Notes on the Occurrence of Multinucleate Cells.

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With eight Figures in the Text.

THE vegetable cell is generally considered uninucleate. Exceptions to this rule are known either in the case of cells of unusual size, e. g. latex tubules and some algal cells, or cells of very special function, such as embryo sacs, pollen tubes, some jacket (4) and tapetal cells (3); and to these must be added certain cases of multinucleate cells occurring in individual plants, i. e. those recorded by Beer for grasses (2), Arber and McLean for several aquatics, (1) and (6). The purpose of the present communication is to record the occurrence of more than one nucleus, not in highly specialized cells, or those of a particular group of plants, but in different tissues of various immature vegetative organs. The details are given in the following table, which also shows that the plants studied are widely separated in habit, habitat, and systematic position :

Plant.	*Organ.*	*Tissue.*
Pteridium aquilinum .	petiole	ground tissue
Marattia sp.	,,	,,
Ophioglossum vulgatum .	sporangiophore	,,
Potamogeton crispus . .	vegetative branch	cortex
Sagittaria sagittifolia .	petiole	ground tissue
Sagittaria lancifolia . .	petiole	ground tissue
Hydrocharis Morsus-ranae	bud	cortex and epidermis
Avena sativa	coleoptile	ground tissue
Zizania aquatica . . .	coleoptile	,,
	mesocotyl	,,
	plumular leaves	mesophyll
Arum maculatum . .	petiole	ground tissue
Calla palustris . . .	,,	,,
Orontium aquaticum . .	inflorescence axis	,,
Carpinus Betulus . . .	hypocotyl	pith and cortex
Ostrya vulgaris	,,	,,
Corylus Avellana . . .	,,	,,
Fagus sylvatica . . .	,,	,,
Morus nigra	bud	,,
Polygonum aviculare .	inflorescence axis	,,
,, *Persicaria* .	,,	,,
,, *amphibium* .	,,	,,
	vegetative stem	,,
	petiole	cortex
,, *orientale* . .	cotyledonary node	pith

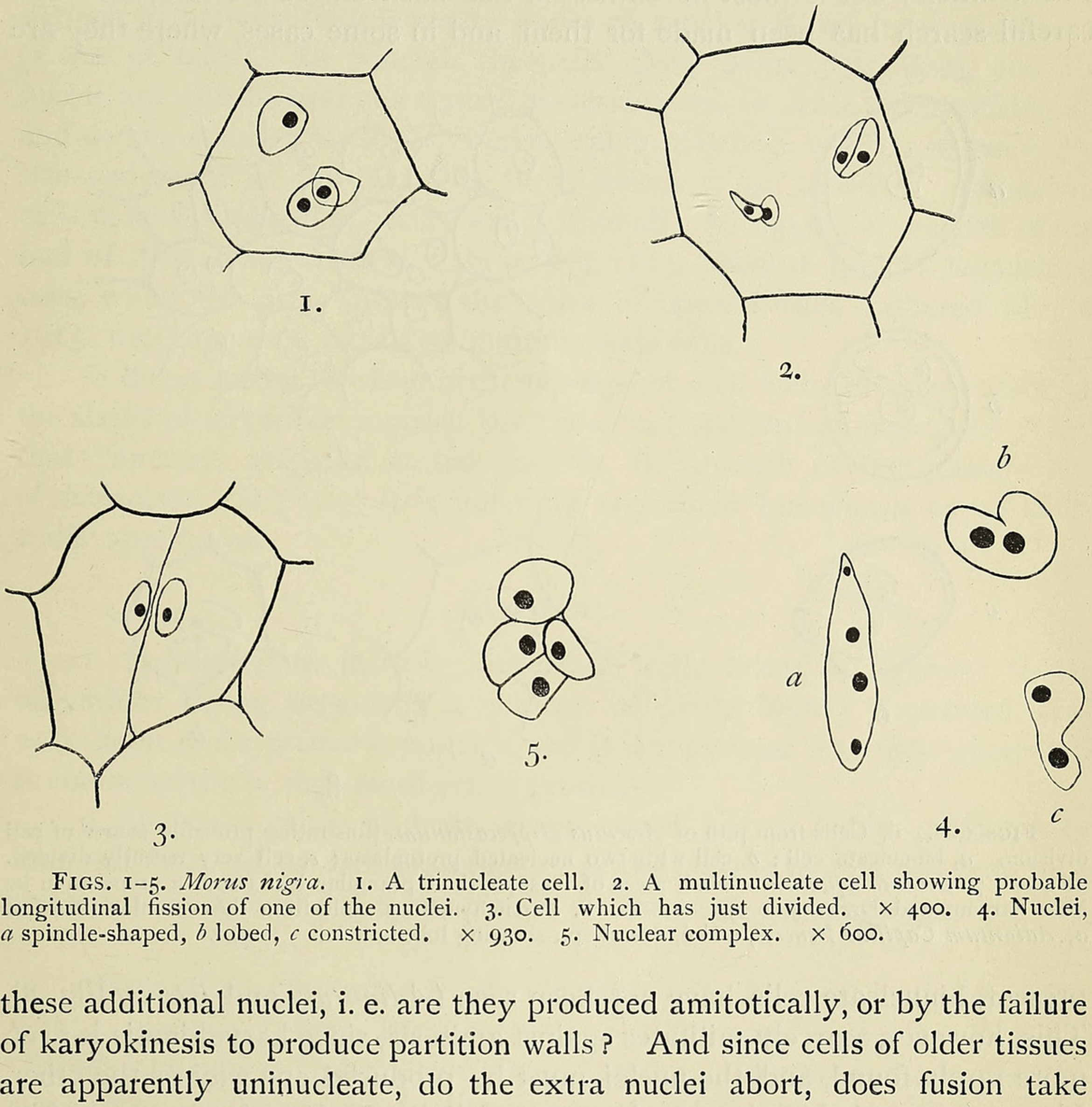
Plant.	*Organ.*	*Tissue.*
Nuphar sp.	peduncle	ground tissue
Anona sp.	cotyledonary node	cortex
Ribes sanguineum . . .	petiole	,,
	inflorescence axis	pith
Vicia Faba	seedling stem	pith and cortex
Linum usitatissimum .	plumule	pith
Acer Pseudo-Platanus .	petiole	ground tissue
Aesculus Hippocastanum	bud	pith and cortex
Tilia europaea	,,	,,
Hedera Helix	petiole	ground tissue
Hottonia palustris . .	bud	cortex
	inflorescence axis	,,
Fraxinus excelsior . .	,,	cortex and pith
Syringa vulgaris . . .	petiole	cortex
Limnanthemum peltatum	,,	,,
Cucurbita Pepo . . .	hypocotyl	,,
Helianthus annuus . .	,,	cortex and pith

In all the above instances the two nuclei were in the same focal plane, cases where they were not so being regarded as possibly due to the effect produced by the superposition of cells. It must, however, be remembered that binucleate cells may be unrecognized by the fact that only one of the nuclei is included in the plane of the section, and this consideration is of greater importance the higher the number of nuclei in the cell, for the less likely will they be all to occur in the same plane (cf. Fig. 7). Nevertheless, trinucleate cells have been recognized with practical certainty in several of the above, e. g. *Arum maculatum*, *Limnanthemum peltatum*, *Zizania aquatica*; and in *Morus nigra* (Fig. 1) they are obvious and frequent.

It is very probable that more than three nuclei occur in some cases, and in *Morus nigra* it would be difficult to say sometimes how many nuclei a particular cell did contain, for we find what can best perhaps be described as a nuclear complex (Fig. 5) where a nucleus is dividing into more than two daughter-nuclei, or, which amounts to much the same thing, where the products of the division of a nucleus are themselves dividing before separation.

Judging from the data at present available, the frequency of occurrence of multinucleate cells will be found to vary considerably in different plants. In some cases, such as that of *Ophioglossum vulgatum*, the occurrence is very likely rare and sporadic, but in most of the plants mentioned above a single section in the appropriate region will reveal several, perhaps many instances. The relative frequency of occurrence also varies in different tissues. In my experience, pith is far the most likely tissue, after this cortex, where they are more often found in the bundle or stelar-sheath—a point to which I attach some significance, and which will receive fuller treatment in another connexion. Binucleate cells are probably very rare in the epidermis, and I have not yet seen them in vascular tissue (see, however, Arber (1) and Thompson (7)). With regard to organs, opening buds have so far proved the most prolific in showing multinucleate cells; Angiospermic seedlings[1] are also good, and after these, inflorescence axes, petioles, &c.

The binucleate cells and their nuclei do not generally differ in size, shape, &c., from the other cells and nuclei of the tissues in which they occur, so that the usual explanation of coenocytic structures (Haberlandt (5)) cannot apply here. The question further arises as to the origin and fate of



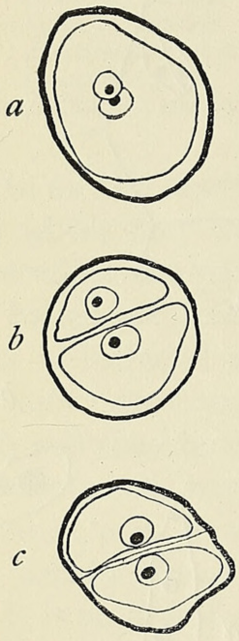
FIGS. 1–5. *Morus nigra.* 1. A trinucleate cell. 2. A multinucleate cell showing probable longitudinal fission of one of the nuclei. 3. Cell which has just divided. × 400. 4. Nuclei, *a* spindle-shaped, *b* lobed, *c* constricted. × 930. 5. Nuclear complex. × 600.

these additional nuclei, i. e. are they produced amitotically, or by the failure of karyokinesis to produce partition walls? And since cells of older tissues are apparently uninucleate, do the extra nuclei abort, does fusion take place, or are walls subsequently formed?

With reference to the first question, the second suggestion is possibly true in certain cases, but in general I think that the multinucleate cells of vegetative tissues are produced by amitosis. In the first place, this is strongly suggested by the position of the nuclei with regard to each other, for they are usually in close contact—a fact difficult to explain

[1] Dr. E. N. Thomas has kindly permitted me to examine a number of her preparations of seedling Gymnosperms, but I was not able to satisfy myself in a single instance of a binucleate cell.

if they are the result of karyokinesis. Stages in their separation from one another can easily be found, and appearances strongly suggestive of amitosis have occasionally been observed (Figs. 2, 4, *b* and *c*, and 8, *b*). The constriction of the nuclei described by Beer for grasses, the longitudinal fission for aquatics by McLean, and the lobing in *Stratiotes* by Arber have all been observed in plants far removed from aquatics in habit, and from grasses in classification; but it must be confessed that such stages are rare, although careful search has been made for them, and in some cases, where they are



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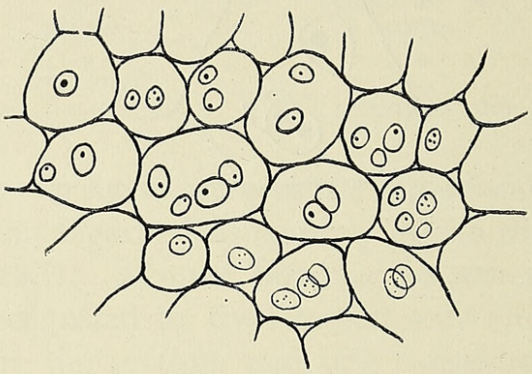

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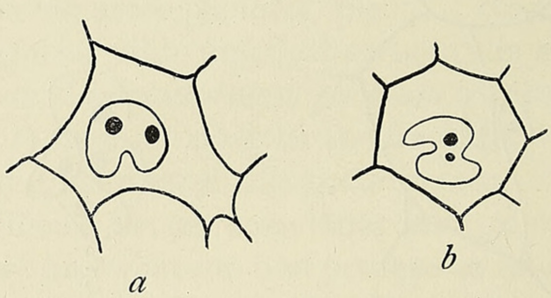

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FIGS. 6–8. 6. Cells from pith of *Aesculus Hippocastanum*, illustrating probable course of cell division. *a*, binucleate cell; *b*, cell with two nucleated protoplasts; *c*, cell very recently divided. × 400. 7. *Zizania aquatica*. Small piece of mesophyll of plumular leaf (from a preparation by Miss Sargant and Dr. A. Arber). × 600. 8. Parenchymatous cells from the vascular tissue of *a*, *Adiantum Capillus-Veneris*, and *b*, *Pteris* sp., showing lobed nuclei. × 930.

present, binucleate cells have not been seen (*Adiantum* and *Pteris*, Fig. 8). This, however, scarcely militates against amitosis, since karyokinesis is even more rarely found, and the nuclei must be produced one way or the other. If, as seems probable, the actual process of division takes place very quickly, or at a special time, the failure to find stages in amitosis at all frequently is easily explained, and further work is contemplated to throw light on this point.

With regard to the ultimate fate of multinucleate vegetative cells, we may surely dismiss the hypotheses of abortion of all but one nucleus, or fusion, since they are improbable on theoretic grounds, and no stage in either process has been observed.[1] Walls, then, must be formed, though evidently

[1] Contrast Carothers' (3) view as to the course of events in the multinucleate cells of *Ginkgo biloba*.

not immediately after direct division of the nuclei. In several different plants, which show binucleate cells, I have observed two nucleated protoplasts within one cell-wall (Fig. 6, *b*), and though this appearance might conceivably be due to artefact, comparison with other cells of the tissues in which they occur makes it seem probable that this apparent cleavage of the protoplasm represents the first stage of wall-formation.

Finally, it may be noted that multinucleate cells tend to occur in regions of activity (cotyledonary nodes of seedlings) and rapid elongation (stems of buds). In general, the cells show dense cytoplasm, and the nuclei are usually near the centre, possessing one or more large, refringent, and deeply staining nucleoli. Considerable plasticity is often shown in the size and shape of the nuclei in these tissues (Figs. 2 and 4). Binucleate cells in actual meristem occur rarely, if at all; for example, the pith of the bud of *Morus nigra*, fixed February 5, 1915, showed typical uninucleate cells, while the pith towards the apex in opened buds gathered May 9, 1915, was composed mostly of multinucleate cells.

If direct nuclear division with subsequent wall-formation does occur, as the above observations suggest, the 'heretical opinion' expressed by Arber that 'amitosis plays an active part in the growth of the young roots of *Stratiotes*' ((1), p. 374) is not only confirmed, but shown to be of far wider application.

Summary.

1. Multinucleate (usually binucleate) cells occur in different tissues of various young organs in a number of plants widely separated both as to habit and systematic position, and it is suggested that their occurrence is characteristic of regions of active growth.

2. In some cases, at least, these nuclei are probably produced by amitosis followed by wall-formation, and the view is maintained that these processes are a means of tissue-formation in rapidly growing organs.

Many of the preparations used in the above were made in connexion with a research I have been carrying out for some years in Professor Oliver's laboratory, University College, London. Microtome series through some grass seedlings were kindly lent me by Miss Sargant and Dr. Agnes Arber, and some other preparations by Dr. E. N. Thomas and several members of her Department at Bedford College, to all of whom I tender my thanks. I am also grateful to Dr. Agnes Arber for the interest she has taken in my work, and to my assistant, Miss G. E. M. Piper, B.Sc., for her sympathetic help.

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LITERATURE CITED.

1. ARBER, A. ('14): On Root Development in *Stratiotes aloides*, with special reference to the Occurrence of Amitosis in an Embryonic Tissue. Proc. Cambr. Phil. Soc., vol. xvii, Pt. 5.
2. BEER, R. ('99): On the Multinuclear Cells of some Grasses. Nat. Science, Dec., 1899.
3. CAROTHERS, I. E. ('07): Development of Ovule and Female Gametophyte in *Ginkgo biloba*. Bot. Gaz., 43, p. 116.
4. CHAMBERLAIN, C. J. ('06): The Ovule and Female Gametophyte of *Dioon*. Bot. Gaz., 42, p. 336.
5. HABERLANDT ('14): The Physiological Anatomy of Plants. Eng. translation.
6. McLEAN, R. C. ('14): Amitosis in the Parenchyma of Water Plants. Proc. Cambr. Phil. Soc., vol. xvii, Pt. 5.
7. THOMPSON, W. P. ('12): The Anatomy and Relationships of the Gnetales. I. The genus *Ephedra*. Ann. of Bot., vol. xxix, p. 1090.

Since the above was written I have discovered multinucleate cells in the pith, just above the nodes, of *Polygonum Persicaria*, in older parts of the stem, where these cells are rare or absent in the internodes. Most, if not all, the cells are at least binucleate, and some show as many as five nuclei in a single plane. This is of interest since it affords a striking instance of multinucleate cells existing in a specially active tissue capable of rapid growth, for it is just at the nodes that geotropic curvatures take place in this plant.