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Cytoplasmic Components of the Broad Bean Root Tip Cells

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Abstract. The cytoplasmic components of broad bean (*Vicia faba* L.) root tip cells were studied light- and electronmicroscopically. Using a light microscope, differences were revealed between cells from the cortex and from the central cylinder. For electron microscope studies the cells near the boundary between the mentioned parts of the root tip were selected at a distance of about 2 mm from the initials. The orientation of objects during embedding made possible fairly accurate localization. No peculiar, strikingly osmiophillic bodies were seen, which could be identified without doubt as osmiophillic platelets. It follows that some of the current cytoplasmic components, perhaps more or less altered were described as osmiophillic platelets. After fixation with $KMnO_4$, in which case the electronmicrographs are most instructive, the leucoplasts show several inclusions, mostly only partially limited against the matrix; it is not clear whether the true membrane is concerned here. The origin of intravacuolar membraneous formations is discussed.

The cells of the broad bean root tip are a classical object of botanical cytology. It proves they are material suitable for biochemical and cytochemical research of cell division, tissue differentiation etc. In comparison with other objects used for such studies, relatively little systematic consideration has been paid to the morphology of the cytoplasm of broad bean root tip cells, though it is fully deserved: e.g. the question of osmiophillic platelets† seems not to be solved definitively (PRESS 1956, 1957), the nuclear localization of DNA has been questioned (BRANTON and RUCH 1964, GAHAN and CHAYEN 1965) etc.

The aim of this study is to start from light microscopic studies and, using various fixatives, to deal with the ultrastructure of this material, especially in connection with the first of the findings mentioned above. Attention has also been paid to the ultrastructure of leucoplasts.

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† Unless otherwise stated, the term osmiophillic platelet has here the same meaning as used by BOWEN (see PRESS 1957).

Material and Methods

Tips of about 3 cm long main roots of germinating broad bean (*Vicia faba* L.) seeds were used (ADÁMKOVÁ and BENEŠ 1966*). For light microscope studies the material was fixed by the fixatives of Regaud (WOLF 1954), ZIRKLE, as modified by O'BRIEN (O'BRIEN 1951) and Němec (chromic acid + bichromate + formol, NĚMEC et al. 1962). Using tertiary butanol series the material was dehydrated and embedded in paraffin. Longitudinal sections 5 μm thick were made. Staining with Heidenhain's iron haematoxyline (WOLF 1954) or with the modified Altmann's technique (BENEŠ, in preparation).

For electron microscopic observation transverse sections about 1 mm thick, taken at the distance of about 2 mm from the tip, were fixed either in a 1·2% solution of $KMnO_4$ in veronal-acetate buffer of pH 7·2 for 4 hours at 0 to 4° C (LUFT 1956), or in a 1% solution of OsO_4 in veronal-acetate buffer of pH 7·2 to 7·5 for 4 hours at room temperature (PALADE 1952), or in 10% formaline (neutralized with $NaHCO_3$) overnight at room temperature; in the latter case the material was washed in water and postfixed with Palade's OsO_4 solution, as given above (double fixation, SITTE 1958). Material fixed with OsO_4 or with formol/OsO_4 was contrasted, when dehydrated, with 0·5%solution of uranylacetate in 70% ethanol overnight at room temperature. Otherwise, the dehydration and embedding (Vestopal W) were carried out in the usual way. Polymerization was performed in flat dishes made from Al foil to achieve the correct orientation of objects in order to obtain longitudinal sections. The cells were studied near the boundary between the cortex and the central cylinder, which concerns cells of the pericycle and adjacent layers of the central cylinder and cells of the endodermis and neighbouring area of the cortex, at the distance of about 2 mm from the tip. The material was sectioned using the ultramicrotome Ultrotome LKB with glass knives. The sections were also contrasted either with 5% watery solution of uranylacetate for 20 minutes at room temperature or with lead citrate (REYNOLDS 1963), or using both treatments. Electronmicrographs were taken by a prototype of Tesla Brno BS 413 electronmicroscope, constructed in the Institute of Laboratory Devices, Czechoslovak Academy of Science, Brno. To ensure accurate histological localization, thick sections from material processed for electron microscopy, were stained when heated with a 2% solution of toluidine blue in phosphate buffer of pH 9·1 and examined by light microscope.

Results

Within the region examined, i.e. at the distance of about 2 mm from the tip, differences between the cells of the cortex and of the central cylinder

* In this paper it is not stated that the water in the dessicator was continuously bubbled by air.

were observed light microscopically when studying the objects prepared by any of the techniques of fixation and staining. The cortex cells are vacuolized. In addition, large starch grains are often visible in these cells. In the vacuoles there are spherical formations, apparently due to precipitation of the vacuolar content. In the cytoplasm, bodies of various shape and size are found, namely mitochondria, proplastids etc. The cells of the central cylinder, especially the cells of the core, are also vacuolized, within the region studied, but there are no striking precipitates in the vacuoles. Leucoplasts are the most noticeable cytoplasmic components in these cells. Cells rich in cytoplasm, still lacking large central vacuoles, located in the proximity of the boundary between the cortex and the central cylinder seem to be most suitable for electron microscopic examination. On the electronmicrographs no special bodies were seen corresponding to osmiophillic platelets. Current cytoplasmic components of root tip cells were found: profiles of the endoplasmic reticulum, ribosomes, spherosomes, dictyosomes, mitochondria, proplastids*, leucoplasts*. Three kinds of bodies, apparently limited by a unit membrane, little smaller than mitochondria, are considered here within the category of spherosomes: 1. bodies comprising material of the same density as the ground cytoplasm; 2. bodies consisting of material of a distinctly greater density; 3. bodies, the central part of which is light on electronmicrograph positives, whereas the periphery is dark. (See formations described as S_1, S_2, S_3 on Fig. 1, 2, 9). Small vesicles, presumably of mutually different character, were revealed in the proximity of both dictyosomes and profiles of endoplasmic reticulum (↗ on Fig. 1). Two types of leucoplasts are clearly distinguishable when material fixed with $KMnO_4$ is examined: leucoplasts with several large inclusions (L_1 on Fig. 1, 2) and leucoplasts with fewer small inclusions (L_2 on Fig. 3). In the former case it is obvious that the inclusions are delimited — though often incompletely — from the matrix. After double fixation with formol/OsO_4 the material of intraleucoplastidic inclusions is distinctly filamentous (↗ on Fig. 5). The OsO_4 fixation apparently causes an alteration of the material so that it is, for example, practically impossible to distinguish between mitochondria and young plastids (Fig. 4). Fig. 5 shows a transverse section of transvacuolar cytoplasmic strand remarkably rich in free ribosomes (↟). In Fig. 6 ribosomes are visible both free (↟) and bound to the outer surface of the endoplasmic reticulum (↗). Compared with Fig. 5, the cytoplasm here is much more "relaxed". Mitochondria bear tubulous type invaginations (Fig. 1, 2, 6, 7, 9). Fig. 7 shows mitochondria or proplastids wedged by a protrusion into a neighbouring one (↗). The larger formation — it is more suitable to describe it as a proplastid — is either budding — or simply — branched. After formol/OsO_4 fixation there are usually membranes visible in the vacuoles (see Fig. 8 and 10). The montage (Fig. 10) helps to show the frequency of

* It seems possible and necessary to distinguish between proplastids and leucoplasts. Leucoplasts are formations much larger than mitochondria, they have a regular round border in section, the inclusions of storage material usually account for a substancial portion of their volume. On the other hand, proplastids (MENKE and FRICKE 1964 noted that this term is not fully acceptable) have approximately the same diameter as mitochondria, their margin is irregular, sometimes they are apparently amoeboid. Cases exist where it is impossible to distinguish proplastids from and mitochondria with certainty.

Longitudinal sections of the broad bean (*Vicia faba* L.) root tip cells, located near the boundary between cortex and central cylinder about 2 mm from initials. Figs. 1, 2, 3, 7, 9 from $KMnO_4$ fixed material, fig. 4 from material fixed with OsO_4, fig. 5, 6, 8, 10 taken from formol fixed OsO_4 postfixed material. The lengths of the abscissas correspond to 1 μm. Abbreviations used: CW cell wall, D dictyosome, ER endoplasmic reticulum, I intraleucoplastidic inclusion, L (L_1, L_2) leucoplast, M mitochondrion. S (S_1, S_2, S_3) spherosome, V vacuole.

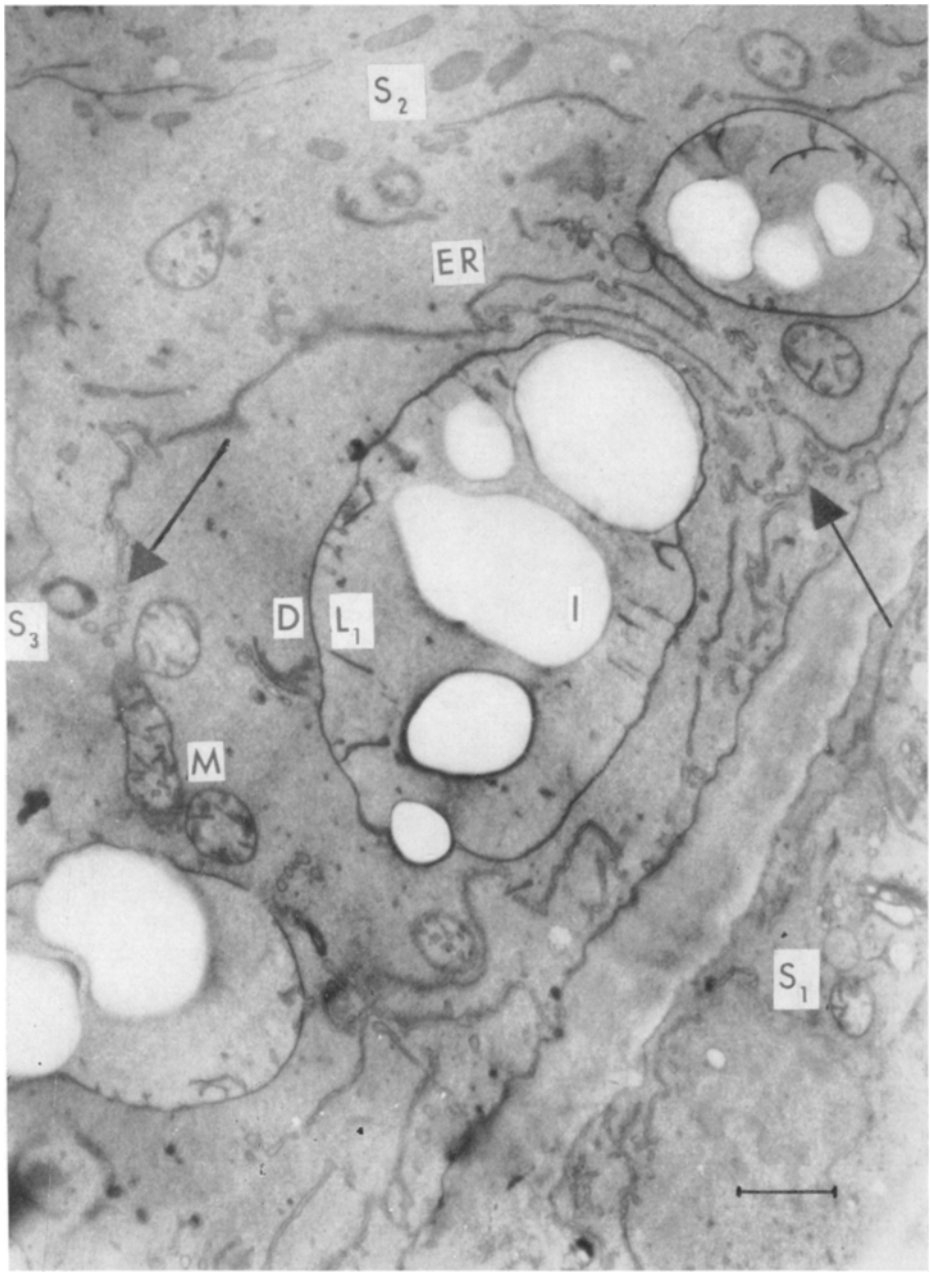


Fig. 1. 3 types of spherosomes (S_1, S_2, S_3), small vesicles at the endoplasmic reticulum (↗), mitochondria, leucoplasts bearing inclusions.

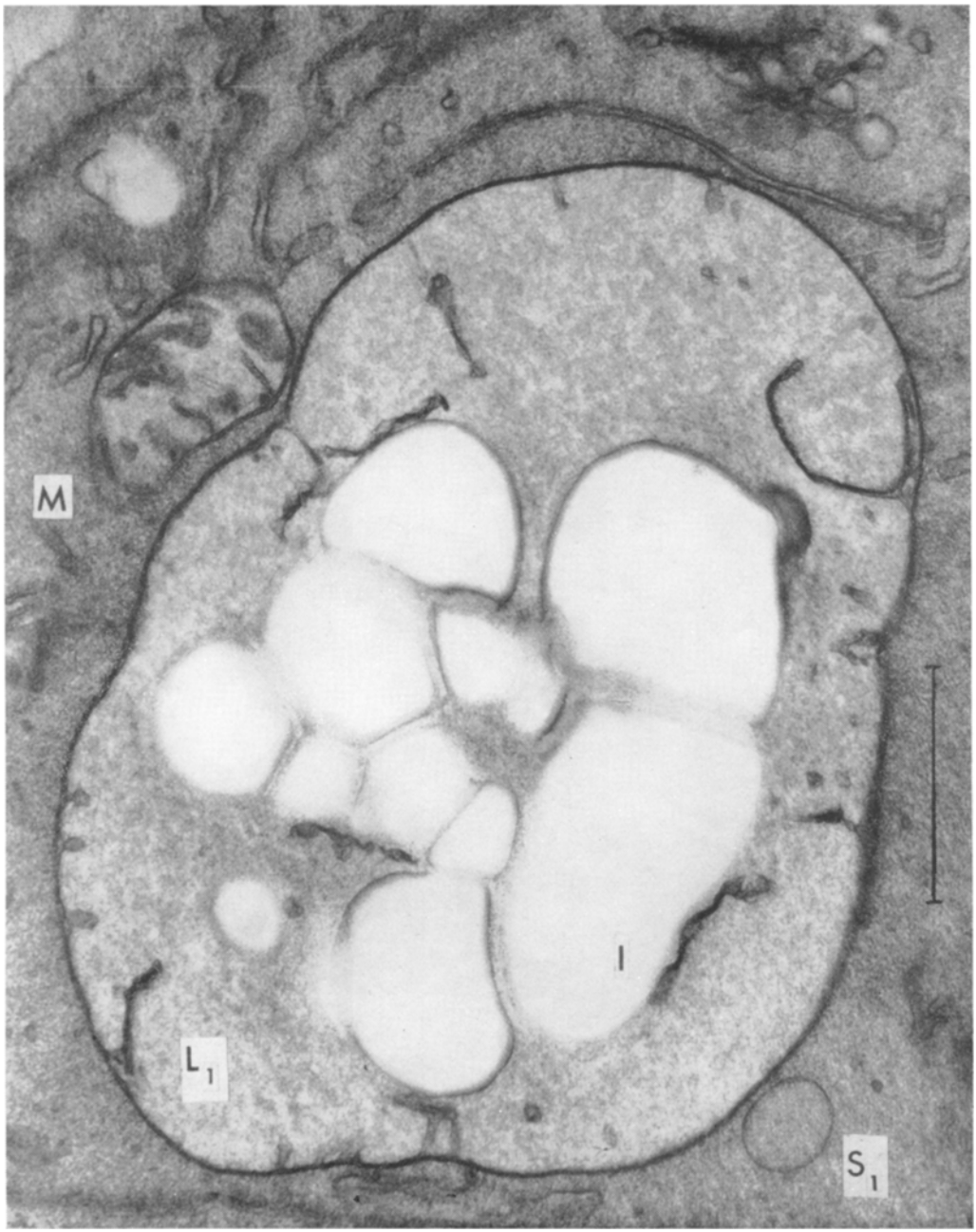


Fig. 2. Leucoplast (L_1) with well developed inclusions. Inclusions partially limited from matrix.

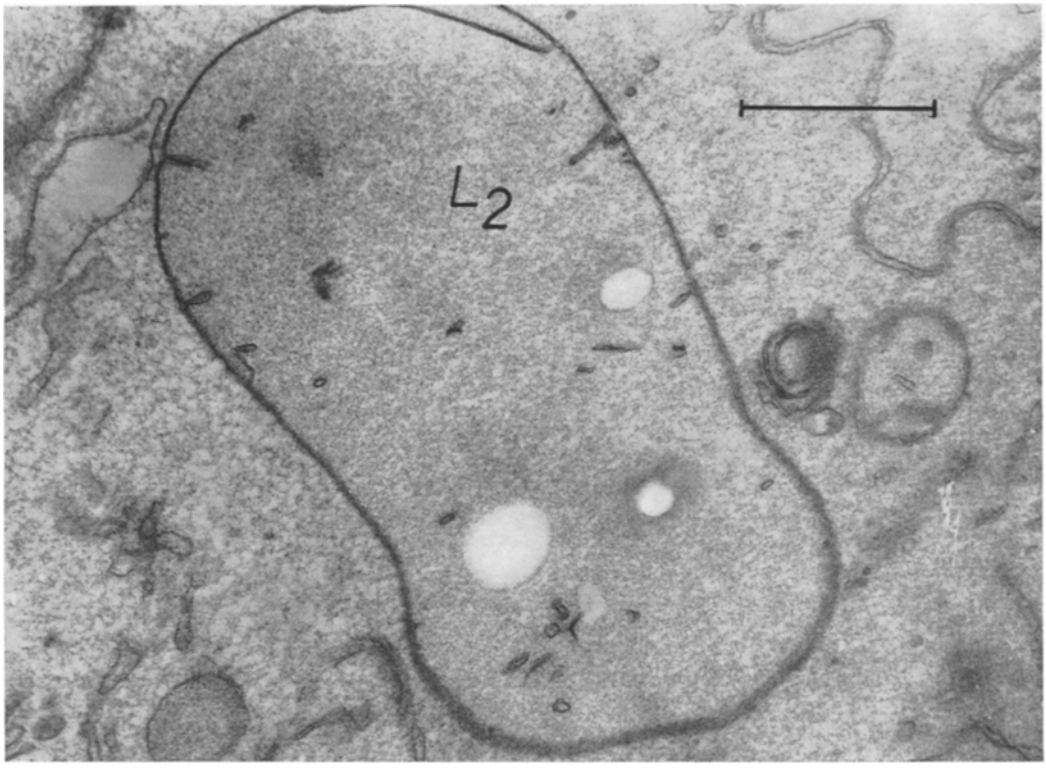


Fig. 3. Leucoplast (L_2) with underdeveloped inclusions.

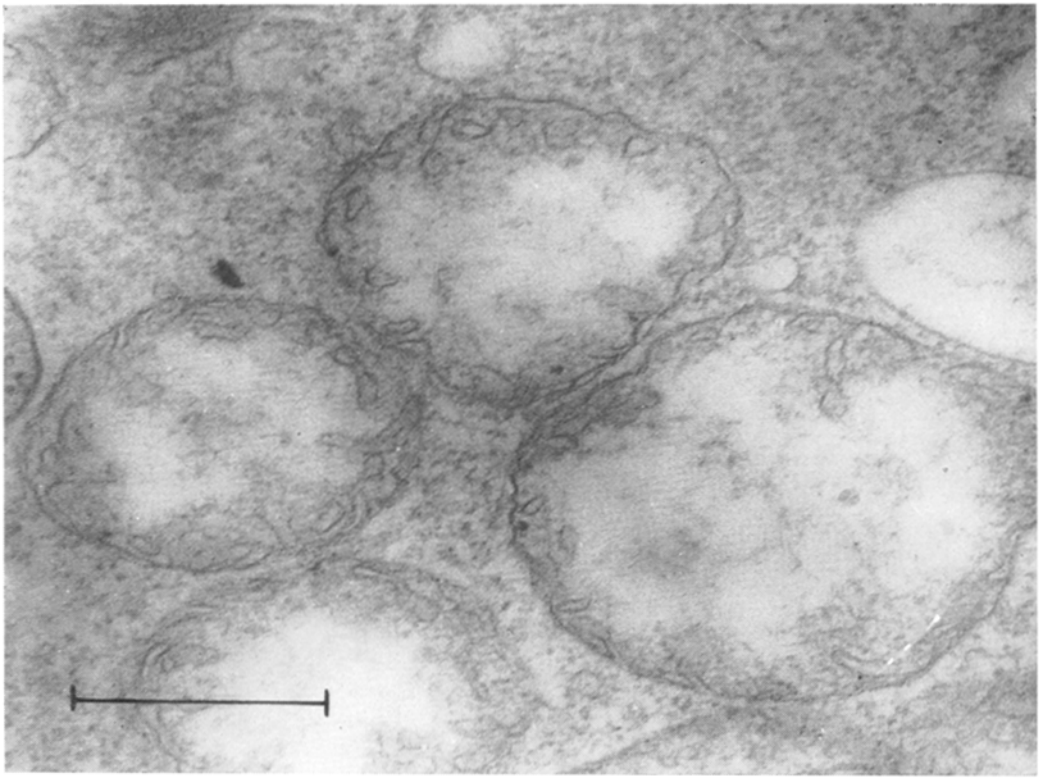
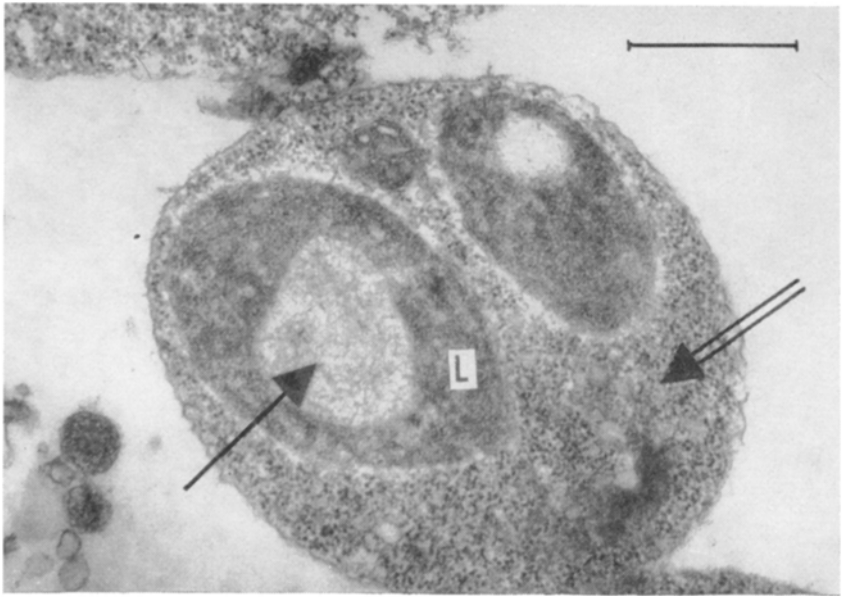


Fig. 4. OsO_4 fixation results in alteration of material so, that e.g. mitochondria are not distinguishable from proplastids.



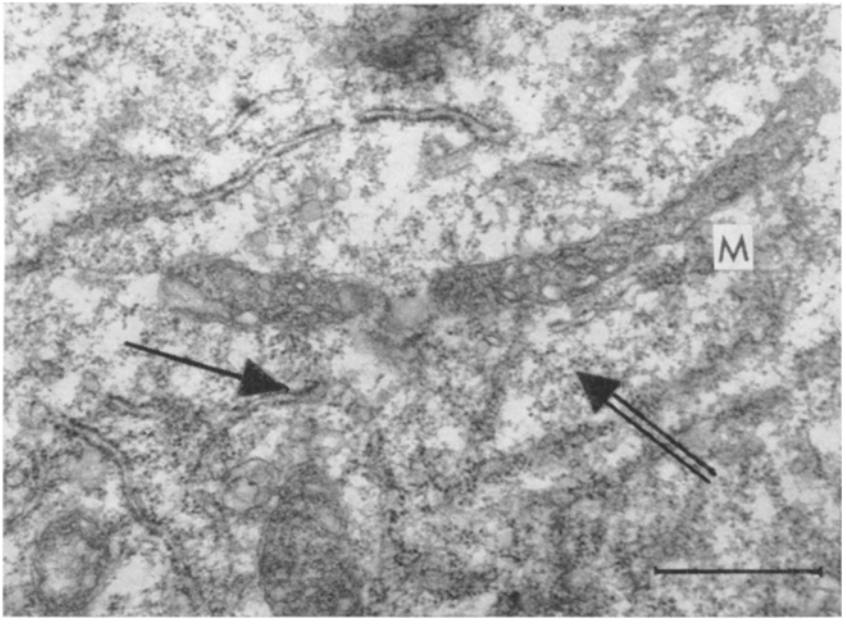


Fig. 5. Double fixation formol/OsO_4 is rather suitable. The preserved inclusion material is filamentous (↗). Note the abundance of free ribosomes in a transvacuolar strand (⇗).
Fig. 6. Ribosomes bound to the outer surface of endoplasmic reticulum (↗) and free ones (⇗).

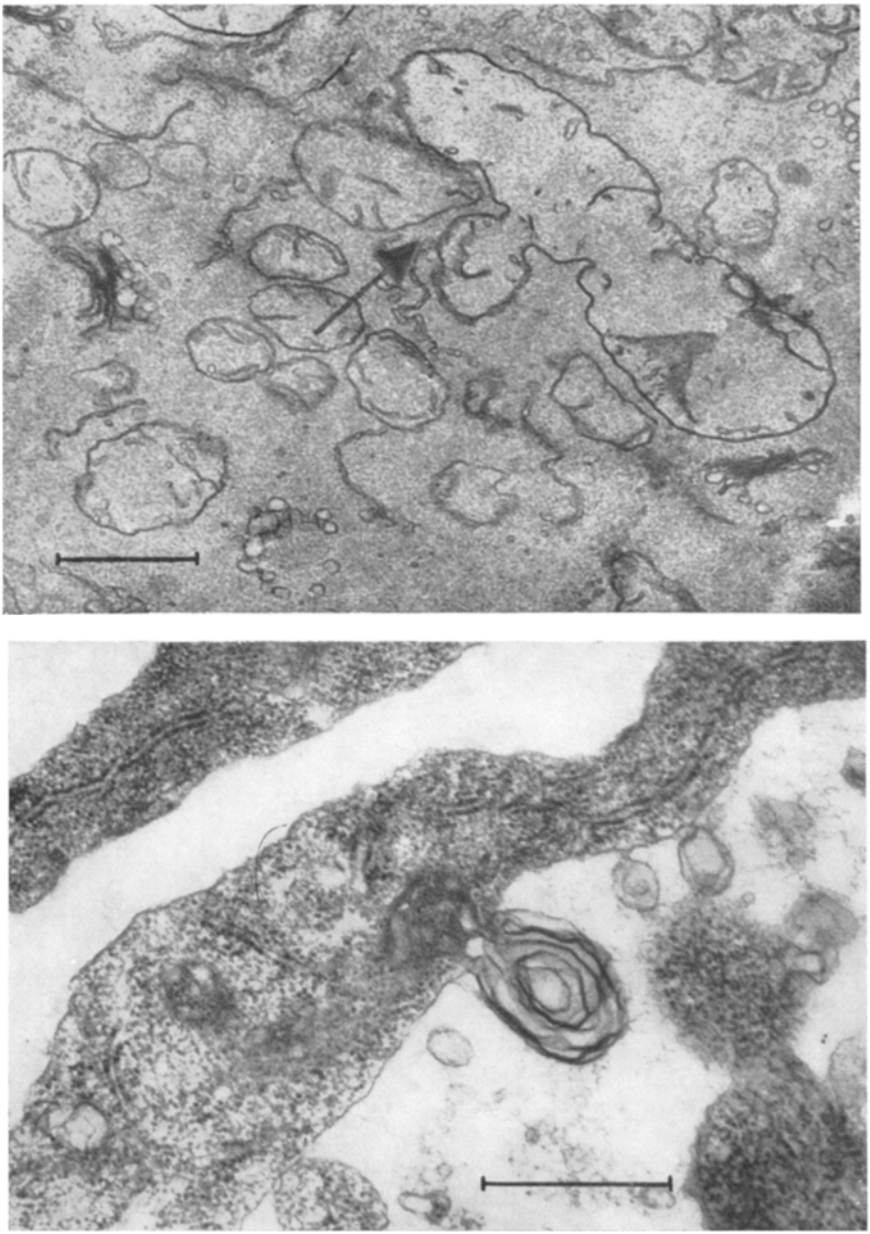


Fig. 7. Mitochondria or proplastids wedged by a protrusion into a neighbouring one (↗). The larger body is either budding or branched.
Fig. 8. Intravacuolar membraneous formation.

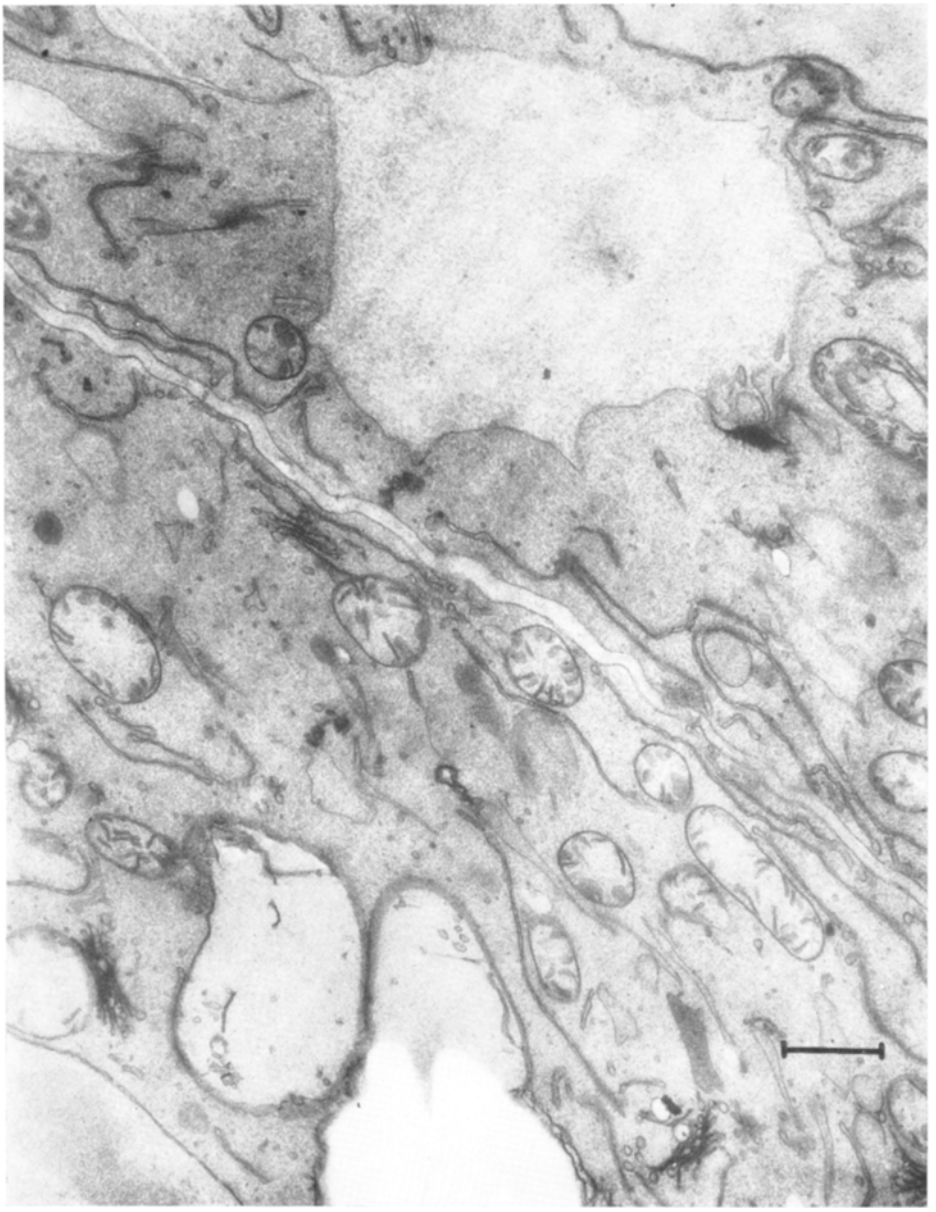


Fig. 9. Cytoplasm of 2 doughter (sister) cells. Note the various types of cytoplasmic bodies.

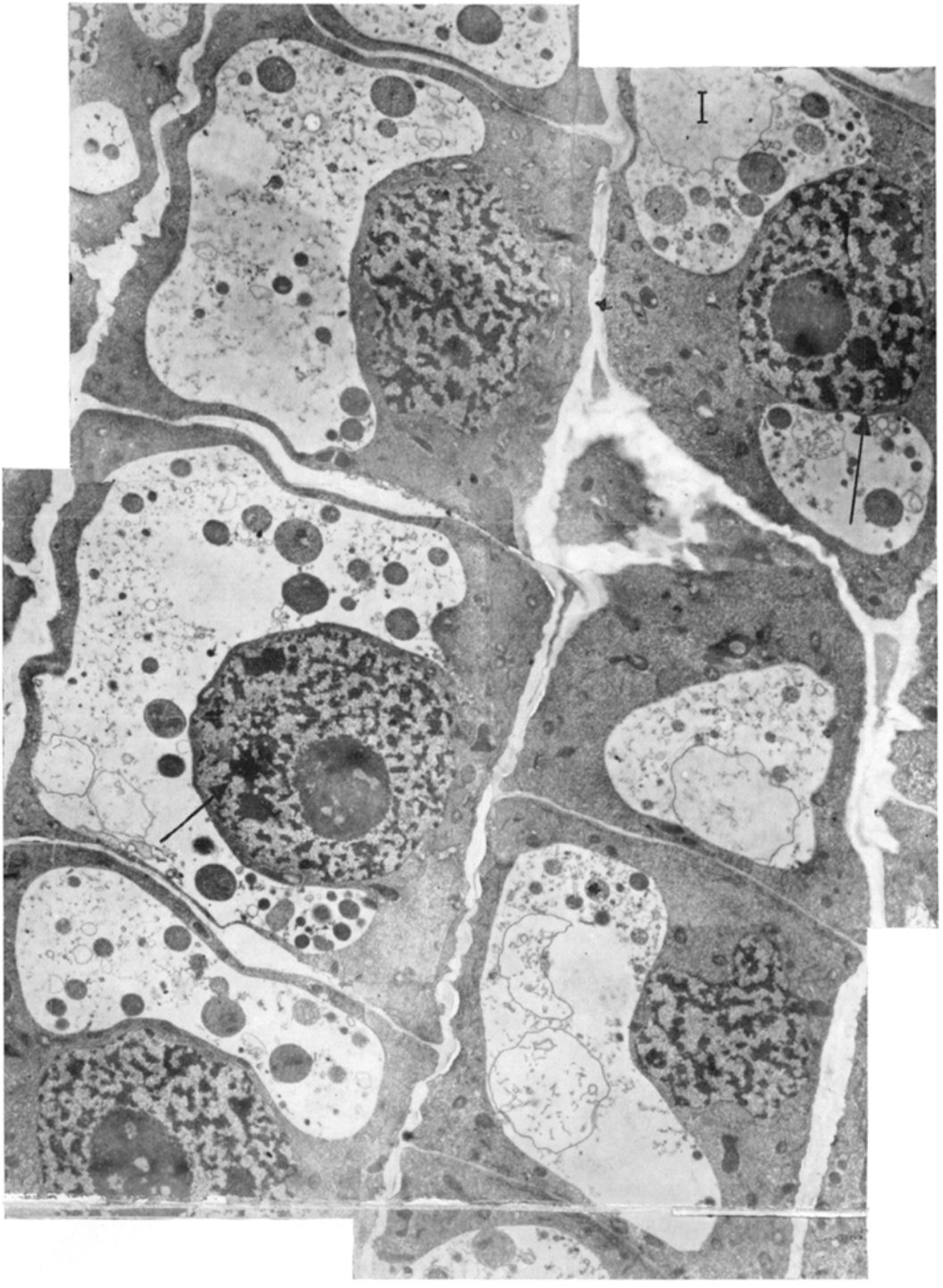


Fig. 10. Montage. Note the frequence of cytoplasmic bodies of different types within a cell. In vacuoles see transvacuolar cytoplasmic strands, membraneous formations and various intravacuolar material. In nuclei, the apparently heterochromatinic chromocentra are well recognizable (↗).

all types of cytoplasmic components within one cell. In addition, within the nuclei chromocentra are visible, apparently heterochromatinic (↗ on Fig. 10).

Discussion

It is apparently quite hopeless to continue discussion of the nature of plant cell cytoplasmic components only on the basis of their light microscopical morphology (see e.g. ZIRKLE 1937, WEIER 1938, NAHM 1940, NEWCOMER 1940, 1951, GUILLIERMOND 1941, DANGEARD 1947, WEIER and STOCKING 1952, PERNER 1953, MILOVIDOV 1962, 1964). Objective findings were often not distinguished from subjective interpretation in these controversies. We refrain from commenting on the relatively recent light microscope findings on broad bean root tip cells (CHAYEN 1952), because, among other reasons, the author did not try to start from the results of previous authors achieved by classical techniques. Only one aspect of light microscopic studies should be mentioned here: inequality or uneven relative frequency of cytoplasmic components in cells of different parts of the root tip. This aspect was stressed by BOWEN (1928) in his paper on osmiophillic platelets. Němec (see MILOVIDOV 1929) explained differences in the effect of a given fixative on various objects by the dissimilarity of intracellular *milieu*, e.g. pH. Inequality of intracellular *milieu* could be considered where there is suspicion of the differential effect of a fixative on cells from different parts of the same object (NĚMEC 1965, personal communication). On the other hand, one can also admit unequal diffusibility of individual components of the fixative during its penetration into the material. In this case there are at least quantitative diferences in the composition of fixative when affecting cells on the periphery and in the middle of the object (BAKER 1958). As far as the present findings are concerned, differences between cells from the cortex and those from the central cylinder, revealed by light microscope, have been caused by tissue differentiation, not by alteration during the treatment. Those cytoplasmic components, found in different regions of the root tip, are of the same appearance without any signs of alteration.

The first electronmicrographs of broad bean root tip cells taken in 1953 (CHAYEN and JACKSON 1957) are a matter of history in electron microscopy. The findings of PRESS (1956, 1957) will be thoroughly discussed further. HEITZ (1957) published, among others, electronmicrographs of cells of the broad bean root tip, distinguishing mitochondria and proplastids, the proplastids lacking *Primargranum*. On this point, he disagrees with the light microscope findings of BARTELS (1955). Both authors are of the same opinion concerning the unstable amoeboid shape of proplastids. The broad bean root tip cells were one of the first object in which dictyosomes were identified (PERNER 1958). In the same object DÖBEL (1964) observed groups of proplastids often wedged into their neighbour by a protrusion.

The electronmicroscopic findings, achieved during this study, correspond quite well to the results given above, concerning the electron microscopy of

the cells in the root tip of broad bean and also to the ultrastructure of similar objects in other species.

In the present work no peculiar, strikingly osmiophillic cytoplasmic components were revealed, which could be considered as osmiophillic platelets. For osmiophillic platelet identification, there is no other way but to take into account the common cytoplasmic components, especially spherosomes, plastids, vacuoles and dictyosomes. Formations that FREY-WYSSLING et al. (1963) regard as phases of developmental changes of spherosomes were observed on electronmicrographs taken during the present study. Particles with dense material on their periphery, which belong to these developmental series from active spherosomes to fat inclusions, could be identified as osmiophillic platelets. A similar view was not definitely rejected by PRESS (1957). Particles stainable with Sudan black B found in broad bean bean root tip cells by SCOTT (1955) and SERRA (1957/8, 1958) could also belong to this category of cytoplasmic bodies. Although the author (PRESS 1957) interpreted it otherwise, young leucoplasts were apparently described as osmiophillic platelets on his electronmicrographs, i.e. formations with a stainable periphery and the middle part filled with reserve material. Young vacuoles with dense material precipitated on their periphery could be regarded as osmiophillic platelets, too. It is known that vacuolar contents of broad bean root tip cells can reduce OsO_4 (BOWEN, see PRESS 1957, SCOTT 1929). Concerning the identity of vacuoles and osmiophillic platelets, PRESS (1957) takes into account broader consequences and his point of view is therefore less clear. Though PRESS (1956, 1957) is of a different mind, the possibility of identity of osmiophillic platelets and dictyosomes seems not to be finally exclused, based on OsO_4 reduction on the periphery of active bodies. The present paper does not give a basis for a certain or final identification or interpretation of osmiophillic platelets. It has been demonstrated with certainty that when the material was well treated using current electron microscope techniques, no peculiarities were revealed and therefore the osmiophillic platelets must be identical with some of the common cytoplasmic components. It follows that osmiophillic platelets are not *sui generis* formations as — apparently — PRESS (1956, 1957) proclaimed: his conclusions are not quite unequivocal in this respect. It is the opinion of the present authors that the osmiophillic platelets need not necessarily correspond to only one particular type of current cytoplasmic particles, but to more types of them. Up to now the possibility has been discussed here that osmiophillic platelets can be identified as some of the normal cell bodies. It is not impossible that Bowen examined more or less altered material. The present work brings no experimental findings supporting a more definite standpoint of this question. Nevertheless the suggestion that osmiophillic platelets are altered cell bodies of one type only, e.g. mitochondria, seems not to be well founded. In this case, as in many others, a more thoroughly elaborated plant cell pathology is urgently needed.

As far as the ultrastructure of leucoplasts is concerned, in the material fixed in $KMnO_4$ inclusion material is delimited from matrix, though mostly incompletely. It could not be claimed from our electronmicrographs whether the true membranes are involved or an accumulation of denser material on the boundary between the stroma and the inclusion, as suggested by BUT-

TROSE (1962). Therefore it is necessary to leave open the question of the origin of intraleucoplastidic inclusions, either free anywhere in the stroma, or by fusion of vesicles, originated by invagination of the inner unit membrane of the leucoplast limiting compound membrane, which are thus homologous to the thylacoids of chloroplasts (CAPORALI 1959, BUTTROSE 1960, BADENHUIZEN 1962). As seen on material fixed with $KMnO_4$, the leucoplasts in broad bean root tip cells belong to those with several inclusions. The presence of leucoplasts rich in reserve material and of leucoplasts poor in this respect — the latter seem to be less frequent — could correspond to CAPORALI's (1959) interpretation of two kinds of leucoplast development, one slower and one quicker.

No further peculiarities were observed that could be considered as relating to mass cytoplasmic localization of DNA. However, this question has not been studied in greater detail in this work.

All cytoplasmic components seen in our electronmicrographs are quite normal and it is not necessary to comment on them. Only the intravacuolar formations are worth mentioning. Besides tiny precipitates of vacuolar contents, membraneous formations were seen, which are either remains of already existing membranes, or artefacts originating 1. from such membranes; 2. completely de novo. Formations like these have already been described e.g. by SITTE (1958). In addition, transvacuolar cytoplasmic strands were often seen. The question of intrusions of cytoplasmic masses into the vacuole (see e.g. HRŠEL and JURÁKOVÁ 1964) deserves further attention, but it is necessary to be more sceptical when interpreting the electronmicrographs: care must be taken to distinguish between the nozzles of transvacuolar strands and formations corresponding to intrusion processes.

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Světelně- i elektronmikroskopicky byly sledovány cytoplasmatické složky buněk kořenové špičky koňského bobu *Vicia faba* L. Světelněmikroskopicky bylo zjištěno, že buňky jednotlivých partií kořenové špičky se liší. K elektronmikroskopickému studiu byly vybrány buňky z rozhraní mezi středním válcem a primární kůrou ve vzdálenosti 2 až 3 mm od špičky. Zvláštní pozornost byla věnována orientaci objektů při zalití. Elektronmikroskopicky nebyly zjištěny žádné zvláštnosti, které by bylo možno uvažovat v souvislosti s masovou cytoplasmatickou lokalisací DNK. Nebyly zjištěny žádné zvláštní, nápadným způsobem osmiofilní útvary, které by bylo možno jednoznačně identifikovat jako osmiofilní destičky. Osmiofilní destičky musí tedy být některé z běžných buněčných složek, snad více či méně alterované. Po fixaci materiálu $KMnO_4$, kdy jsou elektronogramy nejinstruktivnější, je v leukoplastech patrno několik nebo větší počet inklusí. Tyto inkluse jsou z části ohraničeny proti stromatu, není však jisté, zda se zde jedná o pravou membránu. Je diskutována povaha intravakuolárních membranosních útvarů.

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С помощью световой и электронной микроскопии исследовались цитоплазматические компоненты клеток верхушки корней конских бобов. При помощи светового микроскопа были установлены различия клеток отдельных частей корневой верхушки. Для электронмикроскопического исследования был выбран слой клеток, находящихся между центральным цилиндром и первичной корой на расстоянии от 2 до 3 мм от верхушки. При обработке объектов внимательно следили за их ориентировкой. Электронмикроскопически не было обнаружено никаких особенностей, которые бы было возможно рассматривать в связи с массовой цитоплазматической локализацией ДНК. Не обнаружено никаких образований, которые можно было бы идентифицировать как осмофильные пластинки. Осмофильными пластинками, повидимому, должны быть некоторые из обычных компонентов клетки, может быть более или менее измененные. После фиксации материала перманганатом калия, когда получаются наиболее отчетливые и содержательные электронограмы, в лейкопластах обнаруживаются включения, различающиеся по количеству. Эти включения по большей части отграничены от стромы, однако не ясно, имется ли у них мембрана в точном смысле слова. Обсуждается характер интравакуолярных мембранозных образований.