THE ROLE OF THE QUIESCENT CENTRE IN THE RECOVERY OF *VICIA FABA* ROOTS FROM RADIATION

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Abstract—The suggestion that cells in the quiescent centre of bean root meristems are relatively insensitive to radiation and promote recovery in irradiated roots is discussed.

Experiments indicate that the cells from which root recovery takes place are not protected by anoxia in normal roots; also that the progeny of these cells have the same radiosensitivity as normal meristems.

Calculations are presented which show that the number of cells in the meristem surviving irradiation is enough to promote recovery without attributing any special degree of protection to any of the cells; but if recovery can only take place from a small region of the root, the possibility cannot be excluded that cells in this region have reduced radiosensitivity.

Résumé—On discute la suggestion suivant laquelle les cellules du centre quiescent des méristèmes radiculaires de la fève sont relativement insensibles aux radiations.

Des expériences indiquent que les cellules à partir desquelles le recouvrement a lieu ne sont pas protégées par l'anoxie dans les racines normales. La descendance de ces cellules à la même radiosensibilité que les méristèmes normaux.

On présente des calculs qui montrent que le nombre de cellules dans le méristème survivant à l'irradiation est suffisant pour faciliter le recouvrement sans attribuer un degré spécial de protection à l'une ou l'autre cellule. Si le recouvrement peut cependant se produire à partir d'une petite région de la racine, on ne peut exclure la possibilité que des cellules de cette région aient une sensibilité réduite.

Zusammenfassung—Die Behauptung, dass Zellen im "ruhenden Zentrum" des Meristems von Bohnenwurzeln verhältnismässig unempfindlich gegen Strahlung sind, und dass diese Zellen die Erholung bestrahlter Wurzeln einleiten, wird diskutiert.

Experimentelle Ergebnisse weisen darauf hin, dass die Zellen, von denen aus die Erholung stattfindet, in normalen Wurzeln nicht durch Anoxie geschützt werden. Ausserdem haben die Nachkommen dieser Zellen die gleiche Strahlenempfindlichkeit wie normale Meristeme.

Berechnungen zeigen, dass die Zahl von Zellen im Meristem, welche eine Bestrahlung überleben, gross genug ist, um eine Erholung zu ermöglichen, ohne dass irgendwelchen dieser Zellen ein besonderer Schutz zugeschrieben werden müsste. Für den Fall allerdings, dass die Erholung tatsächlich nur von einer kleinen Region der Wurzel aus stattfindet, kann die Möglichkeit, dass Zellen in dieser Region verringerte Strahlenempfindlichkeit haben, nicht ausgeschlossen werden.

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INTRODUCTION

FROM a study of the pattern of division in apical mersistems, CLOWES[2] postulated the existence in root apices of *Zea* of a quiescent centre, a region in which cells divide rarely if at all, in the normal growth of the root. The cells of the quiescent centre are carried forward passively by the growth of the surrounding meristem. They are quiescent not because of any inherent disability, but because of their position within the apex.

The presence of such a quiescent centre has been demonstrated experimentally in many apices, including that of *Vicia faba*,[3] Roots were fed with tritium-labelled thymidine, which is incorporated into the D.N.A. of cells preparing for divisions. In auto-radiographs prepared from sections of the root, the quiescent centre stands out from the rest of the meristem because the labelled D.N.A. precursors are incorporated at a much slower rate, indicating relatively infrequent cell division. In *Vicia faba*, the quiescent centre consists of about 1000 cells, grouped together in a volume whose shape approximates to a hemisphere (Fig. 1). CLOWES[3] has also shown that if roots are exposed to 360 r of X-radiation, most of the meristematic cells stop synthesizing D.N.A. and stop dividing. But cells which were previously quiescent may now start to synthesize D.N.A. and to divide. Autoradiographs prepared several days after irradiation from sections of roots fed with tritiated thymidine showed the reverse pattern to that described above for unirradiated roots. The cells of the quiescent centre incorporate labelled D.N.A. precursors at a much faster rate than those of the surrounding meristem.

He interpreted this evidence to mean that the quiescent centre is a reservoir of cells that are sufficiently undamaged by the irradiation to be able to start dividing, form a new meristem, and lead to the recovery of the root. DAVIDSON[4] has suggested that the reduced radiosensitivity of the quiescent cells may be due to their slow rate of division.

The number of cells required to initiate a root has been studied by a number of workers. BRUMFIELD[1] induced chromosome aberrations in young radicles of *Vicia faba* by exposure to 300 r of X-radiation, and allowed the primary
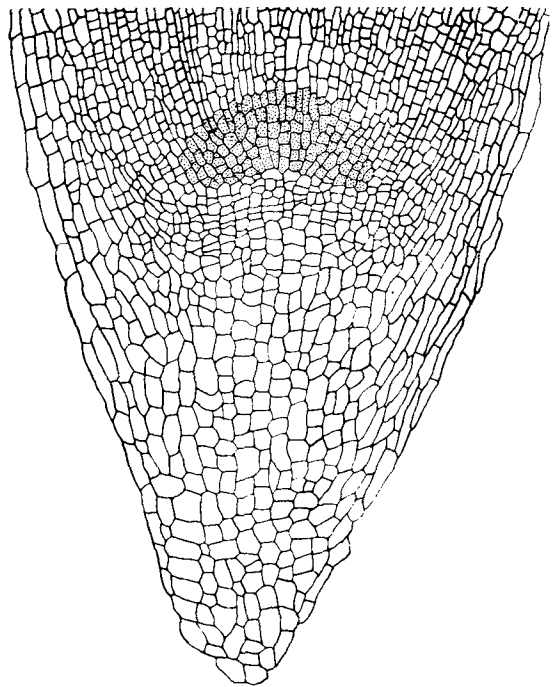

FIG. 1.   Median section of root apex of *Vicia faba* showing the position of the quiescent centre (shaded).

root to grow. After a period considered sufficient to eliminate cells not derived from the initials existing at the time or irradiation, he examined sections of the root to find the position of aberrant cells. He found that some of the roots were sectorial chimeras, in which particular aberrations existed in sectors of the root extending from the stele to the cap, and for about ⅓ of the circumference. He concluded that there were three initial cells each of which had produced a sector of tissue.

DAVIDSON[4] used 600 r of X-radiation to produce chromosome aberrations in *Vicia faba*, and followed these aberrations in the lateral side-roots of the irradiated primary root 3 to 4 weeks later. He found that one lateral root had cells with five different chromosome complements as well as normal cells. He concluded that at least six different kinds of cells contributed to the formation of the primordium of the lateral root, and the actual number of cells involved was likely to be greater than six. This result implies that six different kinds of cells exist

together within a very small region of the primary root, and is therefore very different from Brumfield's interpretation of his sectorial chimeras.

In later experiments, DAVIDSON[5] irradiated primary roots with 600 r of X-radiation and used aberrant chromosome complements as cell markers to deduce the number of initial cells from which the root had been able to regenerate. Three weeks after irradiation, he could recognize an average of eight types of abnormal cells and a ratio of about three normal to one atypical cell. Assuming that no two cells would suffer identical aberrations, and assuming that the ratio of normal to atypical cells remained unchanged, he estimated the average number of meristem initial cells to be thirty-two —composed of eight abnormal and twenty-four normal cells. In a later publication, DAVIDSON[6] has revised the figure to between forty and fifty.

EXPERIMENTS

(a) *The sensitivity of bean roots to a second dose of radiation after recovery from an initial dose*

A large number of seeds were germinated and cultured by the methods previously described in detail.[8] On the seventh day after germination, thirty roots were reserved for controls, and the remaining 144 roots exposed to a dose of 360 rad. The length of each root was measured daily, and the growth-rate expressed as cm. per day. Of the irradiated roots, fifty (34 per cent) stopped growing or developed split roots, and were therefore discarded. The average daily growth-rates of the remaining ninety-four are plotted as crosses in Fig. 2 up to the 15th day
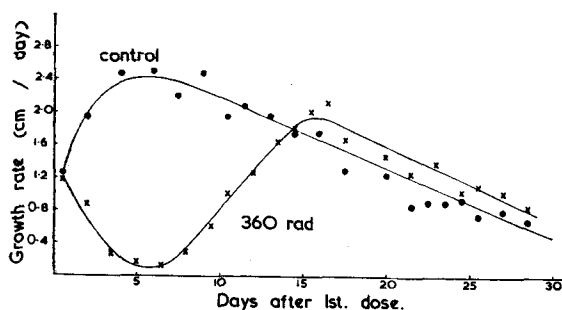
after irradiation. By this time, their growth-rate had recovered to equal that of the unirradiated controls.

The roots that had recovered were divided into six groups, five of them were irradiated a second time with doses in the range 100 to 200 rad, and the remaining group served as control for the second irradiation (group C2). The length of each root was measured daily for a further two weeks. The crosses in Fig. 2 for day 16 onwards represent the mean daily growth rate for the control group C2.

The effect of the second irradiation is illustrated in Fig. 3, in which the daily growth increments of the groups of roots exposed for a second time are expressed as a fraction of control group C2. Radiation damage was scored by two
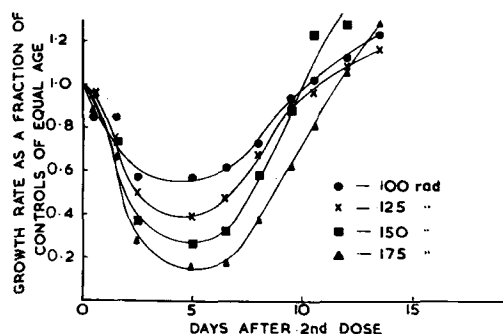

FIG. 3.   Growth rate of roots exposed to a second dose of radiation after recovery from an initial dose.

independent parameters; firstly the "minimum growth rate" reached by the roots after irradiation, expressed as a fraction of the control value; secondly, the "growth in 10 days", defined to be the increment added to the length of irradiated roots in the 10 days following irradiation expressed as a fraction of the same quantity for controls. Both parameters are plotted against dose in Fig. 4, and compared with the corresponding quantities obtained in previous experiments in which roots were irradiated once only. Fig. 4 then, compares the sensitivity of roots exposed a second time after they had recovered from an initial dose of 360 rad. There is evidently no gross difference in sensitivity when judged by either "minimum growth rate" or "growth in 10 days".

FIG. 2.   Growth rate of roots irradiated with 360 rad, compared with control roots.
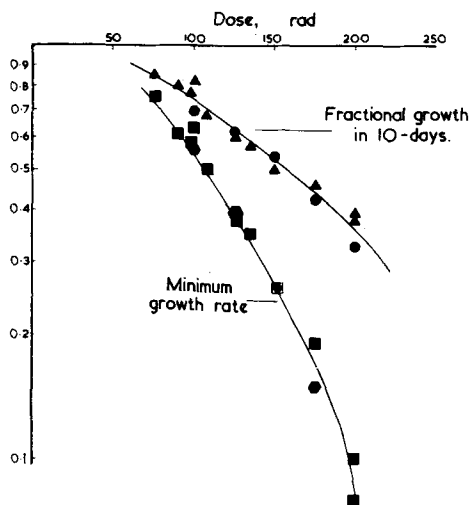
FIG. 4.   Comparison of the sensitivity of roots exposed a second time after recovery from an initial dose, with roots irradiated only once.
Hexagons and circles refer to the effects of a second irradiation; squares and triangles refer to roots exposed once only.

FIG. 5.   Comparison of the effect on growth-rate of 360 rad delivered in aerated water, and 1000 rad in nitrogenated water.

(b)   *The influence of oxygen on the effect of large doses of radiation*

A large number of seedlings that had been cultured together, were divided into three groups. One was reserved for control purposes; the second received a dose of 360 rad of X-radiation in aerated water, and the third a dose of 1000 rad under anoxic conditions. The length of each root was measured daily for two weeks. Of the sixty-eight roots irradiated under aerobic conditions, forty (fifty-nine per cent) recovered; of the fifty-one roots irradiated anaerobically, forty-three (sixty-three per cent) recovered. The mean daily growth-rates of each group of roots are plotted in Fig. 5.

It may be concluded from these results, that the ratio of doses delivered in the presence and absence of oxygen which result in the same proportion of roots recovering and the same rate of recovery is close to 2·8 (1000/360) i.e. the oxygen factor is approximately the same for lethal effects on roots as for reduction in root growth produced by much lower doses.

CONCLUSIONS AND DISCUSSION

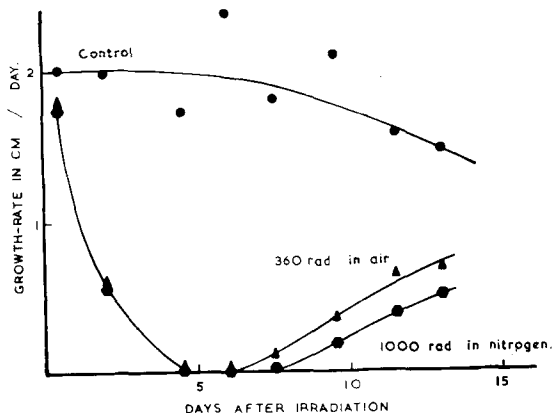Two definite conclusions may be drawn from these experiments. Firstly, the cells which promote root recovery (whichever they may be) are not protected by anoxia when the root is exposed in aerated water. This is evident because the lethal effect on roots is depressed if oxygen is excluded from the root, and cells which are already anoxic could not be further protected by making the whole root anoxic. Secondly, the descendants of the cells which promote recovery have the same radiosensitivity as normal meristem cells.

However, the present evidence does not exclude the possibility that cells of the quiescent centre are highly protected by some other (at present unknown) means, and after becoming meristematic when the surrounding cells are sterilized, they and their descendants acquire normal radiosensitivity. The number of quiescent cells is so small compared with the surrounding meristem (10^3 compared with 2×10^5) that the degree of protection would need to be high before the number of cells surviving in the quiescent centre would add significantly to the number retaining their reproductive integrity in the remainder of the meristem. The following calculation gives some information about the need to regard the quiescent cells as peculiarly resistant.

DAVIDSON[5] has estimated that a minimum of thirty to fifty viable cells are needed for root recovery to take place. Brumfield's experiments with sectorial chimeras suggest that it is possible

occasionally for a root to recover from as few as three cells. It is reasonable to suppose, therefore, that on average, thirty to fifty cells retain reproductive integrity in roots exposed to the mean lethal dose. HALL, et al.[9] have published the dose-response curve for reproductive integrity of *Vicia* meristem cells, illustrated in Fig. 6. This may be extrapolated to predict the number of cells from the whole meristem which survive the mean lethal dose. (Reported values for the X-ray L.D.$_{50}$ vary from 435 r by GRAY, et al.[7] to 368 r by SPALDING, et al.[10] The result is twenty and ninety-eight cells respectively for the two values of the mean lethal dose, a total meristem population of 2×10^5 cells being assumed in each case.

It is evident, therefore, that a sufficient number of cells from the meristem would be expected to survive to satisfy Davidson's estimate, without ascribing any special powers of protection to the quiescent centre.

The possible objection to this evidence is that survivors scattered throughout the meristem may be less effective in promoting recovery than a small number of cells surviving in the quiescent centre, where they would be in a particularly favourable position to develop a new meristem without much disruption of the apex.

However, if Clowes' suggestion is true that it is the quiescent centre that provides for the recovery of the root, then it is still necessary to show how it does this. The overall conclusion at this stage must be that the special role of the quiescent centre in the recovery of roots after irradiation is no better understood than its function in normal root growth. It is a topic that needs further investigation.
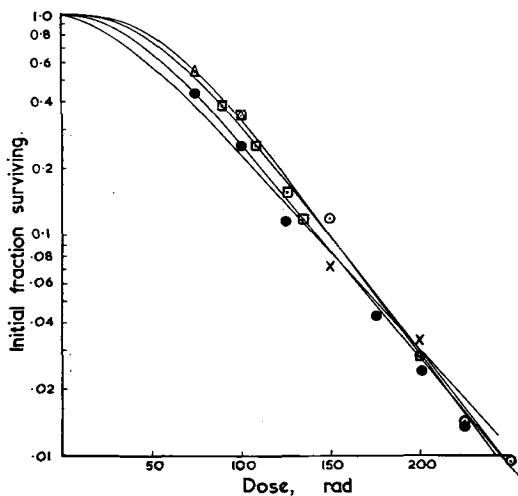

FIG. 6. Initial surviving fraction of cells in the meristem plotted against dose; the different symbols refer to separate experiments. The curves drawn are all of the form: fraction surviving $= 1-(1-e^{-\lambda D})^n$
The extrapolation number (n) and the 37 per cent dose slope $(1/\lambda)$ for the four curves, from upper curve downwards, are:

$$n=5; \quad 1/\lambda=39 \text{ rad.}$$
$$n=4; \quad 1/\lambda=40 \text{ rad.}$$
$$n=3; \quad 1/\lambda=43 \text{ rad.}$$
$$n=2; \quad 1/\lambda=48 \text{ rad.}$$

The curves for $n=3$ or 4 are a reasonable fit to the data. In fact, it was the $n=3$ curve that was extrapolated to obtain the number of cells surviving the mean lethal dose referred to in the script.

REFERENCES

1. BRUMFIELD R. T. (1943) Cell-lineage studies in the root meristems by means of chromosome rearrangements induced by X-rays. *Amer. J. Bot.* **30**, 101.
2. CLOWES F. A. L. (1954) The promeristem and the minimal constructional centre in grass root apices. *New Phytol.* **53**, 108.
3. CLOWES F. A. L. (1959) Apical meristems of roots. *Biol. Rev.* **34**, 501.
4. DAVIDSON D. (1959) Changes in the chromosome complements of cells of *Vicia faba* following irradiation. *J. Exp. Bot.* **10**, 391.
5. DAVIDSON D. (1960) Meristem initial cells in irradiated roots of *Vicia faba*. *Ann. Bot. Lond.* **24**, 287.
6. DAVIDSON D. (1961) Mechanisms of reorganization and cell repopulation in meristems in roots of *Vicia faba* following irradiation and colchicine. *Chromosoma* **12**, 484–504.
7. GRAY L. H., READ J. and POYNTER M. (1943) The effect of ionizing radiations on the broad bean root. Part V. The lethal action of X-radiation. *Brit. J. Radiol.* **16**, 125.
8. HALL E. J. (1961) The relative biological efficiency of X-rays generated at 220 kVp and gamma radiation from a Cobalt 60 therapy unit. *Brit. J. Radiol.* **34**, 313.

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9. HALL E. J., LAJTHA L. G. and OLIVER R. (1962) The X-ray dose-response relationship for reproductive integrity of *Vicia faba*. *Brit. J. Radiol.* **35**, 388.

10. SPALDING J. F., LANGHAM W. and ANDERSON E. C. (1956) The relative biological effectiveness of tritium beta radiation with the broad bean root (*Vicia faba*) as a test system. *Radiat. Res.* **4**, 221.