THE STRUCTURE OF PLASTIDS AND OTHER CYTOPLASMIC BODIES IN FIXED PREPARATIONS OF EPIDERMAL STRIPS

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(Plate 1)

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Summary

The fixation of the stromatic structure of plastids was found possible by the use of mixtures designed for the fixation of viruses in infected plant tissues. Other features of plastids seen in fixed material are described.

Bodies formerly assumed to be protein crystals are fixed in a form that suggests a less simple structure and possibly a more important function than that of reserve protein.

At times there seems to be an association between plastids and bodies that is partly dependent on incident light.

I. Introduction

The structure of plastids has been discovered mainly from observations on living material (Weier 1938; Jungers and Doutreligne 1943). Most of the established fixatives seriously distort the stroma, and in doing so they destroy the plastids' most characteristic structural feature (Zirkle 1926). During experiments with fixatives intended to facilitate the staining of viruses in infected plant tissues (Bald 1948*b*), it was found that the stromatic structure of the plastids was sometimes preserved. Fixatives were developed that consistently preserve this and possibly other essential features of the plastids.

In addition, granules that have been in one of their forms called by virus workers "cuboidal bodies" (Rawlins and Johnson 1925; Goldstein 1926; Holmes 1928; Clinch 1932; Woods 1933) have appeared as portions of composite structures that superficially were somewhat like immature plastids. If the whole structures have not previously been observed and described, it is because portions of them are artefacts due to these newly-developed fixatives; or else the more delicate parts are easily destroyed by other types of fixation. At present, the latter explanation seems more likely, because the frequency with which the whole structures are seen in fixed preparations increases as the accuracy with which the details of plastid architecture are fixed. The images of plastids and the so-called "cuboidal bodies" as they appear after fixation will be briefly described.

II. Materials and Methods

The test material used in the development of fixing and staining methods for viruses (Bald 1948*a*) was largely epidermal strips from tobacco and occasionally from other plants. Generally some parenchymatous cells were stripped off with the epidermis, and the fully developed chloroplasts they contained were available

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for comparison with the smaller plastids of the epidermis. The descriptions and illustrations in this paper are taken from healthy control tobacco plants.

Two fixatives will be described here. The first may be used for the preparation of material to demonstrate the stromatic structure of the plastid, the second to demonstrate also the structure of the cuboidal bodies. Further particulars of fixation and staining methods may be found elsewhere (Bald 1948*b*).

Fixative 1b may be made by mixing 50 ml. alcohol, 20 ml. Lugol's iodine, and 30 ml. 1.5 per cent. chromic acid immediately before use. Lugol's iodine consists of 6 g. potassium iodide and 4 g. iodine in 100 ml. water. Fix epidermal strips 20 minutes or longer in the dark. Wash in several changes of 50 per cent. alcohol in the dark; or in 0.5 per cent. sodium thiosulphate in 50 per cent. alcohol if precipitates tend to form. Pass through graded alcohols to 95 per cent., and leave 1 hour or longer to harden before staining.

The other fixative is 3a plus 1 per cent. carminic acid (Bald 1948*b*). It is made with 50 per cent. alcohol, 40 per cent. glacial acetic acid, and 10 per cent. water by volume, 1 g. cerium nitrate and 1 g. carminic acid. If necessary, heat to dissolve the carminic acid; and filter if the mixture is not clear. Fix epidermal strips 15 minutes or longer, transfer through several changes of 95 per cent. alcohol, and after an hour or more pass through 85 per cent. to 70 per cent. alcohol. Leave overnight before staining. The carminic acid is not used as a stain but as a fixative. Possibly it has some mordanting action for trypan blue (McWhorter 1941; Bald 1948*b*), which, of the stains tested, was the one that best reveals the full structural details of the so-called "cuboidal bodies."

III. The Plastid

Figure 1 illustrates the structure of the plastid of the tobacco plant as it appears after fixation. It is semi-diagrammatic in that no staining schedule has yet been found to differentiate with such clarity in one preparation all the structural features shown in this figure. The stroma, properly stained, appears more or less clearly according to the size and condition of the plastid (Fig. 1 and Plate 1, Fig. 1). Large chloroplasts in tissues fixed early in the morning reveal the stroma most clearly. The stromatic structure is probably permanent, although it may be obscured in living plastids of plants submitted for some time to bright daylight (Weier 1938*b*). It is present in the smaller plastids of the epidermis and the larger chloroplasts of the mesophyll.

The stromatic structure is confined to an outer shell. In tobacco and several other plants examined, the peripheral continuity of the stroma seems often (possibly always) to be broken by at least one circular gap. More than one gap is sometimes found, but whether the extra gaps are normal or a result of fixation has not been determined. The presence of gaps in the stroma of living chloroplasts was deduced by Zirkle (1926). At the edge of this gap, or occasionally elsewhere on the plastid, is a single refractile deeply-staining granule, too small to be resolved except under the highest power of the light microscope. Although it cannot be seen on every plastid or in every preparation, heavy staining, particularly with Giemsa, and subsequent destaining to a light colour will reveal it in

the majority of plastids. Iron-alum haemotoxylin, and other stains may also be used to demonstrate it. There is difficulty because of its small size in detecting the colour it may assume. It is sometimes clearly situated in a round lighter-coloured area larger than could be attributed to diffraction effects, but the clear space might be a result of fixation. The refractile granule can be seen on conveniently placed plastids, to be situated no deeper than the stromatic layer.

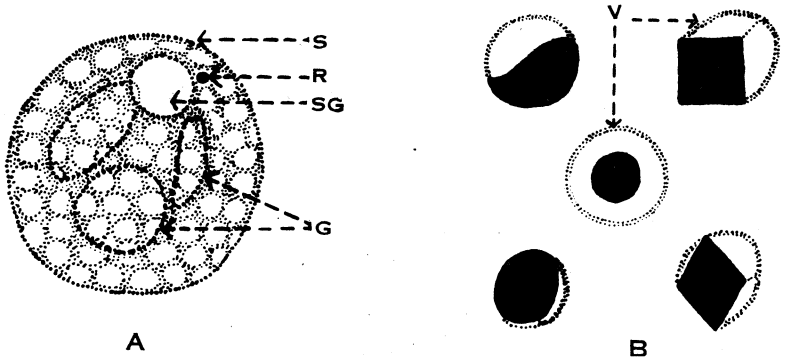

Fig. 1A.—Chloroplast from sub-epidermal mesophyll cell of tobacco leaf, showing stroma (*S*) containing starch grains (*G*) surrounded by cytoplasmic material, and penetrated by stromatic gap (*SG*) beside which is a minute refractile granule (*R*).

Fig. 1B.—Various forms of so-called "cuboidal bodies" at x 1¼ to x 2 the magnification of the plastid. A structure like a delicate vesicle (*V*) is attached to each body.

The starch may be revealed by the haematoxylin iodine method (Bald 1948*b*) after 1b fixation. The starch grains do not always stain uniformly. Sometimes the centre is light coloured, and parts or all of the periphery darker. Seen at right angles to their broadest surface, they appear dark and rod- or slightly crescent-shaped. If they are fairly small compared with the size of the plastid that contains them, they may be grouped in a ring inside and around a somewhat enlarged stromatic gap.

Cytoplasmic material, in which the starch grains are embedded, appears to occupy the centre of the plastid, and is surrounded by the stroma. It may sometimes be seen even when the network of the stroma is definitely stained, and thus the starch grains may be more or less clearly outlined. When the plastids are gorged with starch the stroma may be barely visible, or invisible, and the material around the starch may outline the grains very clearly.

The presence of a containing semi-permeable membrane around the whole plastid has been deduced from the vesiculation of plastids under hypertonic conditions (Weier 1938*a*; Esau 1944). Zirkle (1926) claims to have shown the presence of a thin clear boundary layer outside the stroma. Occasionally during the present studies it has seemed that there was a layer outside the stroma of many plastids, sometimes more, sometimes less deeply stained than the stroma.

IV. Cuboidal or Spherical Bodies

The granules, of which the "cuboidal bodies" are one form, are preserved in part by the majority of fixatives, whether or not the plastids are well fixed or

the mitochondria are preserved. The granules accept a number of stains, but are most brilliantly coloured by the acid fuchsin of combinations containing it (Woods 1933). They are not always cuboidal in form; more often they are spherical, sometimes irregular, and they vary in size. They never appear to be as small as spherical mitochondria. Many are much larger than the 0.8 micron given by Woods as a maximum measurement. They are free in the cytoplasm or adhere to plastids. They may be observed in the streaming cytoplasm of living hair cells or in the elongated epidermal cells from beneath the veins, if the cells are stained *in vivo* with dilute Janus green according to the method given by Sorokin (1941).

As methods of fixation employed in the virus studies (Bald 1948*b*) became reasonably adequate, some images were obtained that suggested variations in staining within a single spherical or cuboidal body. Often a clear area showed around a deeply staining cuboid or sphere or the central part was clearer than the outer part. Finally, preparations were obtained in which the walls of what might be interpreted as a fine vesicle were stained (Fig. 1B and Plate 1, Fig. 2). This appeared surrounding or at one side of the deeply staining granule. When the granule was spherical, the image was somewhat like that occasionally given by immature plastids. The vesicle was best preserved and stained by fixative 3a plus 1 per cent. carminic acid, followed by trypan blue and orange G (Bald 1948*b*). Other stains, including iron-alum haemotoxylin sometimes differentiated the fine vesicle wall. In some preparations a considerable proportion of granules appeared to have vesicles attached, while others had no sign of them. Some of the forms they took are shown in Figure 1B. They included, besides, vesicles surrounding only a dot of deeply staining material, and apparent division figures.

In some preparations these bodies were plentiful in the cytoplasm. No more adhered to plastids than might have been expected from random contact during streaming and adhesion until the cytoplasmic currents pulled them apart again. In other preparations many more of the bodies adhered to plastids than would be expected from random contact (Plate 1, Fig. 1). In others again there seemed to be fewer bodies in the cells. Examination of plastids in some of these preparations often revealed images that might be interpreted as indicating a release of the stainable material of the granule into the plastid, or collection of stainable material in the plastid to form a granule.

It would be possible to illustrate these appearances as a sequence, and suggest a functional association between plastids and bodies. However, the adequacy for this purpose of the fixing and staining techniques would have to be examined more carefully, and critical experiments would be needed to test such an interpretation. Following is the principal evidence so far accumulated that might be interpreted as suggesting an association.

In epidermal strips from leaves of healthy plants collected in the greenhouse about midday or early in the afternoon of bright sunny days, the bodies were mainly distributed throughout the cytoplasm. In strips from leaves collected at 8 or 9 a.m. on dull mornings, there were many examples of adherence, and of the presence of material in the plastids that stained in the same way as the cuboidal or spherical bodies.

Leaves from healthy tobacco plants were collected about 3 p.m. on sunny days, and split into two pieces down the midrib. Epidermal strips were taken from half of each leaf and fixed immediately. The other half leaves were kept damp in a dark cupboard for 2 hours before strips were taken from them and fixed. In the epidermal strips from half leaves kept in the dark, there appeared to be more frequent association of plastids and bodies than in those submitted to bright light.

A phenomenon that might lead to the alternative explanation, that these appearances are, at least in part, artefacts of fixation, is described by Dufrenoy, Stamatinis, and Sarejanni (1929). They describe a dissociation of protein and lipoid material in diseased plastids during fixation with Nemec's and other mixtures. Some of the mixtures evolved for the fixation of viruses (Bald 1948*b*) produced similar effects in healthy plastids. It is possible that the best fixation so far attained may fail to prevent this dissociation, and many of the images indicating association of plastids and protein or cuboidal bodies may have been due to poor fixation of the living structure. Only further critical work can decide the point.

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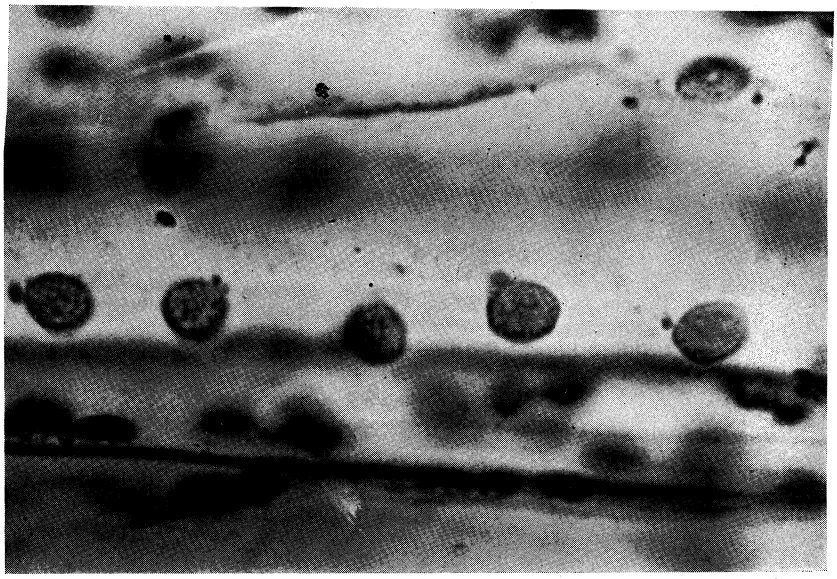

Fig. 1

Fig. 2

BALD.— THE STRUCTURE OF PLASTIDS AND OTHER CYTOPLASMIC BODIES IN FIXED PREPARATIONS OF EPIDERMAL STRIPS

Explanation of Plate 1

Fig. 1.—A row of 5 plastids showing fixation of stromatic structure. So-called "cuboidal bodies" adjacent to four of the plastids. Fixation 1b, stain Giemsa. *c.* x 850.

Fig. 2.—Two plastids with the spherical form of "cuboidal bodies" attached. The vesicular portion of these bodies is well fixed. The stromatic structure of the plastids is faintly evident, but the staining is too deep to demonstrate it clearly. Fixative 3a plus 1 per cent. carminic acid. Stain, trypan blue and orange G. *c.* x 850.