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MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

MEETING OF THE SECTION FOR PLANT PHYSIOLOGY ON JUNE 1, 1973

C. KOLLÖFFEL (*Botanisch Laboratorium, Utrecht*)

Subcellular distribution and activity of some urea cycle enzymes from the cotyledons of broad beans during maturation and germination

The storage proteins of *Vicia faba* are characterized by a high proportion of arginine (BOULTER & DAVIS 1968). The biosynthesis of arginine in plant tissues occurs by a pathway similar to the Krebs-Henseleit cycle of mammalian tissues (REINBOTHE & MOTHES 1962). During germination the storage proteins are hydrolyzed and the arginine released is metabolized mainly in situ (JONES & BOULTER 1968). The present study deals with the subcellular distribution and the activity of two enzymes of this Krebs-Henseleit cycle from the cotyledons of broad beans during maturation and germination. One of the enzymes – ornithine carbamyltransferase – is concerned with the synthesis of arginine whereas arginase catalyses the breakdown of arginine in urea and ornithine.

Extracts were prepared from cotyledons of broad beans germinated for 2 or 3 days. The extracts were fractionated by differential centrifugation. The fractions containing tightly coupled mitochondria showed hardly any ornithine carbamyltransferase activity but a very high arginase activity. The mitochondrial fraction was further fractionated on a sucrose gradient. The distribution of the arginase activity closely followed that of the succinate dehydrogenase activity. The arginase activity of the mitochondrial fraction was enhanced by freezing and thawing it by treatment with Triton X-100 or by exposure to an osmotic shock. These experiments clearly indicate that the arginase activity from broad bean cotyledons is located mainly in the mitochondria whereas ornithine carbamyltransferase activity is located outside the mitochondria. A quite different localization is found, however, in mammalian tissues and in *Neurospora*. Here, the arginase activity is located in the cytosol and ornithine carbamyltransferase activity in the mitochondria.

During maturation the ornithine carbamyltransferase activity from the cotyledons sharply decreased. It declined further during subsequent germination. Extracts from cotyledons of developing seeds show arginase activity. The activity decreased to about one third of its maximal activity during maturation but increased over 10-fold during germination. Thus, the activity of the enzyme concerned with arginine synthesis is high in developing cotyledons when the system is directed to arginine synthesis, conversely its activity is low in cotyledons of germinating seeds, which are actively degrading arginine. On the other hand, the maximum arginase activity from cotyledons of germinating seeds is much higher than that from developing seeds.

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J. ROMBACH (*Afdeling Plantenfysiologisch Onderzoek, Landbouwhogeschool, Wageningen*)

Growth stimulation by cytokinins, thiamine and phytochrome in Lemna minor L. in darkness and in light

Axenic cultures of *Lemna minor L.* were grown on a medium with sugars and amino acids. Multiplication rate, meristematic growth, and rate of frond expansion were increased by light. At intensities of continuous illumination above 1000 erg/cm^2 sec the growth rate increased in proportion to the photosynthesis. At low light intensities the maximum effectivity of the light was in the red. Carbohydrate assimilation from the medium contributed to dry matter accumulation per unit of frond area by a fixed amount, independent of light intensity but dependent on the sugar concentration and cytokinin content to the medium.

Thiamine was required for the continuation of growth in continuous darkness. An illumination of two minutes every 48 hours was sufficient to make growth independent of external thiamine supply.

Kinetin 3×10^{-6} M and 6-benzyl-amino-purine 1×10^{-6} M increased the rates of cell division, frond expansion, and frond multiplication. Proportionally the stimulation by cytokinin was the same in darkness, in darkness interrupted by brief illuminations, and in low-intensity light. With continuous illumination at light intensities above 100 erg/cm^2 sec the effect of kinetin decreased as the light intensity increased.

Growth rate was also stimulated by periodically-repeated brief illuminations. A treatment with far red radiation reduced the effect of the preceding illumination. The part of the light effect that could be reduced by far red is assumed to be due to the action of phytochrome P_{FR} during the dark intervals between the illuminations. The part impossible to reduce by far red given directly after the light treatment is named the "irreversible part of the light effect".

When the dark periods between the illuminations were shortened the irreversible part of the light effect increased. This increase was reinforced by cytokinins.

A light period once a day equal to the sum of brief illuminations per day had practically no effect on the irreversible part of the light effect.

The irreversible part of the light effect probably results from interaction during the illumination between the photoreceptive pigment and a substrate that is replaced during the dark intervals. It may be that cytokinins promote the replacement of the substrate.

MEETING OF THE SECTION FOR VEGETATION RESEARCH ON OCTOBER 16, 1973

D. M. PEGTEL (*Biologisch Centrum, Afdeling Plantenoecologie, Rijksuniversiteit Groningen, Haren*)

Effect of crop rotation on the distribution of two ecotypes of Sonchus arvensis L. in The Netherlands

Sonchus arvensis, a geophyte with superficially spreading thickened roots, has a world-wide distribution. The species occurs especially on arable land and is considered a troublesome weed.

In The Netherlands *Sonchus arvensis* is not restricted to arable fields but is also found in coastal dune areas. Inland and coastal individuals differ in leaf morphology. Sowing and transplantation experiments carried out under different environmental conditions proved the observed morphological differences to be genetically determined. Individuals from the two habitats may therefore be regarded as ecotypes. Taxonomically, the inland or arable type is referred to as *Sonchus arvensis L. var. arvensis* and the dune or coastal type as *Sonchus arvensis L. var. maritimus G. F. W. Mey.*

The fact that the coastal type has never been found inland raises the question how this is brought about. In other words, what is the cause why this type in not adapted to the inland (arable) habitat. It is likely that farming practices, associated with cropping, have caused a selection. The analogous problem related to the absence of the arable type in dune areas will not be discussed here.

Germination tests have shown that the coastal type can establish itself from achenes on arable land. However, in regularly cultivated fields few or no seedlings of the arable type have. been found. Those occurring were limited to ditch sites, to low-lying places and to field edges. Starting from such places, cultivated parts of the fields may be invaded through vegetative propagation (sprouting of root buds).

Cultivation practices entail ploughing and the removal of shoots by either hoeing or spraying with herbicides. Weeds are adapted to such activities by means of generative and/or vegetative mechanisms. The question arises what the mechanisms are that make perennials become weedy species.

Subterranean parts, intact or fragmentated should have the capacity to sprout rapidly and extensively. Moreover, net assimilation rates must be high in order to restore losses of food reserves and to compete with crop growth. Thus distribution patterns of assimilates and growth-forms may play an important role.

With this in mind the inland absence of the coastal type was investigated. Three experiments were carried out to test the two ecotypes in their response to:

1) removal of shoots;
2) fragmentation of thickened roots;
3) cropping (oat and potatoes) with relevant agriculture practices.

The first experiment was intended to trace the effects of shoot removal upon dry matter content of thickened roots. Dry matter contents (dry weight/fresh weight × 100) were determined at intervals of three weeks. If shoots were not removed contents of the arable type were 6% higher than those of the coastal one throughout the year. For *variety arvensis* values ranged from 18% in early spring to 28% in the late summer. Corresponding values for *variety maritimus* were 12 and 22%, respectively.

Shoot removal affected both ecotypes in the same way; when applied frequently at intervals of three weeks dry matter contents of the thickened roots dropped. Rates of exhaustion are nearly the same. Since the initial dry matter content of the arable type is higher, shoot removal is withstood better. Regrowth of the arable type was faster than of the coastal type. It showed more and larger leaves resulting in a greater shoot production. The speed of regrowth may be determined by dry matter content of the thickened roots, particularly during the spring and

early summer. In the late summer and autumn regrowth is not correla ted with dry matter content. Possibly due to a day length effect, growth is inhibited and finally stopped.

Contents of mono-, di- and polysaccharides (inulin) were also found to fluctuate. Polysaccharides and dry matter contents show a high positive correlation. No such correlation was found for mono- and disaccharides. Maximum values of the latter were found during winter and minima during spring and summer. The sprouting of root buds in spring may be preceded by maximum values of mono- and disaccharides. During winter dissimilation causes a slight decrease in total water soluble carbohydrates (mono-, di- and polysaccharides combined).

When corrected for total water soluble carbohydrates macro-nutrient contents of the thickened roots were the same for both ecotypes. The same holds not for crude-protein contents (N organic) during the months May to August when thickened root growth is most pronounced. During this period the arable type requires more nitrogen for the formation of thickened roots. For the remainder of the year no such negative correlation with total water soluble carbohydrates was found. This applies to both ecotypes.

In the second experiment, carried out in a greenhouse during spring and autumn, the formation of new plants from thickened root fragments of various weights was studied. In spring the arable type regenerated faster and more abundantly than the coastal type. It produced more dry weight of shoots per root fragment. However, in autumn regeneration is about the same for both ecotypes. Near to all fragments formed shoots. The weight of newly produced shoots was higher for the coastal type.

The highest production rates were found in spring. This finding also points to inhibited regrowth in autumn. In autumn root buds are predormant. During winter these buds become afterripened by low temperatures. True dormancy was not observed. Therefore, predormancy is followed directly by postdormancy.

The third experiment, perpetuated four years, revealed that after three years of cultivation nearly all coastal-type individuals had disappeared. Neither the physiognomy of the crop (oat or potatoes) nor the surface coverage had any effect. This indicates that the coastal type is excluded from the inland habitat by mechanical disturbances only. The arable type was affected by crop height and density but did not disappear.

From the experiments described it was concluded that the inland absence of the coastal type is caused by agricultural practices and not affected by the crop as such. Cultivation results in the fragmentation of thickened roots. Following frequent removal of shoots, thickened roots of the coastal type are exhausted sooner than those of the arable type.

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MEETING OF THE SECTION FOR PLANT MORPHOLOGY AND ANATOMY ON OCTOBER 26, 1973

W. A. VAN HEEL (*Rijksherbarium, Leiden*)

The ovules of Adansonia digitata (Bombacaceae)

The African Baobab has a large ovary which is one-celled and contains many septs projecting inwards. The ovules are inserted along the lateral margins of these septs. They are carried by 3 to 4 times dichotomously forked funicles forming small bunches. In young stages they show circinnation. Thus, isotomy in funicles can be added to the well known isotomies in the filaments of Malvales. A similar phenomenon is known in Cactaceae, possibly also in Orchidaceae. A tentative review of dichotomies in vascular plants is presented. It is thought that isotomy of the funicles is a means toward increase of the number of ovules (seeds). Here this is effectuated by a potency which the ovules – very ancient organs as they are – can still possess, namely dichotomous forking.

A. D. J. MEEUSE (*Hugo de Vries Laboratorium, Amsterdam*)

Phylogenetic origin and homology of laminiform floral parts

In terms of the "classical" phytomorphology, the sterile laminiform parts of the, by postulation, uniaxial flower are (1) of basically one kind only, (2) equivalent to (= homologous with) all other floral members, and (3) considered to be "appendicular" or "phyllomic". Theoretically, in this train of thought, trophophylls (= vegetative, *i.e.*, assimilatory phyllomes), cataphylls, bracts, bracteoles, sepals, petals, stamens and carpels pass into one another, which idea is supposed to be supported by a number of cases in which "transitions" (morphological intermediates) occur between organs belonging to at least two successive categories of this sequence. There are several "exceptions" to the "rules" emanating from the theory of the uniaxial flower, such as the incidence of opposition of whorls instead of alternation (*e.g.*, obdiplostemony), the arrangement of androecial members often associated with centrifugal stamen maturation, and certain incongruous developmental and anatomical features. Such "aberrations" necessitated the introduction of, sometimes rather involved and dubious, ancillary hypotheses (*e.g.* a secondary multiplication or "splitting" = chorisis, and tangential shifts of primordia during floral ontogenesis) to make the theory fit in with the actual floral organisation and to resolve some of the apparent discrepancies. It is, therefore, not surprising that sooner or later the incongruity of the classical theory and the factual floral architecture resulted in more or less fundamentally different views concerning the nature and the possible evolutionary history of the flower and of its appendages.

About 1900 ČELAKOVSKY revived some older views and accepted an origin of the petals by the secondary modification of stamens into optical lures, *i.e.*, into laminiform intrafloral semaphylls. His interpretation implies that there are *two* categories of laminiform floral parts, *viz.*, *direct* derivatives of vegetative phyllomes (the sepaliferous, perigonous, or tepaliferous elements already present in a primitive flower type), and *secondarily* modified "microsporophylls" (= stamens), *i.e.*, semaphylls of androecial derivation. This hypothesis was never categorically rejected by contemporary phytomorphologists and was revived and emended by, *e.g.*, MATTFELD in 1938, and by HIEPKO in 1965. The concept of the uniaxial flower was not rejected by such workers, however, and all floral appendages were still interpreted as basically homologous, phyllomic organs. One conclusion stands: certain categories of perianth members are semophyletically older than the petaloid floral parts of androecial derivation. This implies unequivocally that, initially, the Flowering Plants and, therefore, their immediate progenitors were "apetalous", and also, apetaly being associated with anemophily, that their flowers can in the beginning not have been pollinated by insects guided by optical signals

(semaphylls). The oldest flower type must, accordingly, have been anemophilous and, hence, also unisexual (diclinous) rather than bisexual (monoclinous). With few exceptions, botanists did not recognise, let alone accept, the consequence of a secondary origin of petaliferous floral parts (*i.e.*, the absence of coloured semaphylls in early angiospermous forms), which does not tally with the conventional ideas regarding primitive Flowering Plants and early flower types (LEPPIK) presupposing an ancestral group of ranalean affinity with monoclinous and showy (phaneranthous, *i.e.*, petaliferous!) flowers pollinated by beetles.

In the anthocorm theory as emended by the present author, the hemi-angiospermous precursors and the early representatives of the Flowering Plants are supposed to have been aphananthous (non-showy, "apetalous") and (at least predominantly) diclinous. In some evolutionary lines leading to mainly (and primarily!) aphananthous and anemophilous recent groups of Angiosperms this morphological and anthecological syndrome was maintained. In lines leading to primarily phaneranthous and zoophilous groups the advent of semaphylls changed the floral region, in its primitive form a multiaxial and unisexual anthocorm devoid of intrafloral petaloid organs but already tending towards ambisexuality, into a showy and insect-pollinated flower. As far as the semaphylls and the stamens are concerned, this process set in with a modification and a divergent evolution of the primitive laminiform, polliniferous organs, the primitive monandra. A monandron differentiated into a group of stamens with filaments and an associated laminiform organ (a *potential* semaphyll), but in exceptional cases (*Nymphaeaceae, Magnoliales* s.s.) the whole monandron remained laminiform and retained some of its sessile androsynangia (= anthers) eventually to become semaphyllous by the total reduction of its pollen sacs. Intrafloral semaphylls derived from monandra are consequently not the homologues of the basically infertile, bracteoid perianth members of the anthocorm contributing, *e.g.*, sepaloid elements (if not obsolete and totally reduced), so that aphananthy ("apetaly") must have preceded phaneranthy. (It can be said in passing that early entomophily was most probably not dependent on semaphylly, and that the more typically zoophilous flowers and their specialised anthophilous pollinators evolved by a process of co-evolution.) This extension of the anthocorm hypothesis not only readily explains a number of traditionally "incongruous" and "aberrant" morphological and anatomical conditions, but also contributes substantially to an understanding of the phylogenetic origin of the Angiosperms from gymnospermous ancestral forms with, inevitably, aphananthous, diclinous and originally anemophilous anthocormoids. Details will be published or have been published elsewhere.

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O. C. DE VOS (*Biologisch Centrum, Afdeling Plantensystematiek, Rijksuniversiteit Groningen, Haren*)

The floral vascular supply in Oenothera

Regarding the interpretation of the inferior ovary and that of the hypanthium of the Onagraceae, contradictory theories have been formulated. For instance, the study of the vascular supply in the flower of *Oenothera* and of other Onagraceae by BONNER (1948) and BAEHNI & BONNER (1949) resulted in their statement that the ovary and the hypanthium had their origin in a concrescence of floral whorls. On the other hand, BUNNIGER & WEBERLING (1968), MAYR (1969), and PANKOW (1966), studying the histogenesis of the flower, concluded that the

tip of the flower apex became invaginated during development. They stated that the outer ovary wall and hypanthium were formed by an axial cup.

To reveal the reason of this contradiction, a renewed study was made of the vascular supply in the flower of *Oenothera biennis* and of its development. About 50 flowers and buds of successive ages were examined. Most of them were serially sectioned. A few of them were bleached and stained. The study resulted in new facts which led to a view on the vascular supply of petals and stamens different from that of BAEHNI & BONNER.

The vascular pattern in the rim of the hypanthium, on which sepals, petals, and the two whorls of stamens are attached, appeared to resemble very closely the vascular pattern of four axial nodes. When superficially observed these four nodes form a ring of vascular tissue, the internodes being very much shortened. The whole system has been bent inward and backward by the invagination of the apex.

These and other facts support the view that the peripheral parts of the ovary and the hypanthium are of axial origin. This view concurs with the results of studies on the histogenesis of the flower.

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C. VAN DER MEULEN-BRUIJNS (*Biologisch Centrum, Afdeling Plantensystematiek, Rijksuniversiteit Groningen, Haren*)

Vascularisation of the ovary of Dorotheanthus bellidiformis (Burm. f.) R. Br.

Dorotheanthus (family Aizoaceae or Mesembryanthemaceae, sub-family Ruschioideae) has a plurilocular inferior ovary with basal to pseudoparietal placentation. On typological grounds the Ruschioideae are assumed to be derived from the Aptenioideae, with a semi-inferior ovary and axile placentation. This assumption is based, e.g., on the fact that in the Ruschioideae as a result of changes in growth pattern a shifting of placentation takes place from axile to basal or parietal. According to the classical theory, placentation in the ovary of *Dorotheanthus bellidiformis* must be interpreted as axile. As placentation is descriptively parietal, it is often correctly called pseudo-parietal.

With this in mind it is interesting to study the vascularisation pattern in the ovary of *Dorotheanthus bellidiformis*, is comparison with an axillary placentation type of, e.g., *Aptenia*.

The vascularisation in the ovary of *Dorotheanthus bellidiformis* was studied on bleached material with stained lignified elements, and on series of microtome-sections. It could be demonstrated, that the ventral vascular bundles are situated in the septal radii, as in the Aptenioideae. In *Aptenia* the ventral bundles are found in the central column, while in *Dorotheanthus bellidiformis*, as a result of the shifting of the placenta, the ventral bundles are found also in the bottom and in the wall of the ovary. On the level of the hypanthium rim two fused ventrals descend in the ovary wall to the central column, separating placental bundles to the adjacent loculi. (The placenta is very large, covering nearly the whole inner surface of the loculus.)

These observations are not in accordance with the results of IHLENFELDT (1961). In this author's view the ventral bundles are found in the central column and, as a result of the shifting of the placenta in the Ruschioideae, these bundles are also found in the middle of the bottom and of the wall of a loculus.

The difference in interpretation of Ihlenfeldt and the present author is that the vascular bundles which Ihlenfeldt interpreted as ventral bundles, are in this study proved to be placental ones, ascending from the ventral bundles in the septal radii.

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M. M. A. SASSEN and M. KROH (*Botanisch Laboratorium, Nijmegen*)

The "cell wall" around the generative cell

Electronmicroscopical studies have taken away all doubts concerning the existence of a generative "cell". During the differentiation into a pollen grain the nucleus of the microspore divides, giving rise to a generative and a vegetative nucleus. Similar to other cell divisions two plasma membranes are formed between the two nuclei. Via a complicated process by which the two membranes turn around the generative nucleus, the generative cell becomes completely surrounded by the cytoplasm of the vegetative cell. We can, therefore, speak in this case of a cell within a cell. (SASSEN 1964). Of the two plasma membranes surrounding the generative cell the internal one belongs to the generative, the external one to the vegetative cell. The question arises whether a cell wall is present between the two plasma membranes. As a working definition for the concept of a cell wall we propose: the cell wall consists of an excretion product of the cell accumulating outside the plasma membranes and consisting mainly of carbohydrates. The formation of the "cell wall" around the generative cell corresponds with the cell wall formation following the division of other cells (ANGOLD 1968, BURGESS 1970, HESLOP-HARRISON 1968). After cell division golgi vesicles accumulate in the equatorial plane, fuse and form the plasma membranes of the two daughter cells. This process starts in the centre and continues centrifugally. The contents of the golgi vesicles fills the space between the two membranes. DIERS (1963) and SASSEN (1964) assumed the electrontransparent space between the two plasma membranes to be a true cell wall without adducing many arguments to support this assumption. DEXHEIMER (1965) and MARUYAMA et al. (1965) assumed the presence of pectin between the two plasma membranes. Their assumption is supported by HORVAT (1969) and ROLAND (1971) on the basis of their cytochemical studies. Roland concluded from these studies that in addition cellulose in the form of microfibrils is present in the cell wall. However, this last statement appears to be very improbable, in particular since the direction of the fibrils is thought to be perpendicular to the cell wall; this is in contrast to microfibril orientation in all other known cell walls. In a number of publications since 1967 GORSKA-BRYLASS demonstrated, by means of fluorescence microscopy in pollen of several plant species, the presence of callose as cell wall material between the two plasma membranes during a short period of time, from the formation of the cell plate up to the detachment of the generative cell from the pollen wall. This sheath of callose is assumed to serve the role of isolating the generative cell by means of a substance with selective permeability that would permit the differentiation of the generative cell independent from the vegetative cytoplasm. After formation of the generative cell the callose wall is assumed to break down.

To check whether a wall is present around the generative cell in mature pollen grains we isolated generative cells from pollen of *Petunia*, *Lilium*, and *Tradescantia*. These isolated cells were studied by means of ultrathin sections and by freeze-etch replicas. The isolated generative cells appeared to be surrounded only by one plasma membrane. Neither in sections nor in freeze-etch replicas cell wall material including microfibrils was found outside this plasma membrane. Also in freeze-etch preparations of intact pollen grains no microfibrils were visible between the plasma membranes of the generative cell. The space between these membranes appeared to be smaller than in thin sections of chemically fixed pollen. In the latter case the larger electron transparent space around the generative cell is possibly an artefact.

We may conclude that in an early stage the generative cell is surrounded by a cell wall containing callose. This cell wall material, however, is broken down. It appears unlikely from

our studies that in a later stage other wall material is laid down. Therefore, one could use the concept of a cell wall around the generative cell only with respect to the first stage.

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P. D. BURGGRAAF (*Botanisch Laboratorium, Leiden*)

Cell divisions in the cambial zone of Fraxinus excelsior L. and a model for cambial activity

Serial radial and transverse sections were cut from samples from the cambial zone of young stems, which were collected at different times during the growth season. Due to careful handling of the material, as described by BURGGRAAF (1973), the cambial zone was very well preserved, as was shown, for instance, by Stereoscan pictures of thick transverse sections. The relatively undisturbed condition of the cambial zone in the sections made it possible to number individual cells according to their position relative to the youngest differentiating phloem cell.

Mitotic figures and cell-division figures were recorded and the respective frequencies were calculated for the cells with identical numbers. Graphs relating these frequencies to the numbered position of the cells within the radial rows, showed several peaks, particularly in material sampled at the height of the growth season.

Cell divisions were present in a large zone, up to cell no. 21. Major peaks were found for cells no. 6 and no. 10: both with 15 to 17% dividing cells. Minor peaks were found for cells no. 0 (= youngest phloem cell) with 5% dividing and for cells no. 16 to 18, with 7% dividing. All these data pertain to material sampled at June 16, 1970.

A model was proposed for cell-division sequences and cell production in the cambial zone. The model was, amongst other assumptions, based on assumed values for the relative rates of cell division and of cell growth, for relative duration of phases of the division cycle, and on the assumption of the occurrence of a uniseriate layer of initials, as originally proposed by SANIO (1873).

Cell-division percentages, calculated for the different cell numbers in this model, gave a multi-maxima graph, which was very similar to the graphs resulting from the counts in the samples.

In the model the numbered cells which showed the input values for the relative duration of

the cell-division phases, expressed as the ratio of their respective frequencies, are the initials. In the graphs of the material such a ratio of nuclear divisions to cell divisions is present in cells no. 4 or 5, which, according to the model, suggests that these cells are the initials, although the absence of special cytological characteristics did not allow the recognition of the initials as such.

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