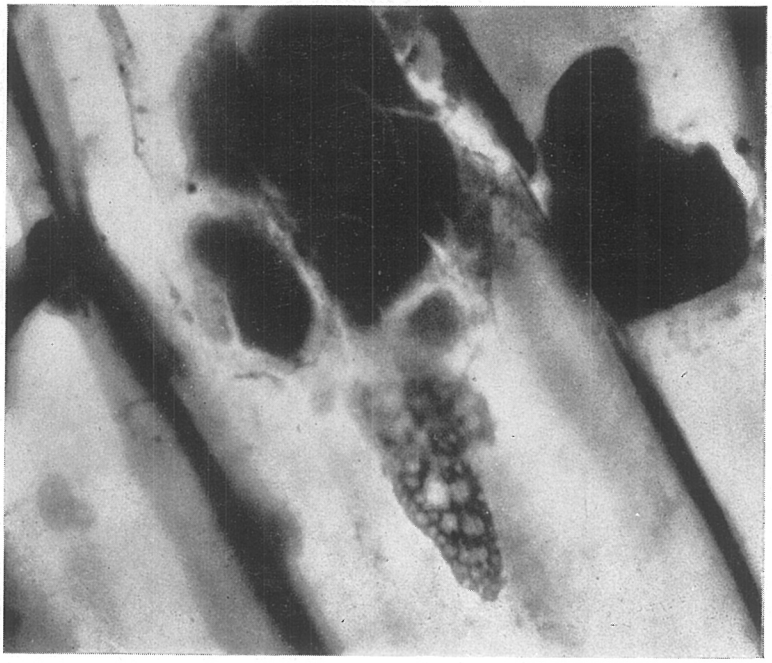

Fig. 1

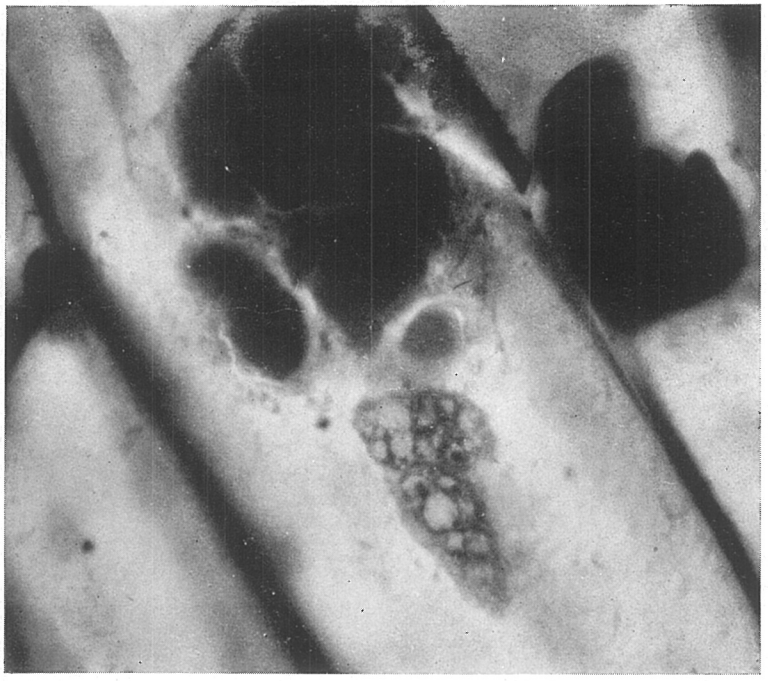

Fig. 2

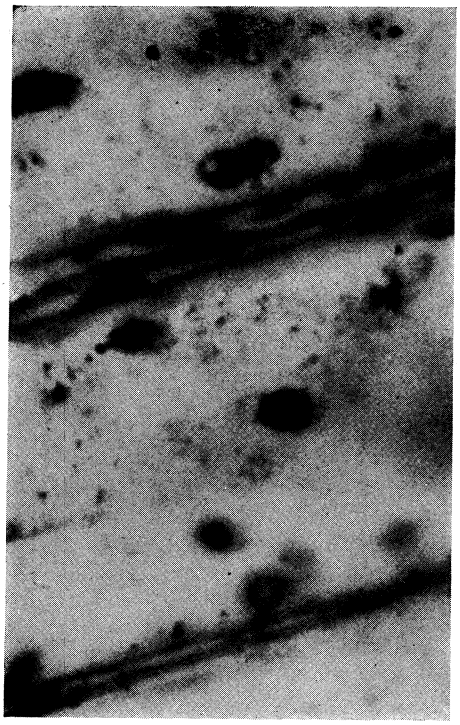

Fig. 1

Fig. 2

BALD.—THE DEVELOPMENT OF AMOEBOID INCLUSION BODIES OF TOBACCO MOSAIC VIRUS

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EXPLANATION OF PLATES 1-2

PLATE 1

Figures 1 and 2 are focused at different levels. Above are massive crystalline inclusions of tobacco mosaic, below an "amoeboid" inclusion body. Figure 1 shows clearly the stromatic structure of the amoeboid inclusion. Figure 2 shows that the inclusion is formed of two masses not yet completely fused. Four "cuboidal" bodies were visible in the whole body. One can be seen in Figure 1 and two in Figure 2, the fourth is out of focus at both levels. Fixative lb plus carminic acid, stain safranin and Giemsa. *c.* x 850.

PLATE 2

Figures 1 and 2 show material staining red with acid fuchsin, in the same way as the granules of cuboidal bodies, clearly visible in leucoplasts. Epidermal cells from the underside of a vein of a recently infected tobacco leaf. The aggregation of virus in stainable quantities is only just beginning in adjacent cells. Fixation 3b, stain aniline-fuchsin Giemsa aurantia. *c.* x 850.