

The concept of the quiescent centre and how it found support from work with X-rays. I. Historical perspectives

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Received on March 13, 2015; Accepted on July 20, 2015

Abstract: Within the tip of roots meristems of angiosperms and gymnosperms there is a small group of cells known as the quiescent centre (QC). The concept of the QC was developed 60 years ago by FAL Clowes, working in the Botany School, Oxford University, UK. To celebrate the Jubilee of the QC, a brief outline of the work that led to its demonstration by autoradiography was presented by Dubrovsky and Barlow (2015). The present article traces Clowes's subsequent experimental studies of the QC, especially with regard to how X-irradiation became an important tool for elucidating the properties and significance of the QC for root development. Also reviewed are some of the consequences that subsequently arose from this work with radiation, in particular the concerns over the use of radioisotopes in attempts to describe the kinetics of cell proliferation in the root meristem.

Keywords: autoradiography, cell cycle, quiescent centre (QC), radioisotopes, root meristem, X-rays

Overview of 'quiescent' zones in root and shoot apices

In the long history of ideas relating to the anatomy, architecture and cytophysiology of root apices (Clowes 1961a), the proposal that there should be a 'quiescent centre' close to the tip of the root meristem must rate as one of the most audacious and, as it turns out, important. It should be remarked straight-away that the adjective 'quiescent' is referring here to the low degree of proliferative activity postulated for cells within this zone, and is contrasting this property with the higher levels of activity expressed by the surrounding cells of the proximal meristem (the meristematic region which provides cells for the body of the root proximal to the cap) and of the distal meristem, or calyptrogen, which supplies cells to the

root cap. The quiescent centre (QC) is sandwiched between these two meristematic zones with hardly any indication of its presence except for the small size of its cells and their low degree of cytoplasmic staining, which indicate its relative inactivity.

It is true that some of the earliest observations on root apices, for example, those of Bohumil Němec (1897), rarely showed cells and nuclei at the pole of the root proper which were in the act of mitosis or cytokinesis. Moreover, this type of observation was amplified by Georges Mangenot (1942) in his study of the mitotic divisions seen in longitudinal sections of *Allium cepa* roots which had been grown in the presence of the stathmokinetic agent, colchicine. He noted the continuing presence of diploid (2x) mitoses and interphase nuclei at the apex of roots treated for up to 192 h with 0.05% colchicine solution, whereas polyploid nuclei (4x, 8x, 16x) populated the remainder of the meristem. Mangenot remarks that, even after 300 h in colchicine "*les noyaux des ces cellules apical sont presque tous de dimensions normales; le rythme mitotique reste toujours assez lent dans cette region pour que la tetraploïdie n'y sont réalisée que dans un nombre des noyaux relativement faible*" [nearly all the nuclei of these cells at the apex have normal dimensions; the mitotic rhythm seems always very slow in this region because the number of tetraploid nuclei formed is relatively small] (Mangenot 1942, p. 71). In other words, in the presence of colchicine the cell cycle could be as long as 12 d in this zone, and that, evidently, divisions at the root apex occur at about one quarter the rate of those in the major portion of the apex.

It took a longer time before shoot apices were examined in a comparable way, with a view to describing the regional distribution of their cell divisions. Instead, attention was being given to structural studies of shoots and to discussions concerning the tunica-corpora theory and the internal zonation patterns of apices. That shoot apices

transform from a vegetative state to a flowering state was an additional diversion. However, in the 1950s, Roger Buvat proposed an integrated view of the shoot apex, suggesting that there was a sub-apical ‘*anneau initial*’ (initiating ring) active in cell proliferation and geared to metamer production, and a region above, which was held to be a ‘*méristème d’attente*’ (waiting meristem) (Buvat 1955). Cells in this ‘waiting meristem’ were thought not to divide during the time when the apex was in its vegetative state, but did so later, when the apex switched to forming flowers. The question was whether the *méristème d’attente* was in any way equivalent to the QC of roots (Clowes 1961a).

In the 1950s, Clowes, like many other anatomists, seemed resistant to the idea of a summit zone where divisions were either absent or infrequent. He proceeded to show that feeding shoot tips with ¹⁴C-adenine and then preparing autoradiographs of sectioned apices, the methods which he had used earlier to demonstrate a QC in roots, did not support the view of Buvat: *Coleus* shoots fed for 8 days (!) with ¹⁴C-adenine showed labelled nuclei at their summits (Clowes 1959a). However, in reviewing apical meristems in general, Clowes (1961a) appears to soften his former tone and concedes that the apical zone could have a low rate of proliferation. On the French side, there was a reciprocal concession, namely, that in its vegetative state, mitoses could occur within the *méristème d’attente* of the shoot apex (see Clowes 1961a, p. 69; and see also Rolinson (1976) for a very brief summary of the position some years later, after further research on shoot apex cell proliferation). It should also be noted that Arlette Nougarede (1965), writing of ‘les réticences anglo-saxonnes’ (Anglo-saxon hesitations), paid tribute to Clowes for his recognition of the work of the French cytologists of Buvat’s school in the following way: “*Clowes admette ultérieurement que les analyses cytologiques de Buvat et de son école aient contribué à mieux connaître l’organisation zonale des apex*” [Clowes later admits that the cytological analyses of Buvat and his school have contributed to a better knowledge of apical zone organization]. Nougarede was no less generous to Clowes when she made him a gift of her publication (Nougarede 1965), with the inscription, “*À Mr FAL Clowes, en l’assurant de ma vive estime pour ses remarquables travaux sur l’apex radiculaire*” [To Mr FAL Clowes, assuring him of my lively esteem for his remarkable work on root apices].

The concept of the quiescent centre

To orient the reader towards the question which we wish to address, namely, how and why Clowes turned

to X-rays to explore the fundamental nature and significance of the QC, we shall briefly summarise the main points of a short article (Dubrovsky and Barlow 2015) recounting the origins of the QC concept, and then go on to describe how the existence of the QC was experimentally validated through the use of X-irradiation of roots. Images of a number of people associated with Clowes’s work in Oxford, as well as of Clowes himself, are to be seen in Fig. 1.

Clowes’s first two publications deal with roots of beech (*Fagus sylvatica*) and their mycorrhiza (Clowes 1950, 1951). Mycorrhiza and plant nutrition were topics of interest to JL Harley who, in 1938, had been appointed Demonstrator in the Botany School, Oxford University, where Clowes was then an undergraduate. After the years of World War II, Harley returned to the Botany School in 1945. And likewise, in 1946, after a spell in the army, Clowes joined Harley as his research student. The topic selected was the study of the anatomy of beech roots, both mycorrhizal and non-mycorrhizal. But in 1947, Clowes took up an appointment at the Department of Botany, University of Manchester, headed by Prof Eric Ashby. With Ashby’s consent, Clowes was able to continue his thesis work on the beech-root system. In the spring of 1949, the thesis was completed (Clowes 1949), and in August of that year Clowes moved back to Oxford to become Demonstrator in the Botany School.

Although Clowes entertained the “hope to be able to design experiments which will both test the hypotheses [relating to the causes of the morphological changes at the beech-root apex due to the mycorrhizal association] and also elucidate some of the aspects of general causal morphology in roots” (Clowes 1951, p. 14), it was this latter avenue of causal morphology – of non-mycorrhizal roots – that Clowes continued to follow for the rest of his career. Apart from some long-term studies of mycorrhizal root growth performed in 1953 (see Harley 1959, p. 110 and plate 7) and a further publication on the mycorrhizal root cap (Clowes 1954a), 30 years were to elapse before Clowes again investigated mycorrhizal apices of *Fagus*, examining their capacity for cell proliferation (Clowes 1981).

In the course of his anatomical work with *Fagus* root apices, Clowes (1950, p. 251) noticed a “conspicuous lack of density of the meristematic cells of the plerome and periblem [which] is not due to the vacuolation of the cells, but to the fact that the cytoplasm itself stains less heavily than in the meristematic cells farther from the promeristem”. This state of affairs was found in both non-mycorrhizal tap roots and in smaller mycorrhizas. The staining procedure used a sequence of haematoxylin, safranin and crystal violet, which gave good



Fig. 1. Senior members of the School of Botany, Oxford University, in 1958, during the years of Clowes's work on the quiescent centre. Shown here are some of his colleagues mentioned in the text who, directly or indirectly, contributed to his work. Front Row: Extreme left: J.L. Harley; third from the left: C.D. Darlington; extreme right: F.A.L. Clowes. Middle row: Fourth from the right: D. Davidson.

definition not only of cell walls but also of nuclei and nucleoli (Clowes 1949). The weakly stained group of cells at the root apex was considered as a 'cytogenetic centre', or as an 'ontogenetic centre' (Clowes, 1950) or, later, as a cytogenetic centre' (Clowes, 1953, 1954b), all these terms being eventually superseded by 'quiescent centre' (Clowes 1956a), as documented by Dubrovsky and Barlow (2015).

Comparative staining intensity of different groups of cells in a root apex would not alone count as sufficient evidence for proposing a 'cytogenetic' or 'ontogenetic' centre; the implication of using such a term is great: that all (or nearly all) cells of the root are ultimately derived from such a centre, or cellular zone. Therefore, a more precise and logical analysis was necessary in order to give the proposition of an ontogenetic centre credibility. From such an analysis it should be possible to deduce that the periclinal and anticlinal divisions needed to construct the cell files of the root apex should originate from this centre. It was only through knowledge of the positioning of these divisions that the architecture of the root could become comprehensible; and this knowledge would also indicate how the divisions of the cells of the ontogenetic centre were coordinated in space and time.

The analytical method used by Clowes was that developed by Otto Schüëpp (1917) for his analysis of root construction in terms of cell lineages. Schüëpp's

method of outlining the cell files seen in median longitudinal section, and then of establishing the sites of periclinal division from the position of 'T' wall junctions within the continuity of the files, could establish where in the apex each of the files had been developed. In theory, the method could also indicate something of the relative rates of division in the small group of cells which putatively co-located within the ontogenetic centre. When this method was applied to the beech roots (Clowes 1950), it was found that the ontogenetic centre was indeed the focus of the various cell files of plerome, periblem, and cap columella. Then, making the assumption that cells did not slide over one another (an assumption that becomes explicit only in Clowes's review of 1959b), it is evident that, for the cellular architecture of the root apex to be maintained, cells within the ontogenetic centre must grow and divide only slowly, or maybe not at all, whereas cells of the surrounding proximal and distal portions of the meristem, which are not subject to such constraint upon their rates of growth and division, could grow faster. The ontogenetic centre was thus deduced to be a structural necessity for the development and stability of the root apex and, hence, of root growth in general.

Without any preliminary remarks, Clowes, in the opening sentence of a paper in 1956, suggests "that there is a quiescent centre in the meristems where cells divide rarely or never". In this paper (Clowes

1956a), he is describing work done earlier (Clowes 1954b) on grass root apices, notably *Zea mays*, where Schüepf's architectural analysis had been applied and where conclusions had been obtained similar to those from *Fagus* roots concerning an ontogenetic centre. With respect to *Zea* roots, Clowes (1954b, p. 114) remarks that, "The cells at the pole of the periblem-dermatogen complex enclosed within the initials, play little or no part in the production of new cells in mature roots where the constructional pattern is preserved". Because of this feature, the zone in question is proliferatively 'quiescent'. Furthermore, continuance of the quiescent property is coupled with a preservation of cellular pattern. The converse of this principle would be where cellular pattern is not preserved; and indeed an alteration of pattern was found after small surgical cuts had been made into the apices of both *Fagus* and *Zea* and the roots had been allowed to regenerate new tissue (Clowes 1953, 1954b). As a result of surgical intervention, evidence was found, again on the basis of cell-file analysis, that the quiescent cells at the pole of the periblem (i.e., files of the epidermis and cortex) had been stimulated to divide, and by so doing these cells in a polar position had thereby brought about the regeneration of the excised portion of root apex.

X-rays and root structure and development

Inevitably, there must have been uncertainty as to exactly which cells were extirpated by surgical intervention at the root tip (Clowes 1953). Moreover, the extent of the damage and the means of its repair could only be inferred retrospectively, from anatomical sections of subsequently re-grown tissue. Also, it was necessary to have some measure, or estimate, of the rates of cell production both before and after surgery. And perhaps surgery, after all, was not the most appropriate means of answering questions about the role of the QC in maintaining root structure: surgery may be suitable to probe the organisation of the more complex shoot apex, but for the apparently more simple, yet more closely organised root apex, a more nuanced means of examining meristem organisation and maintenance was required. Incidentally, surgical operations on apices were the speciality of two of Clowes's colleagues in these early years: at Manchester, in the 1947, the year in which Clowes arrived there from Oxford, Claude Wardlaw began to publish the first of a series of papers describing the results of surgery performed on the large shoot apices of ferns (Wardlaw 1947); and in Oxford, Robin Snow, Fellow of Magdalen College, of which Clowes was also a member (and Snow must surely be regarded as one of the last 'gentleman' exponents of botanical research (see Clapham 1970)), was a strong propo-

nent of surgical methods to study growth processes (some of the experimental surgical work of both Wardlaw and Snow is summarised by both Clowes (1961a) and Sachs (1991)).

It was at this juncture, with the appointment in 1953 of Cyril Darlington as Sherardian Professor of Botany at Oxford University, that the idea of utilizing X-rays as a means of probing the properties of the QC began to take shape. In the mid-1940s, while still at the John Innes Institute at Merton Park in south-west London, Darlington and his assistant, Len La Cour, had investigated patterns of X-ray-induced chromosome breakage in roots and other tissues of a number of species possessing large chromosomes, including *Vicia faba* (Darlington and La Cour 1946). Moreover, during the 1940s and '50s radioisotopes were being employed as tracers of the movement of mineral ions, as well as markers of synthetic processes, such as those relating to nucleic acids and, hence, of chromosome structure. I shall describe first the X-ray data gathered by Clowes and others and then pass to a discussion of his utilization of radioisotopes.

Soon after Darlington's arrival in Oxford, Douglas Davidson entered the Botany School as a post-graduate doctoral student of Darlington. His thesis work continued the investigation of X-ray-induced chromosome breakage, using roots of *Vicia faba* as his main material (Davidson 1956). Davidson completed his thesis work in 1956 but, on receiving a fellowship from the Agricultural Research Council, he continued to work in the Botany School until 1958, when he departed for Oak Ridge National Laboratory, Tennessee, USA, where he remained for two years before returning to UK. The Botany School had acquired a Newton Victor X-ray machine, and with this apparatus Clowes and Davidson performed their separate researches on X-irradiation and roots, Clowes studying the reorganisation of *Vicia* and *Zea* root meristems after irradiation, Davidson (1960) investigating the fate of those cells within *Vicia* roots whose chromosome complements had been altered by X-rays, a study which was an early form of clonal analysis and cell-fate mapping. In this respect, Davidson's work was similar to earlier work of Robert T Brumfield (1943) who, by X-rays, had induced sectorial chimeras in roots of *Crepis capillaris*: in one of these cases, cells with a particular aberrant chromosome complement occupied a minor sector of a recovering root, cells with the normal complement occupied the remaining major sector. From this single observation, Brumfield deduced that there was a minimum of three initial cells from which the root was constructed. This was the very problem – the number of root initial cells – which Clowes had been trying to

address through his surgical experiments using *Fagus*, *Vicia*, and *Zea* (Clowes 1953, 1954b). Davidson, now at Oak Ridge, was likewise attempting to estimate initial-cell number in the apical meristem. Like Brumfield, he was approaching this question through X-ray-induced chromosome aberrations but was using *Vicia* roots, which are much broader than those of *Crepis*. He estimated that, after X-irradiation, a root could re-grow from approx 30-50 cells (Davidson 1960, 1961). Later, Hall et al. (1962a), at the Churchill Hospital, Oxford, published a dose response curve for the reproductive integrity of *Vicia* meristem cells from which it could be extrapolated that between 20 and 98 cells (the different values depended on what was considered to be the lethal dose of X-rays for this material, for which two different values had been published) survived out of a total of 2×10^5 meristem cells and, hence, were potentially available to restore root growth. Hall's estimate of survivor cells is similar to the number of root initials estimated by Davidson (1960, 1961).

Both Davidson (1959) and Clowes (1959b) noted that irradiated *Vicia* roots (which are more sensitive to X-rays than *Zea* roots) occasionally formed two or three new meristems close to the tip. By the time these new roots emerged the original irradiated root had ceased to grow and the former meristem had become disorganised. Remarkably, Davidson (1959) discovered a case where each root tip of a pair of new roots arising from the tip of an irradiated apex contained cells bearing the same chromosome aberration. This was a reciprocal translocation between a nucleolar M chromosome and a non-nucleolar S chromosome; the respective chromosome complement was considered to be the product of a single irradiation event. The pair of roots was sampled 9 d after the irradiation event. From one root of the pair 27 mitotic figures contained the reciprocal translocation, in the other root 39 such mitotic figures were found. The fact that both roots contained the same aberrant chromosome complement suggested that they had a common origin, from a single irradiated cell within the original apex. This cell was probably located in the QC before the irradiation event and the subsequent splitting of the apex. The pair of roots revealed another important feature: the two clones of cells with the chromosome translocation yielded a total of $(39 + 27) = 66$ mitoses in 9 d of growth. This indicated that the mother cell of this clone had undergone 7 chromosome-doubling cycles and division cycles: that is, approx. one cycle per 1.3 d, or 31 h. According to Davidson (1959), the first mitoses in recovering roots occurred 2 d after irradiation, which would mean that the mentioned interdivisional time was actually shorter, about every 24 h. This rate of division was similar to that found in

normal, unirradiated *Vicia* roots. The root which had revealed 27 aberrant mitotic figures had evidently undergone one cycle less. Cells of irradiated root meristems generally failed to divide for at least 10 d after the irradiation event; only then did the roots slowly begin to re-grow, using the few surviving cells, mentioned above, for recovery. The cells from which the pair of roots had descended within a space of 2 d had presumably occupied some position in the root tip which was somehow protected from the effect of X-rays.

Autoradiography and the defining of the quiescent centre

The time was now right to undertake more detailed work on cell division in the QC and to form an understanding of the QC's contribution to the re-growth or replacement of the irradiated apex. The circumstances were fortunate because a few years earlier Stephen Pelc, at the Medical Research Council's Radiotherapeutic Research Unit, London, UK, and George A. Boyd at Arizona State College, Tempe, AZ, USA, were both, independently, experimenting with photographic emulsions applied to microscope slides to detect 'autoradiographs' of biological materials which had been fed with isotopically labelled ^{32}P -phosphate and ^{14}C -adenine (Pelc 1947, Boyd and Williams 1948). Whereas Boyd was interested in animal material (though he even applied autoradiography to meteorites of which he had a large collection!), Pelc was keen to use bean roots (they were used in his Research Unit as a model system for radiotherapy research) and study how they incorporated these radioactively labelled precursors into their cells and nuclei (Howard and Pelc 1951a). Already, at the macroscopic level, Scott Russell, in the Agriculture Department of Oxford University, was using autoradiography to study the movement of ^{32}P through barley seedlings (Russell and Martin 1953) by pressing their radioactively labelled leaves and stems against X-ray film and recording the resulting silver grains deposited in the emulsion in response to the β -particle emissions from the isotope.

With radiobiologist Alma Howard, Pelc devised a method to detect the synthesis of nuclear DNA in root meristems of bean (Howard and Pelc 1951b, c, 1952). Clowes (1956a) adopted their method, acknowledging his discussions with Howard and Pelc with regard to the labelling and autoradiography of *Zea* roots, and his autoradiographic images of longitudinally sectioned roots led to the now classic depictions of the QC (Clowes 1956a, b, 1959a), as shown in Fig. 2. Even after only 24 h of feeding with ^{14}C -adenine, the definition of the QC by autoradiography was remarkable, especially the sharp

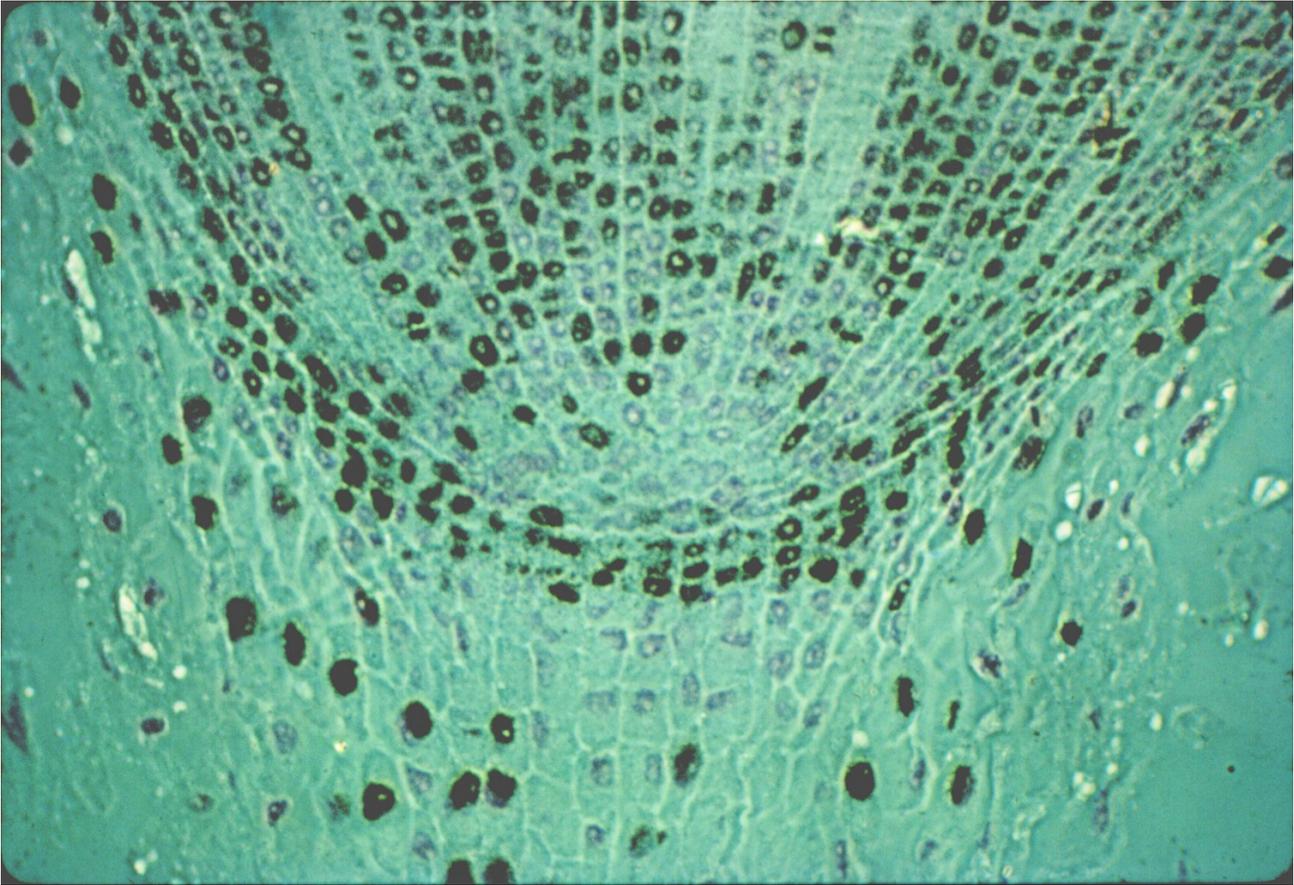


Fig. 2. Autoradiograph of a median longitudinal section of a root apex of *Zea mays* fed for many hours with ^3H -thymidine. Silver grains cover nuclei that are, or which have, engaged in DNA synthetic activity during the mitotic cell cycle. Silver grains are absent over nuclei within a semicircular area which corresponds to the quiescent centre.

boundaries between unlabelled QC and labelled neighbouring proximal and distal parts of the meristem.

Already it was evident to Clowes (1956a) that the average interdivisional period in the *Zea* root meristem was less than 24 h, whereas it was clearly much longer in the QC. The size of the QC was unexpected, however. Although it had been suggested, on the basis of results from surgical experiments, that the cytogenenerative centre of *Vicia* (and of *Fagus*) roots “must be broad and not confined to one or a few axial cells ... there was no reason then to suspect that some of the cells were quiescent” (Clowes 1956b, p. 310). That is, the QC as defined by autoradiography was actually larger than what might have been deduced on the basis of surgical experiments.

Cell kinetics in the QC and meristem before and after X-irradiation

The stage was now set for a further phase of Clowes’s research, namely, the detailed kinetics of cell division in various zones of the meristem, especially in relation to the QC and its response to

X-rays. The kinetic approach to meristematic cells was already evident, though barely acknowledged, in the papers of Howard and Pelc (1951a, b). Using autoradiography of cells from successive segments of bean root tips labelled for 2 – 48 h with ^{32}P , the boundary between cells with labelled nuclei and with unlabelled nuclei was displaced away from the apex by about 3 cm in a day due to cell proliferation within the labelled meristem. Moreover, the fraction of labelled nuclei varied along the root tip and, furthermore, this fraction increased the longer the precursor was available. Although displacement of labelled cells away from the tip was treated as a feature of cell kinetics at a later date (Barlow 1985), Howard and Pelc (1951c) immediately realised that it was possible thereby not only to estimate the cycle time of cells within the meristem, but also to infer that there were two periods during interphase when nuclear DNA was not being synthesised. These became known as the G1 and G2 phases of the cycle (Howard and Pelc 1953).

Thus, it was now possible, using continuous labelling with the more specific DNA precursor, ^3H -thymidine (which also gave a superior resolution

to the autoradiographs due to the shorter path-length of the β -particles), to estimate the timing of the mitotic cycle in various zones of the *Zea* apex (Clowes 1961b). The results so gained could also be checked by a second method, using the stathmokinetic agent, colchicine, to accumulate metaphases (c-metaphases), the mathematical methodology to obtain cycle times having been designed by John Evans and colleagues at the Radiation Biology Research Unit, Harwell (Evans et al. 1957). On the whole, the two methods gave similar results for the cell-cycle durations in the *Zea* root meristem (Clowes 1961b). Importantly, the QC was shown (but only by the colchicine method) to have a cycle time about 8 \times longer than the stele region immediately proximal to it, and 17 \times longer than the neighbouring cap initials (Clowes 1961b).

C-metaphase accumulation was also used to show that, after X-irradiation, the cell cycle of the QC of *Zea* was considerably shortened, whereas the cycle was lengthened in the two mentioned surrounding zones (Clowes and Hall 1962, Clowes 1963). Earlier work (Clowes 1959c) with X-irradiated *Zea* roots fed with ^{14}C -adenine during the recovery period, showed that after 4 d the QC was smaller, and after 6-8 d it was "often completely absent and the cells in the position of the quiescent centre may be almost the only ones with nuclear autoradiograph" (Clowes 1959c, p. 207). A similar picture emerged from X-rayed *Vicia* roots: "a smaller meristem is found either in a lateral position near the apex ... or in the quiescent centre" (Clowes 1959c, p. 207). Clowes therefore concluded, "that the quiescent centre, which consists of about 500 cells in *Zea* and 1,100 cells in *Vicia*, behaves as a reservoir of cells which will become meristematic when surrounding cells have lost their ability to divide at a normal rate" (Clowes 1959c, p. 208). The value of 1,100 cells in the QC of *Vicia* is clearly vastly different from the minimal number of 30-50 cells, estimated in other ways by Davidson (1960, 1961) and Hall et al. (1962a), that were considered necessary for regeneration of the *Vicia* root following X-irradiation.

Problems encountered in subsequent meristem research

Two problems remained. 1) How was it possible that some cells, like those of the QC, could remain proliferatively viable after X-irradiation whereas most other cells of the meristem were rendered non-viable? 2) How reliable were those techniques which used radioisotopes for assessing cell proliferation when X-irradiation had been shown to disturb

cell division?

Differential responses to X-rays.

In addressing the first problem, it should be recalled that, naturally enough, Clowes and Davidson, in Oxford, were not the only ones interested in how X-rays disrupted the proliferative integrity of roots. Another who shared this interest was John Thoday, at Sheffield University (Thoday 1954). Thoday, who later became Professor of Genetics at Cambridge University and, whilst there, made significant experimental contributions that supported the theory of evolution, had worked, in the immediate post-war years, in the radiobiology department of Mount Vernon Hospital and Radium Institute, in Northwood, London. The head of the department was the physicist, LH Gray, whose name has been given to the ISI unit of radiation dose, the gray or Gy (where 1 gray = 1 rad in non-ISI terms). Besides contributing to Gray's long study (Gray and Scholes 1951) of the effect of ionising radiation on *Vicia* roots (Thoday 1951), Thoday's most relevant finding with respect to Clowes's work was his discovery that, in the presence of oxygen, the susceptibility of chromosomes to X-rays was increased, whereas this effect of X-rays was mitigated when the roots were anoxic (Thoday and Read 1947, 1949). At that time in the 1950s, chromosome damage was considered to be the reason for the impairment of root growth by X-rays. Thoday's discovery of an oxygen effect on chromosome radiosensitivity led Clowes to consider the possibility that the QC might be resistant to X-rays because of a relatively low oxygen status at that particular location of the root, a higher oxygen status being a feature of the more radiation-sensitive meristem proper. However, through an experiment performed at the Churchill Hospital in Oxford by Clowes, with the collaboration of Eric Hall, a radiation physicist, and Lazlo Lajtha, a haematologist, it was argued that the cells which promote recovery of root growth after X-irradiation (i.e., those of the QC) are not protected by an inherent anoxia (Hall et al. 1962b); nor did further experiments really clarify this, such as those by the husband-and-wife team of Michael Ebert and Alma Howard (1961) or of Ebert and David Barber (1961), the latter at the Agricultural Research Council's Letcombe Laboratory, Wantage, UK, of which Scott Russell (mentioned earlier) was now director.¹ Unfortunately, the experiment did not directly address the behaviour of the QC in the context of their hypothesis, and the authors' reasoning and conclusions are not particularly convincing. Moreover, they could not exclude, as they themselves

¹ Local geography may have had an effect on root research: Wantage is 7 miles from Harwell (location of HJ Evans), and both are 11 miles to the south of Oxford, which itself is a relatively short journey by train (44 miles) from Hammersmith, London (location of Michael Ebert, Alma Howard, and Stephen Pelc).

admitted, the possibility that the cells of the QC “are protected by some other means (as yet unknown)” (Hall et al. 1962b). The experiment of Hall et al. (1962b) also showed that, when released from their quiescence, the descendants of the QC were as radiosensitive as the majority of root meristematic cells. However, HJ Evans, at the Medical Research Council’s Radiation Biology Research Unit at Harwell (Evans later became Director of the Medical Research Council’s Clinical and Population Cytogenetics Unit in Edinburgh, Scotland), in a review of the radiation biology of roots (Evans 1965), thought that the significance of chromosome damage had been over-emphasised and that an impairment of cell growth and function *per se* was a more likely reason for the radiation-induced reduction of root growth. So, it may be that the cells of the QC are, after all, protected from X-rays through some property that was not related to chromosome sensitivity.

Probably, it was the possible anoxic status of the QC that prompted Clowes and Barrie Juniper (at that time the latter was Research Officer and electron microscopist in the Botany School) to investigate the ultrastructure of the *Zea* root meristem, and it may be no coincidence that they paid especial attention to the mitochondria of the QC, which were in low numbers in cells of this zone (Clowes and Juniper 1964). They took note that, in some other cellular systems, those cells which were especially radiation-sensitive also had the fewest mitochondria, though they were forced to admit that the evidence was only circumstantial, and that any causal connection between mitochondrial numbers and radiosensitivity of the QC was not known. In any case, it was not clear whether the QC was sensitive or resistant to X-rays: if the meristem was sensitive, and ceased to proliferate after X-rays, then surely the QC was likely to be resistant.

For a long while, Clowes considered the resistance of the QC to X-irradiation to be in accordance with the Law of Bergonié and Tribondeau (1906), which states that X-rays are more effective on cells that divide actively, and less so on cells that divide slowly (Clowes 1963). Moreover, because the cells of the QC are mainly held in G1 phase and have a smaller nuclear volume than those of G2 cells, they present a smaller target to X-rays (Clowes 1965) and, hence, their resistance to radiation. However, later work by Clowes (1970) on the radiation-response of the QC threw this conclusion into question because he found that X-rays could provoke from cells an immediate, but transient, entry into both DNA synthesis and mitosis. Janet Thompson, who had completed a PhD thesis under the supervision of Douglas Davidson at St Andrews University, Scotland, and who, in 1967, was working in Clowes’s laboratory, where she

defined the duration of the cell cycle of the QC of roots of *Allium sativum* (Thompson and Clowes 1968), had also noticed that many nuclei of the QC of this species arrived at prophase a few minutes after an acute dose of X-rays (unpublished, but reported by Clowes 1970, p. 1). Clowes therefore concluded that “something other than the DNA content of the cells may be partly responsible for the differential response of the meristem to irradiation (Clowes 1972a, p. 896).

Clowes’s work with X-rays and meristems led to invitations to speak at two symposia on these topics in the USA – at Brookhaven National Laboratory, Upton, NY, in June 1963, and at the University of Rochester, NY, in August 1971. In the conference proceedings of both these events, he summarised his main findings regarding X-rays and meristems (Clowes 1964, 1972b). Mention should also be made of an invitation from the Science Students’ Council of the University of Witwatersrand, South Africa, to write for their journal, *Probe*. For this publication (Clowes 1966), he wrote an article about his radiation experiments on meristems.

Radioisotopes and cell proliferation.

Although labelling with ^{14}C -adenine had been helpful in confirming the presence of the QC in all species examined up to 1959 (Clowes 1959a), there was, nevertheless, a doubt about its suitability for tracing the behaviour of the QC. This stemmed from the report of McQuade and Friedkin (1960) that when either ^3H -thymidine or ^{14}C -thymidine was incorporated into nuclear DNA of onion (*Allium cepa*) seedlings, each was able to induce chromosome breaks in the root meristems. The breaks were similar to those induced by X-rays. Therefore, it seemed uncertain that the usage of radioactive precursors could give a true picture of cell kinetics, given that the cells being studied were themselves sensitive to the radioisotope which was being fed to the root. Clowes himself was able to observe that ^3H -thymidine (admittedly with a specific activity many times greater than would have been normally used for experimental purposes) brought about the stimulation of DNA synthesis and cell division within the QC of *Allium* roots 3 d after an 18-h exposure to ^3H -thymidine (Clowes 1961c). This was much the same result as would later be found for the X-ray response in *Zea* (Clowes 1970), where the damage from the β -emissions was substantial enough to stimulate the organisation of a new meristem which later replaced the former ^3H -labelled meristem.

The problem of sensitivity to incorporated tritium radioactivity was studied extensively by Clowes and Consuelo de la Torre, the latter visiting Oxford while

on leave from the Consejo Superior de Investigaciones Científicas, Madrid, Spain. They knew it was possible to estimate cell flow through the mitotic cycle either by marking nuclei with radioactivity or by accumulating c-metaphases with colchicine, methods which Clowes had used years earlier (Clowes 1961b). They therefore exposed roots of *Zea* to either non-labelled thymidine or ^3H -labelled thymidine and measured the rates of accumulation of either labelled or non-labelled c-metaphases using colchicine (de la Torre and Clowes 1974). Differences in the cell-flow rates of the respective cell populations would be expected if there were an effect of the radiation. From their estimates of cell flow, it was possible to conclude that the ^3H with its β -particle emissions, which had been fed to the roots as ^3H -thymidine, did indeed interfere with the cell cycle, accelerating it in the QC and delaying it in other regions of the meristem. This, again, pointed to the sensitivity of the QC to radiation.

Meristem growth fraction

It may be fair to say that uncertainties about the possibility of artefacts introduced by the cell-cycle measurement techniques led to another, more general, uncertainty of the exact structure of the meristem in terms of its cell-cycle kinetics. Were there, for example, long and short cycles mixed together in the various zones examined? This additional problem prolonged Clowes's experimental work with apices. There was also the problem of the 'growth fraction': this was being encountered elsewhere in studies of cell kinetics in complex proliferative systems (Mendelsohn 1962). Hitherto, it was an unknown feature of root meristems, and where, if it were true, growth fractions of less than unity would be responsible for distorting the estimated mean rate of regional cell proliferation (Clowes 1971). This topic of whether there were some cells which were 'out-of-cycle' and were simply being carried passively forward within a proliferating population, came to the fore in relation not only to portions of the meristem where certain cells were ceasing their proliferation as root histogenesis proceeded, but also to much smaller regions such as the QC itself. Here, for example, it was possible to see nuclei in different parts of the QC with different degrees of chromatin compaction and rates of ^{14}C -thymidine labelling (Barlow 1978, Fig. 1).

As Mendelsohn later said, "It should now be obvious that any wide variety of growth patterns can be produced by either varying cell cycle time or varying the fraction of cells proliferating. Nor is there any reason why these parameters could not change simultaneously" (Mendelsohn 1963, p 198). While

this degree of importance of the growth fraction may not be so relevant to understanding the steady, rectilinear growth of a root meristem, it would, however, be relevant in regions of more complex meristems (shoot apices, for example), where the form of the apex varies throughout a plastochron (Clowes 1961a). Growth fraction would also have been a variable to reckoned with had Clowes (1957) analysed shoot and leaf chimaeras with the diligence that he applied to root meristems.

With regard to the QC, the long mean cycle time of its cells prompted the question of whether some cells merely had a long G1 phase, as the classical model of cycling populations would suggest, or whether some cells had left the cycle and entered a G0 phase of indeterminate length. Another possibility was that the QC might even contain some fast cycling cells as, indeed, Clowes (1971) believed to be the case: 16 % of QC cells were estimated to have a cycle time of 40 h compared to a mean doubling time for the whole QC of 230 h. However, the realization that the proliferative structure of plant root meristems with their QC bore a striking resemblance to certain animal proliferative systems, such as the mouse intestinal epithelium with its population of crypt cells with long cycles (Fry et al. 1963, Potten 1977), meant that the possibility of a G0 phase could not be ignored, either in the QC (Clowes 1982) or elsewhere (Clowes 1976). Clowes continued to hold the view that some QC cells were out-of-cycle (Clowes 1982), though he did not identify where they were located. Probably, these non-cycling cells occupy a site within the QC which is at the pole of the stelar complex, a zone which seems to have been ignored in his examination of cell kinetics in and around the QC (Clowes 1982, but see Barlow 1978, Fig. 1).

Exit from the mitotic cycle

It is ironic that Clowes entitled his paper in 1983 "Exit from the mitotic cycle in root meristems of *Zea mays* L.", it was his last work in which ^3H -thymidine labelling was used to study cell kinetics (Clowes 1983). [The last paper in which use of X-rays was described was published nine years earlier (Clowes and de la Torre 1972).] In 1984, however, ^3H -thymidine labelling coupled with autoradiography was finally used as a means of defining the dimensions of the QC in 23 species; this parameter was then related to root diameter. It was discovered that the smaller the meristem, the smaller the QC, also the smaller the difference between the cell cycle time between cap meristem and QC. Ultimately, the correlation between the size of the QC and the degree of its quiescence would seem to "approach the condition in small roots of pteridophytes where the

site of the QC is occupied by a meristematic cell” (Clowes 1984, p. 20).

Thereafter, from 1987 until 2000, Clowes was able to return to a study of the morphology of apices, publishing six articles on this theme, five of them dealing with the origin and behaviour of the root epidermis, the sixth, co-authored with Margaret Macdonald, dealt with the morphology and proliferative activity of cells in the shoots and buds of potato (Clowes and Macdonald 1987). The final paper (Clowes 2000) was devoted purely to root morphology. It considered the different cellular architectures found in roots of angiosperm species, focussing on the various forms of open and closed meristems and, in particular, the way in which epidermis, cap and cortex were developed.

Thus, in the intervening 30 years, Clowes had devoted himself to an experimental exploration of the root meristem. The techniques used were ones initiated and developed during the same period when he was working on the problem of the QC, and to which he actively applied the new methodologies. These included: autoradiography and microdensitometry (used by Clowes (1968, 1970) in relation his studies on nuclear DNA contents in the QC of normal and X-irradiated roots), radioisotopes for marking nucleic acid synthesis (and then applying this method in conjunction with pulse-labelling to analyse the cell cycle), and the use of colchicine and other chemical agents to study rates of cell flow through the mitotic cycle. In some ways, it may be said that, as soon as each new technical innovation arose, Clowes applied it to problems of root organisation: X-rays led to his use of radioactive isotopes, isotopes to autoradiography, autoradiography to cell cycle studies, cell cycles to microdensitometry. It was a concatenation of opportunities which any conscientious investigator could not ignore; and these led to a rounded and satisfying body of research.

The wider significance

In the 1960s, root meristem research and the results from X-irradiation were very much allied with similar research on animal systems, particularly in relation to cancer and radiotherapy. In the context of research in the 1950s and ‘60s, the bean root meristem served as a useful model system in the hands of Gray, Evans, Read, and others, from which concepts, methods and ideas could be transferred to the relatively less accessible *in vivo* animal models (see Read 1959). The therapeutic effect of X-ray-induced cell death was evident, even if the mechanisms, at least as far as plant meristems were concerned, were still obscure (Evans 1965). It was also clear that X-rays allowed proliferative systems of both plants

and animals to be ‘opened-up’ for analysis of the respective cell populations and sub-populations, and how these systems were inter-connected not only between themselves but also with the whole body (Lajtha 1963). But it was Clowes’s results with root apical meristems which led to the more fundamental realisation of their complexity in relation to organ growth; and these insights were matched by similar insights of other workers into the complexity of both cancerous tumours and normal tissues of animals and humans. Moreover, as Wardlaw (1952) said in the preface of his book, ‘Phylogeny and Morphogenesis’, “In the realm of science, botany may rightly be held to be one of the great cultural disciplines. It is well, then, that botanists should show proper pre-occupation, not only with the integrated wholeness of the organism, but with the integrated wholeness of their science”. Clowes’s body of work exemplified this precept, but went one step further, pointing out, to those able to appreciate them, the analogies between animal and plant proliferative systems.

Afterword

In May 2015 the Internet search facility ‘Web of Science’ recorded >500 research paper when the term ‘quiescent+centre’ was used as the ‘Topic’ entry, with the rate of citation suddenly accelerating after the year 2005 and continuing to increase thereafter. Naturally, it is intriguing to examine what has given rise to this surge of interest in the small group of cells within the root meristem called ‘quiescent centre’. One might hazard the opinion that it has not been because of advances in cell cycle research, as might be thought, given that the ‘quiescence’ of the QC relates to its proliferative properties but, rather, has been due to advances in genetics of plant embryogeny (of *Arabidopsis thaliana*, in particular) (Mayer et al. 1991, Scheres et al. 1995) and of gravitropism, especially in relation to PIN proteins and auxin transport (Müller et al. 1998, Friml et al. 2002). A brief survey of recent research into the QC and its role in root growth will be given in a further paper.

Acknowledgements

I am grateful to Mrs D Winson (née Clowes) for information about her father, Dr FAL Clowes, and to Professors Douglas Davidson and Eric J Hall for recollections of the early years of their own research in Oxford. I am also grateful to Professor Joseph G Dubrovsky for a careful reading of the manuscript. Thanks are also due to the Department of Plant Sciences, Oxford University, and to Serena Marner, in particular, for making available the photograph

shown in Fig. 1, which was originally taken by FAL Clowes but has been slightly modified by the present author for the present publication. Fig. 2 is from a photomicrograph in the collection of FAL Clowes and was kindly made available by Mrs D Winson.

References

- Barlow PW 1978 The concept of the stem cell in the context of plant growth and development. In: Lord BI, Potten CS, Cole RJ, Eds., Stem cells and tissue homeostasis. Cambridge University Press, Cambridge, pp. 87-113.
- Barlow PW 1985 The nuclear endoreduplication cycle in metaxylem cells of primary roots of *Zea mays* L. J. Exp. Bot. 55: 445-457.
- Bergonié J, Tribondeau L 1906 Interprétation de quelques resultants de la radiothérapie et essai de fixation d'une technique rationnelle. C. R. Acad. Sci., Paris 143: 983-985.
- Boyd GA, Williams AI 1948 Stripping film technics for histological autoradiographs. Proc. Soc. Exp. Biol. Med. 69: 225-232.
- Brumfield RT 1943 Cell-lineage studies in root meristems by means of chromosome rearrangements induced by X-rays. Am. J. Bot. 30: 101-109.
- Buvat R 1955 Le méristème apical de la tige. Ann. Biol. 31: 595-656.
- Clapham AR 1970 George Robert Sabine Snow. 1897-1969. Biographical Memoirs of Fellows of the Royal Society 16: 498-522.
- Clowes FAL 1949 The morphology and anatomy of the roots associated with ectotrophic mycorrhiza. D.Phil. Thesis, Oxford University.
- Clowes FAL 1950 Root apical meristems of *Fagus sylvatica*. New Phytol. 49: 249-268.
- Clowes FAL 1951 The structure of mycorrhizal roots of *Fagus sylvatica*. New Phytol. 50: 1-16.
- Clowes FAL 1953 The cytogenetic centre in roots with broad columellas. New Phytol. 52: 48-57.
- Clowes FAL 1954a The root-cap of ectotrophic mycorrhizas. New Phytol. 53: 525-529.
- Clowes FAL 1954b The promeristem and the minimal construction centre in grass root apices. New Phytol. 53: 108-116.
- Clowes FAL 1956a Nucleic acids in root apical meristems of *Zea*. New Phytol. 55: 29-34.
- Clowes FAL 1956b Localization of nucleic acid synthesis in root meristems. J. Exp. Bot. 7: 307-312.
- Clowes FAL 1957 Chimeras and meristems. Heredity 11: 141-148.
- Clowes FAL 1959a Adenine incorporation and cell division in shoot apices. New Phytol. 58: 85-88.
- Clowes FAL 1959b Apical meristems of roots. Biol. Rev. 34: 501-529.
- Clowes FAL 1959c Reorganization of root apices after irradiation. Ann. Bot. 23: 205-210.
- Clowes FAL 1961a Apical meristems. Blackwell Scientific Publications, Oxford.
- Clowes FAL 1961b Duration of the mitotic cycle in a meristem. J. Exp. Bot. 12: 283-293.
- Clowes FAL 1961c Effects of β -radiation on meristems. Exp. Cell Res. 25: 529-534.
- Clowes FAL 1963 X-irradiation of root meristems. Ann. Bot. 27: 343-352.
- Clowes FAL 1964 The quiescent center in meristems and its behavior after irradiation. Brookhaven Symp. Biol. 16: 46-57.
- Clowes FAL 1965 The duration of the G₁ phase of the mitotic cycle and its relation to radiosensitivity. New Phytol. 64: 355-359.
- Clowes FAL 1966 Radiation experiments on meristems. Probe (Journal of the Science Students' Council, Witwatersrand): 27-31.
- Clowes FAL 1968 The DNA content of cells of the quiescent centre and root cap of *Zea mays*. New Phytol. 67: 631-639.
- Clowes FAL 1970 The immediate response of the quiescent centre to X-rays. New Phytol. 69: 1-18.
- Clowes FAL 1971 The proportion of cells that divide in root meristems of *Zea mays*. Ann. Bot. 35: 249-261.
- Clowes FAL 1972a Cell cycles in a complex meristem after X-irradiation. New Phytol. 71: 891-897.
- Clowes FAL 1972b The control of cell proliferation within root meristems. In: Miller MW, Kuehnert CC, Eds., The dynamics of meristem cell populations. Plenum Press, New York-London, pp. 133-147.
- Clowes FAL 1976 Estimation of growth fractions in meristems. Ann. Bot. 40: 933-938.
- Clowes FAL 1981 Cell proliferation in ectotrophic mycorrhizas of *Fagus sylvatica* L. New Phytol. 87: 547-555.
- Clowes FAL 1982 The growth fraction of the quiescent centre. New Phytol. 91: 129-135.
- Clowes FAL 1983 Exit from the mitotic cycle in root meristems of *Zea mays* L. Ann. Bot. 51: 385-393.
- Clowes FAL 1984 Size and activity of quiescent centres of roots. New Phytol. 96: 13-21.
- Clowes FAL 2000 Pattern in root meristem development in angiosperms. New Phytol. 146: 83-94.
- Clowes FAL, de la Torre C 1972 Nucleoli in X-rayed meristems. Cytobiol. 6: 318-326.
- Clowes FAL, Hall EJ 1962. The quiescent centre in root meristems of *Vicia faba* and its behaviour after acute X-irradiation and chronic gamma irradiation. Rad. Bot. 3: 45-53.
- Clowes FAL, Juniper BE 1964 The fine structure of the quiescent centre and neighbouring tissues in root meristems. J. Exp. Bot. 15: 622-630.
- Clowes FAL, Macdonald M 1987 Cell cycling and the fate of potato buds. Ann. Bot. 59: 141-148.
- Darlington CD, La Cour LF 1946 Chromosome breakage and the nucleic acid cycle. J. Genet. 46: 180-267.
- Davidson D 1956 Experimental observation of chromosome behaviour. D.Phil. Thesis, Oxford University.
- Davidson D 1959 A method for estimating mitotic rates in *Vicia* roots after X irradiation. Brit. J. Radiol. 32: 612-614.
- Davidson D 1960 Meristem initial cells in irradiated roots of *Vicia faba*. Ann. Bot. 24: 287-295.
- Davidson D 1961 Mechanisms of reorganization and cell repopulation in meristems in roots of *Vicia faba* following irradiation and colchicine. Chromosoma 12: 484-504.
- de la Torre C, Clowes FAL 1974 Thymidine and the measurement of rates of mitosis. New Phytol. 73: 919-925.
- Dubrovsky JG, Barlow PW 2015 The origins of the quiescent centre concept. New Phytol. 206: 493-496.
- Ebert M, Barber DA 1961 The effects of X-rays on the extension growth of barley roots. Internat. J. Rad. Biol. 3: 587-593.
- Ebert M, Howard A 1961 Relative radiation sensitivity in the

- study of oxygen supply to root meristems. *Internat. J. Rad. Biol.* 3: 619-625.
- Evans HJ 1965 Effects of radiations on meristematic cells. *Rad. Bot.* 5: 171-182.
- Evans HJ, Neary GJ, Tonkinson SM 1957 The use of colchicine as an indicator of mitotic rate in broad bean root meristems. *J. Genet.* 55: 487-502.
- Friml J, Wiśniewska J, Benková E, Mendgen K, Palme K 2002 Lateral relocation of auxin efflux regulator PIN3 mediates tropism in *Arabidopsis*. *Nature* 415: 806-809.
- Fry RJM, Leshner S, Kisielski WE, Sacher G 1963 Cell proliferation in the small intestine. In: Lamerton LF, Fry RJM, Eds., *Cell proliferation*. Blackwell Scientific Publications, Oxford, pp. 213-233.
- Gray LH, Scholes ME 1951 The effect of ionizing radiations on the broad bean root Part VIII. Growth rate studies and histological analyses. *Brit. J. Radiol.* 24: 82-92, 176-180, 228-236, 285-291, 348-352.
- Hall EJ, Lajtha LG, Oliver R 1962a The X-ray dose-response relationship for reproductive integrity of *Vicia faba*. *Brit. J. Radiol.* 35: 388-397.
- Hall EJ, Lajtha LG, Clowes FAL 1962b The role of the quiescent centre in the recovery of *Vicia faba* roots from irradiation. *Rad. Bot.* 2: 189-194.
- Harley JL 1959 *The biology of mycorrhiza*. Leonard Hill Books Ltd., London
- Howard A, Pelc SR 1951a Synthesis of nucleoprotein in bean root cells. *Nature* 167: 599-600.
- Howard A, Pelc SR 1951b Nuclear incorporation of P³² as demonstrated by autoradiographs. *Exp. Cell Res.* 2: 178-187.
- Howard A, Pelc SR 1951c Synthesis of deoxyribose nucleic acid and nuclear incorporation of ³⁵S as shown by autoradiographs. In: Wolstenholme GEW, Ed., *CIBA Foundation conference on isotopes in biochemistry*. J & A Churchill, London, pp. 138-148.
- Howard A, Pelc SR 1952 Techniques of autoradiography and the application of the stripping-film method to problems of nuclear metabolism. *Brit. Med. Bull.* 8: 132-135.
- Howard A, Pelc SR 1953 Synthesis of desoxyribonucleic acid in normal and irradiated cells and its relation to chromosome breakage. *Heredity* 6 (suppl.): 261-273.
- Lajtha LG 1963 The use of radiation in studies of cell proliferation. In: Lamerton LF, Fry RJM, Eds., *Cell proliferation*. Blackwell Scientific Publications, Oxford, pp. 80-91.
- Mangenot G 1942 Action de la colchicine sur les racines d'*Allium cepa*. *Actualités Scientifiques et Industrielles*. No. 915: 1-120. Hermann et Cie, Paris.
- Mayer U, Torres Ruiz RA, Berleth T, Misera S, Jürgens G 1991 Mutations affecting body organization in the *Arabidopsis* embryo. *Nature* 353: 402-407.
- McQuade HA, Friedkin M 1960 Radiation effects of thymidine-³H and thymidine-¹⁴C. *Exp. Cell Res.* 21: 118-125.
- Mendelsohn ML 1962 Autoradiographic analysis of cell proliferation in spontaneous breast cancer of C3H mouse. III. The growth fraction. *J. Nat. Cancer Inst.* 28: 1015-1029.
- Mendelsohn ML 1963 Cell proliferation and tumor growth. In: Lamerton LF, Fry RJM, Eds., *Cell proliferation*. Blackwell Scientific Publications, Oxford, pp. 190-210.
- Müller A, Guan C, Gälweiler L, Tänzler P, Huijser P, Marchant A, Parry G, Bennett M, Wisman E, Palme K 1998 *AtPIN2* defines a locus of *Arabidopsis* for root gravitropism control. *EMBO J.* 17: 6903-6911.
- Němec B 1897 Cytologická pozorování na vegetačních vrcholech rostlin. *Sitzungsber. K. Böhm. Ges. Wiss.* 1897: 1-26.
- Nougarède A 1965 Organisation et fonctionnement du méristème apical des végétaux vasculaires. Masson et Cie, Paris.
- Pelc SR 1947 Autoradiograph technique. *Nature* 160: 749-750.
- Potten CS 1977 Epithelial proliferative subpopulations. In: Lord BI, Potten CS, Cole RJ, Eds., *Stem cells and tissue homeostasis*. Cambridge University Press, Cambridge, pp. 317-334.
- Read JB 1959 *Radiation biology of Vicia faba in relation to the general problem*. Blackwell Scientific Publications, Oxford.
- Rolinson AE 1976 Rates of cell division in the vegetative shoot apex of rice (*Oryza sativa* L.). *Ann. Bot.* 40: 939-945.
- Russell RS, Martin RP 1953 A study of the absorption and utilization of phosphate by young barley plants I. The effect of external concentration on the distribution of absorbed phosphate between shoots and roots. *J. Exp. Bot.* 10: 108-127.
- Sachs T 1991 *Pattern formation in plant tissues*. Cambridge University Press, Cambridge.
- Scheres B, di Laurenzio L, Willemsen V, Hauser M-T, Janmaat K, Weisbeek P, Benfey PN 1995 Mutations affecting the radial organisation of the *Arabidopsis* root display specific defects throughout the embryonic axis. *Development* 121: 53-62.
- Schüepp O 1917 Untersuchungen über Wachstum und Formwechsel von Vegetationspunkten. *Jb. Wiss. Bot.* 57: 17-79
- Thoday JM 1951 The effect of ionizing radiations on the broad bean root Part IX. Chromosome breakage and the lethality of ionizing radiation to the root meristem. *Brit. J. Radiol.* 24: 572-576, 622-628.
- Thoday JM 1954 Radiation-induced chromosome breakage, desoxyribose-nucleic acid synthesis and the mitotic cycle in root-meristem cells of *Vicia faba*. *New Phytol.* 53: 511-516.
- Thoday JM, Read J 1947 Effect of oxygen on the frequency of chromosome aberrations produced by X-rays. *Nature* 160: 608-609.
- Thoday JM, Read J 1949 Effect of oxygen on the frequency of chromosome aberrations produced by alpha-rays. *Nature* 163: 133-134.
- Thompson J, Clowes FAL 1968 The quiescent centre and rates of mitosis in the root meristem of *Allium sativum*. *Ann. Bot.* 32: 1-13.
- Wardlaw CW 1947 Experimental investigations of the shoot apex of *Dryopteris aristata* Druce. *Phil. Trans. R. Soc. London B* 232: 343-384.
- Wardlaw CW 1952 *Phylogeny and morphogenesis*. Contemporary aspects of botanical science. Macmillan and Co, London.



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