ULTRA-STRUCTURE OF CEREAL GRAINS

IN RELATION TO GERMINATION

Thesis for the Degree of Doctor of Philosophy in
The University of Edinburgh.

Presented by

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To: My Mother, Aunts, Professor Garth Chapman and a Friend.

".... whoever could make two ears of corn or two blades of grass to grow upon a spot where only one grew before would deserve better of mankind, and do more essential service to his 'country' than the whole race of politicians put together."

Gulliver's Travels: Swift.

I N D E X

INTRODUCTION

General Introduction

At the inception of this investigation (1965) a fellow student informed me that the impetus for research on the role of the embryo in barley germination was exhausted. In fact, I was left with the impression that since commercially-available gibberellic acid (GA_3) could release hydrolytic enzymes from embryo-less barley endosperms, the embryo no longer merited serious study.

Alarmed and immediately pensive at the possibility of been allocated an "exhausted" topic to investigate, a thorough examination of the available literature on barley germination and subsequent seedling growth was undertaken prior to any experimental work.

On the completion of this literature review it was concluded that since the classic memorandum on the Germination of some of the Graminae (Brown & Morris, 1890) there has been remarkedly little published information on the correlative histochemical, anatomical and physiological changes associated with germination and subsequent seedling growth of barley grains.

Brown & Morris (1890), after painstaking observations and careful experiments found that the storage starch of barley endosperms, deprived of their embryos, failed to fragment into simple reducing sugars after long incubation periods. In short,

as far as endosperm modification* in the intact grain was concerned, no embryo growth meant no modification, and as a consequence, no mobilisation of food reserves. This ability of the embryo to secrete diastase into the endosperm was attributed to the scutellar epithelial cells - a unitary layer of columnar cells at the embryo-endosperm junction.

Taken together with the usually neglected report of Haberlandt (1890), later substantiated by Brown & Escombe (1898), that the aleurone-layer also contributed to the total diastatic secretions which caused endosperm dissolution, the physiological ground-plan for embryo-endosperm inter-relationships was set, some three-quarters of a century ago. Although unextended in its entirety and correlative purpose, one aspect of this ground-plan immediately attracted much research. Between 1897 and 1941 a host of investigators have attempted to reach a final unequivocal decision on the site-epithelial or aleurone cells - from which hydrolytic enzymes emanated. The balance of opinion though not always convincing, favoured the epithelial cells, with the aleurone cells having a minor role - a situation not brought into question until after 1961 (Harris, review, 1961).

The question as to why the "feeble" secretory abilities of the aleurone cells were conditioned by the presence of the growing embryo was not answered until 1958, and heralded the

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* Modification is a convenient term used by maltsters to describe the changes which occur in the endosperm during the transformation of barley into malt.

first new and important contribution to embryo-endosperm inter-relationships since the last century.

Working in Japan, Yomo (1958, 1960) found that a water-soluble hormonal-like factor emanated from excised germinating barley embryos, passed through a semi-permeable membrane and possibly induced embryo-less endosperm halves to modify. This embryo factor caused a release of the important hydrolytic enzyme, α-amylase. Paleg (1960), aware of Hayashi's (1940) finding that gibberellin "A" increased α-amylase activity in germinating cereals, reported that gibberellic acid (GA_3) could substitute for Yomo's "embryo factor" in catalysing the dissolution of embryo-less endosperm slices.

The advent of gibberellic acid (GA_3) resulted finally in the cessation of systematic developmental studies on barley germination and subsequent seedling growth. Researches on "barley germination" continued along two main lines: that of the maltster, mainly for economic reasons and that of the biochemist, to whom embryoless endosperm pieces are merely tractable materials for studies on the mechanism of hormonal action - despite a sometimes misguided belief that they are working on germination.

The malting scientist, in a desired effort to curtail the growth of the embryo and/or to increase the rate of endosperm modification, sometimes judiciously employs the oddest array of physical and chemical factors towards this end. For

example, the growing grains may be subjected to a second immersion in water at ambient (Pollock, 1960) or elevated temperatures (Lubert & Pool, 1964) in order to kill the growing rootlets, or steeping may be accomplished not by immersion of the grain but by calculated increments of water (Reynolds & MacWilliam, 1966) which in total, are sufficient for growth to be induced and accompanying modification of the endosperm to be achieved.

With respect to additives, H_2S may be added to overcome dormancy (Pollock et al, 1955), gibberellic acid may be used to accelerate enzyme formation alone (Macey & Stowell, 1961) or after treatment of grains with sulphuric acid to kill the emerging rootlets (Kurth Malting Company patent, 1963); acetic acid and acetoacetates (Griffiths et al, 1965) and the fungal metabolite helminthosporol (Briggs, 1966) may produce effects similar to though less pronounced than gibberellic acid. Bromate may be used to minimise proteolysis and respiratory loss (Macey & Stowell, 1961) and formaldehyde (Macey et al, 1966) can be used to reduce the subsequent susceptibility of the beer to non-biological haze.

Here it is clear that interest in "barley germination" lies in the technological improvement of the finished malt and not in the botanical changes which the treated grain sustained. This is an understandable point of view but it is surely desirable also to appreciate the reasons for the changing behaviour of barley which is being malted in modified

conditions (with or without additives) and to relate any observed differences in the malt to the morphology, physiology and biochemistry of malt which has been grown in the usual fashion.

The biochemical line of study has been equally detached, being almost entirely confined to endosperm with the ultimate desire of "solving" the hormonal action of gibberellic acid on hydrolytic enzyme release from aleurone cells.

An important contribution is that of MacLeod & Miller (1962) who found that gibberellic acid acted upon living aleurone cells to catalyse the release of hydrolytic enzymes, such as, α-amylase and endo-β-glucanase. Briggs (1963) verified this finding and relegated about 86.5% of the α-amylase formed during germination to the endosperm portion of the grain. Varner (1964) and Varner & Ramchandra (1964) demonstrated that α-amylase thus formed actually possessed labelled amino acids (which were fed to the slices) within the protein molecule and not merely at the terminal portions of polypeptide chains, - an observation which is highly indicative that gibberellic acid may act to catalyse the synthesis of this and other hydrolytic enzymes released into the endosperm. However, although Varner & Ramchandra (1964) have suggested, from evidence using metabolic inhibitors such as actinomycin D and puromycin, that gibberellic acid may trigger-off the DNA- controlled synthesis of specific RNA molecules which in turn direct the synthesis of enzymically active proteins, there are other workers (MacLeod, 1967a) who

believe that, taken together, this explanation is not complete because actinomycin D in combination with gibberellic acid fails to inhibit α-amylase release from endosperm slices which had been pre-soaked. This implies that, in this case, the "specific RNA messenger" coding for enzyme protein was already formed before addition of gibberellic acid. It would appear that not only gibberellic acid (GA_3), of the familiar gibberellins, can release hydrolytic enzymes from endosperm slices. Griffiths et al (1967) and Coombe et al (1967) have shown that: A_1, A_2, A_4, A_5, A_6, A_7, A_8, A_9 and two derivatives of A_3, gibberellenic and allogibberic acid, can also catalyse the release of hydrolytic enzymes.

Concurrently with this body of evidence on isolated endosperm pieces, MacMillian et al (1959) and Radley (1967) have shown that the gibberellins, A_3, A_4 and possible A_7 may occur in immature barley grains. Duffus (1965) has suggested that in the matured, dry and ungerminated barley embryo, A_1 and possibly A_3 may be present.

However, although the possible natural occurence of gibberellins in barley grains may help to justify the addition of gibberellic acid to malting barley grains, it is not proof that the hormonal-like factor which emanates (Yomo, 1958) from germinating embryos is a gibberellin, although it is highly suggestive.

To conclude therefore, it cannot be said that the

correlative botanical aspects of embryo growth and development which must control germination and subsequent seedling growth are any better understood than seventy-five years ago; except for the contribution of the "embryo-factor" of (Yomo, 1958). The important inference here is that the growing embryo may hormonally control the synthesis and release of hydrolytic enzymes into its endosperm and that gibberellic acid can imitate this factor (Paleg, 1960; Yomo 1960; MacLeod & Miller, 1962; Varner & Ramchandra, 1964). One may also include as another inferred contribution that since excised scutella of germinating barley embryos can take up labelled glucose and convert it to sucrose (Edleman et al,1959), the scutellum of the intact grain does the same for glucose residues - this, incidentally, was suggested by Brown & Morris in 1890.

Mindful of the long neglect of systematic investigation on the physiological and structural aspects of the embryo of barley grains and the obviously dominant role the growing embryo plays in endosperm modification,I decided that this investigation was justified, not only for a better understanding of this specialised aspect of plant development but also so that practices such as the Malting and Agricultural industries may take a fuller and possibly more rewarding interest in the barley grain.

Historical Survey

Brown & Morris (1890) used the extensive momograph of Holzer & Lerner, Beitrage zur kentniss der Gerste (see Brown & Morris 1890) as the anatomical basis for their physiological studies. At the commencement of their investigations Brown, a most imaginative botanist, was reported to have made the following statement, which, in a narrower context, is equally true today: "It has been a matter of wonder to me that, having gone so far, these observers should not have extended their botanical studies to investigating the physiological meaning of what they saw and drew so well." In rectification of that situation Brown & Morris (1890) left a host of important observations which, although now taken for granted, laid the basis for nearly all subsequent studies on physiological and biochemical changes which accompany growth of barley embryos.

They recorded that the embryo's food store sufficed for its germination and that the axis and scutellum of the intact grain were able to accumulate starch granules by 24 hr. germination time. This starch accumulation increased rapidly with the commencement of endosperm modification. Embryos, excised from their endosperms after 2 hr. soaking accumulated only a "paltry" amount of starch granules when grown on water; however, when supplied with "cane sugar", maltose or dextrose, starch accumulation equalling that of the intact embryo occurred. Relating this observation to the dry weights of excised embryos grown on water, compared with that of embryos with attached

endosperms (taking note of the loss in dry weight of the endosperms) they concluded that the growing embryo was dependent on the endosperm for its nutrition and that the granular starch of the axis and scutellum was mainly derived from that source.

Continuing their observations they noticed that immediately after root emergence the cytoplasm of the scutellar epithelial cells changed from granular to pellucid, a situation reminiscent of secretory cells in the animal kingdom. The epithelial cells then protruded into the starchy endosperm by individual elongation. Endosperm dissolution commenced over the scutellar epithelial surface (embryo-endosperm interface) at the onset of protrusion and progressed uniformly away from the embryo-end of the grain, except for a slightly more rapid progression on the dorsal (non-furrowed) side of the endosperm. This disproportionate progression of dissolution was attributed to the possible "thiness" of the endosperm storage cell walls on this side of the grain.

From observations, Brown & Morris proceeded to experiments and showed, using novel dissection techniques, that the excised embryos of barley grains secreted a diastase which, in solution, degraded starch and, when the epithelial cells were removed or damaged by gentle scraping over the epithelial surface with a micro scalpel this diastase secretory ability was impaired. Furthermore, when excised embryos were placed on solid starch paste or foreign endosperm pieces, liquefaction occurred.

These findings convinced Brown & Morris that the scutellar epithelial cells were responsible for endosperm dissolution by virtue of the fact that they secreted diastase into the endosperm of the intact grain. These same cells, from strong inference, appeared also to function in absorbing soluble endosperm nutrients for early embryo growth.

An additional observation of sheer genius was their record that during endosperm dissolution in matured grains starch grains were either "pitted" or "cracked" as opposed to the "smooth" dissolution of starch grains, removed from an area at the embryo-endosperm junction (the Intermediate layer) during maturation of the barley grain on the parent plant. Here, remarkably enough, was the first record of an empirical difference between β and α-amylase.

Now, Haberlandt (1890), working concurrently in Germany published his findings that in rye grains the aleurone cells, could secrete diastase into the starchy endosperm. Brown & Morris (1890) in their addendum stated that from their investigation isolated barley aleurone or endosperm pieces, with attached aleurone cells, always failed to show any independent diastase secretion. Only the embryo had this ability, they emphasised. However, Haberlandt's proposal of aleurone participation in the diastase pool hinged on the necessary presence of the embryo, to which, like (Brown & Morris, (1890) he allocated the dominant role of diastase secretion. None the less a few years later, Brown & Escombe (1898) were able

to show that the aleurone layer of barley grains was also capable of "rather feeble" diastatic activity.

Possibly because of the well-earned authority Brown and his co-workers had in this field of cereal germination subsequent workers concerned themselves mainly with obtaining unequivocal proof that the scutellar epithelial cells possessed the dominant role in endosperm dissolution, for example: (Mann, 1908; Mann & Harlan, 1916; Horning & Petrie. 1927; Shade, review, 1937; Dickson & Shands, 1941; Dickson & Buchart, 1942; Engel, 1945; Engel & Heins, 1947; Kirsop & Pollock, 1957; Dure, 1960a,b; and Harris, review, 1961).

The conclusions, on the secretary role of the scutellar epithelial cells, reached by some of the above workers were sometimes inferred, (Mann. 1908; Shade, review, 1937; Dure, 1960a,b - indirectly from unverified work on maize; and Harris, review, 1961). However, direct histochemical and anatomical evidence came from Mann & Harlan (1916); Horning & Petrie (1926) and Dickson & Shands (1941), while biochemical support came mainly from analysis on the proximal and distal halves of the germinating grain. Dickson & Buchart (1942) localised amylolytic activity mainly in the embryo-half of the germinating grain; while α-amylase, but not β-amylase, was present in the scutellum (Engel, 1945); proteinases were also localised in the scutellum (Engel & Heins, 1947). Kirsop & Pollock (1958), in contrast to Engel (1945), recorded a decline in the β-amylase

content of endosperms from which germinating embryos had been removed. With regard to these biochemical reports it is clear that these workers were merely comparing one half of the grain which had modified with the other half which had not - in fact, no direct proof that the epithelial cells were involved was deducible from their results.

The histochemical and anatomical evidence in support are also open to question. For example, Mann & Harlan (1927) drew their conclusions from only transverse sections of the germinated grain, thus casting doubt on the accuracy of their conclusion while Dickson & Shands (1941) went to the other extreme of drawing their conclusion from small areas of longitudinal sections which had already undergone extensive modification. Horning & Petrie (1927) from histochemical evidence, concluded that "tiny migratory mitochondria particles" passed from the scutellar epithelial cells to the endosperm. These "particles" were therefore the instrument of endosperm modification. However, there seems to be no available evidence at the moment to substantiate the diastatic properties of mitochondria, and, with the low resolution microspope these workers possessed it might have been difficult to ascertain whether or not the observed "particles" were not just inside and outside those epithelial cells, with no migratory properties or modificatory role.

To make a brief mention of the modification pattern as a whole; only Mann & Harlan (1916) and Miller (1962)

have put forward ideas for the cause behind the disproportionate pattern of modification observed by Brown & Morris (1890). Mann & Harlan suggested that the reported thinner cell walls on the dorsal (non-furrowed) side of the endosperm allowed more rapid diffusion of enzymes from the scutellar epithelial cells, while Miller (1962) suggested that the number of aleurone cells on the dorsal side of the endosperm were in greater abundance than on the furrowed-side. Although Miller's idea seems more reasonable, both ideas require anatomical verification.

The shaky merits of the above mentioned investigations could be attributed to the preconception that the scutellar epithelial cells should secrete hydrolytic enzymes because their position in the grain coincides with the accepted "pattern of modification" given by Brown & Morris (1890).

At the present time it is still believed that these cells, although on a reduced scale, make a useful contribution to the hydrolytic enzymic pool (MacLeod & Miller 1962; Briggs 1963; 1964; Laufer 1964). With the same unfounded belief, Nieuwdrop (1963) and Nieuwdrop & Buys (1964) have made detailed ultrastructural interpretations on the function of sub-cellular organelles in the scutellar epithelial cells. The continued acceptance of this so far unsubstantiated belief could lead to wasted research and, therefore, in respect to the whole modification phenomenon, early experimental clarification on the ability of scutellar epithelial cells to secrete hydrolytic enzymes seems

desirable.

None the less, with this line of research "assumeably settled", the discovery (Yomo, 1958; 1960) that growing barley embryos, separated from their endosperms by a semipermeable membrance in liquid culture, could effect dissolution and increase the α-amylase content of these endosperms, came as a welcomed advance.

Although this hormonal-like factor has not yet been characterised chemically or biologically it has led to the tentative conclusion that it may be gibberellic acid-like in action (Paleg, 1960) and acts on the living aleurone cells of endosperm slices (MacLeod & Miller, 1962) to catalyse the synthesis (Varner & Ramchandra, 1964) and release of α-amylase and possibly other hydrolytic enzymes as well.

These findings (1958 - 1962) were instructive as to how the embryo exercised a unique control in the attainment of its own endosperm food reserves, and should have led to structural and physiological researches on the growing embryo. However, the impetus for research remained with the aleurone cells for reasons already mentioned, and, as a consequence, correlative studies on barley germination progressed little from that undertaken in the last century. This has resulted in conflicts between old and new experimental findings in addition to a gross neglect of many novel observations left by Brown & Morris.

For example: We now have the situation where the aleurone cells (endosperm) account for 86.5% of the α-amylase

produced after 7 days "germination time" (Briggs, 1964) yet the pattern of modification, as indicated by past and present research (Brown & Morris, 1890; Laufer, 1964), still seems to implicate the scutellar epithelial cells as a major site of hydrolytic enzyme production. There is as yet no adequately substantiated explanation for the disproportionate pattern of endosperm modification. The nutritional inter-relationships between the growing axis and scutellum in early germination, when no food supply from the endosperm is available, has not been examined since first studied by Brown & Morris (1890). Neither is anything known about hormonal actions in the growing embryo nor is it known where in the tissue complexes of the embryo the proposed gibberellic acid-like factor emanates, or more provocatively, whether it is, at all, a gibberellin.

Being confronted with such a chaotic situation it was decided that the available literature should serve mainly as a guide for elucidation of "pitfalls" and to illuminate suspect assumptions and analogies which, in biology, can be dangerous things indeed. Since the greatest progress in research on the grain has been made on the functioning of the aleurone cells and the release of hydrolytic enzymes it therefore seemed desirable that the proposed investigation on the embryo should be closely related to physiological aspects of endosperm modification.

The experimental approach to this investigation was modelled on that of last century's pioneers where observations

preceded experimentation. This approach seemed the most useful in a situation where deductions and assumptions from past work could lead to wrong conclusions.

In conclusion, even at this early stage of the investigation, I found it possible to inform my "fellow student" that the impetus for research on the embryo declined mainly as a result of the excellence and comprehensiveness of the work of Brown & Morris and Haberlandt; however, for the future, the embryo will be providing exciting problems long after the already saturated interest in the aleurone cell overspecialises and becomes extinct.

SECTION I

HISTOCHEMICAL, ANATOMICAL and ULTRASTRUCTURAL INVESTIGATION

INTRODUCTION

Surprisingly, few photomicrographs or modern descriptive accounts of the structure of the embryo of matured barley grains appear to be available in the botanical literature, though other cereals (MacMasters, 1965) particularly wheat and maize are well documented. There is also a similar lack in information on the overall histochemical changes which take place during the early stages of seedling development and growth.

Brown & Morris (1890) based most of their physiological interpretations of germination and seedling growth on the anatomical ground-plan of Holzer & Lerner (see, Brown & Morris 1890) which described only the dry ungerminated grain.

Since it was our intention to investigate the embryo over a period of 72 hrs. after the commencement of germination, it appeared desirable not only to verify the overall structure of the dry embryo, but also to follow important changes in its anatomy and morphology over this growth period.

Now, it is known that the cotyledons of many seeds contain large reserves of food materials which become mobilised, on germination, to support seedling growth (Mayer & Poljakoff-Mayber, 1963). Whether barley grain scutellum - the cotyledon of cereal grains (Brown, 1965) - functions in the same capacity is not known. Histochemical analysis may throw some light on this matter. In addition, the acquired histochemical information may also be useful in contemplated studies on the controversial role of this organ in barley germination and seedling growth.

In this section, therefore, interest was not in the function of the embryo per se but in the acquisition of an anatomical and histochemical ground-plan for further physiological and biochemical studies.

Ultrastructure:

It was decided that if the anatomical and/or histochemical studies revealed any information which appeared to need further resolution, judicious electron microscopic analysis was to be attempted. The primary aim being the acquisition of relevant ultrastructural information which could possibly assist in physiological interpretations of germination and subsequent seedling growth processes. Mindful of this aim, in addition to the temperamental age (Akashi TRS 50 EI) and the tight interdepartmental schedule of the electron microscope the sometimes "wasteful practice" of collecting and presenting micrographs merely because they were available or "looked interesting" was avoided.

In short, it was hoped to use this ultrastructural information; not only to illuminate crude histochemical or anatomical findings but also to lend relevant guides to physiological or biochemical lines of study if they were to materialise.

MATERIALS AND METHOD:

Dehusking:

Unless mentioned otherwise all the barley grains (Hordeum vulgare L var. Proctor, 1964) used in this investigation were dehusked by 50% sulphuric acid thus: About 500 g. of dry barley grains were placed into a 3 litre beaker, covered with cooled 50% sulphuric acid and allowed to stand for about 3 hrs. in a sink of water. Thereafter, excess of sulphuric acid was poured off and tap water allowed to wash the grains. The separated husks were washed away and the dehusked grains remained. After 5 min. vigorous washing anhydrous calcium carbonate was added with gentle agitation and the washing continued for another maximum period of 10 min. With the grains neutral to litmus paper they were quickly dried on sterile blotting paper and stored away at 5°C for use.

Grains dehusked in this way showed no deleterious effects on subsequent germination. However, careless dehusking resulted in damage to the coleorhiza and roots. When sulphuric acid-dehusked grains were compared with hand-dehusked grains the only visible difference in germination was that quicker emergence of roots occurred for the acid-treated grains. Light microscopic examination revealed that the outer coverings (pericarp testa) of the acid-dehusked grains were somewhat fragmented.

Selection of grains for germination:

Only visually sound grains with endosperm diameters between about 2-3 mm. were used. In short, visual selection on a

quasi-objective basis was employed.

Embryo germination

For histochemical and anatomical analyses grains were evenly spaced and germinated at 25°C in the dark on two pieces of distilled water-saturated filter paper rings in 7 inch petri dishes. After appropriate germination times samples were removed, and, depending on tests, were thinly sliced or vertically cut in half by a clean new razor blade (Fresh Hand Sections); or fixed in Formalin (40% commercial formalin)/Glacial acetic acid/Ethyl alcohol (50%), 90/5/5(V/V), F.A.A;or cleared for Whole-Mount examination.

Although some of the following histochemical methods are given in Jensen (1962) and Baker (1963), slight variations of these schedules sometimes proved more suitable for the tissue being used. It was therefore considered necessary to describe these methods in detail.

HISTOCHEMISTRY - Light microscopy:

A. Fresh Hand Sections

Procedures: 0 hr. embryos only

(a_1) Total Carbohydrates - Insoluble Polysaccharides - Periodic Acid - Schiffs (PAS) reaction:

(VLS) = Vertical Longitudinal Sections.

(1) Stain (VLS) of embryo for 5-30 min. in 0.5% periodic acid

(2) Wash in running water for 10 min.

(3) Stain in Schiffs reagent for 10-15 min.

(4) rinse in water

(5) rinse in 2.0% sodium bisulphite for 1-2 min.

(6) wash in running water

(7) dehydrate and mount

Reaction: Insoluble polysaccharides stain intense purplish red. Soluble polysaccharides washed out.

(a_2) Cellulose - Iodine - Pot.Iodide-Sulphuric acid method-

(1) Place embryo (VLS) in IKI solution (2% KI to which 0.2 g. I_2 is added) for 15 min.

(2) add one drop of 65% sulphuric acid and allow to diffuse under cover glass

Reaction- Walls containing cellulose stain dark blue; lignin, orange.

(a_3) Callose - Analine Blue - Visible light method for β-1:3 linked glucosans such as Callose:

(1) Place embryo (VLS) in 0.005% solution of analine blue in 50% alcohol for 24 hr.

(2) rinse sections in water and mount in gelatin

Reaction: Callose should stain blue.

(a_4) Pectin - Ruthenium Red method-

(1) Place embryo (VLS) into aqueous ruthenium red (1:5000) until red appears (12 hr.)

Reaction: Pectin stains red.

Pectin - 0.5% Commercial Pectinase:

(1) Place embryo (VLS) into 0.5% pectinase solution, at 37°C for 24 - 48 hr.

(2) mount sections on clean slide, cover with cover-slip, tap gently and examine.

Reaction: cells separate.

B. Fresh Hand Sections and Half Grain (Cut longitudinally) Examination.

Procedures- 0. 12, 20, 24, 48, 72 hr. growth periods

(b_1) Lignin - phloroglucinol test:

(1) Mix together one drop of a saturated solution of phloroglucinol and one drop concentrated hydrochloric acid on a slide

(2) add embryo (VLS) and examine.

Reaction: Lignin stains red.

(b_2) Total Lipids - Sudan III or Sudan Black in 70% alcohol:

(1) Place embryo (VLS) in 50% ethyl alcohol for 5 min.

(2) stain in sudan III or sudan black for 15 min.

(3) wash quickly with 50% ethyl alcohol, about 1 minute

(4) mount in dilute glycerol.

Reaction: Sudan III stain Lipids red, Sudan Black stains them black.

(b_3) Starch - Iodine - Potassium - Iodide (0.2 g. of iodine in 2 g. potassium iodide to 100 ml.):

(1) Mount embryo (VLS) in a drop of iodine solution on slide

(2) add a drop of dilute glycerine solution and examine.

Reaction: Starch grains stain blue-black - newely formed starch red to purple.

(b_4) Total Protein - Ponceau-De-xyledene, 0.1%:

(1) Add embryo (VLS) stain for 15 min.

Reaction: Protein bodies supposed to stain red

Total Protein - Croceine orange (4.6 g. Croceine orange, 100 ml. distilled water, 200 ml. Glacial acetic acid, 1 ml. Proprionic acid; mixed held at 90°C for 30 min. cooled and diluted as necessary):

(1) Add embryo (VLS), stain for 10 - 15 min.

Reaction: Protein bodies orange.

Total Protein - Ninhydrin-Alloxan-Schiffs, Fixed microtome sections used - 10 to 15μ (method given below in Anatomy section):

(1) Embryo (VLS), deparaffinze on slide with xylene

(2) place slides with sections in petri dish, cover with 0.5% ninhydrin in absolute alcohol at 37°C for 24 hr.

(3) rinse with two changes of absolute alcohol, then distilled water,

(4) place in Schiffs reagent for 20 min.

(5) rinse in water, add drops of 2% sodium bisulphate for 2 min.

(6) wash with drops of tap water for 20 min.

(7) dehydrate, and mount in canada balsam.

Reaction: Protein bodies appear pinkish-red.

Deamination control:

(8) place deparaffinized sections in 60% sodium nitrate: 1% acetic acid, 1:3 (V/V)

(9) repeat from (2)

Reaction: No pinkish-red; if this colour occurs then compounds other than proteins having α-amino and α-carboxyl groups are present.

C. Mitochondria test - Janus Green B, 0.005%:

(1) Add fresh hand sections of embryo with endosperm attached (VLS) to stain, until blue colour develops (12 hr.)

Reaction: Mitochondria and especially protein bodies are supposed to stain blue.

D. Length of Scutellar Epithelial cells:

Measured with the aid of a slide micrometer at X 400.

E. Dissolution of Endosperm - Half grain examination:

Total extent of dissolution was assessed visually after grains were cut in two halves by a vertical longitudinal slice, and gently sprayed with water to wash away dissolved area.

ANATOMY - Light microscopy:

The fixative Formalin/Acetic acid/alcohol (F.A.A.) was used because it left barley embryo tissue as life-like as possible - nuclear and cytoplasmic inclusions were stabilised and the fixation artifact of "spongeworks" in the protoplasm was not observed.

Formaldehyde is the most important constituent of this fixative. It not only fixes the nuclear material and stabilizes lipid bodies but it also hardens protein and as a result fixes them. F.A.A. appeared to leave the tissue in a state where basic dyes are rapidly taken up by acid inclusions like storage proteins and nucleic acids.

Stains

Heidenhain's heamatoxylin + Eosin was the double stain used. Heidenhain's haematoxylin is the principal basic dye and dyes nucleoli, nuclei, mitochondria and protein strongly blue-black. This gave, against the pinkish background of the weekly acid Eosin dye, excellent contrast for photomicrography.

Procedures:

Preparation of permanent (VLS) sections of barley embryos were made at 0, 12, 20, 24, 48, 72 hr.

Fixation:

Distal halves of whole grains (Embryo + Endosperm) were removed by a sharp scalpel at appropriate germination times, and fixed in F.A.A. for at least 24 hr. The cut surface on these "half grains" facilitated solvent and solute penetration.

Dehydration:

After fixation, tissues were removed and dehydrated in tertiary butyl alcohol (TBA), ethyl alcohol, and water series at 25°C.

(1) 50 ml. H_2O, 40 ml. 95% alcohol, 10 ml. T.B.A.: 2 hr.
(2) 30 ml. H_2O, 50 ml. 95% alcohol, 20 ml. T.B.A.: 2 hr.
(3) 15 ml. H_2O, 50 ml. 95% alcohol, 35 ml. T.B.A.: 2 hr.
(4) 45 ml. 95% alcohol, 55 ml. T.B.A.: 2 hr.
(5) 25 ml. Absolute alcohol, 75 ml. T.B.A.: 2 hr.
(6) 100% T.B.A.: 2 hr.

Paraffin Infiltration and Embedding:

Tissue from 100% T.B.A. then transfered to:

(1) 50% paraffin (56°C) + 50%, 100% T.B.A.: 12 hr.: 60°C
(2) 75% paraffin (56°C) + 25%, 100% T.B.A.: 12 hr.: 60°C
(3) 100% paraffin : 24 hr.: 60°C

Tissue were then blocked-out in paraffin blocks in the usual way; one, "half grain" per block.

Sectioning:

Vertical longitudinal sections (VLS) were cut at 10 - 15μ on a Cambridge rocker microtome.

Staining:

(1) Sections were deparaffinised in xylol and taken down to water thus- absolute alcohol, 90% alcohol, 70% alcohol, 50% alcohol, 40% alcohol, 30% alcohol (10 min. each) and then to distilled water.

(2) stain in Heidenhain's haematoxylin for 25 min.

(3) wash in running tap water to blue and destain in 2% ferric chloride with constant watching if necessary,

(4) wash thoroughly in running tap water,

(5) take sections up alcohol series to absolute alcohol,

(6) counter stain for 1 - 2 min. with 0.5% Eosin in clove oil,

(7) differentiate in a solution of equal parts clove oil/absolute alcohol,

(8) place in three changes of xylene (15 min. each)

(9) mount in canada balsam.

Reaction: Chromosomes, nuclei, nucleoli, plastids, mitochondria and protein bodies stain dark-blue. Cytoplasm and cell-walls, light pink.

Safranin - Fast Green stain was also used on fixed embryo materials primarily to locate vascular tissue.

(1) Deparaffinise as above and take up the alcohol series to 50% alcohol,

(2) stain in alcoholic safranin for 2 hr. (1% safranin in 95% alcohol and dilute with an equal amount of water for use),

(3) destain if necessary with acidified 70% alcohol, then stop reaction immediately 95% alcohol,

(4) counterstain with fast - green (0.5% solution in 50% clove oil/50% alcohol) for 30 seconds,

(5) differentiate fast-green by placing in a mixture of 50% clove oil/25% absolute alcohol/25% xylene - two changes of 10 min. each,

(6) clear in two changes of xylene, 15 min. each,

(7) Mount in canada balsam.

Reaction: Vascular elements (lignified cell walls) and cuticle stains red. Cytoplasm stains dark green.

Whole Mount Preparation - light microscopy

Whole embryos from 72 hr. germinated grains were removed and placed in 5% potassium hydroxide for 48 hr. at 60°C, then gently washed with distilled water to remove hydroxide, cleared in 80% chloral hydrate for 48 hr., stained in basic fuchsin and examined and photographed in dilute glycerol (.88 ammonia may be used to remove excess of basic fuchsin). For photography it was necessary to dissect away the entire shoot and parenchyma cells that obscured the vascular elements of the scutellum. The fuchsin dye stained these vascular elements red.

Photo micrographs were taken where facilities presented themselves: Queen Elizabeth College, London University; Taxanomy department, Edinburgh University and The Heriot-Watt University.

ULTRASTRUCTURE - Electron microscopy: 0, 12, 24, 48, 72 hr, growth periods.

Since good electron micrographs are available on the ultrasturcture of the scutellar epithelial cells during germination and seedling growth (Nieuwdrop & Buys, 1964), it was decided to confine the studies mainly to the scutellum, and, to a less extent, to that tissue region between the acrospire and the roots, termed the scutellar node.

Preparation of material for fixation

Grains from appropriate growth periods were selected for soundness. With the aid of a sterile 1 mm. cork borer-like instrument a plug of tissue was taken from the scutellar node region. This included cells from the: Scutellar node, Vascular system, Scutellum, Epithelial cells and, Endosperm, when this latter tissue was in association with the embryo - for example, at 0-hr.

Here, 0-hr. means dry ungerminated grains.

Formulae and Schedules for Operations-

Fixation: The two different fixation techniques used were chosen mainly because of their simplicity and there is some information as to the possible manner in which they fix some of the cytoplasmic inclusions.

Luft's potassium permanganate fixative (Mercer & Birbeck, 1961) is supposed to react strongly with nucleic acids, proteins and phospholipids but not

with polysaccharides or fats. Therefore, limiting membranes, the important outlines of subcellular inclusions, are clearly defined. On the other hand, however, Caufield's sucrose balanced osmium tetroxide fixative reacts strongly with fats and phospholipids. In both instances the cell wall should appear electron transparent.

Fixation formulae and schedule:

Buffer solutions; Solution A

Sodium Veronal (sodium bicarbonate)	14.7g.
Sodium Acetate $3H_2O$	9.7g.
Distilled water	500 g.

Kept in refrigerator, 5°C

Solution B

Hydrochloric acid	0.1N

Lufts Permanganate Fixative:

Solution A	20 ml.
Solution B	22 ml.
Potassium permanganate	2 g.
Distilled water	50 ml.

Care was taken to dissolve all the permanganate and the fixative was adjusted to pH 7.4 and stored at 5°C until ready for use.

Canfield's Balanced Osmium Fixative:

Solution A	5 ml.
Solution B	5 ml.
2% OsO_4 solution	20 ml.
Sucrose	0.015 g/ml
Distilled water	10.0 ml.

pH was 7.45 and the solution was stored at 5°C until ready for use.

The plugs of embryo tissue were quickly placed into cold potassium permanganate or osmium tetroxide fixative in small 1½" x ¼" glass tubes. The tubes were then closed and placed into the freezing compartment of the refrigerator: potassium permanganate tubes for 35 or 80 min. and osmium tubes for 2½ hr. Fixatives were then gently poured off tissue plugs and the latter given quick washes with 25% alcohol to remove excess surface stain. The tissue was then ready for dehydration.

Dehydration:

(1) 25% alcohol 15 min.
(2) 50% alcohol 15 min.
(3) 75% alcohol 30 min. (overnight if necessary)
(4) Absolute alcohol 2 hr.
(5) Xylene 30 min.
(6) 50:50 xylene: araldite mixture 30 min: 37°C.
(7) Araldite mixture (37°C) 30 min: 37°C.
(8) Araldite mixture (37°C) 30 min: 37°C.
(9) Tissue to gelatin capsules (size 00), filled with araldite mixture (37°C) and orientated horizontally

at the base of capsule.

(10) Capsules covered and allowed to polymerise for 48 hr. at 60°C.

(11) Polymerised capsules for different growth times, 0, 12, 24, 48 and 72 hr. were then filed and stored at room temperature for sectioning.

Schedule for Araldite mixture:

Araldite M	20 ml.
Resin 964B	20 ml.
Accelerator 964C	0.6 ml.
Dibutyl Phthalate	2.0 ml.

Accelerator volume is critical. Mixture made fresh when required.

Sectioning:

Polymerised blocks were trimmed and sectioned onto water at 300 - 500 A° by a Cambridge ultramicrotome. Chloroform vapour was sometimes used to flatten-out sections which were lifted onto copper grids (Athene, type 200) and coated with a thin layer of carbon on the upper surface only. The sections were examined and photographed in an Akashi TRS 50 EI electron microscope fitted with a modified D.C. filament supply and a 50 k.v. E.H.T.

RESULTS AND DISCUSSION

HISTOCHEMICAL ANALYSIS

Despite the simplicity and obvious limitations of most histochemical techniques, the observations listed in Tables I and II proved very interesting. Table I shows that the cell wall of the storage parenchyma cells of the scutellum appeared to be mainly cellulose. Pectinaceous material was located in the middle lamella region. Although the cell wall of the epithelial cells was positive for cellulose no pectinaceous material was detected in the middle lamella region and, unlike the storage parenchyma cells of the scutellum, these cells failed to separate when treated with pectinase.

MacLeod & McCorquodale (1958) suggested that the dead endosperm cells of barley and Bromus were not cemented together by pactinaceous material, as is usually the case in higher plants, but by proteinaceous substance. Unlike these endosperm cells papain failed to effect separation of the epithelial cells and thus the possibility of a 'third' cementing material in barley grains seems not unlikely.

No positive indication of β-1:3 linkages (e.g., callose) was observed for the embryo tissue despite a weak response on the cell wall of endosperm storage cells, which have been reported by MacLeod & Sandie (1961) to contain hemicellulosic material. Johnston (1964) suggested that the dye analine blue reacted best with pure β-1:3 glucans but poorly with mixed hemicelluloses like that found in barley endosperms. Therefore it is quite possible

that the negative result obtained in Table I for β-1:3 linked polysaccharides might have resulted from inability of the dye to detect the material sought.

Table II depicts histochemical time-course changes of certain important storage and structural components whose integrity was strongly influenced during the 72 hr. growth period of the grain.

Lignification appeared in the embryo before 20 hr. and was confined to provascular cells which, in the scutellum, were located towards the acrospire-end. By 24 hr. extensive vascular contact between the scutellum and the growing axis was established.

Extensive fat deposits in both the scutellum and the epithelial cells declined at the inception of seedling growth and the epithelial cells lost their deposits after 24 hr. Protein deposits (discrete bodies, 1-2μ diameter) were in less abundance and disappeared by 24 hr. The important morphological feature of the epithelial cells was their ability to elongate rapidly between 20-72 hr. Scutellar parenchyma cells showed no signs of elongation.

In agreement with Brown & Morris (1890) there was a close association between epithelial elongation and the extent of modification in the adjoining endosperm. However, at this stage, it was impossible to determinate whether modification was being influenced by these elongating epithelial cells or vice versa. Despite this, it was observed that endosperm modification appeared to commence at the acrospire-end of the epithelial-endosperm

interface and not over the entirety of this tissue association (Brown & Morris, 1890; Laufer, 1965), but further evidence on this matter is required.

Table II also shows a close parallel between the appearance of starch granules and the disappearance of fat deposits from especially the scutellum. Although it is tempting to suggest that here barley scutellum was behaving as other oil-bearing seeds, where fat deposits are reported to be converted to starch (Kornberg & Beevers, 1957; Carpenter & Beevers. 1959; Marcus & Velasco, 1960; Cherry. 1963) careful examination showed that there was also a parallel between endosperm modification (viz., possible transfer of hexose residues to the scutellum) and starch accumulation. However, mindful of the intended exploratory nature of this histochemical section and the accepted limitations of histochemical techniques in general, this fat-starch parallelism, for the moment, must remain as such.

At the completion of this histochemical investigation two points were assumed to be of interest with respect to further work.

Firstly, the observations in Tables I and II suggested that general metabolism of important structural and reserve materials commenced after germination was completed (i.e., after 17 hr.)- note the rapid coleorhizal and root elongation over this period. The second point relates to uncertainties raised in Table II. If the cementing material between the epithelial cells is neither pectin or protein, what is it and how does it feature

in the exceptional elongative properties of these cells? Is lignification an orderly process in the acrospire end of the scutellum and could there be any significance in this one-sided orientation of vascular elements? Could the apparently close association between epithelial cell elongation and endosperm modification be a direct result of hydrolytic enzyme secretion by these cells (Brown & Morris, 1890; Laufer, 1965) or is the association merely fortuitous with respect to this function really signifying other physiological events? And, finally, was the close parallelism between fat disappearance and the appearance of starch granules related to the well documented cases of oil-bearing seeds (e.g., castor oil and peanut) where the obligatory participation of the glyoxylate pathway supposedly mediated this conversion (Kornberg & Beevers, 1957; Carpenter & Beevers, 1959; Cherry, 1963)?

In my estimation it appeared that these important but inconclusive points could be at least partially resolved by the use of techniques which would give greater structural resolution and thus facilitate more meaningful interpretation of the acquired data.

This therefore led to anatomical and electron microscopical analysis on barley grains during germination and seedling growth.

ANATOMICAL ANALYSIS

As stated before in the Introduction, surprisingly few photomicrographs or modern discriptive accounts of the structure of the barley embryo appear to be readily available in the botanical literature, though other cereals (MacMasters 1965), particularly wheat and maize, are well documented. Although it was my intention to rectify this situation for barley grains, information on the functional implications of structural changes, crystallized from histochemical analysis, was also being sought.

A vertical longitudinal section from dry ungerminated grain is shown in Fig. I. Four structurally distinct regions can be seen: (i) the root system with a primary root R^1 (one of usually five) covered by the coleorhiza and, R^2, one of the subsidiary root initials; (ii) the acrospire, which includes the coleoptile and, inside this cylindrical organ, the stem apex and leaf primordia; (iii) the scutellar nodal region between root and shoot and laterally (iv) the scutellum, which a clear distinction can be drawn between the superficial single layer of flat topped column-like epithelial cells and the main bulk of the organ. The body of the scutellum consists of irregular non-vacuolated parenchyma cells which lack distinguishing features and appear to be storage cells. Permeating the acrospire-end of the scutellum is a centrally orientated strand of elongated provascular cells. This strand joins the provascular system of the axis at the scutellar node. The embryo is separated from the storage cells of the endosperm by a bold layer of apparently squashed endospermic

material and is labelled the Intermediate layer. The aleurone layer is limited to the periphery of the grain and does not in any way continue as a 'separating layer' between the embryo and endosperm as drawn by Cooke & Pitt (1964).

It is not my intention in this section to engage in controversy relating to the phylogenetic interpretation of different parts of the embryo as the suggestions of Brown (1963) on this matter are well documented: I therefore equate the scutellum with a single cotyledon, the coleoptile as an embryonic organ or innovation in its own right and the coleorhiza as the residual base of the proembryo, in which the primary root differentiates endogenously. The scutellar nodal region is treated as a single connective structure between root and shoot.

Plate 1 shows the embryo at a pre-germination condition, i.e., 12 hr. after initial wetting of the grain. The most elongated of the five primary roots is about to escape from the coleorhiza which has completed its own elongation. Fragments of the pericarp-testa can still be seen attached to the embryo.

Plate 2 shows the embryo at a post-germination condition, i.e., 24 hr. after initial wetting of the grain. Note that the foliar shoot is still encased in the coleoptile, both organs elongating at about the same rate.

In both plates the endosperm with its intermediate layer has moved away from the embryo. However, the only significance in this is that the endosperm-embryo association is weakened by soaking and the tensions of microtome sectioning effected

the separation. Undoubtably here lies the basis for the easy separation of embryo from endosperm after dehusked grains have been soaked for 2 hr. This technique for the separation of embryo from endosperm was discovered by Brown & Morris (1890) and is still used today. None the less in Plate 3 it is evident that careful microtoming of a 20 hr. seedling still leaves intact the association between embryo and intermediate layer.

Elongation of Epithelial Cells

Plates 3, 4 and 5 depict morphological changes of the epithelial cells during elongation. Soon after 20 hr. a wave of elongation of these cells quickly spreads over the entire concave surface of the scutellum. Nucleoli are prominent during the early stages of elongation and the nucleus of the elongating cell becomes more prominent and ellipsiod-like.

As observed by Brown & Morris (1890), during the elongation process, the epithelial cells became separated from each other, changed their cytoplasm from granular to pellucid and their shape from rectangular-like to finger-like.

Owing to the small dimensions of these cells in addition to the fact that Nieuwdrop & Buys (1964) have made a concise electron microscopic study of ultrastructural changes of these cells during germination and seedling growth, no information on the cellular inclusions was recorded. However, Nieuwdrop & Buys (1964) in their micrographs showed that fat and protein declined and the appearance of starch granules roughly coincided with that observed in the histochemical analysis; in addition, these

micrographs of Nieuwdrop & Buys indicated that the epithelial cells elongated by tip-growth as opposed to stretching of their lateral walls.

Anatomical time-course study of Endosperm Modification

Plates 6, 7, 8, 9 and 10 comprise a series of virtually self-explanatory photomicrographs on the pattern of endosperm modification which occurred during barley germination and seedling growth (0 - 48 hr.). Interest was confined to the acrospire-end of the endosperm-embryo junction because intense and lengthy observations on fresh and fixed grains, sliced through a variety of planes, left me convinced that this area of the grain was important with respect to the entire pattern of endosperm modification.

Plate 6 shows a vertical longitudinal section of the unimbibed grain. Here it is evident that the intermediate layer separates the scutellar epithelial from the starch endosperm - note the position of the aleurone layer, which possesses three cells at its greatest width.

Plate 7, for the 20 hr. seedling, shows the first signs of endosperm modification. There is a definite weakening of the endosperm tissue under the aleurone layer, presumably from enzyme attack; however, note that no apparent attack on the intermediate layer is evident.

Plate 8, for the 24 hr. seedling, shows an obvious removal of endosperm tissue from under the aleurone cells, while the remainder of the intermediate layer and its associated endosperm

tissue remains intact. This can be more fully appreciated in Plate 2 where the entire 24 hr. seedling is shown.

Plate 9, for the 48 hr. seedling, shows extensive depletion of endosperm material from that area immediately below the aleurone layer. However, note the pronounced remains of the intermediate layer although it is directly opposite elongated epithelial cells. Plate 10 shows the scutellar heel (i.e., the furrowed surface of the grain) of the same grain shown in Plate 9. Here, despite obvious elongation of the epithelial cells no modification is apparent.

Available micrographs for the 72 hr. seedling merely continued this trend.

The above results, in my estimation, would appear to implicate the aleurone layer as the main instigator of endosperm modification which commenced under the aleurone cells at the acrospire-end of the germinated grain and progressed, concurrently, downwards over the surface of the scutellar epithelial cells and outwards towards the distal non-embryo end of the grain.

Such a conclusion seems feasible in view of the photomicrographic evidence presented in conjunction with the findings (MacLeod & Miller. 1962; MacLeod et al, 1964) that the living aleurone cells of endosperm slices were capable of secreting hydrolytic enzymes (e.g., α-amylase, endo-β-glucanase and proteinase) which effected endosperm modification.

The important point here is that if one had only made observations on the 48 hr. seedling then one would possibly

conclude that the scutellar epithelial cells featured in the modification process. A mere biochemical analysis on the proximal and distal halves of the grain for enzymic actions which are known to feature in the modification process would also give a similarly misleading conclusion (cf., Dickson & Buchart, 1942; Engel 1945; Engel & Heins, 1947; Kirsop & Pollock. 1958).

The micrographs presented here do not cast any doubt on the authenticity of the micrographs of Mann & Harlan (1916) or Dickson & Shands (1941). Mann & Harlan (1916), unfortunately, examined only transverse sections of the acrospire-end of modified grains. Such a line of cut would 'correctly' imply that the epithelial cells were entirely responsible for endosperm modification. The photomicrographs of Dickson & Shands (1941), in my estimation, merely represented a small longitudinal sector from the acrospire-end of about a 24 hr. seedling. In this sector only a few epithelial cells and, interestingly enough, a prominent portion of the intermediate layer, was shown. None the less, the free end of the intermediate layer appeared identical to the free end of the intermediate layer shown in Plate 2 for the 24 hr. seedling. In short, all the signs indicated the overlying presence of the aleurone layer.

Now, Nieuwdrop (1963) and Nieuwdrop & Buys (1964) interpreted the ultrastructural changes of the epithelial cells on the assumption that the epithelial cells secreted hydrolytic enzymes; however, in the light of the above evidence where no such role was deducible, reinterpretation, with respect to the extraordinary

elongative properties of these cells, might be more meaningful.

Epithelial cells and Cytolytic Particle migration.

With the evidence that aleurone cells were principally involved in normal endosperm modification of the intact grain it was decided to reinvestigate the proposal of Horning & Petrie (1927) that the epithelial cells of the intact grain secreted mitochondria-like particles with cytolytic activities.

Plate 11 shows unimbibed aleurone cells and Plate 12 shows aleurone cells from a region of extensive modification in a 48 hr. seedling. Note that, unlike epithelial cells, the protoplasm of these cells, with age, disintegrates and 'tears' easily. This light microscopic observation is supported by the electron microscopic work of Paleg & Hyde (1964) on the aleurone and Nieuwdrop & Buys (1964) for epithelial cells.

O'Brien (1942) using Heindenhain's haematoxylin to stain the scutellar epithelial cells of maize embryos, arrived at the conclusion that no migratory particles passed from the scutellar epithelial cells to the endosperm.

Using the same stain as Horning & Petrie (1927), i.e., Janus Green B, a close time-course examination (0, 12, 24, 48, 72 hr.) was made on the 'particle secreting role' of the epithelial cells of barley grains. Surprisingly enough, blue-stained particles as reported by Horning & Petrie (1927), were detected at the epitheliail tips where endosperm modification was taking place. These particles were first apparent at

24 hr. and varied in shape and size (10-2.5μ) as seedling growth progressed towards 72 hr. On the basis of size alone these blue particles cannot be mitochondria; however, the mechanism of the staining action of the dye used offered useful information on the nature of these blue particles.

Lazaron & Cooperstein (1953) made an extensive study of the dying mechanism of Janus Green B and concluded that although the mitochondria-staining properties of this dye depended on the cytochrome C/cytochrome oxidase system of the mitochondria being able to maintain this dye in the oxidised (blue) form; however this dye was more or less readily taken up by diverse proteins. Using casein, I confirmed this latter property of the dye.

In conclusion it seems quite possible that the 'particles' observed by Horning & Petrie (1927) were merely endosperm storage protein undergoing degradation by proteinases (MacLeod et al, 1964).

This finding, in conjunction with the anatomical observations on endosperm modification, left me almost convinced that there remained no known reference which could adequately substantiate a hydrolytic enzyme secretory role for the epithelial cells. But why almost convinced? Well, there remains the well-known fact that excised barley embryos can secrete hydrolytic enzymes, and if epithelial cells do not secrete hydrolytic enzymes and since recent published diagrams of the position of aleurone cells in barley grains show that these cells abut on the embryo but do not penetrate it (Bergal & Clements. 1962;

Cooke & Pitt, 1964), from where within the tissue complex of excised embryos did these hydrolytic enzyme emanate?

After a close examination of excised embryos and intact grains it was noticed that the aleurone was three cells deep over the endosperm but this same layer penetrated the outer margins of the scutellum as a single layer of cells to a depth of about 500μ (see Plate 15). The presence of this remnant of aleurone makes it probable that when embryos are excised traces of aleurone are present.

This is obviously a matter of importance when the relative enzymic contributions of embryo and endosperm are assessed (Briggs, 1964); however, biochemical support for the non-enzymic participation of epithelial cells in endosperm modification will be included in the physiological section of this thesis (Section III).

Pattern of Modification and Vascular Morphology

Although anatomical results already given here on endosperm modification failed to attribute any enzyme secretory role to the epithelial cells and thus conflicted with the findings and suggestions of past workers since Brown & Morris (1890); the aged observation of Brown & Morris (1890) that modification was more rapid on the dorsal non-furrowed surface of the endosperm seems absolutely correct (see Plate 2). This asymmetric pattern of endosperm modification has been variously explained as due to thinner endosperm cell walls in the attacked region (Brown & Morris, 1890), to less dense packing of starch grains, so

allowing more rapid diffusion of enzymes in the modified area (Mann & Harlan, 1927) and to poor development of the aleurone layer on the non-modified surface of the grain (Miller, 1962).

Extensive observations failed to convince me that these suggestions were wholely correct and that the possible reason for this asymmetric modification phenomenon rested entirely on the basic structural architecture of the embryo. To explore this possibility, two series of dissections were performed.

In the first, the region of the scutellum containing the potential vascular system (i.e., acrospire-end half) was excised, after a 2 hr. soak, without damaging the scutellar node, and the grains were incubated for 72 hr. and then sliced vertically into longitudinal halves and examined for extent of endosperm modification. It was found that the normal pattern of advancing modification in the endosperm altered; not only was the rate of modification diminished but in these grains had progressed more rapidly on the unusual furrowed-surface of the endosperm. Although, at this stage there is no desire to discuss the hormone potentialities of the growing embryo, it was surmised that since Yomo (1958) has produced good evidence that a hormonal-like factor was transported from the growing embryo to the endosperm during seedling development and since Paleg (1960) and MacLeod & Miller (1962) showed that commercial gibberellic acid could imitate this factor, there was the possibility that the orientation of vascular elements in the acrospire-end half of the scutellum preferentially directed this hormonal-like factor,

in intact grains, to the dorsal surface of the grain.

Mindful of this, in the second experiment the embryos were excised, inverted and re-affixed on their endosperm by minute pins so that the vascularised acrospire-end was directed towards the furrow. Grains treated in this way modified much more rapidly near the furrow than in any other part. These two sets of experiments suggested that the vascular system of the scutellum may be concerned with the transport of the hormonal-like substance which catalyses the release of hydrolytic enzymes in the aleurone cells.

Plates 13 and 14 show a vascular trace from the acrospire-end of the scutellum for the unimbibed grain and for the 72 hr. seedling respectively. Although lignified xylem elements and other associated non-vascular elements can be seen no typical phloem elements were observed.

Being fairly convinced that the orientation of vascular elements in the acrospire-end half of the scutellum conditions the pattern of endosperm modification and, realising that sections in one plane give no idea as to the true distribution of vascular material, a whole-mount preparation of the scutellum (72 hr.) was attempted using the potassium hydroxide/chloral hydrate/basic fuchsin method. Despite the frailness of the prepared material photomicrographs were obtained and these results are represented diagramatically in Fig. 2. The important point to note is that the vascular tissues are confined exclusively to the acrospire-end of the scutellum as two distinct

collections of individual strands.

Function of the Scutellum

Edleman et al. (1959) suggested that the scutellum of barley embryo absorbed degraded food materials from the endosperm. Since endosperm modification commences at the acrospire-end of the endosperm and since there is the possibility that a hormonal-like factor is directed by the one-sided orientation of vascular elements to the endosperm-embryo junction it appears likely that the initial area of induction of endosperm modification is also the initial area of food absorption. Once absorbed, transport to the growing axis is effected.

The 'sensible' biological correlation between, orientation of vascular elements, modification and subsequent absorption of degraded food materials seems to be an important but indirect function of the scutellum in germination and subsequent seedling growth. Whether the scutellum in its function as a cotyledon directly nourishes the growing axis will be considered later in Section II.

Anatomical studies on the Scutellum storage parenchyma cells

In an effort to gain further resolution on the intracellular time-course histochemical changes of the scutellum during germination and subsequent seedling growth (Table II) and as a directive prelude to electron microscopic studies on the scutellum and axis, light microscopic investigation on the storage parenchyma cells was performed.

Plates 16, 17, 18, 19, and 20 depict light microscopic changes in the storage parenchyma cells during germination and seedling growth (Heidenhain's haematoxylin and Eosin were the stain used). Only Plate 20 was not taken from centrally placed scutellar cells.

To recap, histochemical analysis revealed (Table II) that of the three major storage products identified, viz., fat, protein and starch, only diffuse fat and discrete protein deposits were detected in the scutellum of the ungerminated grain; starch appeared after 20 hr. growth.

Now, in Plate 16 (0 hr. ungerminated grain) the light transparent areas could be equated to that area occupied by fat deposition. The light dense areas range from less than 1μ to about 3.5μ. They are definitely basophilic with respect to the dyes used and are identical in every respect to protein deposits in the cotyledon and perisperm of Yucca seeds (Horner & Arnott, 1965). Even the morphology of these barley protein bodies is similar to that of the protein bodies of Yucca seeds where a usually concentrated core of dense material is permeated by accompanying light transparent pores. As was the case for Yucca seeds, no protein deposits were observed in the provascular elements of the scutellum of dry barley grains (see, Plate 13).

Plate 17 (20 hr. seedling) shows what would appear to be a disorganisation and association of protein bodies. Occlusion of their light transparent pores also occurs. The general cytoplasm now contains well defined light transparent bodies

which have a dark staining periphery. The interesting thing about these bodies is that they sometimes show dark tri-radiate band which is usually regarded as a morphological feature of developing starch grains.

Plate 18 (24 hr. seedling). Here the protein bodies appear much reduced in size and are at the point of disappearing, but, on the other hand, the cytoplasm now contains a heavy deposit of starch grains.

These plates give support to the protein-starch histochemical findings in Table II.

Plate 19 (48 hr. seedling). Although rare protein-like bodies can be seen, these cells are virtually packed-out with starch grains. At 72 hr. the seedling's scutellum showed storage parenchyma cells completely filled with starch grains.

Plate 20 (48 hr. seedling). These cells were photographed from the same scutellum as Plate 19, not, however, from the central region but from the base of the scutellum, i.e., the scutellar-heel. Here, complete cell 'ghosts' are evident as a result of complete disorganisation and loss of cytolpasmic content. Only the dislodged nuclear apparatus is present.

For obvious reasons only cells from the central core of the scutellum were considered suitable for future electron microscopic studies.

In conclusion, although there was no information on the decline of fat deposits in the scutellar parenchyma cells confirmation of histochemical findings was obtained for the

appearance of starch granules and the disappearance of what may be protein bodies, in at least the top-most three-quarter portion of the scutellum.

However, before the physiological implications of these structural changes in the scutellum can be fully contemplated and assessed, information is required on these changes during the first 17 hr. period after the initial recommencement of general metabolic activity in the quiescent grain. It is believed that with this information physiological deductions should be possible on the functional role of the scutellum in germination and seedling growth as: nutritional organ viz., cotyledon, transport organ for hormone, absorption organ for food materials and their subsequent transport, and as a biochemical entity merely for judicious conversion of stored foor products.

Since this is basically a problem of structural resolution, electron microscopic analysis of the whole scutellum was undertaken.

ULTRASTRUCTURAL ANALYSIS on the Scutellum and Scutellar Node.

Although histochemical and anatomical analyses have so far given some insight into some of the structural changes which take place in the scutellum and the adjoining endosperm during late germination and subsequent seedling growth, it was considered that the physiological consequences of many of these changes, especially during the period of germination, would be impossible to assess without the use of the electron microscope. For example, there is the question of estimating more precisely the

appearance and disappearance of cellular structures and organelles in the scutellum and the scutellar node which, in chance preliminary examinations, appeared to have some influence on the extent of endosperm modification. There is also the additional matter of the nature of the cementing material which binds the epithelial cells in the dry grain. This material appeared to be neither pectin nor protein. Nieuwdrop & Buys (1964) in their extensive electron microscopic study on the epithelial cells confirmed the original observation of Brown & Morris (1890) that during elongation this cementing material disintegrates. Although Nieuwdrop & Buys (1964) suggested that the disintegrating material was possibly pectinaceous in nature they showed no micrographs on the original (dry) state of the cementing material. Nieuwdrop (1963) '0 hr.' grains were pre-soaked for an undefined period (over 72 hr. by Nieuwdrop & Buys (1964) which makes it impossible to assess the credibility of Nieuwdrop's conclusion. The nature of this cementing layer in the dry ungerminated grain must also be examined in the electron microscopic analysis.

Scutellar parenchyma cells

0 hr. (dry grain). Potassium permanganate fixation

FAT (Oils) deposits. For Plate 21, one notices a large number of electron transparent inclusions which occupy most of the ground material. Each inclusion is delimited by a limiting membrane. With Osmium tetroxide staining (see Plate 22) these electron transparent areas become electron dense. According to Horner &

Arnott (1965) potassium permanganate fixation empties oil-bearing inclusions and make them appear electron transparent: conversely, osmium tetroxide fixation is supposed to render oil-bearing inclusions partially electron dense (Mercer & Birbeck, 1963). Therefore, these electron transparent inclusions, with respect to their abundance, diffuseness, and histochemical reactions at both macro and ultrastructural levels possible represent fat deposits.

PROTEIN deposits. Plate 21 shows that two distinct types of electron dense bodies are clearly discernable and in work on peanut cotyledons (Cherry, 1963), on pea cotyledon (Bain & Mercer, 1965) and on Yucca seeds (Horner & Arnott, 1966), these have been called protein bodies. Both types of bodies are usually delimited by a unit membrane. The most frequent in the dry grain (Plate 21) is the one called the 'meshwork-type'. The other is the 'core-type' (Horner & Arnott, 1966; Paleg & Hyde, 1964) which has mostly smooth electron dense material with accompanying electron transparent areas and small electron dense spheres called 'inclusions'.

Osmium tetroxide is supposed to be a poor protein stain (Mercer & Birbeck, 1963). As expected, Plate 22 shows the proposed protein areas electron transparent, in fact, a direct 'negative' of potassium permanganate fixation.

Further information on protein reaction was obtained with respect to the detection of protein-like material in the endosperm, already indicated to be present by the Janus Green B test.

Plate 23 shows a conspicuous area of electron dense material within which starch grains are encased. Plate 24, for similar dry grains, using osmium tetroxide as opposed to potassium permanganate fixation shows virtual absence of the electron dense areas of Plate 23. Johnston (1964) has also identified similar protein areas in the endosperm of Bromus and maize. In Plate 23 typical concentric lemellation of cereal grain starch can be seen.

As is evident in Plate 21, the parenchyma cells of 0 hr. scutellum do not contain starch and familiar metabolic organelles of the cytoplasm; e.g., mitochondria, er-membranes and plastids are evidently not visible. Cherry (1963) Bain & Mercer (1963) and Horner & Arnott (1966) found a similar situation in their respective studies on the cotyledons of peanut, pea and Yucca seeds.

12 hr. Potassium permanganate fixation

FAT (Oils) deposits. Plate 25 shows a general profusion of electron transparent inclusions as at 0 hr; however, notethe inclusions are reduced in size which may be indicative of a reduction in oil content. Such a reduction, if real, should able to be detected by biochemical analysis on total fat content of the scutellum; this information will be reported later.

PROTEIN deposits. Although Plate 25 shows only one large 'core-type' protein body numerous micrographs are available which indicate that this type of protein body is predominant at this germination time. A "transformation', resulting from hydration from, ,meshwork-type' to 'core-type' might have occurred.

24 hr. Potassium permanganate fixation

FAT (Oils) deposits. Plate 26 shows a drastically different but rather interesting picture with respect to fat inclusions. The spherical electron transparent inclusions have now disappeared but have given way to as large a number of smaller stellate inclusions with limiting membrane. Frey-Wyssling & Muhlethaler (1965) suggested that spherosomes, not dissimilar to these stellate inclusions, are transformed to larger spherical bodies containing oil during development. If this is the case, then we now appear to have the reverse process where oil containing inclusions are transformed to small stellate bodies.

Jacks et al. (1967) found that isolated 'spherosomes', which in fact looked very much like large fat inclusions, contained 98.1% total lipids, 0.77% phospholipids and 1.27% protein by dry weight. Lipase and fatty acyl-Coenzyme A synthesase activities were not associated with these isolated peanut cotyledon 'spherosomes'. This implies that the high lipase activity of ungerminated barley embryos (MacLeod & White, 1961) was possibly external to the fat inclusions.

Paleg & Hyde (1964) found similar stellate bodies in the oil-bearing aleurone cells of barley endosperms after 24 hr. seedling growth.

PROTEIN deposits. Plate 25 shows no signs of protein deposits at 24 hr.

STARCH deposits. Amyloplasts are now present (Plate 25) housing electron transparent areas which, themselves, have no limiting membranes. These electron transparent areas, found in extensive reports on starch grain ultrastructure, are evidently starch deposits. Membranes are beginning to appear in the cytoplasm.

Interestingly enough, at 12 hr. electron microscopic analysis seems to indicate a decline in fat content and the appearance of starch before any such change was detected by ordinary histochemical analysis. As may be remembered from Table II, endosperm modification was not apparent until about 20 hr. and therefore the possibilities of a genuine fat-starch parallelism in terms of a transformation of fat to starch seems more plausible. If this observation is as plausible as it seems then one would expect the glyoxylate pathway, which has been found to be obligatory (Carpenter & Beevers, 1959) in 24 oil-bearing plants capable of this fat-starch conversion, to be present in the scutellum of barley grains - especially during this 0 - 12 hr. period of germination. This investigation will be reported later.

The limitations of ordinary histochemical techniques are clearly exemplified with respect to the situation where a negative finding is not necessarily indicative of absence of the material sought.

STARCH deposits. Amyloplasts with definite double membranes (Plate 26) can be seen and their interiors are heavily packed with growing starch grains.

MITOCHONDRIA and ER-membranes. In Plate 26, mitochondria and er-membranes are visible for the first time. It is worth noting that this late appearance of mitochondria in these food storage cells in not unusual among storage organs and Cherry (1963) could only find 'mitochondria-like' organelles after pellets from peanut cotyledons were subjected to electron microscopy.

72 hr. (48 hr. was about identical). Potassium permanganate fixation FAT (Oils) deposits. No obvious decline in spherosome number. (Plate 2

PROTEIN deposits. None visible.

STARCH deposits. Starch grains are now more electron dense than at 24 hr. and have just about filled the inner spaces of their amyloplasts.

Mitochondria and er-membranes can still be seen.

Nature of Cementing Material between Epithelial Cells

Plate 28 shows ultrastructural detail of scutellar epithelial cells and associated intermediate layer for the dry grain. This amorphous intermediate layer is part of the endosperm system of cell wall material, which makes it mainly hemicellulosic in structure. From this micrograph it is clear that the hemicellulosic intermediate layer (I) passes down between the epithelial cells as (I^I), thus binding these cells together.

For the 24 hr. seedling (see Plate 29) one observes signs of separation of elongating epithelial cells, in addition to the appearance of spherosomes, starch bodies in amyloplasts and interesting plasmodesmatal-like 'openings' on the lateral walls of these cells. Absorption of degraded food materials could possibly be effected through these openings if they are functional. Subsequent transport to the vascular system of the scutellum could then occur.

It therefore seems that the conclusion reached by Nieuwdrop (1963) and Nieuwdrop & Buys (1964) that the cementing material between the epithelial cells was pectinaceous, lacks ultrastructural support. The evidence here seems to implicate hemicellulosic extensions of the intermediate layer as the cementing material. Therefore, just as the intermediate layer was found to separate easily from the epithelial cells and was dissolved during the modification process, these intermediate extensions might suffer the same fate in the intact grain during seedling growth.

Additional Ultrastructural aspects of the Scutellum

Plasmodesmata. In Plate 21 plasmodesmata are present as electron dense pores traversing the electron transparent cellulosic walls of scutellar parenchyma cells. These channels may serve to facilitate the passage of degraded endosperm food products to the vascular strands of the scutellum for eventual transport to the growing axis.

Vascularisation - Xylem formation

Lignin deposition. Light microscopic observation revealed bands of provascular elements which extended towards the acrospire-end of the scutellum. After 12 - 20 hr. reticulate, spiral and annular bands were detected. Although non-lignified cells with dense cellular contents were observed, in close association with the lignified cells, no features typical of conventional phloem were observed.

Electron microscopic analysis revealed excellent features of lignin deposition.

It is known that the structure of full grown cell walls can be stabilised and strengthened by incrustation with lignin, a non-cellulosic polysaccharide. Lignification can be confined to a pathway of cells (Plate 30) or to a localised association of cells (Plate 31). Lignin deposition can begin within the confines of the primary wall but not as a result of cytoplasmic stratification, as Hepler & Newcombe (1963) proposed. Wooding & Northcote (1964) observed that lignin deposition began as a 'halo' of electron transparent material situated above electron dense areas within the primary wall where lignification is expected to commence : see 'halo' (h) in Plate 32 and progressive lignification (l_1, l_2, l_3, l_4) in Plate 33.

With respect to the mechanism of lignin deposition, Frey-Wyssling & Muhlethaler (1965) suggest and explained diagrammatically, that lignin becomes incrusted onto the fibrillar framework of non-contrasted cellulose material of the cell wall,

which, as a result, becomes fibrillar. This is clearly shown in Plates 32 and 33. However it is worth noting that the fibrillar pattern of cellulosic material can only be seen clearly during the early stages of lignification: compare Plates 30 and 31 with, for example, Plate 33.

It would therefore appear that, quite unexpectedly, electron microscopic analysis on vascularisation in the scutellum of barley embryos revealed features of the lignification process which in some parts have been confined to descriptive models. There is also the additional observation that at the onset of active lignification disorganisation and disintegration of the protoplasmic cell content begins (see Plate 32).

As in anatomical analysis, typical phloem elements were not observed. However, a type of unspecialised conducting cell, somewhat like a sieve cell (Esau, 1957), may be present. These cells have mitochondria and internal membranes and their end walls are perforated by a collection of large pores (P) which are not electron dense like plasmodesmata and are much wider (see Plate 32A for enlargement of end wall in Plate 32). These large pores may function in rapid transport of food materials from endosperm to the growing axis.

From the above, it would appear that the scutellum is well equipped to carry out the important role, in germination and seedling growth, of rapid bidirectional transport of food and hormonal substances. However, since germination is completed by 17 hr. and since vascular development and

endosperm modification start at about 20 hr. it would appear that the seedling growth process would derive the greatest benefits from the transport of materials in the scutellum.

Scutellar Node. Cells from this region (whose injury or removal appeared to retard the rate of endosperm modification) are about 4-5 times as small as the scutellar parenchyma cells. Plate 34 shows them at 0 hr. to possess mitochondria without cristae, plasmodesmata and smooth deposits which may be proteinaceous. After 12 hr. germination (Plates 35, 36) many normal mitochondria, golgi bodies, free ribosomes and er-membranes can be seen but no starch granules are present and the protein-like deposits have disappeared. At 24 hr. (Plate 37) the mitochondria begin to swell - a possible sign of ageing - and at 72 hr. a general disorganisation of cellular integrity is apparent, see Plate 38.

Ultrastructural evidence therefore seems to imply that these cells of the scutellar node, unlike the storage parenchyma cells of the scutellum, are basically metabolic rather than nutritional in function. In fact, as one moves from the scutellum to the nodal region (Plate 39, 24 hr. seedling) amyloplasts appear to lose their capacity to synthesise starch and as observed for cells of the scutellar node itself, even amyloplasts were not present.

Thus it would appear that the mere presence of a sub-cellular organelle is not proof that it is capable of normal metabolic activity in vivo. From Plate 39 it would appear the 'fine' positioning of apparently similar cells in the same

organ strongly dictates certain aspects of subcellular function: in this case, starch production. Plate 40 shows detail of empty amyloplast from scutellar node-scutellum transition region.

GENERAL CONCLUSION

Histochemical analysis during germination and seedling growth revealed the following: endosperm modification, scutellar lignification and elongation of scutellar epithelial cells commenced after germination. i.e., by 20 hr. growth ; pectinase or proteinase failed to separate the epithelial cells; abundant fat and discrete protein deposits were found to decline in the scutellum and the scutellar epithelial cells of the growing embryo. Increase in starch granules paralleled fat decline. A fat to starch conversion was tentatively intimated.

Extensive anatomical analysis not only confirmed the 20 hr. commencement of endosperm modification and epithelial cell elongation but also showed convincingly that modification commenced under the aleurone cells at the acrospire-end of the endosperm-embryo junction and progressed concurrently outward and downward over the epithelial cell surface, thus casting doubt on the accepted belief that elongating epithelial cells can secrete hydrolytic enzymes into the endosperm. No migrating mitochondria particles with hydrolytic enzyme activity were observed to pass from epithelial cells into the endosperm. Vascularisation of the scutellum was observed to take place in discrete provascular strands orientated only to the acrospire-end

of the scutellum and, as a result, directed hormonally-induced endosperm modification to that endosperm area where vascular strands terminated in close association with the aleurone layer. No information was obtained on the nature of the cementing material that bound the epithelial cells. The decline of protein bodies was again observed and the 20 hr. appearnace of starch granules was again evident in the scutellar parenchyma cells.

The extensive resolving power of the electron microscope revealed that in the scutellar parenchyma cells fat deposits declined and the appearance of starch granules was much earlier (viz., 12 hr. germination time) than indicated by ordinary histochemical or anatomical techniques. Protein bodies were still present at 12 hr. but disappeared by 24 hr. Fat bodies at 24 hr. gave way to equally numerous spherosomes. Starch-filled amyloplasts, mitochondria, and er-membranes were visible at 24 hr. Since endosperm modification commenced at 20 hr. there is the possibility that in the scutellum of barley grains a fat to starch conversion could occur at least during the germination process, viz., 0 - 17 hr.

Electron microscopic analysis also revealed that the cementing material that bound the epithelial cells at 0 hr. was an extension of the mainly hemicellulosic intermediate layer of the endosperm. Separation of these epithelial cells occurred at the onset of elongation and endosperm modification in the intact grain.

The structural mechanism of lignification was verified and the cells of the scutellar node, unlike the larger storage cells of the scutellum, during germination and early seedling growth (0 - 72 hr.) contained various metabolic organelles, e.g., mitochondria, er-membranes, golgi bodies and free ribosomes.

In conclusion, although there is the possibility that many more observations could have been made in this section, it was felt that certain interesting observations had been made especially with respect to structural relationships in the intact grain during its germination and seedling growth. However, these structural relationships can only be regarded as expressions of physiological and/or biochemical involvements. Therefore, mindful of this, I decided to try by experimentation to equate some of these structural growth changes with their possible physiological and/or biochemical meanings.

This attempt constitutes the remainder of this thesis.

TABLE I

Histochemical tests of Ungerminated Dry Grains

Germination time (hr.).	Insoluble polysaccharide in embryo	Cellulose in embryo	Callose in embryo	Pectin in embryo	
				scutellum	epithelium
0 hr.	+++	+++	0	++	0

\+ trace; +++ = maximum amount observed.

+, not equivalent for different tests.

TABLE II

Histochemical changes in the Embryo and Endosperm during Germination (0 - 17 hr.) and Seedling growth (17 - 72 hr.). Growth Temperature = 25°C.

Time after wetting grain (hr).	0 hr.	12 hr.	20 hr.	24 hr.	48 hr.	72 hr.
Acrospire length (hr).	0.10	0.15	0.20	0.46	0.98	3.40
Coleorhiza and root length (cm).	0.50	0.20	0.25	0.40	1.62	2.70
Vascular differentiation in scutellum .	0	Cell elongation	Lignin +	Lignin ++	Lignin +++	Lignin ++++
Fat in scutellum.	++++	++++	++++	+++	++	+
Fat in scutellar epithelium.	++	++	+	±	0	0
Starch in scutellum.	0	0	+	++	+++	++++
Starch in scutellar epithelium.	0	0	0	+	++	++
Protein in scutellum.	++	++	+	±	0	0
Protein in scutellar epithelium.	±	+	±	0	0	0
Length of scutellar epithelial cells.	30 μ	30 μ	30-40μ	40-50μ	70-75μ	75-80μ
Modification of Endosperm	0	0	+†	++	+++	++++

+ = trace; ++++ = maximum amount observed + only equivalent along one line.

† = at acrospire-end only.

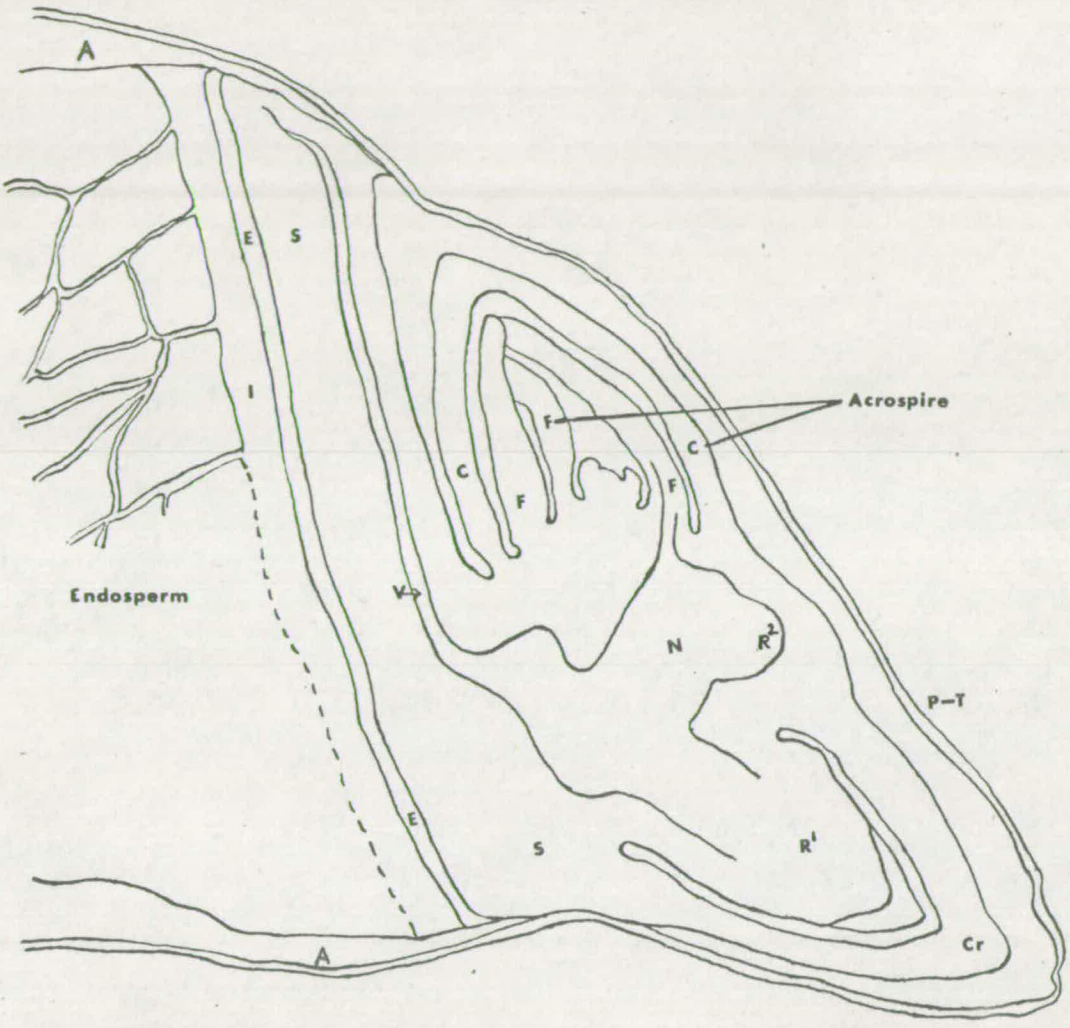


Fig. I. Diagram of median longitudinal section of the ungerminated barley embryo.

P-T - Pericarp-Testa.

Axis

C - Coleoptile.
F - Foliar shoot.
N - Node (scutellar node).
R^2 - Seminal root.
R' - Root.
Cr - Coleorhiza.

S - Scutellum.
V - Vascular trace.
E - Epithelial cell layer.
I - Intermediate layer.
A - Aleurone cell layer.

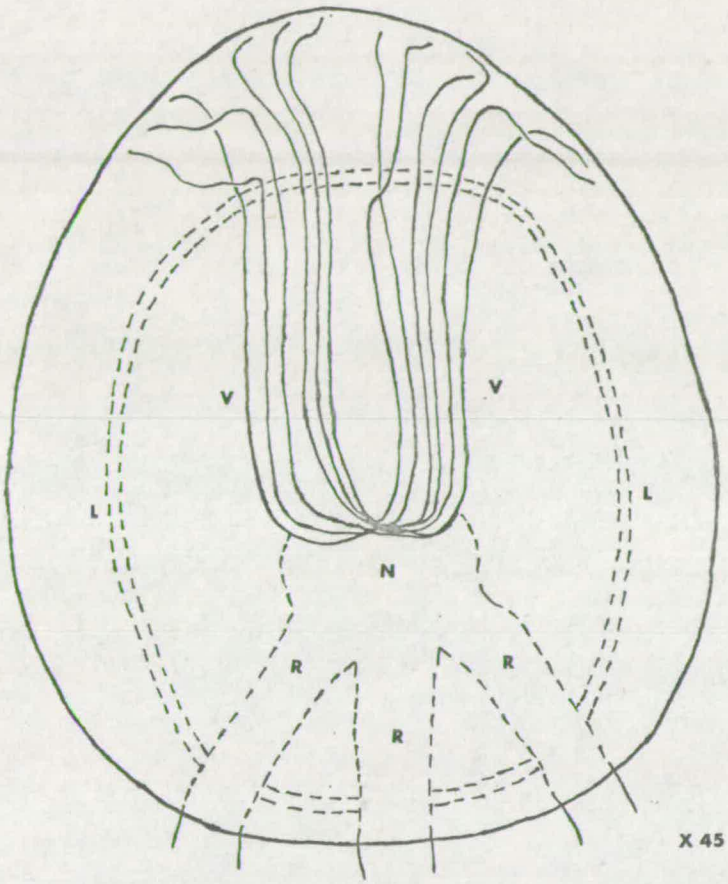


Fig. 2. Diagram of entire scutellum, dissected and stained with basic fuchsin to show vascular system. Grain grown for 72 hr.

V - Vascular traces leading from scutellar node(N) to apex of the scutellum.

R - Vascular supply to roots.

L - Line of lignin - like material deposited on cell walls.

SECTION II

ANALYSIS ON FUNCTIONAL ASPECTS OF STRUCTURAL AND METABOLIC CHANGES DURING BARLEY GERMINATION AND SEEDLING GROWTH.

INTRODUCTION

In Section I analysis indicated that many structural changes were apparent in the embryo even before germination (root emergence) had begun; changes with respect to lipids, starch, protein, lignin, cell elongation, endosperm modification and general metabolic organelles were recorded. Most of these changes were recorded for the scutellum and this organ is supposed to be the cotyledon of barley grains (see Brown, 1965). It is generally assumed that during the germination of seeds with food reserves in cotyledons, drastic break-down of these food reserves occur with the loss of soluble products to the developing axis (Mayer & Poljakoff-Mayber, 1963). Now, with the possibility that at least during germination a fat to starch conversion occurred in the scutellum of barley grains, the validity of a nutrition relationship between scutellum and axis will be investigated especially during the germination process (0 - 17 hr.); i.e., before any visible signs of endosperm modification (20 hr.).

Mindful of this, physiological studies were made to determine whether the glyoxylate pathway was functional and also the fate of important nutritional reserves such as, fat, sucrose, raffinose, amides and amino acids in the scutellum and axis during embryo growth. The mechanism of raffinose degradation was investigated. Important changes in dry weight and water content were also recorded.

It is hoped that this section will give some insight into the metabolic independence of inter-dependence of the two major embryo organs (scutellum and axis) especially during germination.

MATERIALS AND METHOD:

1. Glyoxylate determination was based on the method of Carpenter & Beevers (1959), with minor modifications owing to difference in experimental material. 100 scutella and axes were carefully removed from germinating Proctor (1964) barley grains at the appropriate times. Scutella and axes were extracted with 2 ml. 0.05M ice-cold phosphate buffer, pH 6.8. The supernatant fraction, after centrifugation at 38,000G for 30 min. at 0°C was then assayed immediately for isocitritase activity. The reaction mixture contained 200 µM phosphate buffer pH 7.8, 15 µM of $MgSO_4$, 6 µM of cysteine HCl adjusted to pH 7.6, 24 µM of isocitrate and distilled water giving a final volume of 2.8 ml. For the assay, 0.2 ml. of supernatant was added and 1 ml. aliquots were removed at 1 minute intervals and placed in a centrifuge tube containing 0.2 ml. of 100% TCA. Precipitated proteins were spun down using a MSE 8 and the supernatant was decanted into a test tube and 0.33 ml. of 0.1% dinitrophenylhydrazine in 2N HCl added. Incubation was in a water bath at 30°C. After incubation 1.67 ml. of 2.5N NaOH was added slowly.

The solution was then placed in a glass cell and read against a standard in a SP 500 at 445mµ. OD readings were then converted into µM of glyoxylate with the aid of a standard graph.

2. Determination of total fat (oil) content at the appropriate times was based on the method given in Peach & Tracey (1955) with minor modifications.

150 embryos at the appropriate growth times were removed from barley grains, dissected carefully into scutella and axes, diced up finely by clean hand razor, placed in fat-free thimbles and extracted for 4 hr. in a Soxhlet apparatus using light petroleum ether (b.p. 40/60). Isomantles were used as heat source. The extracts were then evaporated under reduced pressure. The last trace of the solvent was removed by heating the flasks at 105°C. The flasks were allowed to cool and substraction of the original weights from the final constant weights of the flasks was recorded as that of the total oil content. It was found that 4 hr. was sufficient for complete extraction.

3. Dry weight determinations were carried out on 50 grains from appropriate growth times. These grains were dissected carefully into scutella, axes and endosperm. Drying extended over 24 hr., in aluminium containers, in an oven set at 105°C.

4. Sugar determinations - Paper chromatography.

The only solvent system used was ethyl acetate/pyridine/water (10/4/3) with the solvent flowing in a descending manner. Chromatograms were developed, usually with silver nitrate and in one case with analine oxalate.

Scutella or axes tissue extraction was carried out with hot 80% ethanol. The tissue was always mashed up in the

extraction fluid and allowed to extract for 2 hr. The whole contents were then filtered and the clear filtrate was reduced to dryness under pressure at 35°C. The dried contents were then taken up in a suitable quantity of distilled water for application to either Whatman No. 1 or 3 chromatographic paper.

Crude enzymic extracts were made by using a hand glass homogeniser. Extraction was in ice-cold 0.1M phosphate buffer pH 6.2. The extracts were centrifuged (5°C) for 20 min. at 9,000 r.p.m. The supernatants were then dialysed against distilled water in the cold room for 6 hr. The dialysed extracts were then incubated with about 3 mg. of substrate in a reaction mixture of 0.2 ml. in small tubes at 37°C for 1½ hr. After incubation small aliquots were spotted onto Whatman No. 1, and the chromatograms were developed and sprayed.

5. Quantitative estimation of raffinose and sucrose.

Scutella and axes from 75 grains were carefully dissected from growing grains at the appropriate times, extracted and applied to Whatman No. 3 as in, 4. Sucrose and raffinose were therefore separated on thick paper, Whatman No. 3, and, after chromatography, the areas containing sucrose and raffinose were cut out and eluted with distilled water into 50 ml. graduated flasks. Eluted solutions were filtered through sintered glass funnels (No. 3 porosity) to eliminate the possible contamination of cellulose fibres from the chromatographic paper. 1 ml. of each eluted solution was then placed into a scrupulously

clean test tube, 2 ml. of 5% phenol (AnalaR) was added and 5 ml. of AnalaR sulphuric acid was then quickly delivered. Test tubes were then cooled and read at 490 mμ on the SP500 using glass cells. Duplicate checks were always carried out. OD readings were then converted into mg. by the aid of a standard graph. Corrections were made from an eluted blank.

6. Determination of total amides and amino acids.

For this determination scutella and axes were dissected from 30 grains at the appropriate times. They were then extracted separately in 3 ml. of hot distilled water in a hand glass homogeniser until the tissue became minute particles. The extract was then made to 60% with respect to ammonium sulphate for precipitation of proteins. The extract was then centrifuged at 9,000 r.p.m. for 20 min. at 10°C.

Reagents: Tri-nitrobenzoic sulphonic acid 0.1% (w/v), sodium bicarbonate 4% (w/v) and hydrochloric acid 2N.

To 1 ml. of the supernatant was added 1 ml. of sodium bicarbonate solution and 1 ml. of tri-nitrobenzoic acid solution. The reaction mixture was then mixed and left in the dark at 40°C for 2 hr. An orange colour developed at the end of this period. The solution was then acidified with 2 ml. of 2N hydrochloric acid solution and read at 340 mμ using silica cells. It was found that the extract made in the way described had to be diluted 5 times before a reading was possible. Calibration using alanine or serine was not attempted. The bulk of this method was communicated to me by Mr. J. Bartlett (Heriot-Watt University, 1966).

RESULTS AND DISCUSSION

PART I

Fat-Starch Relationship in the Scutellum

Murlin (1934) showed that during the germination of oil-bearing seeds, such as peanut, the decrease in oil content was associated with an increase in carbohydrate.

Kornberg & Beevers (1957) for castor beans, Marcus & Velasco (1960) and Beevers (1961) for both pumpkin and peanuts and Cherry (1963) for peanuts have all shown that isocitritase, one of the key enzymes in the glyoxylate pathway, is always associated with oil-bearing seeds. Carpenter & Beevers (1959) also showed that in 24 plants examined isocitritase was always found in the region of fat breakdown. Beevers (1956), using non-green cotyledons of castor beans from 5 to 6 day old seedlings, has demonstrated that when labelled glycerol was applied the metabolic equilibrium was transferred towards anabolism and labelled sucrose was detected.

1. Since in Section 1 the proposed fat to starch conversion was based on structural observations it was first decided to follow the decline of fat chemically, and, more precisely, relate it to the electron microscopic appearance of starch grains on a cellular basis. Fig. 3 shows a drastic decline in scutellar fat content at the very onset of germination with a levelling-out from 24 - 72 hr. Fat content of the axis also falls drastically during the entire germination process but begins to reappear

after 24 hr. Fig. 4 on the other hand depicts, from ultrastructural observation, an increase in starch granules per single cell profile in scutellar parenchyma cells. Here starch grain deposition, as was the case for fat decline, is well under way by 12 hr. germination time.

As far as the scutellum is concerned the decline in total fat content (Fig. 3) appears to be directly related to the ultrastructural organelles which were labelled as being associated with fat deposition. For it may be remembered that by 24 hr. the diffuse deposits of electron transparent bodies (Potassium permanganate fixation) gave way to small stellate spherosomes. It is therefore possible that these electron transparent bodies, shown in Plate 21, 0 hr. grain and Plate 25, 12 hr. grain, represent a decline in fat content while Plate 25, 24 hr. seedling represents a virtual depletion of these electron transparent bodies content of fat. These bodies at 24 hr. might thus appear shrunken and the small stellate bodies could represent this shrunken form.

Now, the associated increase in starch granules by 12 hr. (before the commencement of endosperm modification) prompted me to investigate the possibility of the glyoxylate pathway operating in the scutellum of these grains. Since important workers in this field have always associated the presence of isocitritase with the functioning of the glyoxylate pathway, the presence of this enzyme was determined in relation to its ability to produce glyoxylate from isocitritase.

Fig. 5 shows that this enzyme is present in the scutellum of the germinated grain and except for a slight lag in activity at the onset of germination the activity of this enzyme coincides remarkably well with the decrease in fat content and increase in starch granules in the scutellum shown in Figs. 3 and 4.

This result, as far as the development of this enzyme during germination and early seedling growth is concerned is consistent with the results of other workers (Marcus & Velasco, 1960; Beevers, 1961; Cherry, 1963). However, Cherry claims to detect high enzyme activity at 0 hr. in the cotyledons of peanut while Marcus & Velasco and Beevers did not. My results did indicate a high initial isocitritase activity in the axis (Fig. 6) but a low initial activity in the scutellum (Fig. 5). Now, had there been axis contamination of the scutellum then a higher activity would have been detected in the scutellum. This may explain the difference in the findings of the other workers.

Taken in conjunction with the evidence of MacLeod & White (1962) that high lipase activity occurred in the embryos of 2 hr. soaked barley grains, it would appear that in the scutellum of growing barley embryos an oil-to-starch conversion might occur through the activities of the glyoxylate pathway.

None the less it seemed worth enquiring whether at the initial (0 - 12 hr.) conversion of fat, starch was the only nutritionally important carbohydrate formed or, was sucrose also formed as in the labelling experiments of Beevers (1956).

2. MacLeod (1957) has shown that the entire embryo from intact barley grains utilised both raffinose and sucrose during germination with sucrose showing a recovered rise at about 9 hr. germination time. Edelman (1959) has shown that the scutellum of germinating barley embryos contains an enzyme system capable of converting hexose residues, resulting from endosperm modification, into sucrose. Mindful of this, it was surmised that since endosperm modification in the intact grain did not begin until after 15 hr. then if there was any marked conversion of fat to sucrose or raffinose in either the scutellum or the axis it should be possible to detect this between 0 - 12 hr.

Figs. 7 and 8 show a detailed time-course analysis of sucrose and raffinose contents in the scutellum and axis over the first 24 hr. period of growth. In both Fig. 7 and 8, over the 0 - 12 hr. period when stored fat declined and starch accumulated in the scutellum, no increases in either sucrose or raffinose contents were observed. However, from the decline of these sugars during the germination process, a possible participation in starch synthesis cannot be excluded (see Leloir, 1964). None the less, sucrose synthesis was evident after 16 hr. in the scutellum and a slower but marked increase was observed in the accompanying axis at 18 hr. (Fig. 7) though not at 9 hr. as was quoted by MacLeod (1957). No increase in raffinose (Fig. 8) was observed and it would appear that in normally germinating grains raffinose synthesis does not accompany sucrose

synthesis on the onset (between 16-20 hr.) of endosperm modification.

To conclude tentatively, it would appear that while the scutellum is capable of synthesising starch, possibly mainly from hexose building blocks provided by fat conversion especially during the early stages of germination, it is incapable of using the same hexose building blocks for the synthesis of either sucrose or raffinose.

However the question remains; was this due to a defect in the sucrose synthetase system of the scutellum during this 0 - 15 hr. period in the scutellum? (see Fig. 7). To answer this in a crude way, 75 barley embryos were excised, after the grains had been soaked for 2 hr. and inserted into the exposed modified proximal ends of endosperms freed from their embryos after 40 hr. growth at 25°C. These 75 reconstructed grains were left for 10 hr. at 25°C and then scutella and axes were excised and assayed for sucrose and raffinose contents.

Table III suggests that the sucrose synthetase system is present and effective in the scutellum during the first 12 hr. period of germination, but in the normal intact grain its expression is limited until endosperm modification begins and transported hexoses are available. Thus it would appear that starch is possibly the main carbohydrate synthesised in large quantities from products of oil degradation during barley germination.

Before going to Part 2 of this section it may be interesting to see how these meagre findings fit into current ideas on the anabolic mechanisms of carbohydrate metabolism.

Edleman (1959) has shown that UDPG and F-6-P function in the synthesis of sucrose in the barley scutellum during germination. De Fekete & Cardini (1958) proposed that starch synthesis is catalysed exclusively by ADPG-starch transglycosylase. Therefore it is possible that from the early degradation of oils in the scutellum ADPG results and facilitates starch synthesis, but when free hexoses from the modifying endosperm are available, the UDPG equilibrium is raised and sucrose synthesis can also go on. UDPG may have an indirect role in starch synthesis from sucrose as starting material, according to Leloir (1964). If there is any truth in the above suggestion, then one would expect to find mostly ADPG during the first 15 hr. with UDPG becoming more abundant after 15 hr. germination.

PART 2

Other Metabolic Aspects of Scutellum and Axis

In the first part of this section it was shown that various metabolic processes take place in the scutellum and axis before root emergence has occurred. It seemed desirable not to leave these data in isolation but see how they fit into that gross event called germination.

It is generally supposed that cotyledons pass their degraded food products to the adjoining axis. In barley grains, in the given conditions, germination is completed by about 17 hr; however, an important question is: could the scutellum limit germination by lack of transport of food materials over the 17 hr. germination period - or is the axis an independant organ?

Fig. 3 suggests that both organs have the enzymic means of degrading their fat reserves from the very onset of soaking.

Figs. 7 and 8 suggest that both the scutellum and the axis, during this pre-germination phase, rely on their own sucrose and raffinose pools.

Fig. 9 suggests the same self reliance for amides and amino acids. With respect to Fig. 9 it is worth remembering that protein-like deposits (Plate 21, 0 hr. grain and Plate 25, 12 hr. grain) disappeared between 12 and 24 hr. in electron microscopic analysis and it is now interesting that during this period of storage protein disappearance in the scutellum there is an associated increase in free amides and amino acids.

In the scutellar node, however, what appears to be protein-like bodies disappear between 0 and 12 hr. (Plates 34 and 35,36). Although the scutellar node is only a small part of the axis, analysis on the whole axis tends to show that there is an associated increase in free amides and amino acids in this 0 - 12 hr. germination period. This increase in amides and amino acids seems to coincide with the disappearance of electron dense bodies which were labelled protein. It is therefore possible that this labelling was correct.

Now, the suggestions in Figs. 3, 7, 8 and 9 for scutellum and axis nutritional self reliance seem reasonable with respect to the fact that the degradation, loss or increase in nutritional products in the one organ is not accompanied by an associated increase in the other. In fact, Figs. 7 shows that at germination (about 17 hr.) while the scutellum is synthesising sucrose the growing axis is still 'on its own' as far as the content of this important metabolic sugar is concerned. It is only after 18 hr. that the real beginning of transport from the scutellum to the axis can be detected.

Taken together, the results tentatively suggest that the axis may have enough food material to exist independently until germination has occurred. This agrees with the findings of Bain & Mercer (1955) for Pisum sativum. Thus the germination process in barley grains appears to have nothing to do with endosperm modification or the transfer of food material from the scutellum to the axis; only seedling growth is dependent

on such translocation.

To verify this matter further it was decided to perform dry weight determinations over the pre- and immediate post-germination periods. Fig. 10 shows that increases in the dry weight of the axis only began to show themselves after 16 hr. when endosperm modification would have commenced. The dry weight of the scutellum changes very little over the 72 hr. growth period and the drastic fall in dry weight of the endosperm strongly imply that the scutellum is mainly an absorbing organ and an important tissue link between the axis and the endosperm for bidirectional transport of hormonal and food materials- especially after germination. Therefore germination is exclusively an embryo event, and materials in the endosperm appear to have no important role in that growth process. From this, it would appear that such descriptions as '7 days germination' are quite meaningless (see Briggs, 1964 ; Van Der Eb & Nieuwdrop, 1966).

Some Aspects of Water Relations in Germination: Fig. 11 shows that the scutellum and the axis, on the basis of their own dry weights, are more efficient water absorbing organs than the endosperm. As shown in Fig. 11 the water content of the scutellum follows that of the axis and the strange 12 - 18 hr. peak can only be correlated with one important event, i.e., the emergence of the root initial from the coleorhiza which has ceased to elongate - see Plate 1. Although root emergence

seems to be the main correlate of this inrush of water, an axis-to-scutellum pathway of water movement during germination is also inferred from Fig. 11. Following Ermilov (1960), it is suggested that the endosperm takes up its own water mainly by inbibition and other physical properties such as diffusion.

At first, this correlation between 'water-rush' and root emergence was thought to be an artifact. However, it was remembered that during the early stages of this thesis some work was performed along with Dr. Greig (from Distillers' Company) on the water relations of barley grains which were steeped along similar lines to those of conventional maltings. Results are shown in Table IV. Again fluctuations in the axis affect mainly the water content of the scutellum; however, now there is no associated rise in the scutellum as there is in 'normal' germination when ample supply of water and air are available. The axis is nevertheless the important organ and at 12 hr. growth (36 hr. after initial steep-in, when the roots are about to appear) there is, as in the immediate pre-germination period in Fig. 11, a 'rush in' and 'falling off' of the water content of the axis. The large water fluctuations of the axis appear to have more influence on the water content of the scutellum than on the water content of the endosperm. Nonetheless the endosperm may suffer slight losses to the scutellum over the 72 hr. growth period.

Despite the similarity in the two types of germination where an immediate pre-germination inrush of water into the axis was observed there is the important difference that in the steeped condition the direction of water movement is now reversed, i.e., from endosperm to axis as opposed to the normal axis to scutellum pathway.

Possible Implications in Malting: The direction of water transport from the endosperm to the axis and the ensuing loss of water from the two important organs (scutellum and endosperm), the junction at which the production of fermentable sugars is initiated, might hold certain disadvantages for the maltster with respect to final extractability. In addition to this there is also the possibility that the transport of the gibberellic acid-like factor which is supposed to pass from the embryo to the endosperm to initiate endosperm modification might be impeded. Nonetheless, on the malting floor, with very little water, the axis is indulging in a transport pathway which is quite 'sensible' as far as its own welfare of survival is concerned but this may not be desirable for the maltster. In my estimation some system of a small constant supply of water would be more advantageous than an initial heavy steep.

In conclusion, it would appear that water plays an important role in the emergence of the root initials from the coleorhiza and the failure of the axis to initiate this pre-germination inrush of water might be important in some types of barley grain dormancy (e.g., water sensitivity).

PART 2A

Some Metabolic Aspects of the Utilisation of Sucrose, Raffinose and Galactose

The obvious rapidity of decline of sucrose and raffinose (Figs. 7 and 8) prompted a study of the localisation of both sugars in the embryo and of the enzymes which hydrolysed them.

From chromatographic evidence it was found that the distribution of sucrose, raffinose and invertase was as shown in Table V at 2 hr. germination; where the shoot (acrospire) contains mostly sucrose, the root (scutellar node + root) mainly raffinose and the scutellum as much raffinose as the root but more sucrose than the shoot. High invertase activity is associated with low sucrose content (viz. root) but the distribution of raffinose seems to be unaffected by invertase.

Over the early germination period, although raffinose decline was chromatographically evident, ethanol extracts showed no presence of galactose or melibiose.

An investigation was then made of the effect of crude enzyme extracts from the scutellum and the axis, on the degradation of raffinose, at 2 hr., 12 hr. and 18 hr. growth times. The results of this investigation are given in Table VI. In Table VI the results suggest that an enzyme capable of hydrolysing raffinose was present in both the axis and the scutellum. However, as would be expected from Fig. 8, the axis appears to possess greater enzymic capabilities, with respects to raffinose degradation, than the scutellum. This hydrolysis appeared to

have occurred without the detection of melibiose. However, if instead of 3 mg. of substrate, 5 - 10 mg. were used then traces of melibiose were always present; but these traces were considered to result mainly from the enzymic action of invertase.

It would therefore appear that in barley embryos there was an enzyme capable of hydrolysing raffinose, other than invertase. But the question of the fate of galactose in the plant and the reason why it was not located in ethanolic extracts of the germinating embryo remained unsolved.

Mindful that galactose is reported not to be metabolically active in promoting root growth (Street, 1962), it was decided to grow barley embryos on all the possible constituents of the raffinose molecule and to examine them for their sugar content.

Table VII gives the results for shoot and root growth. Galactose lived up to expectations by not stimulating root growth but glucose, although less effective than sucrose, was found to be quite effective in promoting root growth. Street (1962) points out that this growth-promotive effect of glucose seems to be limited to cereal roots. However, from this exercise, the real surprise was that in the galactose-fed embryos an unparalleled accumulation of glucose was found. It was then thought that possibly the embryos of barley could convert galactose to glucose. Mindful of this possibility the following experiment was conducted.

Crude dialysed enzyme extracts from the axis and scutellum of barley grains (2 hr., 12 hr. and 18 hr. growth) were incubated as before at 37°C but only with galactose. Results are shown in Table VIII. From Table VIII it appears that the extracts from both the axis and the scutellum are capable of producing glucose from galactose and, as expected from Fig. 8 and Table VI, the axis was more efficient at this conversion than the scutellum. From Table VIII it would also appear that this apparent epimerisation was much less intense at 18 hr. growth, which coincides with the growth time for the intact grain (Fig. 8) when raffinose degradation begins to level off. The crude dialysed enzyme extract did not affect melibiose.

After the completion of this work it was realised that a very complex conglomerate of galactose-metabolising enzymes was found in germinating soybeans by Pazur et al. (1962). They found, among other enzymes, galactokinase, UTP:Gal-1-Uridyltransferase and UDP:Gal-4-epimerase. These workers did find that melibiose was slowly hydrolysed by their enzyme system but only after 12 hr. incubation.

The tentative conclusion drawn from the above results is that raffinose is metabolised during the early stages of germination as is the case in soybeans. It would appear that in the intact grain, raffinose is not degraded into melibiose and fructose but possibly into galactose and sucrose. The galactose is then rapidly epimerised, possibly by a UDP:Gal-4-epimerase system, to glucose and the sucrose enters the general sucrose

catabolic pool. Such an explanation would account for the non-detection of galactose in ethanolic extracts. Since galactose is of little immediate metabolic use to the plant its quick in vivo removal would be desirable.

The function of raffinose is still doubtful but being the next abundant oligosaccharide to sucrose found in plants (French 1954) it seems that it must be of some importance. Since galactose in the free state is possibly harmful then this may be a way of binding up galactose and also a way of storing sucrose. In passing, it was noted that when galactose and sucrose were used for growing excised embryos only in that treatment was a positive raffinose spot detected on the chromatograms, and growth was increased over that of galactose alone (Table VII).

GENERAL CONCLUSION

In this section biochemical support was given to sub-structural observations in Section 1 that a possible fat to starch conversion took place in the scutellum of germinating barley embryos. The fluctuations of lipid, amides and amino acids contents of the scutellum during germination and seedling growth were in keeping with proposed subcellular organelle labellings.

Raffinose was mainly found in the root, sucrose in the shoot and the scutellum had about as much sucrose as the shoot but more raffinose than the root. In the embryo raffinose degradation was more rapid than in the axis but general raffinose degradation was about completed at the commencement of seedling growth and involved enzymic epimerisation of its galactose moiety to glucose. Sucrose on the other hand underwent drastic degradation by the embryo until the onset of endosperm modification whereupon sucrose synthesis in the scutellum commenced with subsequent sucrose transfer into the growing axis.

Dry weight determinations on the scutellum, axis and endosperm supported the possibility that despite phylogenetic evidence that the scutellum was the cotyledon of cereal grains it would appear that at least during the germination process in barley grains the scutellum merely converted one major storage product into another and catabolised other available food products for its own metabolic needs. This implies that as far

as nutrition during germination was concerned the axis was self-reliant.

None the less, in Section 1 it was shown that the scutellum was structurally equipped for absorption and transport of degraded endosperm food products to the axis. The sucrose results of this section give some vindication of that structural proposal.

A rapid inrush of water into the embryo during the immediate pre-germination period was observed and this may be an integral part of germination, with respect to the actual escape of the root from the coleorhiza. However, there is much scope here for further work.

An important point which emerged from Section I and this section was that germination was completed at the very commencement of endosperm modification and that during this germination period many profound structural and metabolic changes took place. In addition to this, anatomical studies (Section I) revealed that the embryo was not a uniform body but a composition of distinct organs which had extreme structural and metabolic differences.

Therefore, in the final section of this thesis it was decided to include studies on the various distinct embryo organs in combination or in isolation, in an effort to deduce physiological information on hydrolytic enzyme production within the embryo and the hormonal performance of the organs concerned with endosperm modification and to a lesser extent with lignification and cell elongation.

TABLE III

Sucrose and Raffinose in Embryos from (a) grains grown normally (12 hr.) and from (b) 2 hr. Excised Embryos Transferred to Endosperms of 40 hr. grown Grains: Further Growth Period of 10 hr. after Reconstruction.

	Sucrose Scutellum	Sucrose Axis	Raffinose Scutellum	Raffinose Axis
(a) Normal Grains	3.90 mg.	2.60 mg.	0.71 mg.	1.10 mg.
(b) Reconstructed Grains	9.60 mg.	4.80 mg.	2.43 mg.	2.43 mg.

Other prominent oligosaccharides found: maltose, usually present after modification had occurred, and maltotriose.

Growth temperature = 25°C.

TABLE IV

Water Content Changes During Steeping and Growth

Mean % Moisture Content per Dry Weight for Samples of 30 Grains after:

	Steeping				Growth (after steeping)				
Part of grain	Dry grains	1 hr	16 hr	24 hr	5 hr	12 hr	24 hr	48 hr	72 hr
Husk	8.6	48.5	50.6	50.6	46.1	21.4	24.9	20.7	20.1
Axis	11.6	44.6	63.7	80.6	70.0	78.8	73.3	76.6	72.9
Scutellum	19.0	39.2	65.6	53.6	61.9	61.4	57.9	55.7	56.4
Endosperm	12.2	18.2	34.7	38.7	39.4	37.5	37.3	36.3	35.2
Whole embryo	12.2	42.2	64.9	65.5	66.3	70.2	64.5	66.3	67.6
Whole grains	12.2	24.7	40.6	42.9	43.2	42.7	42.7	42.6	42.3
Time from 0hr	0	1	16	24	29	36	48	72	96

Steeping and Growth at 25°C.

Grain pieces dried in aluminium containers at 120°C for 24 hr.

TABLE V

Presence of Raffinose, Sucrose and Invertase in Different Organs of 2 hr. Soaked Embryos.

	Shoot	Root	Scutellum
Sucrose	++	+	+++
Raffinose	+	++	++
Invertase	+	++	+

Symbols for sucrose and raffinose not equivalent: the results are valid only along one line. Soaking at 25°C.

TABLE VI

Effect of Crude Enzyme Extracts from Axes and Scutella on Raffinose

(1½ hours Incubation at 37°C.)

Extract from	Sugars Present at Given Times		
	2 hr.	12 hr.	18 hr.
Scutellum	Raffinose	Raffinose *Galactose (trace) Glucose	Raffinose
Axis	Raffinose *Galactose (trace) Glucose Fructose	Raffinose *Galactose (+) Glucose	Raffinose

*A Product of Raffinose degradation. Note absence of melibiose.

+ = more than a trace.

Growth temperature = 25°C.

TABLE VII

Growth of 2 hr. Excised Embryos in Various Sugars

Media	Mean Root Length	Shoot Length
Water	4.0 mm.	4.0 mm.
Galactose = 1%	4.5 mm.	4.0 mm.
Melibiose = 1%	6.0 mm.	4.0 mm.
Sucrose = 1%	12.5 mm.	4.5 mm.
Sucrose = 1% + Galactose = 1%	8.0 mm.	4.0 mm.
Glucose = 1%	10.2 mm.	4.5 mm.
Fructose = 1%	11.8 mm.	4.0 mm.
Raffinose = 1%	8.0 mm.	4.0 mm.
Glucose = 2%	9.0 mm.	4.0 mm.
Glucose = 2% + Sucrose = 1%	13.0 mm.	4.0 mm.

Growth temperature = 25°C.

Note non-stimulative action of Galactose.

TABLE VIII

Effect of Crude Enzyme Extracts from Scutellum and Axis on Galactose.

	Length of Growth Period		
Source of Extract	2 hr.	12 hr.	18 hr.
Scutellum	*Glucose + Galactose Raffinose ?	*Glucose ++ Galactose Raffinose ?	*Glucose + Galactose --
Axis	*Glucose ++ Glactose Raffinose ?	*Glucose ++ Glactose Raffinose ?	*Glucose + Glactose --

*Sugars found after 1½ hr. incubation of galactose with enzyme extract.

Growth temperature = 25°C.

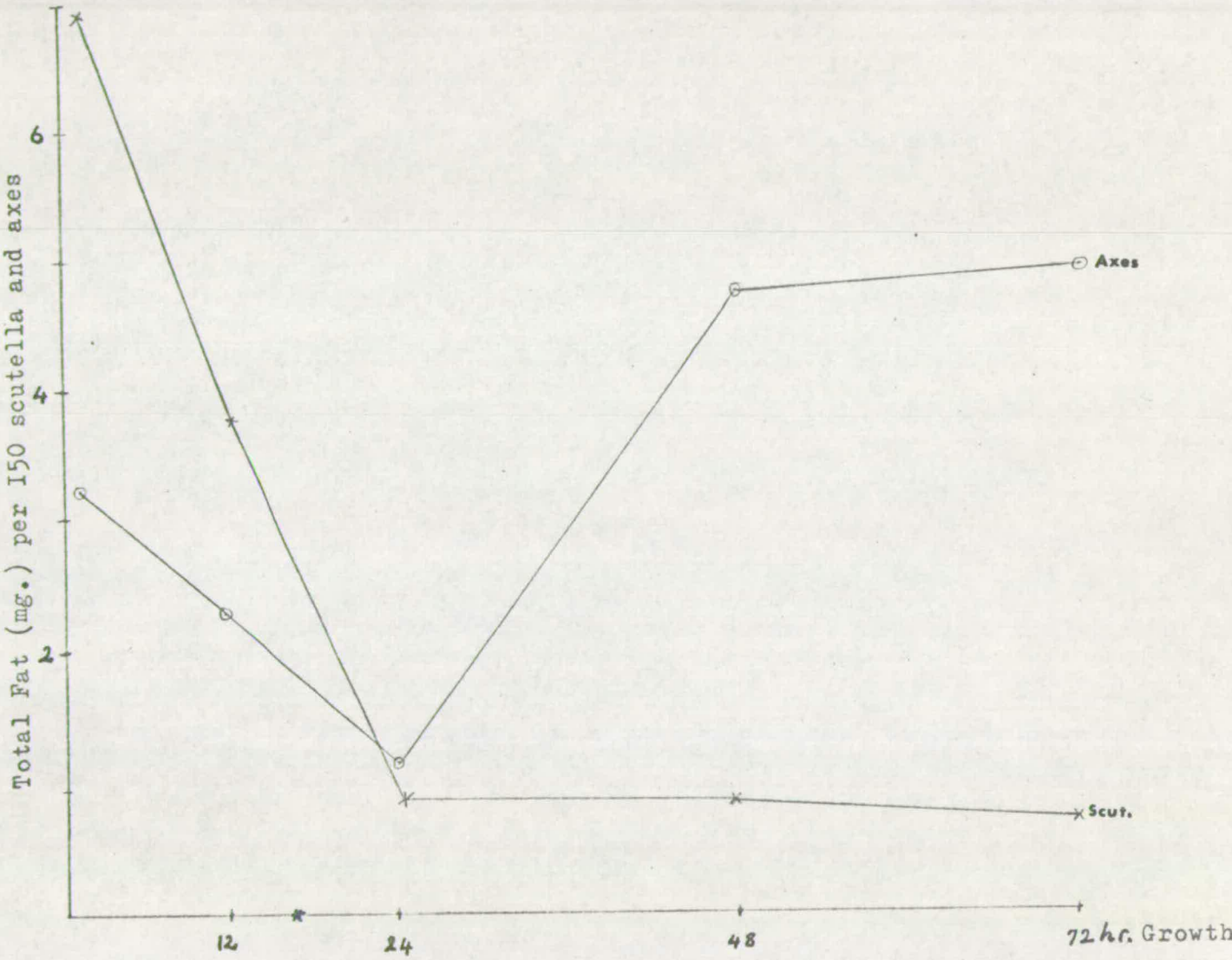


Fig. 3. Changes in Fat content during germination and seedling growth.

* Germination.

Temperature = 25°C.

Scut. = Scutella.

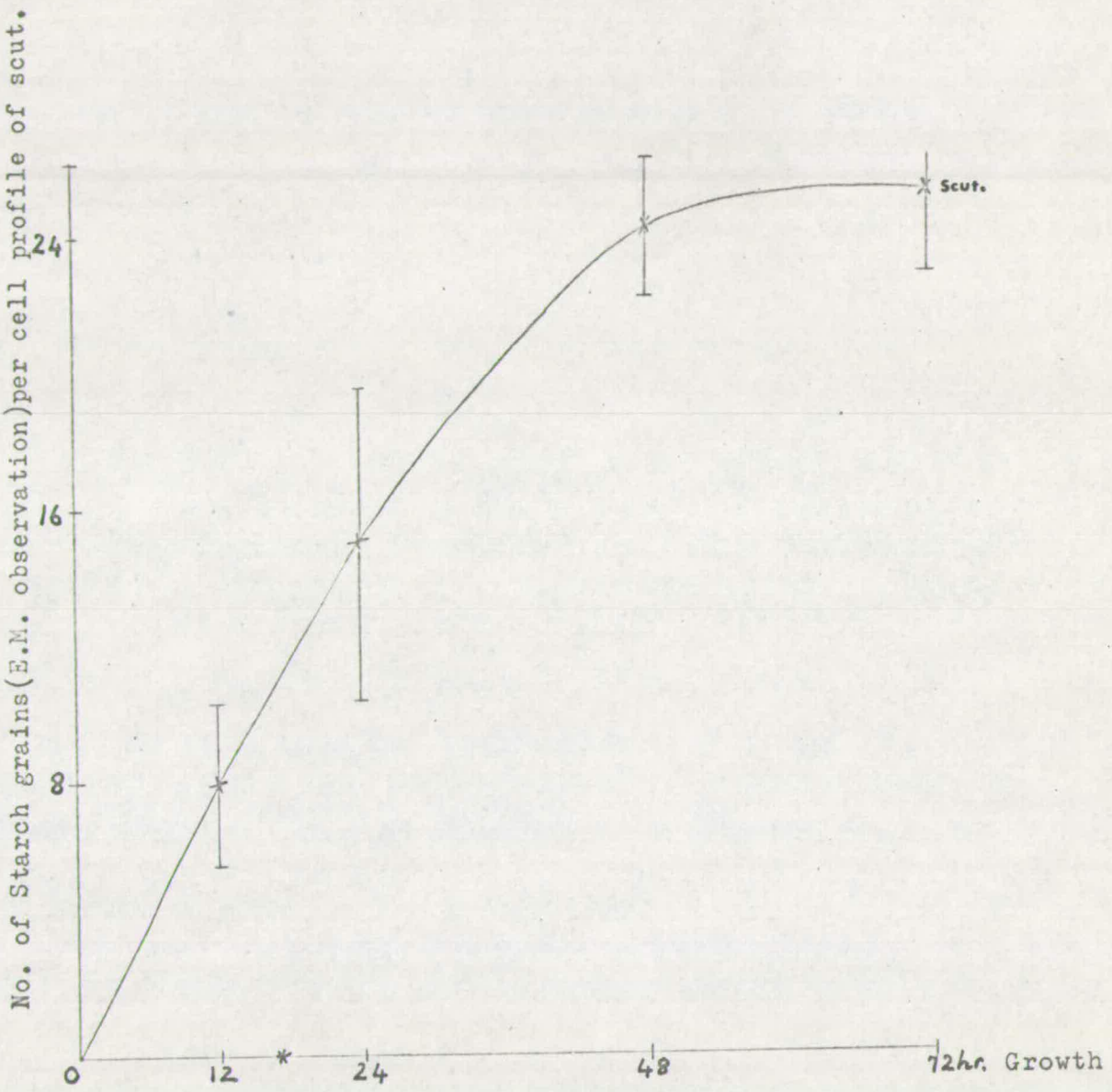


Fig. 4. Starch grain accumulation in scutellum during germination and seedling growth.

* Germination.

Temperature = 25°C.

Scut. = Scutellum.

Vertical rod = Standard Deviation from mean.

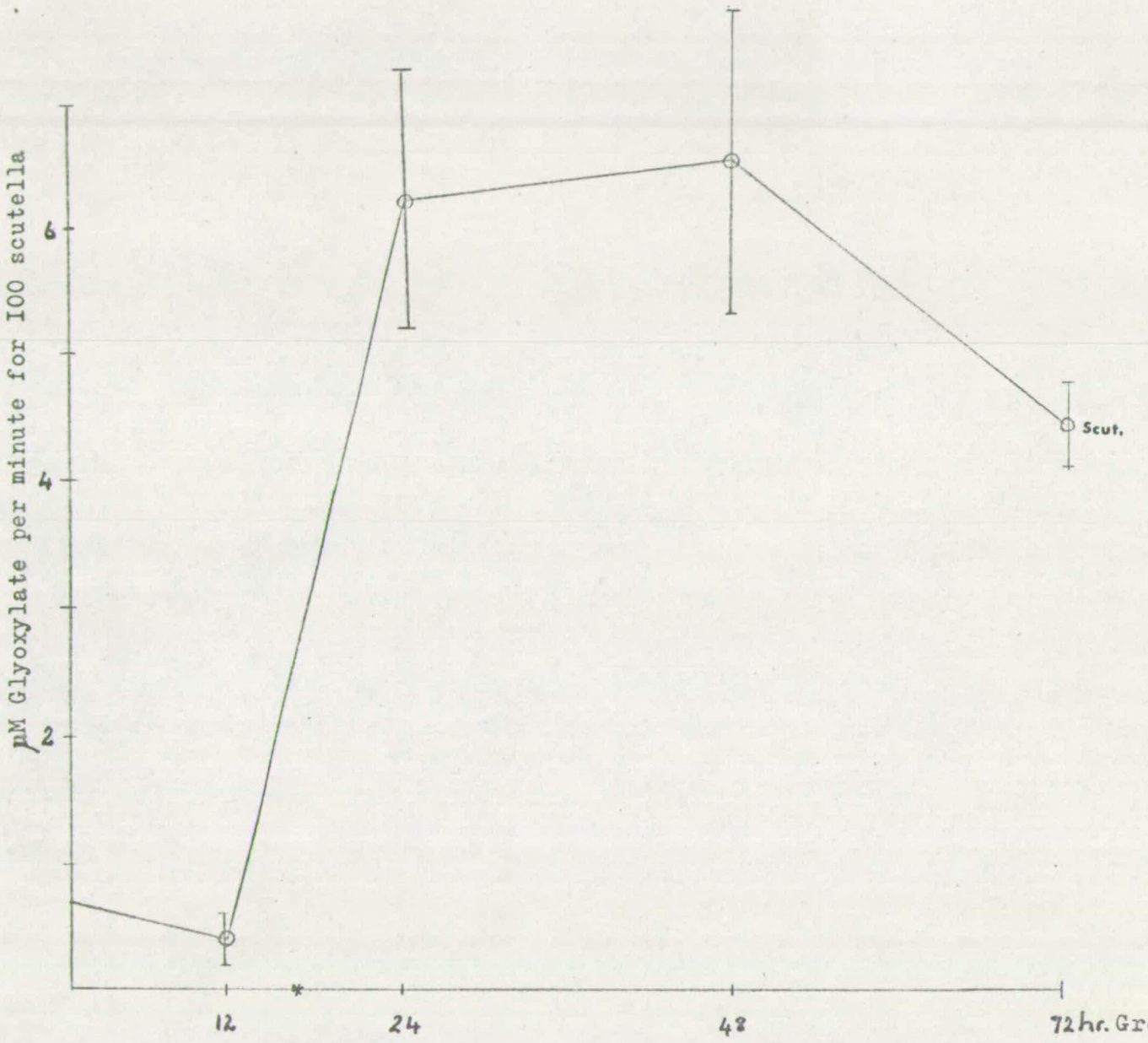


Fig. 5. Changes in Isocitritase activity during germination and seedling growth.

* Germination.

Temperature = 25°C.

Scut. = Scutella.

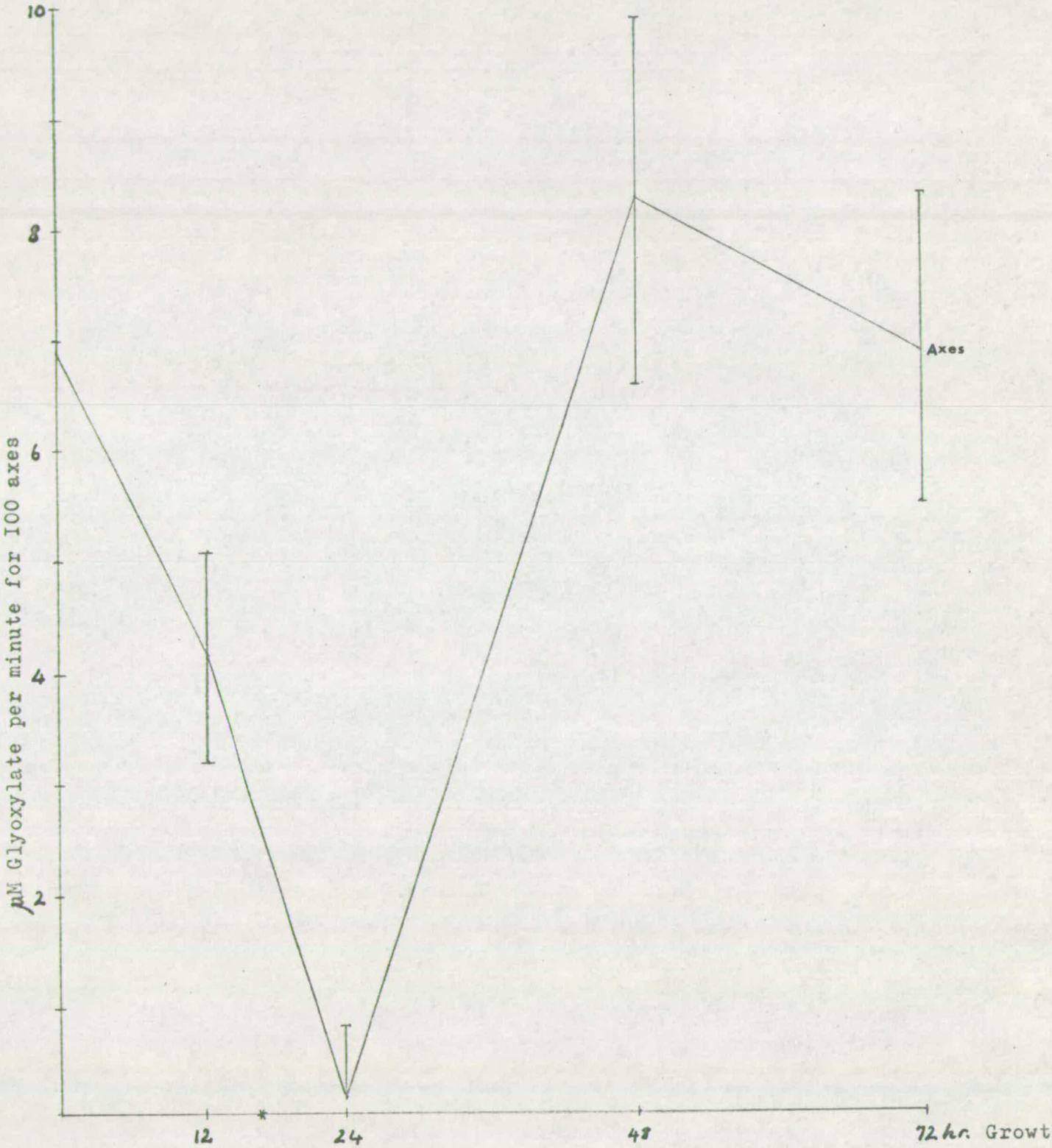


Fig. 6. Changes in Isocitritase activity during germination and seedling growth.

* Germination.

Temperature = 25°C.

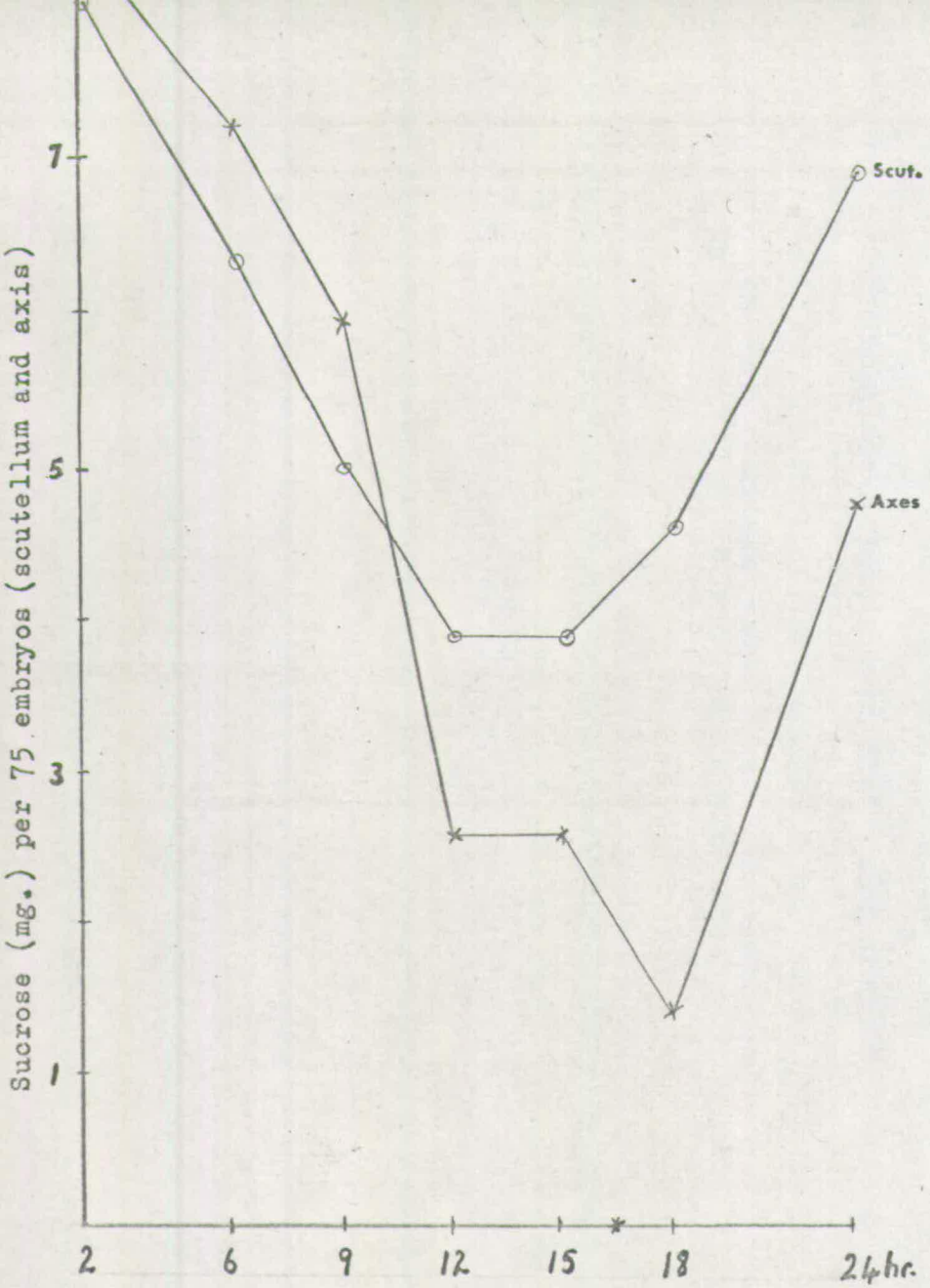


Fig. 7. Sucrose metabolism during germination and seedling growth.

* Germination and start of Endo. Modification.

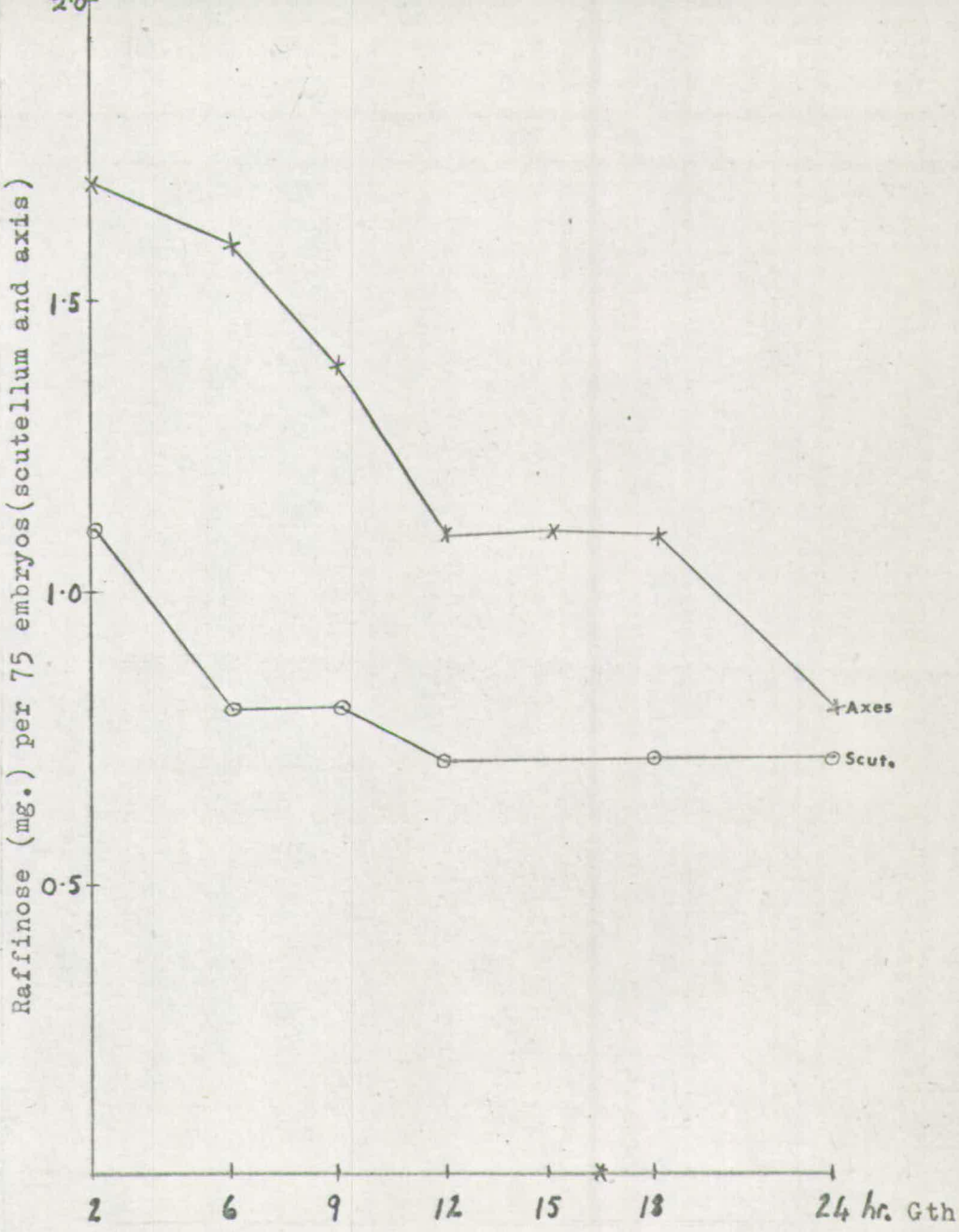


Fig. 8. Raffinose metabolism during germination and seedling growth.

* Germination and start of Endo. Modification.

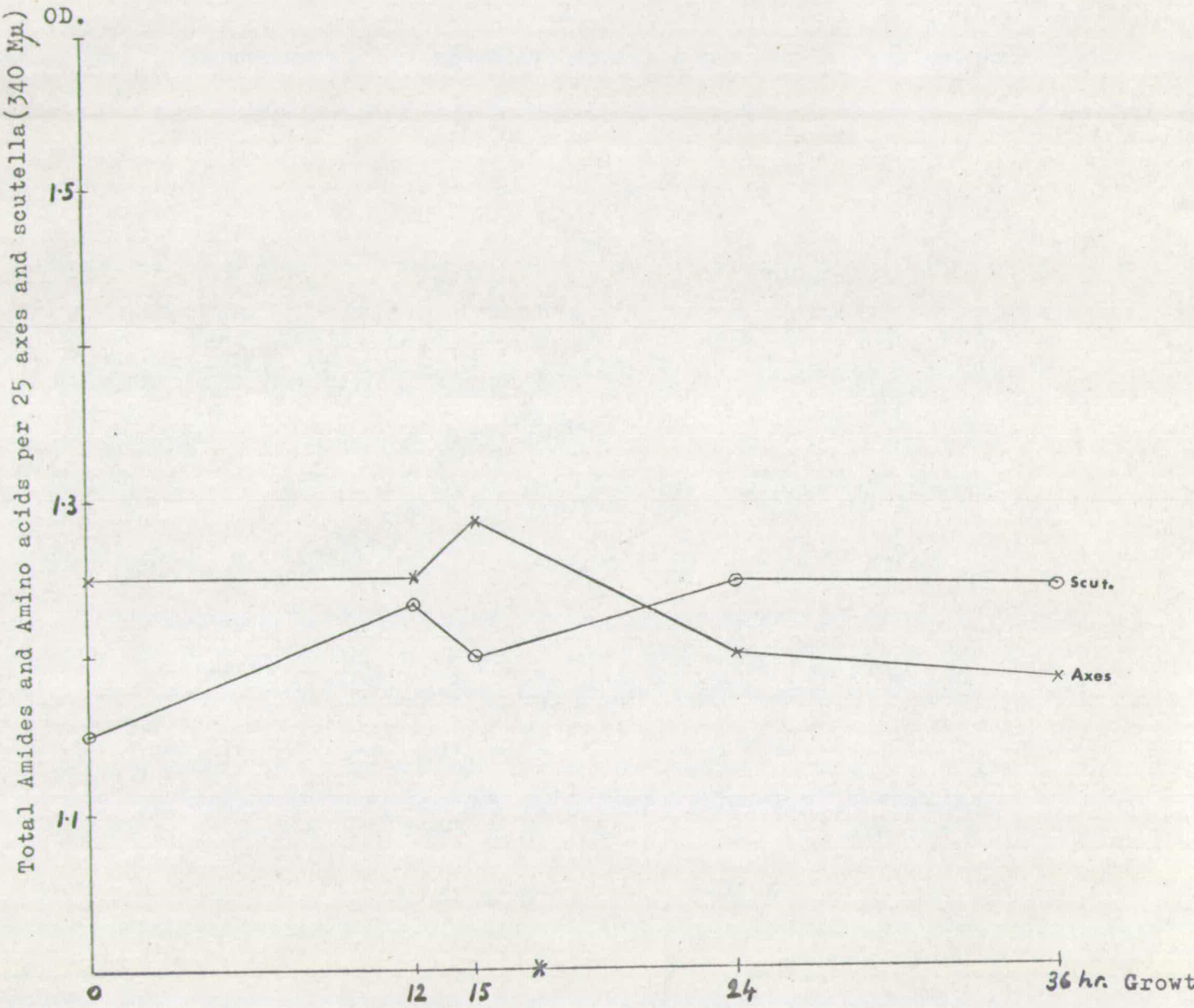


Fig. 9. Changes in Amides and Amino acids during germination and seedling growth.

* Germination.

Temperature = 25°C.

Scut. = Scutella.

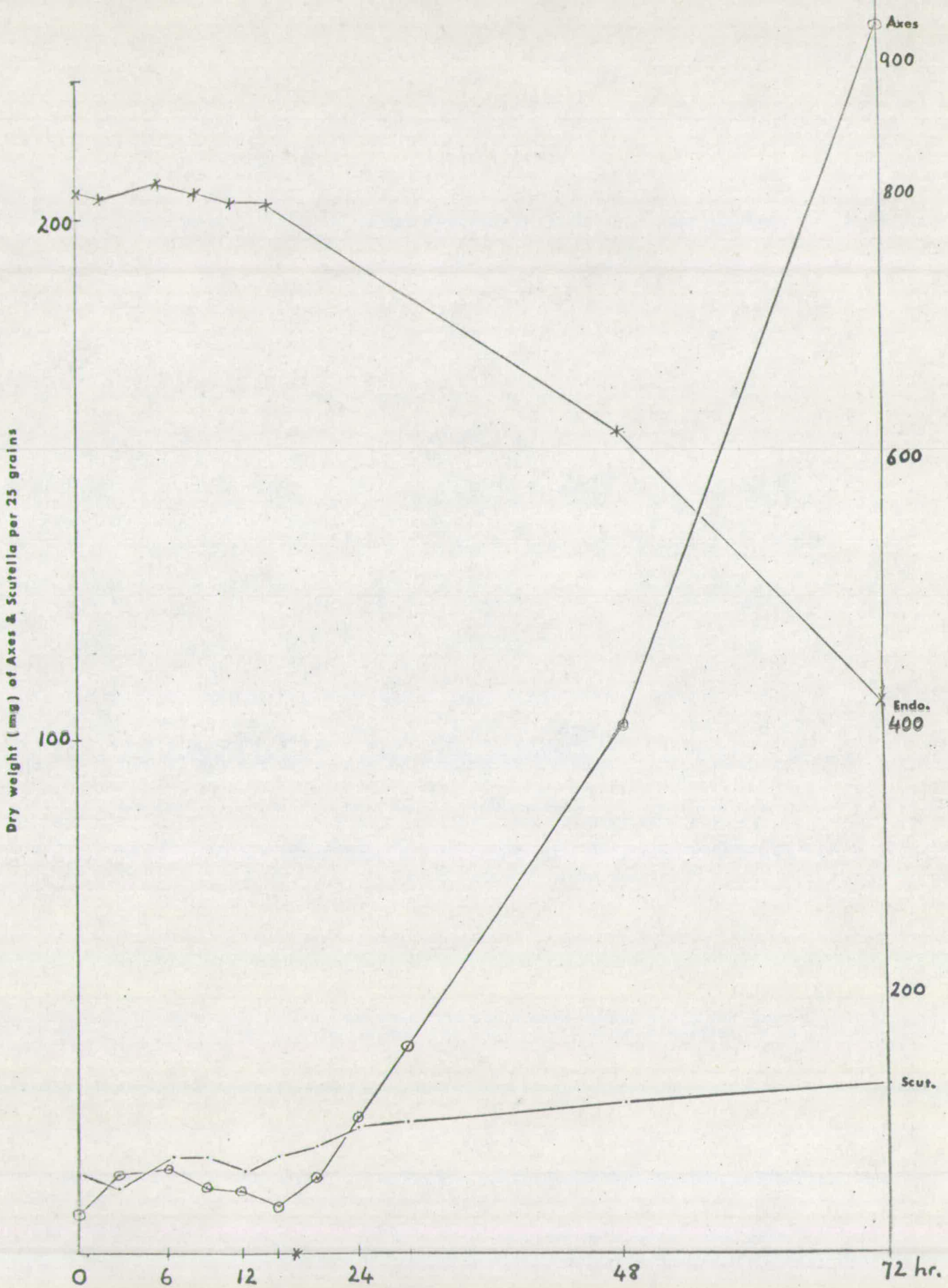


Fig. 10. Dry weight changes for endosperm, axis and scutellum during germination and seedling growth.

* Germination.

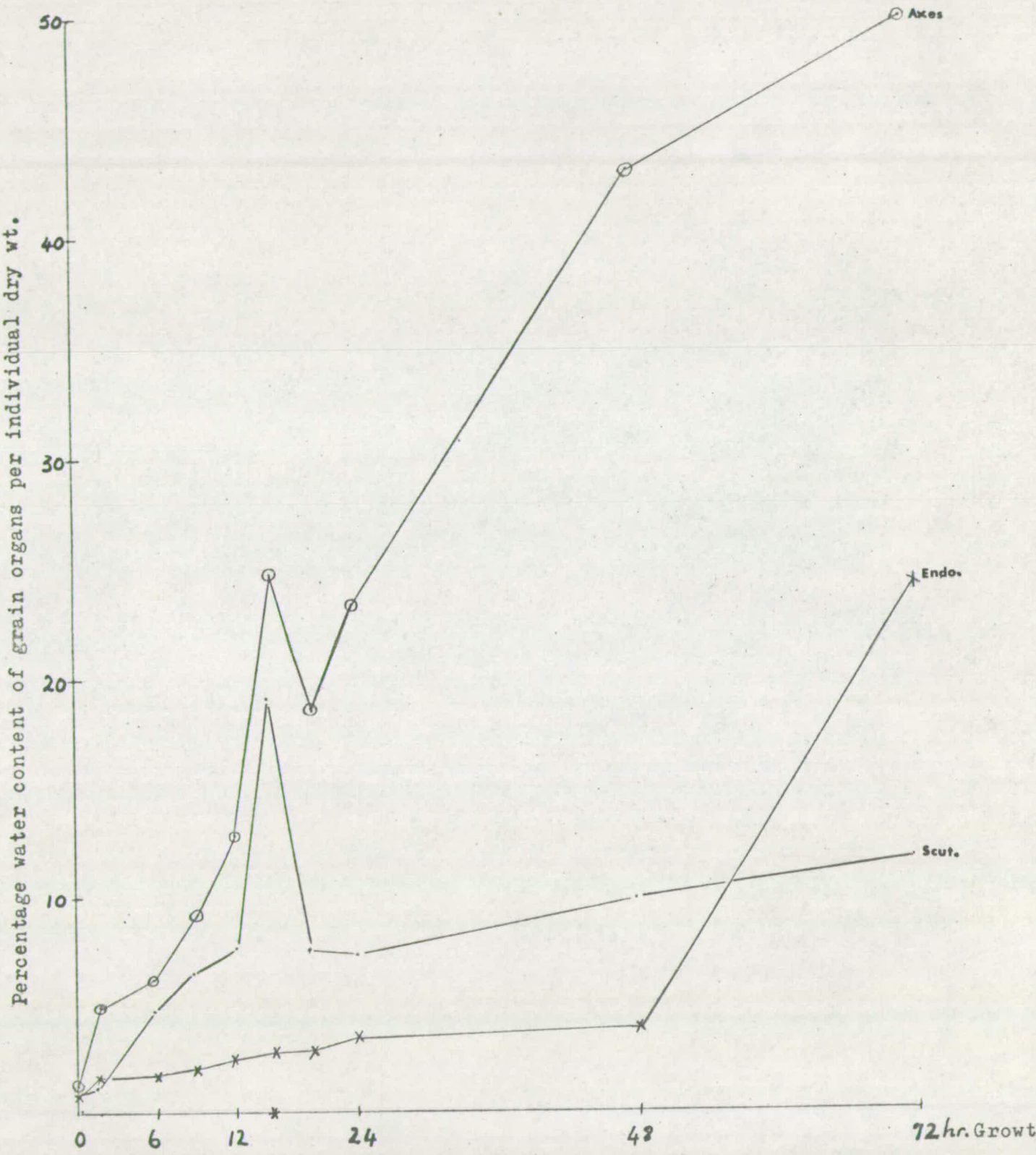


Fig. II. Fluctuations in water content of grain during germination and seedling growth.

* Germination.

Temperature = 25°C.

SECTION III

HORMONAL ASPECTS OF BARLEY GERMINATION AND SEEDLING GROWTH.

INTRODUCTION

It has long been recognised by plant physiologists that as a necessary prerequisite for the understanding of correlative growth and developmental processes in intact plants, it is essential to gain some insight into the physiological importance of individual organs. Brown & Morris (1890) were early pioneers of this approach and by separating the barley grain into embryo and endosperm they did much to establish many of the facts about barley germination and subsequent seedling growth which are now accepted as commonplace. Although some of their ideas have undergone extension and modification (see Sections I and II of this thesis) it was shown that many of their basic observations are still sound. None the less, one must recognise both the usefulness and limitations of any surgical approach because 'wounding' can cause the appearance of traumatic acid, (Bonner & English, 1938) and this has recently been reported to cause slight endogenous Indoleacetic acid destruction in Lens root tissue (Pilet, 1965). Surgical treatments were, however, used in this hormonal section in an attempt to gain some insight into the possible hormone role of the various organs of the embryo especially in the process of endosperm modification. The surgical method was employed because no better approach presented itself.

From this study it is hoped that sufficient information will be forthcoming to indicate: (i) possible hormonal aspects of epithelial elongation and hydrolytic enzymic secretion by

these cells, (ii) the embryo site of origin of the gibberellin-like factor which has been reported to move from the germinating embryo to the endosperm to effect modification (Yomo, 1958; Paleg, 1960; MacLeod & Miller, 1962), (iii) whether indoleacetic acid has any useful role in the germination and seedling growth processes and, finally, (iv) the chemical and physiological nature of the proposed gibberellin-like factor of the matured germinating grain.

The desired aim is the acquisition of useful physiological information which, in conjunction with the results from Sections I and II, may serve to give a better understanding of the correlative developmental aspects of barley germination and subsequent seedling growth processes. Here, therefore, the proposed emphasis is not on a "pharmaceutical" approach where isolated barley grain tissues such as coleoptile, foliar shoot, scutellum or endosperm are subjected to external additives in liquid or solid media, mainly for the sake of gaining physiological data on the additives used.

In this section each major experimental study is presented on its own and the whole represents a sequential development and analysis of ideas.

PART 1

EPITHELIAL CELL ELONGATION

Observational evidence, given in Section I shows that the epithelial cells, over a 72 hr. growth period (see Table II and Plates 3, 4, 5), elongated from 30 to about 80 μ. Since this dramatic elongation might be associated with the transfer of degraded food reserves from the endosperm to the scutellum and thence to the axis (see Section II, Part 2) it was decided to investigate the effects of a small variety of growth-regulating substances on the elongative properties of these cells. According to most plant physiologist (see Leopold, 1964.) Indoleacetic acid (IAA) or Gibberellic acid (GA) can be important hormones of cell elongation while in special circumstances Tripalmitate (Stowe, 1961) or Adenine (Bonner & Galston, 1952) can also stimulate elongation. Kinetin seems to be mainly concerned with cell division (see Leopold, 1964); while Triiodobenzoic acid (TIBA)can function as a competive inhibitor of indoleacetic acid (Audus & Thresh, 1956a,b; Audus, 1963).

Although it was hoped to gain some insight into the hormonal feature of epithelial cell elongation it was also decided to make observations on the response of the scutellum to two extreme concentrations of indoleacetic acid, 10^{-5} and 10^{-11}M. According to Thimann (1937) the higher concentration generally stimulates an elongative response in shoots while the lower concentration usually promotes an elongative response in

roots. In the Material and Method section the significance of the removal of the entire axis has its basis in the well known fact that coleoptile apices are efficient producers of indoleacetic acid (Went, 1928; Thimann, 1963).

MATERIALS AND METHOD

Elongation of epithelial cells in excised cores of scutellum

Dehusked grains were soaked for 2 hr. in glass-distilled water, the axes were dissected away using a No. 11 sterilised scalpel blade, and 1 mm. cores of scutellar tissue were removed from the central region of the scutellum. These cores on anatomical investigation were seen to comprise storage parenchyma cells, ramification of provascular cells and epithelial cells. For the removal of these cores a minature cork-borer-like instrument, originally designed and constructed for another series of experiments, was used.

After removal, 12 cores were placed in heat sterilised 3 x 1 inch glass tubes containing 1 ml. of the relevant incubation medium. 'Incubation medium only' was a full complement of Knops nutrient mixture dissolved in glass-distilled water and sucrose at 1.5% concentration. The whole medium was buffered with 1 mM acetate buffer, and had a final pH of 6.2. The other media were identical with respect to the concentration of Knops nutrient mixture, sucrose, acetate buffer and final pH. These other media contained the following substances at the indicated molarity and concentrations: IAA 10^{-6}M, IAA 10^{-11}M, IAA 10^{-11} + GA 10^{-5}M, TIBA 10^{-5}M, Kinetin 10^{-6}M, Adenine 10^{-6}M, and Tripalmitate 0.05 mg./l (after Stowe 1961).

The tubes were plugged with non-absorbent cotton wool and shaken in a water bath at 25°C. The initial epithelial cell lengths after 24, 48 and 72 hr. incubation were checked

with the aid of a slide micrometer. The cores were thinly sliced with a sharp razor blade and temporary mountings were in glycerol.

All glassware and filter paper used in this series of experiments were soaked in alcohol and oven sterilesed (120°C) and cooled before use.

Elongation of epithelial cells in whole excised embryos

Grains were soaked for 2 hr. and whole embryos were separated by careful manipulation of an alcohol-flamed No. 11 scalpel blade. The whole ringing operation was performed at the scutellum-endosperm junction. The incubation media relevant to this experimental section were of the constitutions indicated above. The growth container was as follows: 2.5 inch lids of small petri dishes were placed (flat surface uppermost) on the bottom of crystallising dishes, 3.5 inch in diameter. 2 rings of clean Whatman No. 1 filter paper were cut to fit the area of the small petri dish lids except at two points, about ½ inch wide, which were bent down so that they touched the incubation media and, as a result, functioned as wicks (petri-wick dishes).

30 ml. of the appropriate incubation medium were placed in separate containers. After mass dissection of embryos, about 25 were placed in each container, scutellar surface downward on the saturated filter paper surface. The crystallising dishes were uncovered as little as possible, and were protected by lids of 4 inch petri dishes. Incubation was in an oven at 25°C.

Elongation of the epithelial cells of embryos partially excised after 2 hr. soaking. Grains were soaked for 2 hr. Ringing and excision was such that the partially excised embryos were left attached to the endosperm by a thin bridge of tissue, viz., the pericarp-tasta - aleurone layer. After surgical treatments the grains were placed on the moistened surface of filter paper rings previously cut to fit into 4 inch petri dish containers. Distilled water was used to moisten these filter paper rings. Incubation was in an oven set at 25°C. Microscopic examinations for elongation, were conducted by the method already described. However, in these examinations the whole scutellar epithelial surface was examined.

Epithelial cell elongation of partially excised embryos previously intact for 24 hr. Grains were placed to germinate on two rings of Whatman No. 1 filter paper, previously cut to fit into a 5½ inch sterilised petri dish. Distilled water was used to saturate the filter paper substrate. As in all other experiments a strict separation of the grains was maintained. It was found that clustering encouraged fungal development. In some preliminary experiments, a member of the Mucorales was invariably the contaminant, but with proper precautions infection was very rare. After 24 hr. growth, grains with the standard 24 hr. acrospire length (mean 0.5 cm.), root lengths (mean 0.4 cm.) and number of initiated roots (usually 5), were selected. Batches of six were transferred to 2.5 inch petri dishes containing two pieces of fitting filter paper. The wads of

filter paper were saturated with the following media: TIBA 10^{-5}M, Kinetin 10^{-6}M, IAA 10^{-5}M, GA 10^{-5}M, IAA 10^{-11}M, GA 10^{-5} + IAA 10^{-11}M, all buffered with ImM acetate buffer, and the final pH was 6.2. A ImM acetate buffer, pH 6.2, was used as control.

Another set of similar experimental treatment was performed, using partially excised scutella (axis removed) instead of partially excised whole embryos.

Lengths of the epithelial cells were determinated at 24 hr. (initial measurement) and 72 hr. (final measurement).

RESULTS AND DISCUSSION

As may be remembered from Section I, in the dry grain, the scutellar epithelial cells are adpressed to the imtermediary layer which represents tightly-packed cell walls which possibly become consolidated during maturation as a result of depletion of cell contents, presumably in association with maturation of the developing embryo. Table IX shows that none of the growth regulatory substances used were effective in promoting usual elongation of the epithelial cells on excised embryo 'cores' removed from 2 hr. soaked grains. Table X shows that even the epithelial cells of entire excised embryos, separated from 2 hr. soaked grains, failed to elongate to the same extent as the intact grain control although the seedlings of these excised embryos grew as well as those of the intact control.

It was then assumed that failure to secure any pronounced elongation of these epithelial cells might rest with the possibility ~~of~~ that some hormonal or nutritional factor was not present in the media used. Thus it was decided to excise the embryo only partially after a 2 hr. soak of the grains, therefore leaving it in contact with its own endosperm nutritional and possibly hormonal supply. Table XI shows that even then epithelial elongation did not occur, despite extensive endosperm dissolution and visually normal elongative growth of the seedlings as compared to the intact control.

At this point it was decided to make careful microscopic observation on the separation of the intermediate layer from the epithelial cells (Plate 28 shows close association between intermediate layer and epithelial cells). Eventually, microscopic observations revealed that there are delicate organic connections between the epithelial cell tips and the intermediate layer. It therefore appeared possible that during this 'apparently safe' 2 hr. separation of the embryo from its entire endosperm, cellular damage was inflicted on the epithelial cells thus rendering them incapable of subsequent elongation.

Now, (Section I) epithelial elongation was observed to be under way by 24 hr. growth (Table II) and it was therefore surmised that once elongation had commenced embryo-endosperm separation might not affect subsequent elongation. It was then decided to grow grains for 24 hr. partially excise them as before and subject these grains to the growth regulatory substances used in Tables IX and X.

In Table XII epithelial elongation comparable to that which takes place in the intact grain over 72 hr. growth is observed for the 24 hr. partially excised embryos. IAA 10^{-5}M, GA 10^{-5}M and Kinetin 10^{-6}M appear to retard elongation somewhat while IAA 10^{-11}M and especially TIBA seem to be without any promotive or adverse effect. This non-toxic TIBA effect is very interesting because in the partially excised scutella treatment (i.e., exclusion of axis and as a consequence its indoleacetic-acid-producing coleoptile tip) TIBA now reduces

epithelial elongation to a noticable extent, possibly signifying a physiological reduction in the endogenous content of indoleacetic acid compounds in the scutellum. Although this explanation is only tentative there is the possibility that epithelial cell elongation may be hormonally conditioned by a low concentration of indoleacetic acid, however further experimentation is required on, for example, the functioning of the competitive property of TIBA. None the less it is very interesting to observe that it is the lower concentration (IAA 10^{-11}) of indoleacetic acid which is without adverse effect on at least one physilogical feature of the scutellum, viz., epithelial elongation.

A chance observation was that IAA 10^{-11} sufficed to keep excised scutellar 'cores' turgid for 72 hr. incubation periods in culture; GA or Kinetin had no such ability.

CONCLUSION

Although there is no intention of suggesting that sufficient evidence was obtained in support of definite hormonal control of epithelial elongation it is tentatively suggested that the tolerance level of the scutellum for indoleacetic acid is within the concentration range suggested for root tissue (Thimann 1937). It therefore follows from this that if this hormone (indolacetic acid) may have a role in epithelial elongation, and there is no evidence to suggest it has not, then the effective indoleacetic acid concentration would be in the 10^{-11} concentration range.

It also appeared that epithelial elongation is not a precondition for the absorption of degraded endosperm food reserves, as partially excised embryos dissected after 2 hr. soak (thus no epithelial elongation, see Table XI) showed no visible signs of nutritional dificiency and, from Section II (Table III) it may be remembered that when 2 hr. excised embryos (therefore no epithelial elongation) were reaffixed to modifying endosperms they accumulated sucrose, presumably from endosperm hexose residues.

Present observations also seem to suggest that the intermediate layer (0 hr. grain) has feeble cell-wall attachments with the tips of the epithelial cells, and once epithelial elongation has commenced (viz., 24 hr. seedling) manual separation of the intermediary layer, unlike at 2 hr. is without subsequent deleterious elongative effect. The physiological significance

of why 2 hr. separation is deleterious may rest with the fact that these epithelial cells elongate by tip growth (Nieuwdrop & Buys 1964) and 2 hr. pre-elongation separation could possibly remove (observational evidence for this available) or irreparably dislodge the epithelial cell wall tip from its underlying cytoplasm (see Plate 28).

Now, if epithelial elongation is not an essential pre-requisite for food absorption, do these epithelial cells - despite the 'almost convincing' negative findings in Section I - in either elongated or non-elongated condition, secrete or produce any important endosperm degrading hydrolytic enzymes? A biochemical examination of this question, based on the anatomical architecture of the embryo, will be dealt with immediately below in Part 2.

PART 2

SCUTELLUM AND HYDROLYTIC ENZYME PRODUCTION

In Section I detailed time-course observations on endosperm modification failed to substantiate the apparently unfounded belief (cf., Brown & Morris, 1890; Laufer, 1964) that the scutellar epithelial cells were enzymically involved in endosperm modification. However there was still the unsolved paradox where, if the aleurone cells were uniquely responsible for the enzymic degradation of endosperm food reserves, how is it that excised barley embryos can secrete hydrolytic enzymes? For example, Briggs (1964) attributed 13.3% of the total α-amylase content of the intact grain, after 7 days 'germination', to the embryo. The question therefore remains - where in the tissue complex of excised embryos does this ability to produce hydrolytic enzymes reside? A biochemical examination of this problem was therefore undertaken.

MATERIAL AND METHOD

Separation of the scutellum into scutellar cores and scutellar rings

Since anatomical observations in Section I (see Plate 15) revealed that the aleurone layer might continue into the periphery of the scutellum as a single layer of small cells, it was decided that surgical separation of the scutellum into scutellar rings and scutellar cores was necessary for 'anatomical purity'.

(a) Miniature core borer. Mindful of the seemingly anatomical basis to the possible physiological cum biochemical problem at hand, a miniature cork borer-like instrument was designed and constructed to perform the surgical tissue separation. This borer had a stainless and rustproof bore, 1 mm. in diameter.

(b) Areas and weights of cores and rings. Grains, after careful selection for uniformity in size and soundness, were soaked for two hours in distilled water. The axes from 24 visually-sound grains were quickly but carefully removed. Each grain contributed only one core, which was removed from that region of the attached scutellum that adjoined the scutellar node. Six scutellar rings were also removed from their endosperms by careful ringing. The rings and cores were lightly blotted and weights for three different trials were as follows: 6 scutellar rings 0.0054 g. and 24 scutellar cores 0.0082 g. The relative areas were: 1 scutellar ring 3.1 sq. mm. and 4 cores total area of 2.9 sq. mm.

(c) α-amylase assay after Briggs (1961). β-amylase solution was prepared by extracting 10 g. soy bean flour with 100 ml. of a solution 0.008M with respects to sodium chloride and 0.0128M with respect to pH 5.3 acetate buffer. Soluble starch solution was prepared as follows: a suspension of 1 g. soluble starch in 10 ml. distilled water was stirred into 50 ml. boiling distilled water and after boiling for a further minute, was cooled rapidly. To the cold solution was added 8 ml. 0.1M sodium chloride and 12.8 ml. 0.1M pH 5.3 acetate buffer and the total volume was made up to 100 ml.

The iodine reagent used consisted of 0.254 g. iodine dissolved in a solution of 4.0 g. potassium iodide and diluted to 1 litre.

The assay was carried out in test tubes in a water bath at 25°C. Substrate was prepared by adding 4 ml. β-amylase solution to 20 ml. starch solution and this mixture was allowed to incubate at 25°C in a water bath for 1 hour. Thus the substrate actually used in the assay was a crude β-limit dextrin, which is not affected by any β-amylase or, over the short incubation period of the intended α-amylase assay (3 minutes), by R enzyme admixed with the α-amylase being assayed. To 1.5 ml. of the substrate solution was added 1 ml. of the enzyme extract which had previously been brought to the same temperature. A 0.2 ml. sample was taken out at zero time and mixed with 10 ml. iodine reagent. Three further samples were taken at one minute intervals over a period of three

minutes and treated identically. The colour produced in each sample was measured in an E.E.L. colorimeter, using an Ilford 608 filter. Blow-out pipettes were used.

In practice, the α-amylase activity was determined as follows. The experimental times and the corresponding colorimeter readings (minus enzyme blanks) were tabulated. The colorimetric reading was then multiplied by a zero time control set at 3.00 (gradient) and divided by the associated substrate blank. Each corrected reading was then read on a standard log graph (after Briggs, 1961) which on the average of three colorimeter readings per extracted enzyme sample gave α-amylase activity in Iodine Dextrin Colour (I.D.C.) units.

(d) <u>α-amylase determination on scutellar rings and scutellar cores.</u> For this determination, rings and cores were dissected out as already described. The assay on the 6 rings and 24 cores was conducted using Briggs' method. The preparation of the extracted enzyme was as follows: the rings and cores were separately homogenised by a hand homogeniser (glass) in 3 ml. 0.6% sodium chloride made up in 0.1M acetate buffer solution pH 5.3. The homogenate was allowed to extract for 1 hour, centrifuged in a MSE 8 centrifuge and the supernatant used for the α-amylase assay. This method of extraction was used through this series of experiments.

The appropriate number of rings and cores were either taken from intact of from excised whole embryos at appropriate growth intervals, (19, 24, 48 and 72 hr.) or initially from

2 hr. excised embryos for incubation of these rings and cores over similar time periods in similar growth containers.

Intact grains were grown in sterile petri dishes on two distilled-water-saturated rings of filter paper.

Excised embryos were grown in petri-wick dishes as described in Part 1, The incubation medium contained Knops nutrient mixture, 1.5% with respect to sucrose, 1 mM $CaCl_2$ buffered by 1 mM acetate buffer with a final pH of 6.2 - this was incubation medium without gibberellic acid (-GA). However, when GA was included the medium is called (+GA).

Each petri-wick dish contained 30 ml. of the respective medium. Again embryos were well spaced out and incubation temperature was 25°C. It took under 1 hr. to excise 200 embryos without visible damage. Results represent mean of three separate experiments.

RESULTS AND DISCUSSION

Fig. 12. for the intact grain, shows that the α-amylase of the rings significantly exceeds the α-amylase content of the cores especially after 24 hr. growth time. Here, it is interesting to reflect that this rapid increase after 24 hr. coincides with the photomicrographical evidence in Section I on the rapid phase of endosperm modification observed immediately after about 24 hr. growth (cf. Plates 7, 8, 9).

A similar disproportionate picture with respect to the relative α-mmylase contents in rings and cores is also observed in Fig. 13 for whole excised embryos grown on the incubation medium (-GA). However, here there is no decline in α-amylase content of the rings after 48 hr. which, in the intact grain, may be due to side effects of accompanying rapid endosperm modification at this growth time. A similar fall in α-amylase content after long incubation periods in Bromus was recorded by Johnston (1964). A possible explanation for this peculiar decline in α-amylase content will be given later. None the less. in Fig. 14, similar whole excised embryos (now with gibberellic acid in the medium)showed the same α-amylase disproportionality between rings and cores as in Fig. 13. Although the presence of gibberellic acid quickened the initial rate of appearance of α-amylase in the rings it somewhat reduced the content of α-amylase after long incubation periods. The reason for this may rest with the possibility that excised embryos (without added GA) can produce an optimal endogenous

supply of gibberellic acid-like substance (Yomo, 1958) and added gibberellic acid may deleteriously exceed this optimum. This seems possible as in Fig. 15, for rings and cores separated from 2 hr. excised embryos and incubated without gibberellic acid, it would appear that the removal of the axis resulted in the lowering of the endogenous gibberellic acid content of the excised rings or the α-amylase producing cells of the rings are somewhat damaged by separating the scutellum into rings and cores and their subsequent long incubation. Fig. 16 shows that the former possibility may be correct because when gibberellic acid is included in the medium the α-amylase content of the rings is greatly increased.

From the above results it would appear that the rings appear to have, especially after 24 hr. growth or incubation, a significantly greater content of α-amylase than the associated cores. In addition to this there is the tentative possibility that the early removal of the axis somewhat reduces the ability of the rings to produce α-amylase and added gibberellic acid appear to put right this deficiency. (This feature of the axis will be investigated in the following part (Part 3) of this Section: verification was obtained).

Now, as shown in Figs. 12, 13 and 14, despite the large differences in α-amylase content, it is evident that especially after the 48 hr. growth period a small rise in α-amylase content of the cores can be detected. Is this due to synthesis or merely due to the associated high α-amylase content of the

rings at this growth period? Figs. 15 and 16 show that the latter reason is possibly more correct as when rings are separated from the cores after only 2 hr. soaking. and incubation is made separately the α-amylase content of the cores falls rather than rises.

In experiments reported in Figs. 15 and 16, it was observed that rings and cores tended to go flaccid after long incubation periods. If the 2 hr. axis excision sufficed to remove the primary source of indoleacetic acid production, viz., the coleoptile tip (Went & Thimann, 1937; Thimann, 1963), then it is interesting to report that (Figs. 15 and 16) indoleacetic acid at 10^{-11}M concentration sufficed to keep these excised scutellar pieces turgid and also increased α-amylase contents of the rings as compared with non-indoleacetic acid treatments. At the moment it is uncertain whether the added indoleacetic acid was not merely affecting the general physiological state of the rings and was not specifically affecting the tissue site of α-amylase production. (This question is extensively examined in Part 4 of this Section).

Now, why should the rings be able to produce (or accumulate) greater amounts of α-amylase than the cores when in both scutellar tissue pieces epithelial cells are present in equal abundance? It seems that undoubtably the answer to this feature of the rings rests with the aforementioned observation that these rings (comprising structurally the peripheral area of the scutellum) are contaminated normally by minute residues

of aleurone cells (see Plate 15).

(Although the sharp fall in α-amylase content in the rings of the intact grain (see Fig. 12) may be due to many unknown factors, it was observed that during the growth of the intact grain a progressive fall in the pH of the modifying endosperm was observed and if this was more acute in the adjoining 'ring' region of the scutellum then α-amylase content (which is adversely affected by pH values below 4) of the rings might be resultantly reduced. Phytin for example, is known to occur in the endosperm in large quantities (Harris, 1961) and its degradation could surely result in effective lowering of the pH values.)

CONCLUSION

In conclusion it would appear that the ability of excised barley embryos to produce α-amylase and possibly other hydrolytic enzymes which are known to be produced in endosperm slices with living aleurone cells (MacLeod & Miller, 1962) resides, not in the functional capabilities of the scutellar epithelial cells (cf., Brown & Morris, 1890; Laufer, 1964) but with that of the aleurone-type cells. This activity of aleurone not only applies to the endosperm but to the embryo as well.

Thus, over 75 years have elapsed since an exclusive role for the embryo in endosperm modification was proposed by Brown & Morris (1890) and despite noble attempts to substantiate this embryo function as an important result of germination, the findings of MacLeod & Miller (1962) and the findings on this thesis on the matter strongly denies the embryo organ per se of any such role.

However, from this conclusion we are left with the uneasy paradox that there is no natural inception of endosperm modification if embryo germination and subsequent growth fails to occur. Fortunately enough Yomo's 1958 finding that the growing embryo possibly secretes an hormonal substance, gibberellic acid-like in action, served to resolve this paradox and opened new exciting possibilities for research on the embryo but, as pointed out in the Introduction, the impetus for further research remained with the use of commercially-available gibberellic acid on endosperm pieces; thus correlative research on the physiology

of this barley gibberellic acid-like factor was lost.

For this reason it was decided to look for the location, or origin, in the tissue complex of the growing embryo, of this gibberellic acid-like factor and secondly (in Part 5 of this Section) to determine whether this factor is a gibberellin. The first question will be discussed immediately below in Part 3 of this section.

PART 3

The Embryo and the Localisation of the Gibberellic acid-like Factor

In Section I it is shown that the pattern of endosperm modification is conditioned by the structural architecture of the embryo (cf., orientation of the vascular elements in the scutellum). From this it was postulated that maybe the proposed hormonal factor of Yomo was preferentially directed to the acrospire-end of the endosperm by similar orientation in the scutellum of the vascular elements (see Fig. 2). An important question which remains is whether the scutellum, or the other distinct tissue regions of the axis - viz., acrospire, scutellar node or root, in various combinations or in isolation, can, from their possible action on the rate of endosperm modification give useful information as to the embryo region primarily responsible for the origination of the gibberellic acid-like factor.

MATERIALS AND METHOD:

For this investigation, where various minute portions of the embryo are to be removed, diligent and careful selection of every grain was especially essential. However, having familiarity with dehusked grains, it is possible to eliminate damaged grains with comparative ease and rapidity. Selection was based on uniformity of grain size, grain firmness and undamaged coleorhiza - an organ which is very susceptible to injury during the dehusking process.

For each experiment a clean 3.5 inch petri dish was filled with dehusked grains, filled with distilled water, covered and then placed in an oven at 25°C for 2 hr. The residual water was then poured off and the selected grains were variously dissected (see Fig. 17) and immediately placed into clean 2.5 inch petri dishes which contained 2 rings of heat-sterilised filter paper saturated with 2mM acetate buffer containing 1 mM $CaCl_2$ pH 6.2. Each 2.5 inch petri dish contained 9 grains from which 6 were selected after 42 hr. growth at 25°C. The relevant growth measurements were recorded and axes or parts thereof were quickly removed and the remainder (scutellum + endosperm) of the grain was assayed for α-amylase (one of the most important hydrolytic enzyme in endosperm modification) by the method of Briggs (1961) as described in Part 2.

Surgical treatments were effected with a used rustless sterile No. 11 scalpel blade.

Observations on Treatments - Fig. 17

Treatment 1

Intact control:- All the various tissues of the embryo are represented, viz., acrospire (coleoptile and foliar shoot), scutellar node (mesocotyl and node with seminal root), scutellum, coleorhiza and initiated roots are present.

Treatment 2

Acrospire + Scutellar Node:- In this treatment the coleorhiza and the initiated roots were picked away carefully. As in all the other treatments, damage to the other remaining organs was carefully avoided. For the first 24 hr. acrospire growth was similar to that of the intact control.

Treatment 3

Acrospire only:- In this treatment it was noticed that if the cut approached the immediate base of the coleoptile then an apparent lag of the foliar shoot growth with respect to the coleoptile occurred. This was avoided.

Treatment 4

Coleorhiza + Scutellar Node:- No regeneration of the acrospire ever occurred. Root emergence coincided with intact control.

Treatment 5

Coleorhiza only:- The root initials emerged from their coleorhiza as quickly as the intact control.

Treatment 6

Without Scutellar Node:- In this treatment, the emergence of seminal roots did not occur. To remove the scutellar node or damage it invariably left about four visible roots at the end of the germination period. Dissection of 2 hour soaked grains showed that there are five root initials, but one of these initials is in close contact with the node and as a consequence gets damaged and does not emerge. The cuts in this treatment were difficult to standardise as is reflected in the table of results (Table XIII).

Treatment 7

Slight Cut below Scutellar Node:- Microscopic observations showed that a slight cut below the nodal region did not penetrate the nodal ball of provascular and meristematic-like cells. Seminal roots rarely emerged but they enlarged to such an extent that the node had a very swollen appearance. General growth was otherwise similar to that of the intact control.

Treatment 8

Deep Cut below Scutellar Node:- A = to above cut and B = to below cut. This cut acted to cut off the root initials from their nutrient supply. Seminal root emergence was very obvious above the cut. This treatment, in a loose way, is not too dissimilar from treatment 2; however, it seems as if the presence of the ephemeral coleorhiza and separated root initials influenced the development of seminal roots.

Treatment 9

Without Coleoptile Tip:- In 2 hr. soaked grains the acrospire was about 1 mm. long. The 5.0 mm. portion which was removed incorporated the coleoptile tip. The foliar apex was not removed. After 18 hr. about 85% showed the usual emergence of 3 initiated roots exhibited by the intact controls. Root emergence of all the grains which were originally sound was complete by 25 hr. The removal of the tip appeared to retard the growth of the foliar shoot. No physiological tip regeneration was microscopically evident, (see Paal, 1919).

Treatment 10

Without Coleorhizal Tip:- Removal of the coleorhizal tip appeared to slow up the early rapid elongation of the root initials and only about 60% showed the usual early rapid elongation of the intact controls. The coleorhiza appears to have some promotive influence over early root elongation. Obvious root elongation did not materialise until after about 25 hr. growth time.

Treatment 11

Without Coleoptile and Coleorhizal Tips:- Removal of both caused further delay in early root elongation. Only about 30% showed the early root elongation of the intact controls.

Using phloroglucinal and conc. hydrochloric acid, a check was made on the extent of vascular differentiation of the node and scutellum when the tips were removed. At the end of

the 42 hr. treatments 9 and 10 had about similar positiveness for the stain used, but, treatment 11 showed reduced vascularisation.

Treatment 12

Without Foliar Shoot:- For this treatment a small incision was made at the point indicated in Fig. 17 and the foliar shoot was gently removed. Grains with visible fragments of foliar shoot after 42 hr. were discarded.

Treatment 13

Median Longitudinal Slit of Axis:- Only a slight incision of the coleoptile and the coleorhiza was made. However, a deeper incision was made in the nodal region. Although roots from the nodal region did not appear, as in the case when the node is removed, the usual five root initials make their appearance as in treatments 5 and 6. From the excellent elongation of the roots it may be assumed that transport of food materials to this structure was not impeded by surgery.

RESULTS AND DISCUSSION

Going through the treatments (see Fig. 17 and Table XIII which gives results of amylase assays) treatments 2 and 3 as compared with 5 show that the presence of roots seem to be a more important aspect of α-amylase production than acrospire growth. Comparing 4 with 5, the removal of the node in 5 appeared to halve the α-amylase content. This could be due, among other things, to faulty translocation of modification stimulant(s) from the developing and growing roots to the endosperm. However, one should not loose sight of the fact that the root lengths in treatments 4 and 5 are very similar.

From these four treatments the important points appear to be:

1. Vigorous root development and growth (viz., treatments 4 and 5) are more necessary for the initiation of α-amylase production than acrospire growth (viz., treatments 2 and 3).
2. Seminal root development (cf. treatment 4) which is eliminated in treatment 5 possibly makes important contribution to the hormonal factor which is supposed to initiate α-amylase production.
3. Despite the obvious virtues of treatment 4, where root length and visible root initiation are very close to those observed the intact control (treatment 1) the removal of the acrospire reduces the α-amylase content far below that of the control.

Since there is a possibility that the scutellar node might be an important site for the origin of the hormonal factor responsible for release of hydrolytic enzymes in the endosperm; the close proximity of the cut, in treatment 4, to the scutellar node might be the cause of the drastic enzymic reduction in this treatment. In treatments 6, 8 and 14, where actual surgical damage to the inner nodal cells was made, drastic α-amylase reduction was apparent. It is interesting to note that in treatment 7 where the surface slit did not penetrate to the underlying nodal cells only slight reduction in α-amylase content was recorded. Although the appearance of seminal roots were invariably inhibited, microscopic observations revealed extensive enlargement of these seminal roots.

Treatment 9, as treatment 7, was designed to take the immediate and direct effect of surgical damage away from the scutellar node. None the less the removal of the coleoptile tip was extensive enough to ensure that there was no question of physiological tip regeneration. An important point which emerged from treatment 9 is that the node is undamaged, yet there is a small but noticeable reduction in the α-amylase content. Therefore in comparison to treatment 7, which can be taken as an assessment of surgical damage, the absence of the coleoptile tip (an important region of auxin (indoleacetic acid) production) might be adversely influencing nodal metabolism and/or aleurone stimulation. Although the

possibility of indoleacetic acid acting in such a capacity will be investigated in Part 4 of this Section. It is interesting to recall that indoleacetic acid did cause an increase in α-amylase in the gibberellic acid response of scutellar rings in Part 2 (cf. Fig. 15 and 16). Bearing directly on the possibility that indoleacetic acid may have a role in aleurone stimulation, MacLeod et al, (1966) have given tentative evidence to suggest that some unknown factor, other than gibberellic acid, moves from the embryo to the endosperm during germination to sensitise the aleurone cells with respect to α-amylase production.

Treatment 12 was conducted with the intention of seeing what kind of effect the foliar shoot had on α-amylase production. This proved to be an interesting treatment. Here it was observed that α-amylase content was greater than that of the intact control. However, it is worth noting that this treatment is very similar to treatment 4 except for the presence of the coleoptile with its tip. This strengthens further the above suggestion that the coleoptile tip may have at least a minor role in the production of α-amylase during barley grain germination. Therefore, despite the surgical damage, the removal of the foliar shoot is effective in increasing α-amylase production. At first glance one is tempted to suggest that the intact foliar shoot was the producer of some inhibitor of enzyme production in the endosperm of germinating barley grains. But consultation of the available literature and experimental

evidence gained in Part 5 of this Section suggests the following explanation.

Radley (1959), Overbeek & Dowding (1961) and Ng & Audus (1965) have reported gibberellic acid as the main hormonal additive necessary for the growth of the excised foliar shoots of wheat and oats and, personal experiments (Part 5) on the foliar shoot of barley acrospires also support this stimulative action of gibberellic acid. In addition, Brian (1961 see Overbeek & Dowding, 1961) also stated that he regards Radley's foliar shoot test for gibberellic acid as very specific.

From this, in conjunction with the assumption that Yomo's factor is gibberellic acid-like in action (cf. MacLeod & Miller, 1962) and that the scutellar nodal region is concerned with its origination, it is quite likely that in the intact grain the foliar short represents a natural consumer of gibberellic acid or other suitable gibberellins. Accordingly, removal of the foliar shoot might therefore naturally cause increased availability of gibberellin-like factor(s) and, as a consequence, increased α-amylase production.

In treatment 10 removal of the coleorhizal tip tended to slow down the initial rate of root elongation and this is reflected in the table of results (Table XIII). Treatment 13 shows quite clearly that the storage parenchyma tissue, viz., the scutellum, has very little stimulating influence on α-amylase production in the adjoining endosperm. Therefore, the gibberellic acid-like factor of Yomo (1958) seems to be

merely transported through the scutellum along vascular tracts to the acrospire-end of the endosperm-embryo junction where endosperm modification is observed to commence in the intact grain (cf. Section I).

Fig. 18 merely shows statistically how significant the respective surgical treatments were in causing deviation, in α-amylase content, from the intact control.

CONCLUSION

To conclude, therefore, this investigation tends to suggest that the axis is the important region of the embryo as far as the incitement of the modification process is concerned. There is evidence to suggest that the gibberellic acid-like hormone has its origins in the active developing region of the scutellar node. Damage to the scutellar node slows down the modification process drastically; none the less, initial root elongation seems to be an important factor of nodal secretion. The coleoptile with foliar shoot in isolation from the scutellar node with root initials, have minimal effect on endosperm modification. However the coleoptile, possibly as a result of its substantiated auxin secretory abilities might indirectly affect modification by its action on the development and growth of the scutellar node and young root initials or by direct action on the aleurone cells.

The foliar shoot for its growth appears to be dependent on the same gibberellin-like hormone that triggers off the hydrolytic enzymes secretory abilities of the aleurone cells.

There is undoubtedly some scientific truth in the old malting practice that if the scutellar nodal region of the barley grain gives a negative tetrazolium staining response then, despite a positive response for the rest of the embryo, it is taken as highly suggestive that this grain will modify poorly. From this investigation it is quite possible that the rate of foliar shoot growth might be a good criterion for discriminating between slow and fast malting barley grains.

Electron microscopic observations (see Section I) on the scutellum and the scutellar node show quite clearly a marked distinction in cell types. The scutellar cells are storage cells while those of the node, even in the dry state, are clearly meristematic in structure. This is not surprising if they are involved in possible hormonal secretion and organ (e.g. root) development.

(It is noteworthy that visual assessment of endosperm modification after identical surgical treatments of the embryo as described in this investigation gave, after 72 hr. growth time, identical results to those obtained from α-amylase assessment.)

Observations on the behaviour of indoleacetic acid in Parts 2 and 3 prompted the following investigation on its possible role, if any, in the process of endosperm modification.

PART 4A

The Barley Embryo, Indoleacetic acid and Gibberellic acid in Endosperm Modification.

Although indoleacetic acid has been called the 'master hormone' in regard to morphological changes and is to be found in abundance in the coleoptile apices of most cereal grains, physiologists have, up to the present, found no important role for this hormone in cereal grain germination.

Went & Thimann (1937), in their classic, Phytohormones, suggested that indoleacetic acid was important for root initiation, but in cereal grains roots are already initiated though Thimann (1936) and Thimann & Lane (1938) suggested that addition of indoleacetic acid to wheat, barley and maize grains might cause more rapid emergence of these initiated roots.

Pollock (1959) found that free indoleacetic acid had very little effect on germinating barley grains and Overbeek (1966) could only suggest, from the knowledge that indoleacetic acid comes from coleoptile tips, that during germination of cereal grains indoleacetic acid functions to bring the coleoptile tip to the surface of the soil.

In the foregoing investigation (Part 3) it is reported that early removal of the coleoptile of barley grains reduced the extent of the vascular link-up between the axis and the scutellum. Only provascular areas and strands are present at the onset of germination (Section I). There is little

doubt that rapid bi-directional transport of various materials must take place between the endosperm and axis during germination and subsequent seedling growth, and, if one assumes, according to the extensive work of Scott & Jacobs (1963), that indoleacetic acid is the hormone primarily responsible for vascular differentiation, then a more direct role for indoleacetic acid, especially in early seedling growth, can be inferred.

Now, a main interest of this study has been in the role of the scutellum during germination and subsequent seedling growth (Sections I and II and Part 3 of this Section) and it is reported in Part 3 of this Section that IAA(10^{-11}M) encouraged scutellar turgidity, ~~and an elongation of the scutellar epithelial cells~~ and increased the α-amylase content of scutellar rings. This was taken to suggest that although the coleoptile may respond favourably to IAA 10^{-5}M concentration (Went & Thimann, 1937), the scutellum, which seems to depend on the axis for its indoleacetic acid, may only receive a concentration of about 10^{-11}M which might be optimal. On this same theme it is interesting to note that, after a 12 hr. soak, personal observations show that the scutellar node area (i.e. the axis-scutellum junction) is histochemically positive for peroxidase activity and, at about this same time, vascular differentiation commences at the nodal region in a basipetal direction, with respect to the shoot apex. Electron microscopic studies show that the nodal area undergoes extensive lignification and cellular disintegration (Section 1) and Siegal (1962) has

pointed out that high peroxidase activity precedes these processes.

This presence of peroxidase, which is a potent destroyer of indoleacetic acid (Ray, 1958; Ray, 1961) at the axis-scutellum junction may function to reduce the IAA concentration received by the scutellum.

Mindful of the close association between the scutellum and the aleurone cells of the endosperm, it was wondered whether this knowledge of the low IAA tolerance of the scutellum could be related to a role for indoleacetic acid in aleurone stimulation and subsequent endosperm modification.

Evidence from the work of Yomo (1958), Paleg (1960), MacLeod & Miller (1962), Varner & Ramchandra (1964) and results from Part 3 seem to imply that a gibberellin-like factor emanates from the scutellar node area of the axis to cause the production of hydrolytic enzymes in the aleurone cells.

Brian (1966) believes, with support from Kefford (1962), that gibberellic acid growth responses in plant tissues may require that IAA is present but agrees with Hillman & Purves (1961) that IAA and GA must act on different sites in cellular response.

Cleland & McComb (1965) tried and failed to find any evidence for an IAA stimulation (synergism) in the gibberellic acid-aleurone response in endosperm slices, though interaction was found by Brian & Hemming (1958) for green pea stem sections, by Wareing (1958) for the breaking of dormancy of woody plants,

by Lang (1959) and Kefford (1962) for shoot growth in intact plants and by Prosser & Jackson (1959) for parthenocarpic fruit setting. From Cleland's results, IAA even at the lowest concentrations (about 10^{-9}M) inhibited the normal GA response, a situation also found by Overbeek & Dowding (1961) for stimulation of growth of excised foliar shoots of wheat.

In spite of this, it seemed desirable to re-examine the joint action of the two hormones in endosperm modification during barley germination.

Endosperm modification in the intact grain begins between 16 and 20 hr. (Section I) and Duffus (1966) has reported that on the onset of modification there is a rapid upsurge of peroxidase activity in that modifying area. From this it was surmised that in the intact grain, once the low concentration of IAA had completed its stimulation on the aleurone cells, resulting modification, with peroxidase action, would quickly affect the IAA. With this proposal in mind it was therefore interesting to note that in the experiments of Cleland & McComb and Overbeek & Dowding, IAA and GA remained in the incubation media for the duration of the experiments.

The possibility that a short-term stimulation by IAA might participate in the aleurone-GA response and that this IAA concentration might operate at about 10^{-11}M was now investigated.

MATERIALS AND METHOD:

Dehusked Proctor from the 1964 harvest was used, and six threequarter grains (i.e. grains from which the extreme distal portions had been removed, to facilitate entry of media) were selected, as indicated in Part 3, from an experimental batch of nine. For the slices experiments, 1 mm. segments of endosperm were normally cut from just behind the scutella of visually sound dry grains. Briggs' method was used for assay of α-amylase and, for the slices, the final volume of the incubation medium was made to 0.6% with respect to sodium chloride in 0.1 M acetate buffer, pH 5.3, before they were ground in an all-glass hand homogenizer. When the slices had to be transferred from one medium to another, they were first blotted gently on the surface.

The slices were incubated in 2.5 in. petri dishes and the three-quarter grains were grown in 3.5 in. petri-wick dishes - i.e. two petri dish lids enclosed in a smaller (2.5 in.) petri dish which formed a platform supporting sterile filter paper cut to dip into the medium in the larger dish, and the grains were allowed to germinate on this platform. This apparatus ensured that fluctuations in the amount of medium reaching the grains were eliminated and growth measurements were more consistent.

Triiodobenzoic acid (TIBA) and gibberellic acid (G.A.) were always used at concentrations of 10^{-4}M. Concentrations of other growth regulators are quoted in the text. It should

be noted that TIBA had no inhibiting effect on the action of pure α-amylase.

Lanolin paste containing additives were made up by dissolving respective additives in 1 to 2 ml. of absolute alcohol and each solution was then mixed into warmed lanolin. The alcohol was allowed to evaporate. The same amount of alcohol was added to plain lanolin for lanolin control treatments (after Thompson & Jacobs, 1965).

To locate sites of peroxidase activity 0.1 M H_2O_2 in phosphate buffer, pH 4.5 + 0.01 M benzidine were added to fresh hand sections of the embryo and the sections were incubated at room temperature for 5 minutes. Sites of peroxidase activity were then blue to brown.

To determine the mitotic index a quick gentle squash was made of 1.5 mm. root tips in 45% lacto-propanol orcein. The number of cells per 1000 counted which showed mitotic figures was recorded.

RESULTS AND DISCUSSION

Fig. 19 records results for three-quarter grains. Here it can be seen that IAA 10^{-10}M or IAA 10^{-4}M in combination with GA and the TIBA + GA treatments all reduced the α-amylase content of these grains, as measured at 36 hr. The IAA results, in the presence of GA, compare well with the findings of Cleland & McComb (1965). For the non-GA treatments (modification now being the sole responsibility of the embryo) there is a slight increase in IAA 10^{-10}M (not significantly different from that of the water control) but it was interesting to observe no marked inhibition. TIBA inhibition is evident but no TIBA: IAA competition. No synergistic response was evident from the results of Fig. 19, but growth measurements taken from the embryos used to give the results of Fig. 19 proved quite interesting (Table XIV). These results indicate a IAA 10^{-4}M + GA root emergence synergism at 17 hr. After 36 hr. growth there is again a growth synergism but now between IAA 10^{-10} and GA.

The point gained from these two observations is that the same organ at different growth stages appeared to need different concentrations of IAA for the synergistic response.

Using three-quarter grains, short IAA pretreatments were given before subsequent transfer to GA. The results are given in Fig. 20. From the inhibitory effect of the TIBA pretreatments it can be assumed that IAA is also getting into the grains. Here neither IAA 10^{-10}M nor IAA 10^{-4}M is

combined with rapid root emergence. With regard to the TIBA results, competitive action with IAA was not observed and TIBA pretreatment reduced significantly the subsequent GA response.

Reassured that at least a short 12 hr. pretreatment was not inhibitory to the subsequent GA response of the aleurone cells, it was decided to use endosperm slices, as the presence of the embryo, although useful for a comparison of the treatments on growth responses, might be unnecessarily complicating the pretreatment response.

Fig. 21 is a repeat of the treatments used in Fig. 19 but now used on endosperm slices. The result is the same, and continuous IAA treatment in the presence of GA caused inhibition of the usual GA response, as found by Cleland & McComb (1965).

More interesting, however, is Fig. 22, which is just a repeat of the conditions reported in Fig. 20, with 1 mm. endosperm slices. In preliminary trials IAA 10^{-4} pretreatments proved erratic and greatly inhibitory and thus only IAA 10^{-10}M was used. Here, (Fig. 22) for the first time, the IAA pretreatment at 10^{-10}M gave subsequent GA responses which were significantly different from these of the H_2O-GA control. Note the similarity between the G-GA (Grain-Gibberellic Acid) treatment and the IAA-GA treatment. The significant difference between the G-GA treatment and the H_2O treatment are similar to the findings of MacLeod et al. (1966) despite the fact that slices were taken from their grains after 24 hr. incubation -

a time when modification had already started (see Section I).

Experiments were now designed to test the longer duration of pretreatments with IAA (10^{-10}M) on subsequent GA-aleurone-α-amylase response. There was one 12 hr. change in the 24 hr. pretreatment media. Comparing Fig. 23 with Fig. 22, note that the longer pretreatments caused a reduction in the GA response but, with regard to Fig. 23 note that a 24 hr. IAA pretreatment is less advantageous than a 12 hr. IAA pretreatment. Now, armed with what appears to be about the right physiological concentration of IAA with regard to the GA-aleurone response, and knowing that this IAA participation is limited to about a 12 hr. pretreatment, as far as α-amylase production is concerned; an experiment was designed which would eliminate the pretreatment by IAA alone. It was reasoned that in the intact grain the first sign of vascular differentiation is at about 12 hr. (presumably following IAA action), while endosperm modification begins before 20 hr. (presumably via GA-like action), thus creating the possibility that a distinct IAA pretreatment might not occur naturally in the intact grain. This led to the trial of various short term IAA + GA combinations, (4 hr. 8 hr. and 12 hr.) with subsequent transfers to GA only. All were given the same total incubation time.

The results of this experiment are shown in Fig. 24. These results are possibly the most interesting recorded in this investigation. Here, the short IAA + GA pretreatments

have completely outstripped the GA-only treatment. However, in the long and continuous IAA + GA treatment (36 hr.) we are back to the inhibition recorded by Cleland & McComb (1965) and in this work (Fig. 19 and Fig. 21). Here lies the reason for the negative results obtained by Cleland & McComb regarding a possibility role for IAA in the GA-aleurone responses. Their experiements, with IAA and GA continuously in the experimental media, ran for 22 hr. The same explanation of the antagonistic effect of even very low IAA concentrations in the presence of GA for too long a period of incubation may also apply to the findings of Overbeek & Dowding (1961) for foliar shoots where the duration of incubation was 50 hr.

Here there seems to be a delicate case of IAA - GA synergism which is based upon the physiology of the living tissue being investigated. Continuous IAA + GA incubation may be more suitable for synergism in green pea stem sections, as shown by Brian & Hemming (1958) but why should it be the same for barley aleurone cells or for the foliar shoot of wheat?

In conclusion it appears tha IAA:GA synergism does occur in barley aleurone cells. A short synergistic period of 8 hr. appears to be optimum for subsequent GA-only α-amylase production. It follows from this that the theory of GA action which speculates that GA acts indirectly on plant materials by sparing IAA, should be re-examined. Having found a stimulative IAA action for aleurone cells it was desirable to find whether TIBA:IAA competition can take place. Cleland & McComb (1965)

failed to show this for PCIB which is also reputed to be an IAA competitor. This failure may have resulted from the long duration of IAA + GA + PCIB treatments (22 hr.). Indeed, in early attempts (Figs. 19, 20, 21, and 22) to show some IAA:TIBA competition my approach was guilty of the same error, as subsequent IAA + GA treatments lasted for 24 hr. This was re-examined, as follows: slices were transferred from TIBA (12 hr.) to IAA^{-10}, IAA^{-8}, IAA^{-6}, IAA^{-4}, 12 hr. and finally to GA for 32 hr. No competitive effects showed (Fig. 25) and it seemed possible that at the low IAA concentration (IAA^{-10}) the TIBA inhibition showed, while at the high IAA concentrations (IAA^{-6} or IAA^{-4}) the IAA inhibition showed. If this was not the case then it might be that the TIBA pretreatment, once having inflicted its inhibitory effect, was not easily reversed. In another set of experiments TIBA was made to compete within the same time period with IAA. This reduced the pre-GA treatment to a more suitable period of 12 hr.

Fig. 26 gives the results of this investigation. Here, it is clearly seen that all the TIBA + IAA treatments showed obvious but varied extents of competition with regard to the subsequent GA-aleurone response. IAA at 10^{-8}M seemed to be the most efficient competitive concentration, as is slightly indicated in Fig. 25. The experiment performed in Fig. 26 was repeated with the same solutions, except for the GA solution, five days later. Here, it appeared (Fig. 27) that an alteration of the IAA concentrations, possibly by oxidation had occurred,

though the solutions were stored in the dark at 5°C. The rather high IAA 10^{-10}M result may be related to the fact that this IAA solution was the only one in a brown bottle.

Hillman & Purves (1961), and Kefford (1962) have reported PCIB to be competitive with IAA while Audus & Thresh (1956a and b), and Sebanek (1963) have reported TIBA to operate in the same capacity. In barley aleurone it seems that TIBA:IAA competition also operates. This experiment also demonstrates that the TIBA concentration was not merely toxic.

Although it was concluded that a peculiar type of sequential IAA:GA symergism can take place in isolated endosperm slices with attached aleurone cells, it remained to be seen whether indoleacetic acid could replace the coleoptile tip (treatment 9 in Part 3) and as a consequence restore α-amylase content to that of the intact control. This should at least strengthen the belief, derived from the endosperm results, that indoleacetic acid may have a natural role in the modification process of intact grains.

Part 4B

Now, treatment 9 in Part 3 of this Section shows that the removal of the coleoptile tips after 2 hr. soaking of the grain resulted in a serious reduction in the α-amylase content and general modification of the endosperm. When only the coleoptile tips remained, with the enclosed foliar shoot removed at 2 hr. (treatment 12), the α-amylase content of the endosperm exceeded that of the intact control. From these two points and a third possibility (that the coleoptile tips of cereal grains are abundant producers of a hormone which is not unlike indole-acetic acid) it appeared that it ought to be possibleto replace, to some extent, the IAA producing action of coleoptile tips, with pure indoleacetic acid. Evidence obtained in Part 4A of this study, denies IAA and independent role in endosperm modification but it was possible that if its short term synergistic role were to operate in the intact grain then an early removal of the coleoptile tip, (the important free IAA source of production) would reduce the α-amylase content of the endosperm, as obtained for treatment 9 in Part 3 (see Table XIII).

It was decided to substitute IAA in lanolin paste for 2 hr.-excised, coleoptile tips to see whether the α-amylase contents of these treated grains could be increased.

Sucrose in lanolin paste was also used for it is recorded in Sect.II (Fig. 7) that the axis of 2 hr. soaked barley grains contained about 8.5 mg. sucrose per 75 axes; of this, the shoot contained, chromatographically (Sect.II, Table V), the greater portion, viz., twice as much as the roots.

RESULTS AND DISCUSSION:

In Fig. 28 only IAA 10^{-4}M and sucrose were tried. The IAA treatment showed an apparent reduction in α-amylase content as compared with the lanolin control and the sucrose treatment. There was no sucrose stimulation. In this experiment, however, all the lanolin pastes were changed after 24 hr. This same experiment was repeated using a wider range of IAA treatments with no renewal after 24 hr.

The results are shown in Fig. 29. The only significant result was that using IAA 10^{-7}M, where, as with 10^{-6}M, there was an improvement in α-amylase content over the lanolin and sucrose treatments. It is rather unusual that 10^{-4}M gave a better result than 10^{-5}M, speaking in terms of an IAA inhibition at higher concentrations. Note also that even at high concentrations (e.g. 10^{-4}M) apical application did not retard root elongation to any great extent as compared to application in the growth media (see Table XIV).

In conclusion, it would appear that IAA, as far as the apparent necessity for the coleoptile tip in endosperm modification is concerned, at about 10^{-7}M concentration can effectively replace the coleoptile tip in respect of maintenance of an about normal α-amylase content. It is possible that 10^{-7}M might be the concentration of the IAA produced by the coleoptile tips.

CONCLUSION

A general picture may be assembled from these two experimental sections (Parts 4A & B) with reference to the role of IAA in endosperm modification.

It seems not unlikely that on the onset of germination the coleoptile tip begins to produce indoleacetic acid and from 12 hr. this IAA begins to manifest its presence at the scutellar node as lignin deposits in differentiating vascular elements. There is also an obvious appearance of peroxidase which Siegel (1962), depicts as a prelignification stimulation of indoleacetic acid. This coleoptilar IAA secretion is in the vicinity of 10^{-7}M but, at the node, as a result of the extensive differentiating process, it becomes reduced to about 10^{-10}M before passing into the scutellum where vascular differentiation is less intense and confined to discrete provascular strands.

Now, since Duffus (1965) recorded that aleurone pieces started to produce α-amylase after 12 hr. incubation in gibberellic acid and also detected α-amylase production after 24 hr. growth in intact grains, it seems not unlikely that about a 12 hr. synthesis time is also needed in the aleurone response of the intact grain. This would require initial synthesis of the GA-like material, (which is believed to come from the scutellar node region) between 0 and 12 hr. germination time.

With IAA and the GA-like hormone leaving the node at about 12 hr. it is quite possible that they get to the aleurone together. The aleurone cells at the apical periphery of the

scutellum become sensitised by a IAA:GA-like synergism and on the onset of endosperm modification, there is a sharp rise in peroxidase activity (Duffus, 1966) which, as a result could remove IAA (see Galston & Dalberg, 1954), leaving the GA-like hormone to give a maximum modification response, on that side of the endosperm which adjoins the apical periphery (i.e., acrospire-end) of the scutellum.

This conclusion has come about from experimental work and observations on endosperm slices and the intact grain and it is highly suggestive that IAA may play a role in endosperm modification in the intact grain; modification itself being a result of hydrolytic enzyme synthesis in the aleurone cells, Varner & Ramchandra, 1964. This IAA role is a synergistic one and, therefore, it is possible for GA to have a dominant but sub-optimal role on its own. If GA and IAA have different sites of action in the cells (Brian, 1966), and GA is supposed to stimulate a type of hydrolytic enzyme-RNA synthesis; the question remains - what in the cell does IAA act upon?

Varner et al. (1965) showed that with isolated aleurone actinomycin D will inhibit the DNA-controlled synthesis of the specific hydrolytic enzyme-RNA but there is also good evidence from Nooden & Thimann (1963); (1966) that this same inhibitor will also reduce IAA induced growth and protein synthesis. From this it seems that the physiological and biochemical data are at variance as far as the sites of action of both these hormones are concerned.

Finally, in the modification of the intact grain, IAA at a low physiological concentration may certainly play an early synergistic role with gibberellic acid. In passing it is worth noting that MacDaniels & Sarkissian (1966) found that oxidative phosphorylation of mitochondria from maize scutella appeared to be maximal with a low IAA concentration, viz., IAA 1.07×10^{-10}M, but was inhibited by IAA 1.07×10^{-4}M. It could be that IAA in the aleurone cells may be performing the same function and thus indirectly, remembering the need of oxygen or aeration for maximal hydrolytic enzyme production, step-up the metabolic machinery for more rapid hydrolytic enzyme production, for which the catalytic stimulus is gibberellic acid.

Part 4C

The Gibberellic acid-like Factor of Barley grains - synthesised or already present?

(2-chloroethyl) trimethylammonium chloride (CCC) and maleic hydrazine (MH) are synthetic dwarfing agents for plant tissue growth.

Bukovac & Wittwer (1956) and Bouillenne-Walrand (1959) have both reported that GA_3 (gibberellic acid) reduces or overcomes MH induced growth inhibition. This was questioned by Brian & Hemming (1957) who showed, as did Harber & White (1960), that GA_3 failed to ameliorate the severity of MH reduced growth.

For CCC, Lockhart (1962), showed from kinetic studies on Phaseolus that CCC and GA_3 interact competitively but, at saturating levels of GA_3, CCC was without effect. Zeevaart & Lang (1963) showed that CCC inhibited long-day flowering and this could be reversed by GA_3, while Kende et al. (1963) reported that CCC inhibited gibberellin production in the fungus Gibberella fujikuroi without any significant effect on growth.

Mindful of this possibility that CCC acts to inhibit GA synthesis but not its action and that barley embryos on germinating dispatch a GA-like substance to the aleurone cells, it was decided to use this compound on three-quarter grains to investigate its effect on α-amylase production in the endosperm. Now, if MH and CCC both reduce the normal growth response of plant tissue it is quite possible that they do not work through the same mechanism, as MH inhibition cannot be reversed by

added GA_3. Harber & White (1960) reported MH as a potent mitotic poison, so a check was made on mitotic index of grains treated with both these compounds.

MATERIALS AND METHODS:

Three-quarter grains, as previously described, were grown in petri-wick dishes and MH and CCC were used at 10^{-3}M and GA at 10^{-4}M. Method for mitotic index determination is already given at the commencement of this Section and α-amylase determination was by Briggs' method.

RESULTS AND DISCUSSION

In Table XV the important point is that CCC, as far as root emergence is concerned, in direct contrast with TIBA (see Table XIV), does not affect initial root emergence in the germination process, while MH reduces this germination property by about 25%. The reason for this MH inhibition is given in Table XVI where in the mitotic index column one sees at a glance that MH is a truly potent mitosis inhibitor. As reported by Harber & White (1960), MH does not appear to affect the process of cell elongation. However, in barley germination (i.e. root emergence) cell division does play some part, but with cell elongation playing the major role. Note that CCC has no effect on cell division while GA-only is a potent inducer.

Two additional points are worth noting. Firstly, GA, (viz., GA + MH) will not reverse MH inhibition of cell division and secondly, a high mitotic index did not give any noticeable elongative growth increases. It follows that cell division may serve to facilitate elongative growth in Thimann's sense but not cause it directly.

Therefore, it is clear that MH and CCC must act on apparently different sites in the plant used. However, we turn now to α-amylase content as an index of gibberellic acid-like substance secreted by the embryo.

Both CCC and MH reduced the α-amylase content of the grains used. However, in combination with GA this inhibition was reversed. This possibly indicates that the aleurone cells are not affected by both these compounds as far as α-amylase production is concerned. None the less, the MH cell division inhibition was not reversed indicating that, at face value, cell with impeded cell division may be playing some role in dissemination of the GA-like substance from the embryo to the aleurone cells. As far as the CCC role is concerned a direct inhibition of GA synthesis in the embryo may be responsible for the reduction of α-amylase.

In conclusion, it has simply been shown that both cell division and root elongation function in the germination process and that the reported MH and CCC reduction of growth in other plants, providing GA is involved, might have completely different physiological bases. From biochemical data Yomo &

Iinuma (1966) reported that the GA-like substance of barley embryos seems to be synthesised. The above results using CCC tends to support Yomo & Iinuma's conclusion.

A Tentative Addendum

Taking points from Table XIVII and XVI, it would appear that if we take root emergence as an index of germination rate then 'added' IAA seems to have a role while 'added' GA seems to make little impression. But things may not be as simple as that with respect to, say, some types of dormancy where GA is more effective than IAA. It could be that in normal grains elongative pre-sensitisation by GA has already taken place during maturation and added IAA will speed up root emergence, while in some dormant grains there has not been the obligatory GA pre-sensitisation and thus added GA is now a prime necessity. This makes IAA presence in both instances merely expedient for the process of root emergence.

So far, experimental evidence in this Hormonal Section seem to suggest that indoleacetic acid, at low concentrations (10^{-7} to 10^{-11}M) has a part to play in barley germination and subsequent seedling growth and that the gibberellic acid-like factor of Yomo (1958) emanates from the scutellar node region of the axis. This factor is possibly synthesised during germination and subsequent growth. Although it is almost certain that this gibberellic acid-like factor bears a close function similarity to GA_3, with respect to promotive actions on endosperm modification, there is still no reported evidence

on whether the secreted embryo-factor of Yomo's is gibberellic acid (GA_3) or one of the other eight known gibberellins or not a gibberellin at all.

The concluding part of this Section deals with some aspects of the physiological and chemical characterisation of this secreted embryo factor.

PART 5

Gibberellin Secretion from Excised Barley Embryos - physiological and chemical characterisation.

Jones, MacMillan & Radley (1963) found that gibberellic acid, (A_3) was present in immature barley grains; however, Jones (1964a, b) found difficulty in demonstrating its presence in mature barley grains. Duffus (1965) tentatively proposed that mature barley embryos contained traces of gibberellic acid and possibly also gibberellin A_1. Radley (1966) has recently reported that A_4 and A_7 may also occur in immature barley grains. Yomo & Iinuma (1966) were able to demonstrate that the ethyl acetate extract from 5-day barley embryos, germinated with suitable carbon source, was able to induce α-amylase production in endosperm pieces. Their failure to demonstrate gibberellic acid-like activity after 3 days germination is rather strange when in normal germination of the intact grain the α-amylase content, by this time, is quite high and the endosperm is extensively modified (cf. Section I Plates, 7, 8 and 9).

Now, these cited instances for the presence of gibberellin-like substances in barley grains and excised embryos are generally taken to support the opinion that a gibberellic acid-like factor moves from the embryo to the endosperm during early germination (see review by MacLeod, 1967).

The easy availability and obvious efficiency of gibberellic acid in inducing α-amylase activity in embryo-less endosperm pieces has tended to cause neglect of the physiological

necessity for actually characterising the proposed endogenous gibberellic acid-like factor. Mindful of this, it was decided to investigate whether or not excised barley embryos could secrete a gibberellic acid-like factor which could be biologically and/or chemically characterised.

MATERIALS AND METHOD

The modified agar diffusion technique of Phillips & Jones (1964) was used. Dehusked grains were germinated in the dark at 25°C. for 24 hours, carbohydrate (e.g. hexoses) transport from endosperm to the embryo thus having begun (cf. Section II Fig. 7). 250 embryos were rapidly excised from these 24 hr. grown grains and placed on the surface of moistened 1.5% agar, in 7 inch petri dishes. These dishes were then placed in a dark incubator at 80% humidity and set at 25°C. Secretion time was for either 36 or 24 hr. After secretion, the embryos were removed and discarded. These "secreted" petri dishes and control dishes, with only agar, were frozen (-15°C.) either for 20 hr. (36 hr. secretion) or for 12 hr. (24 hr. secretion). With the water frozen out, the agar was repeatedly extracted by redistilled acetone, reduced to a small volume under vacuum by a rotary evaporator (30°C.), acidified with hydrochloric acid, pH 2.4, and extracted three times with distilled ethyl acetate. The ethyl acetate fractions pooled, reduced to dryness and taken up in suitable volumes of 0.1% Tween. These solutions (secreted and non-secreted control) were then used for biological

tests or for thin layer chromatography.

Kieselguhr or silica gel plates were usually spotted with test solutions and A_3 (gibberellic acid) run with benzene/acetic acid/water, 8/3/5, dried at room temperature, sprayed with 70% sulphuric acid, dried at 120°C. and examined under UV light. Half the volume ofthe 24 hr. extract was streaked on to Kieselguhr and the approximate area was eluted with ethyl alcohol, reduced to dryness under vacuum and taken up in 0.1% Tween for biological assay.

The cucumber and lettuce hypocotyl tests for gibberellins were as described by Brian, Hemming & Lowe (1964). Excised foliar shoots (excluding the scutellar node) from 48 hr. dark germinated barley grains were also tested. The excised foliar shoots were floated on a small volume of the test solutions (at 450 f.c. and 100% humidity) and measured after 18 or 20 hr. This latter test, at the present moment, is not to be regarded as entirely reliable. Further investigation on this test is required.

8, 1 mm. endosperm slices, after an 18 hr. soak in distilled water, were also tested for α-amylase secretion with the test Solutions.The Briggs (1961) method was used for α-amylase assay as in Part 4A of this Section.

Dosage of test solutions- 40 ml. for cucumber. 1.5 ml. for lettuce. 1.5 ml. for foliar shoot and 2 ml. for endosperm slices.

RESULTS AND DISCUSSION

From two repeats on Kieselguhr and one attempt on silica gel, there was only one distinct spot that fluoresced. On Kieselguhr the Rf was about 0.8 and for silica gel about .20 (see Table XVII). None the less, on the Kieselguhr plates this fluorescent spot was always linked to the solvent front by a fluorescent streak which coincided with a average Rf of about 0.92. The extract from the agar control showed no similar fluorescence.

For the thin layer system used, MacMillan & Sutter (1963) found that on Kieselguhr: A_6 Rf was 0.76, A_5 was 0.88 and A_3 was 0.38. For silica gel: A_6 Rf was 0.21, A_5 was 0.35 and A_3 was 0.00.

From the high value for GA_3, (0.490 in Table XVII) it is possible that the fluorescent spot of the 24 hr. secreted extract was running too quickly. Taking this into consideration, with an Rf value of o.20 for the extract, on silica gel, it is possible that this fluorescent spot corresponds chemically to the gibberellin A_6. The fluorescent streak, with an average Rf of 0.92, might be traces of A_5.

Results from biological tests are given in Tables XVIII, XIX and XX.

In Tables XVIII and XIX it is evident that the ethyl acetate extracts had no marked biological activity except in the α-amylase response of the endosperm slices. No hypocotyl lengths were recorded for lettuce as all the extracts

caused a general decay of this region. The extracts also caused a gradual loss of chlorophyll in the foliar shoot tests. These effects on the test materials, as far as elongative growth is concerned, made interpretation impossible. However, the effect of these ethyl acetate extracts on the cucumber hypocotyl and α-amylase production was more rewarding. Despite the failure of the extracts to stimulate growth no harmful effects were observed. If anything, ethyl acetate has been found by Coombe, Cohen & Paleg (1967) to stimulate the enzyme secretory abilities of the aleurone cells slightly. In Table XVIII and XIX an increase in the α-amylase content for the secreted extracts over the agar controls is evident.

Therefore, with respect to Tables XVIII and XIX, it is only possible to say that the 36 and 24 hr. secreted extracts contained a factor which had no elongative effect on cucumber hypocotyl extension but which caused an increase in α-amylase content of endosperm slices.

Owing to the suspected harmful action of minute traces of ethyl acetate in these tests, comparison with GA_3 action is not possible. This was remedied by streaking the ethyl acetate extracts onto Kieselguhr and elution of the zones of appropriate Rf (see Table XX).

For this 24 hr. secreted extract with respect to the agar control no significant growth increases were observed despite the healthy appearance of all the test materials. However, again, as in Tables XVIII and XIX there is an obvious

increase in α-amylase content for the 24 hr. secreted extract as compared with the agar control. GA_3 had no elongative effects on lettuce hypocotyls and the foliar shoots. The well-known stimulative action on α-amylase release is again recorded.

Now, at first sight we have a situation where chemical tests on the secreted extracts revealed a substance which occupied an equivalent Rf to gibberellin A_6 and possibly A_5. However, (Table XX) materials of these Rf areas have no action on elongative growth - only on α-amylase release to a very limited extent. From the table of biological activities of gibberellins A_1 to A_9 given by Brian (1966), we are, however, confronted with the somewhat rewarding possibility that if the fluorescent area was mainly A_6 we are in fact dealing with a gibberellin of generally poor biological activity. The lettuce hypocotyl test is recorded, in this table, as being one of the most efficient for A_6. There was no growth response at all from the secreted extracts; maybe for concentration reasons (experimental evidence for this is given later in Figs. 30 and 31).

Brian's Table did not include the endosperm test and it is most interesting that recently Coombe, Cohen & Paleg (1967) produced figures which indicate that in the endosperm bioassay test, at 10^{-9}M, A_3 is more than 13 times as active as A_5 and about 10 times as active as A_6: A_1 was as active as A_3. However, at 10^{-8}M, A_3 was only about 7 times as active as

A_6 and, at 10^{-7}M about 3 times as active; with A_3 at a plateau and A_6 still rising. A_5 was generally less active than A_6.

Brian (1966) in table on the relative activities of the 9 gibberellins in biological assays indicates that A_6 (disregarding A_8, which seems to have no biological activity) is the most generally inactive, while the figures of Coombe, Cohen & Paleg (1967) make A_6, above 10^{-7}M concentration, nearly as active as A_3 and A_1.

CONCLUSION

Therefore, taking the present chemical and biological tests as a whole the tentative conclusion may be that the factor which is transported from the germinating embryos to the endosperm is a gibberellin. This gibberellin-like substance is chemically not unlike A_6. The general biological inactivity of this gibberellin, except on endosperm enzymic mobilization, may also implicate A_6 as being the transported factor (further confirmation using commercially-available A_6 is given later).

The physiological lesson from this preliminary investigation is that the biochemical solution to enzymic release in the aleurone cells of barley endosperms may have to take into consideration that A_3 may be naturally foreign to the endosperm and that normally another gibberellin may synergise with some other factor to produce maximal efficiency. For example, a GA_3-indoleacetic acid synergism, based on important

time durations and concentrations of indoleacetic acid did occur in endosperm slices (see, Part 4A of this Section).

Being worried about the report of Yomo & Iinuma (1966) that they failed to obtain any substantial quantities of gibberellin-like material from 3-day old embryos, it was decided to perform this simple experiment:

Dehusked grains were allowed to germinate for 24 hr. as usual, and embryos or scutella were excised and placed on 18 hr. soaked endosperm slices. One embryo or scutellum was used per slice. This embryo-slice or scutellum-slice association was left in the dark on moist filter paper in a closed petri-dish for 24 hr. at 25°C. The embryos and scutella were then removed and the slices left for 24 hr., after which they were assayed for α-amylase as before. Results are given in Table XXI.

From this it would appear that excised embryos or the scutella (remembering that the latter structures came from 24 hr. embryo) can and do possess the ability to secrete a gibberellin-like factor within 3 days germination. The 5-day gibberellin-like factor extracted from embryos by Yomo & Iinuma (1967) may be a gibberellin, but the question as to whether a gibberellin-like factor is secreted by germination embryos into their endosperms by 24 hr. germination was not enlightened by Yomo & Iinuma's results.

Physiological and Chemical Studies using Commercial A_6.

At the completion of the above investigation which suggested that the secreted embryo factor of Yomo (1958) was possibly A_6 and not gibberellic acid (A_3), a minute sample of commercially-available A_6 was obtained. This was undoubtably very fortunate as A_6 is very scarce indeed.

Excised barley embryos were allowed to secrete on an agar base for 24 hr. as previously described, and likewise, the extract chromatographed on Kieselguhr with A_6 mixed-in as standard or spotted separately in the same capacity.

When the plates were sprayed, dried and examined under UV light the commercial A_6 had an Rf of 0.867 while the secreted extract had an Rf of 0.835. There was only one spot with an Rf of 0.87 when A_6 was mixed with the chromatographed extract. It may be remembered that the extract samples in Table XVII have a mean Rf value of 0.832.

From this analysis it would appear that the gibberellin which is secreted by excised barley embryos is not chemically dissimilar from the gibberellin, A_6.

Now, it may be recalled that failure to obtain a growth response for lettuce hypocotyl (Table XX) with the 24 hr. embryo secreted extract was attributed to the possibility of too low a concentration. Since an α-amylase response was obtained for endosperm slices with this same extract it is quite possible that if this extract contained A_6 then commercially-available A_6 used over a sensible range of concentrations

might give some information of the relative sensitivity to this hormone by endosperm slices and lettuce hypocotyl.

MATERIAL AND METHOD:

8, 1 mm endosperm slices from dry grains were incubated in 2.5 inch. petri dishes with various concentrations of GA_3 or GA_6 dissolved in ImM acetate buffer pH 6.2. Each petri dish contained, as usual, 4 ml. of 0.6% sodium chloride in 0.1 M acetate buffer pH 5.3 was added. The Briggs assay method was used for α-amylase assay as in Part 4A of this Section.

Lettuce hypocotyl assay was conducted differently from the method of Brian Hemming & Lowe (1964) previously used in the earlier investigation where the growth container was 2.5 inch petri dishes. Here, lettuce (Carters) were grown as usual in the dark for 24 hr. at 25°C in 3.5 inch. petri dishes. 6, germinated seeds of equal dimensions were then supported on 2 small rings of filter paper at the bottom of 5 x 1 cm. tubes and then saturated with two drops of the test solution. Each vial was covered with parafilm, in which a pin hole was made, and allowed to stand for 48 hr. at 450 f.c. in continuous light at 100% humidity at 25°C. Hypocotyl lengths were measured after 48 hr. growth period. This method requires very little test solution, is very simple to construct, and gives superb visual observation of the changes in elongative lengths.

RESULT AND DISCUSSION

As was hoped, (compare Fig. 30 with Fig. 31) at 10^{-8}M, GA_6 elicits a pronounced α-amylase response in endosperm slices while this same concentration fails to stimulate a hypocotyl elongative response in lettuce seedlings. Surely, if a pure sample of commercial GA_6 fails to elicit a growth response in these hypoctyls then it is quite possible that too low an A_6 concentration in the extract from embryo secretion was not an improbable suggestion (cf., Table XX). None the less in Fig. 30 it is observed that GA_6 elicits an α-amylase response at 10^{-8}M concentration which seems to suggest that the lettuce hypocotyl sensitivity test for A_6 (Brian, 1966) is far less sensitive than the α-amylase response of endosperm slices. It goes without saying that A_3, in both biological tests, is far more active than A_6.

CONCLUSION

These subsequent chemical and physiological tests tend to indicate that the earlier suggestion that A_6 may be the gibberellic acid-like factor of Yomo (1958) which is supposed to be secreted into the aleurone cells by the growing embryo and there catalyses hydrolytic enzyme release and, as a consequence, endosperm modification.

Although the question as to why the embryo should choose to export such an apparently singularly inefficient menber of the known gibberellins seems unanswerable at the moment. None the

less, Dr. J. N. Turner, Plant Protection Ltd., provided valuable information on this gibberellin which are highly suggestive as to how A_6 could become the secreted embryo factor of endosperm modification.

The formula of A_6 differes only slightly from that of A_1 or A_3 (see Fig. 32). Gibberellin A_1 is the same as A_3 except that it has no double bond. A_6 is the same as A_1 except that the hydroxyl group nearest the lactone ring has moved to some other (undefined) position. It is very easy, therefore, to visualise changes from A_3 to A_1 to A_6 such as may be caused on transport out of the embryo - or for that matter, the method of extraction. In fact, the natural occurrence of A_6 is rarely reported though Sambduer (1964) and MacMillan et al (1961) have both reported the presence of this gibberellin in Phaseolus.

Now, owing to the low biological activity of A_6 (Nitsch, 1962; Brian, 1966) it seems not unreasonable to regard it as an end-product of the generally highly active A_3 or A_1 rather than being a hormone sensu stricta - but, to substantiate this possibility extensive chemical analysis would be necessary.

However, since the purpose of this investigation was merely to obtain information on the physiological and chemical identity of the secreted embryo factor, all can be said about the above idaa of gibberellin conversion is that, since both A_1 (matured embryos) and A_3 (immature grain) have been tentatively reported to occur in barley grains (Jones, MacMillan & Radley, 1963; Duffus 1965), the only grace of this 'conversion

possibility' is that it is reasonable. None the less, at a guess, A_6 may be more amenable to polar transport (e.g. from the scutellar node to acrospire-end of the scutellum-endosperm junction, see Section I) then either A_3 or A_1 which, with special reference to A_3, appear to move in a non-polar manner in plant tissues (Brian, 1966). On reaching the aleurone cells this A_6-like gibberellin might synergise with other compounds (of which indoleacetic acid may be one. See, Part 4A of this Section) to promote optimal catalytic synthesis of hydrolytic enzymes; however, ideas or guesses without experimentation can only remain as such, scientifically. It is hoped that scientific substance was given to at least some of the idea more directly concerned with this Thesis.

GENERAL CONCLUSION

Although no attempt is being made to impress on the reader that a summary of the entire events of barley germination and subsequent seedling growth is being attempted the work conducted here by the aid of various simple and more complex structural, biochemical and physiological techniques seems to suggest the following:

At the recommencement of intense metabolism, as a result of wetting the matured grain under suitable growth conditions, root emergence by cell elongation and division and vascular differentiation are major morphological processes. Associated with the event of root emergence from the coleorhiza are intense

ultrastructural changes in the scutellar node and scutellum. In the scutellum these ultrastructural changes concern storage products while in the scutellar node they involve various subcellular organelles which, in such quantities, are usually intrinsically concerned with secretory events. Differential dissection showed that a gibberellic acid-like substance had its origins at this scutellar node region and that transport to the aleurone cells was through the acrospire-end of the scutellum, thus causing a disproportionate pattern of endosperm modification. This acrospire-end orientation would seem to depend on the fact that in that end of the scutellum a well developed system of vascular strands is present. Endosperm modification is an aleurone event commencing at about when germination is completed (17 hr.) and the elongating scutellar epithelial cells do not contribute to the hydrolytic enzymic pool of endosperm degradation.

However, it is not only the aleurone cells which require this gibberellic acid-like stimulation for metabolic action- the foliar shoot seems to be also a natural consumer of this gibberellic acid-like factor which emanates from the scutellar node.

There is also a possibility that low indoleacetic acid concentrations (10^{-10} to 10^{-11}M) may, in the intact grain, synergist with the gibberellic acid-like factor in a short term optimal presensitisation of the aleurone cells to subsequent gibberellic acid-like action. In fact, the

scutellum (epithelial elongation and vascular differentiation) may be influenced by a decreased indoleacetic acid concentration, this hormone originating mainly in the coleoptile tip at about 10^{-7}M concentration.

Chemical and physiological characterisation of the gibberellic adic-like factor seems to indicate that this secreted substance is not gibbereilic acid A_3 but possibly the gibberellin A_6.

Germination seems to be an axis event with no important contribution of food products by the scutellum or endosperm as opposed to seedling growth where utilisation of degraded endosperm food reserves takes place.

The approach throughout this thesis is basically a biological approach where selected observation are collected and subjected to physiological analysis in the hope of gaining useful information on the functioning of the embryo as an integrated whole. Although the reported work may appear only to scratch the surface of problems relating to barley germination and subsequent seedling growth an important point must be that the problems of germination and endosperm modification (both commercially important) are all packed into the first 17 hr. after initial wetting of the mature grain.

Although the biochemical and physiological events within this 17 hr. time span are too numerous to elucidate completely, in my estimation, a limited attempt at this task

would certainly not be wasted. Surely, with valuable information on the correlative physiology of tissue interaction in the embryo during germination the solving of at least some of the problems of dormancy, inviability and malting efficiency may have greater scientific possibilities rather than an haphazard approach of indiscrimately subjecting barley grains, viable or otherwise, to the oddest array of chemical or physical factors whose resulting actions sometimes escape useful explanation. In short, how can we hope to rectify for example a certain type of dormancy when no information is available on the physiology of the normally germinating seed or grain?

To conclude this thesis it would appear that the whole problem of the correlative physiology of barley germination needs finer analysis despite the often held but invariably unfounded supposition that the biology of this commercially important grain is 'well understood'. It is hoped that at least some of the information in this thesis will be useful in further physiological explorations on problems of barley germination and subsequent seedling growth.

TABLE IX

Elongation of Cells of Scutellar Epithelium in Cores Removed after 2 hr. soak (25°C).

(Incubation media: Potential growth factors)

Incubation Time	2 hrs.	24 hrs.	48 hrs.	72 hrs.
Medium	Mean Length of Cells			
A Basal Incubation Medium	30 μ	35 μ	35-40 μ	35-40 μ
IAA 10^{-11}M	30 μ	35-40 μ	40-45 μ	45-50 μ
IAA 10^{-5}M	30 μ	30-35 μ	30-40 μ	30-40 μ
IAA 10^{-11}M + GA 10^{-5}M	30 μ	30-35 μ	30-35 μ	30-35 μ
GA 10^{-5}M	30 μ	30 μ	30 μ	30 μ
TIBA 10^{-5}M	30 μ	30 μ	30 μ	30-35 μ
Kinetin 10^{-6}M	30 μ	35 μ	35 μ	35 μ
Adenine 10^{-6}M	30 μ	35 μ	35 μ	35 μ
Tripalmitate 0.05 mg/l	30 μ	35 μ	35 μ	35 μ
Intact Grain	30 μ	40-50 μ	70-75 μ	75-80 μ

Basal Medium = Knops solution + 1.5% sucrose in 1 mM acetate buffer pH 6.2

IAA = Indoleacetic acid

GA = Gibberellic acid

TIBA = Triiodobenzoic acid

TABLE X

Elongation of Cells of Scutellar Epithelium in Entire Excised Embryos (25°C).

Incubation time	2 hours	24 hours	48 hours	72 hours
	MEAN LENGTH OF CELLS			
A BASAL INCUBATION MEDIUM	30 µ	30 - 35 µ	30 - 40 µ	40 - 50 µ
IAA 10^{-11}M	30 µ	30 - 35 µ	35 - 40 µ	40 - 50 µ
IAA 10^{-5}M	30 µ	30 µ	30 - 35 µ	35 - 40 µ
GA 10^{-5}M	30 µ	30 - 35 µ	30 - 35 µ	30 - 35 µ
KINETIN 10^{-6}M	30 µ	30 - 35 µ	30 - 35 µ	30 - 35 µ
TRIPALMITATE 0.05 mg/l	30 µ	30 - 35 µ	30 - 35 µ	30 - 35 µ
INTACT GRAIN	30 µ	40 - 50 µ	70 - 75 µ	75 - 80 µ

Measurements made in areas used as "cores" in Table IX.

Basal medium = Knops solution + 1.5% sucrose in 1 mM acetate buffer pH 6.2.

IAA = Indoleacetic acid.

GA = Gibberellic acid.

TABLE XI

Elongation of Cells of Scutellar Epithelium in Embryos

Attached to Endosperm by Pericarp-Testa and Aleurone Only.

Growth Temperature = 25°C.

2 hours	24 hours	48 hours	72 hours
30 μ	30 - 35 μ	30 - 40 μ	40 - 50 μ

TABLE XII

Behaviour of Cells of Scutellar Epithelium, and of Entire Embryo, in Grain Grown for 24 hours and Treated as in Table IV. Growth Temperature = 25°C.

Medium	Buffered Water Control	TIBA 10^{-5}M	IAA 10^{-11}M	IAA 10^{-11} + GA 10^{-5}	IAA 10^{-5}M	GA 10^{-5}M	Kinetin 10^{-5}M
INITIAL EPITHELIAL ELONGATION (24 Hours Growth)	40-50 µ	40-50 µ	40-50 µ	40-50 µ	40-50 µ	40-50 µ	40-50 µ
(Partially excised embryos) EPITHELIAL ELONGATION AFTER 72 hours Growth.	75-80 µ	75-80µ	70-75 µ	70-75 µ	50-60 µ	50-60 µ	50-60 µ
(Partially excised scutella) i.e. axis removed. EPITHELIAL ELONGATION AFTER 72 hours Growth.	70-75 µ	50-60 µ	65-75 µ	40-50 µ	50-60 µ	40-50 µ	50-60 µ

Water Control = 1 mM acetate buffer pH 6.2

IAA = Indoleacetic acid

GA = Gibberellic acid

TIBA = Triiodobenzoic adid

TABLE XIII

Showing the Effects of Various Surgical Treatments on the α-Amylase content and Growth Behaviour of Barley Grains after 42 hr. growth (25°C)

Experimental Treatments (Condition of Embryo)	Mean α-amylase content in IDC units	Mean Coleoptile length in cm.	Mean Root length in cm.	Mean number of visible roots
1. Intact grain control	117±11.8 (3)	1.6±0.2 (4)	1.9±.14 (3)	6.2±.38 (4)
2. Acrospire + Scutellar node	19.2±7.0 (4)	1.1±0.2 (6)	(0-0.2) (6)	(0-2) (4)
3. Acrospire only	2.7±1.8 (4)	0.9±1.4 (6)	0 (6)	0 (4)
4. Coleorhiza + Scutellar node	45.7±4.4 (4)	0 (6)	1.7±.5 (4)	6.0±.44 (4)
5. Coleorhiza only	26.7±5.6 (4)	0 (6)	1.7±.46 (4)	4.2±.31 (4)
6. Without Scutellar node*	33.0±7.0 (4)	0.9±.3 (4)	2.1±.56 (4)	3.6±.9 (4)
7. Slight cut below scutellar node	97.0±7.8 (3)	1.7±0.2 (3)	2.4±.14 (3)	4.4±.5 (3)
8. Deep cut below scutellar node	55.0±3.0 (3)	1.2±.02 (3)	A=0.2±0.2(3) B=0.7±.03(3)	A=2.1±.3(3) B=1.4±.12(3)
9. Without Coleoptile tip*	92.6±7.5 (3)	0.8±.002(6)	2.4±.37 (4)	6.4±.10 (4)

TABLE XIII (cont.)

Experimental Treatments (Condition of Embryo)	Mean α-amylase content in IDC units	Mean Coleoptile length in cm.	Mean Root length in mm.	Mean number of visible roots
10. Without Coleorhiza tip	85.0 ± 7.6 (4)	1.5± .02 (4)	1.4 ± .25(4)	6.2 ± .10 (4)
11. Without coleoptile and Coleorhiza tips*	76.5 ± 16.5 (4)	0.6 ± .002 (3)	1.4 ± .25(4)	5.5 ± .5 (3)
12. Without Foliar shoot	165.5 ± 15.8 (4)	0.9 ± .14 (4)	2.6 ± .26(4)	5.7 ± .29 (4)
13. Scutellum only ∓	10.0 ± 0.01 (3)	0 (3)	0 (3)	0 (3)
14. Median longitudinal slit of embryo axis	66.4 ± 6.6 (3)	1.1 ± 0 (3)	2.3 ± .5 (3)	4.9 ± .1 (3)

* - Treatments where foliar shoot showed reduced growth with respect to that of the coleoptile.

∓ - I.D.C. Units for 48 hr. incubation

Original α-amylase content for 2 hr. soaked grains = .25 ± .08 I.D.C. Units (13).

I.D.C. - Iodine Dextrin Colour Units ≡ Arbitary unit of α-amylase

Number of experiments given in parenthesis.

TABLE XIV

Effect of Indoleacetic Acid, Gibberellic Acid and Triiodobenzoic Acid on Root Emergence (17 hr.) and Mean Root Length (36 hr.). Growth in Petri-wick Dishes at 25oC.

MEDIA / TIME	H_2O	∓ GA^4	$TIBA^4$	IAA^4	$TIBA^4$ + IAA^4	IAA^{10}	$TIBA^4$ + IAA^{10}	$TIBA^4$ + GA^4	IAA^4 + GA^4	IAA^{10} + GA^4
17 hr. (Emergence) (Mean Number)	1.1	1.3	0.31	1.7	1.0	1.0	0.6	1.2	2.0*	1.3
36 hr. (Length)	1.30	1.50	1.00	0.25	0.8	1.4	1.2	1.25	0.6	1.8*

∓ GA - Gibberellic acid

TIBA - Triiodobenzoic acid

IAA - Indoleacetic acid

Superscripts: $4 = 10^{-4}M$; $10 = 10^{-10}M$

* = Significant differences.

TABLE XV

Effect of 2-Chloroethyl Trimethyl Ammonium Chloride (CCC) and Maleic Hydrazine (MH) on Germination of Barley

Both were used at 10^{-3}M.

Medium	Germination at 18 hr. (25°C.)
H_2O	93.3%
CCC	93.3%
MH	68.3%

TABLE XVI

Effect of MH and CCC (10^{-3}M) on α-amylase Content, Mitotic Index and Early Growth after 36 hr. at 25°C - using 6, "¾ grains" in Petri-wick Dishes.

Media (36 hr.)	α-Amylase - IDC units	Mitotic Index	Root Initiation	Root Length (cm.)	Shoot Length (cm.)
H_2O	112.8 ± 13	80	6.0	1.30	0.90
MH 10^{-3}M	79.9 ± 17.1	0	5.7	0.90	0.70
CCC 10^{-3}M	61.7 ± 11.3	80	6.0	1.26	0.90
GA 10^{-4}M	257.0 ± 58.7	120*	6.0	1.50	1.02
GA^4 + MH^3	253.6 ± 71	2	5.7	1.26	0.90
GA^4 + CCC^3	258.5 ± 85.2	120	6.0	1.43	0.90

* Most of the other cells, with nuclear content were in incipient prophase.

CCC - 2-chlorethyl Trimethyl Ammonium Chloride

MH - Maleic Hydrazine

I.D.C. - Iodine Dextrin Colour Units = to α-Amylase content

TABLE XVII

Rfs for Fluorescent Spots of Embryo

Secreted Agar Extract and for GA_3.

	Rf Values			
	A 36 hr. extract	B 24 hr. extract	A GA_3	B GA_3
Kieselguhr	0.804	0.860	0.390	0.490
Silica gel	0.20	-	0.000	0.000

GA_3 = Gibberellic acid.

TABLE XVIII

Showing Final Hypocotyl Length for Cucumber and Lettuce.
Original Length of Foliar Shoot, 1.5 cm.

	36 hr. agar control	36 hr. extract	$GA_3 10^{-5}M$
Cucumber hypocotyl (64 hr.)	1.27 cm.	1.40 cm.	1.39 cm.
Lettuce hypocotyl (64 hr.)	-	-	2.00 cm.
Foliar shoot (18 hr.)	1.6 cm.	1.7 cm.	2.40 cm.
Endosperm slices	15 IDC	19.5 IDC	30 IDC

-amylase content as I.D.C. Units.

GA_3 = Gibberellic acid in 1% tween.

I.D.C. = Iodine Dextrin colour units.

Acetone - Ethyl acetate extracts.

TABLE XIX

Showing Final Hypocotyl Length for Cucumber and Lettuce.
Original Length of Foliar Shoot, 1.5 cm.

	24 hr. agar control*	24 hr. extract*	$GA_3 10^{-5}M$
Cucumber hypocotyl (72 hr.)	1.25 cm.	1.28 cm.	1.30 cm.
Lettuce hypocotyl (48 hr.)	-	-	2.00 cm.
Foliar shoot (20 hr.)	1.7 cm.	1.7 cm.	2.70 cm.
Endosperm slices (54 hr.)	16 IDC	22 IDC	50 IDC

α-amylase content as I.D.C. units.

GA_3 = Gibberellic acid in .1% tween.

I.D.C. = Iodine Dextrin Colour Units.

*Acetone - Ethyl acetate extracts.

TABLE XX

Showing Final Hypocotyl Length for Cucumber and Lettuce.
Original length of Foliar Shoot, 1.5 cm.

	24 hr. control agar *	24 hr. extract*	$GA_3 10^{-5}M$
Cucumber hypocotyl (64 hr.)	1.25 cm.	1.18 cm.	1.20 cm.
Lettuce hypocotyl (64 hr.)	0.75 cm.	0.70 cm.	1.90 cm.
Foliar shoot (18 hr.)	1.7 cm.	1.90 cm.	1.70 cm.
Endosperm slices (46 hr.)	10.0 IDC	18.0 IDC	40.0 IDC

α-Amylase content as IDC Units.

GA_3 = Gibberellic acid

I.D.C. = Iodine Dextrin Colour Units.

* Acetone - Ethyl acetate extracts : then Ethyl alcohol elution from Rf's = 0.7 to 0.95 on Kieselguhr.

TABLE XXI

Showing the Effect of the Embryo and Scutellum on the Release of α-Amylase from Endosperm Slices after a Secretion Time of 24 hr. for 48 hr. Embryos and Scutella

	Embryos + slices	Scutella + slices	Water control slices	Embryo + Dead slices *
Endosperm slices	14 IDC	9.6 IDC	6.6 IDC	0 IDC

I.D.C. Iodine Dextrin Colour Units to α-Amylase content.

8, 1 mm. slices per duplicate treatment

* Dead slices (heated at 150°C for 24 hr.)

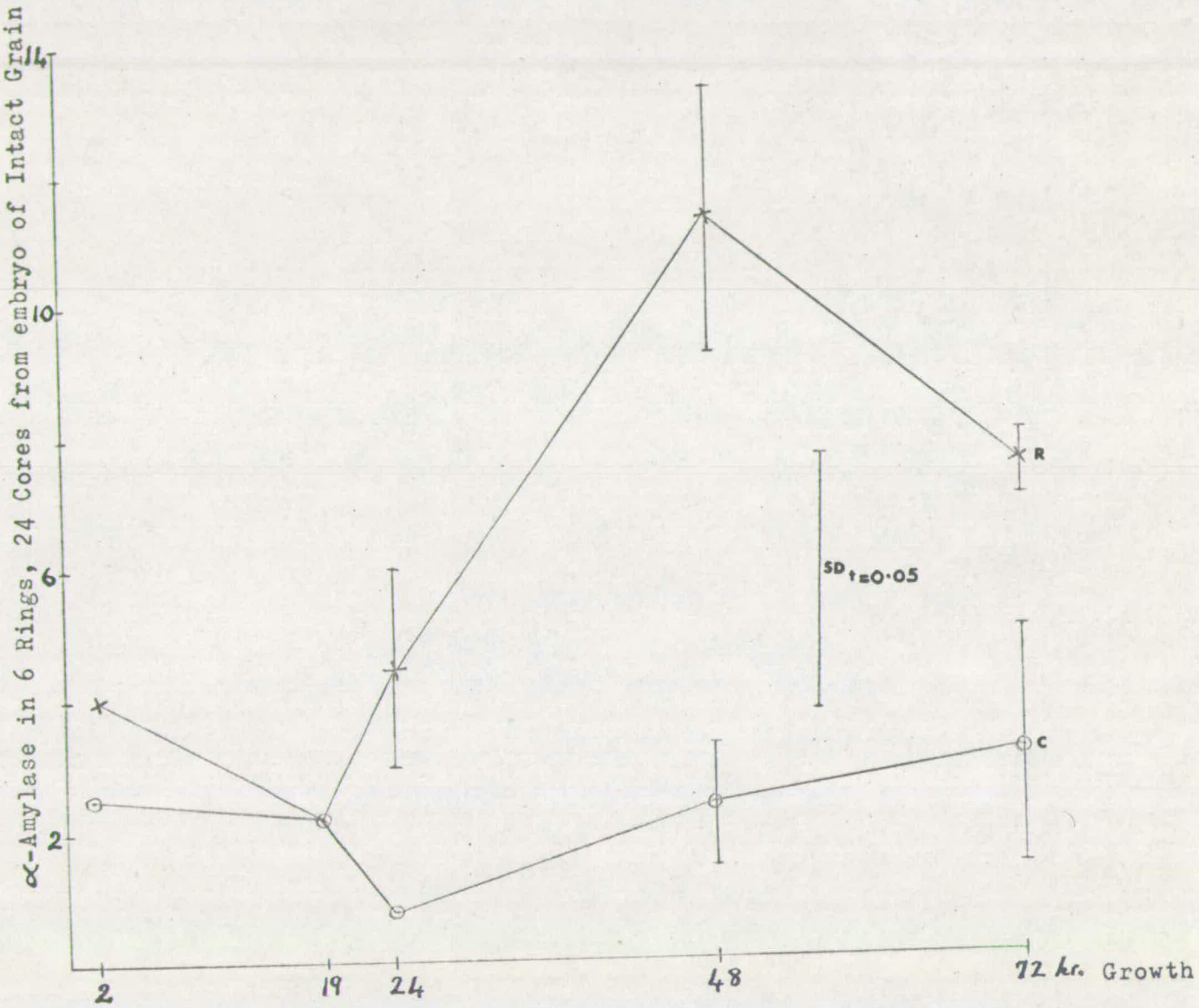


Fig. I2. Intact Grain. Appearance of α-amylase in the scutellum during germination and seedling growth. Rings contaminated with aleurone cells.

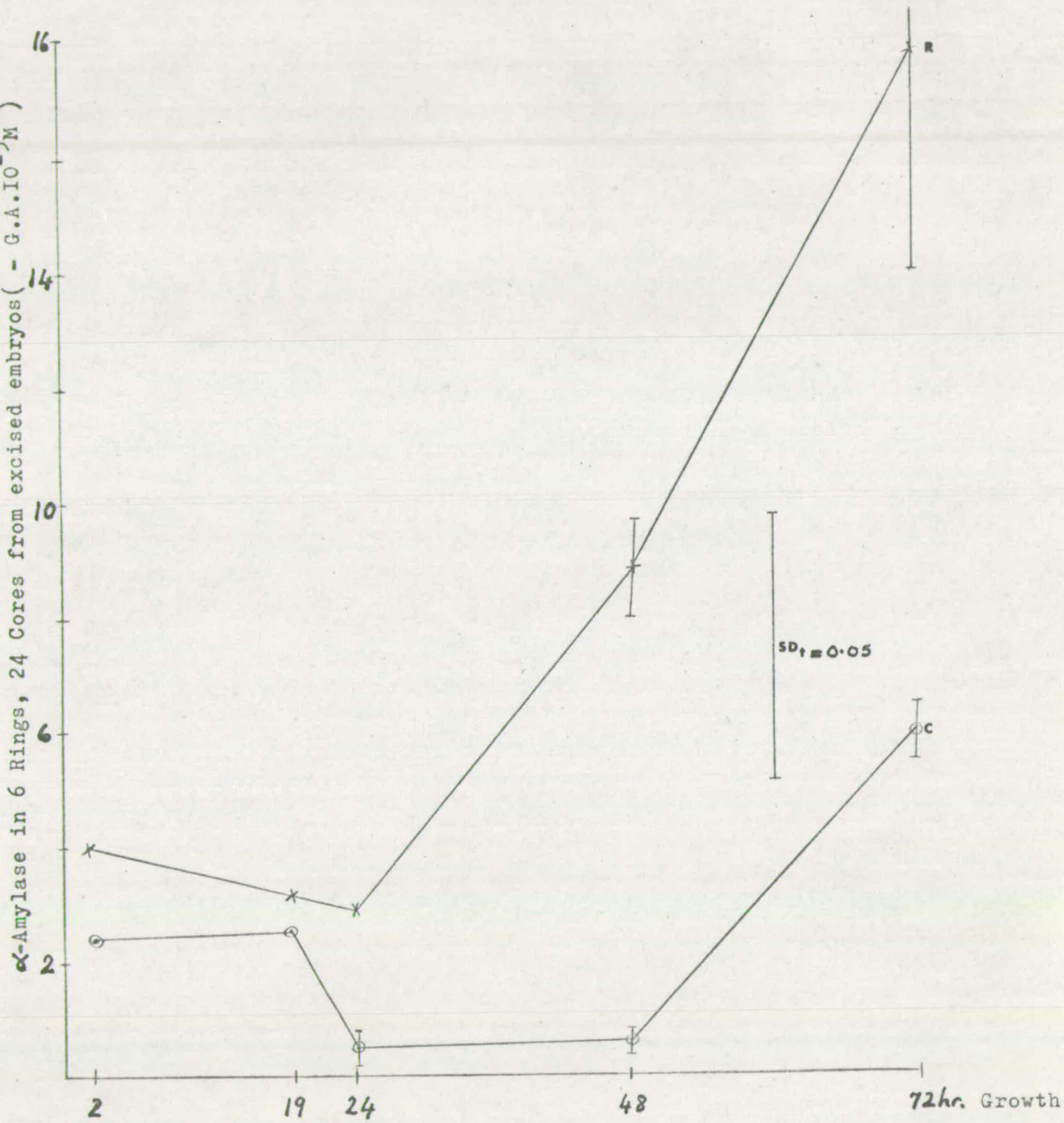


Fig. 13. Excised embryos(-G.A.). Appearance of α-amylase in the scutellum during embryo growth. Rings contaminated with aleurone cells.

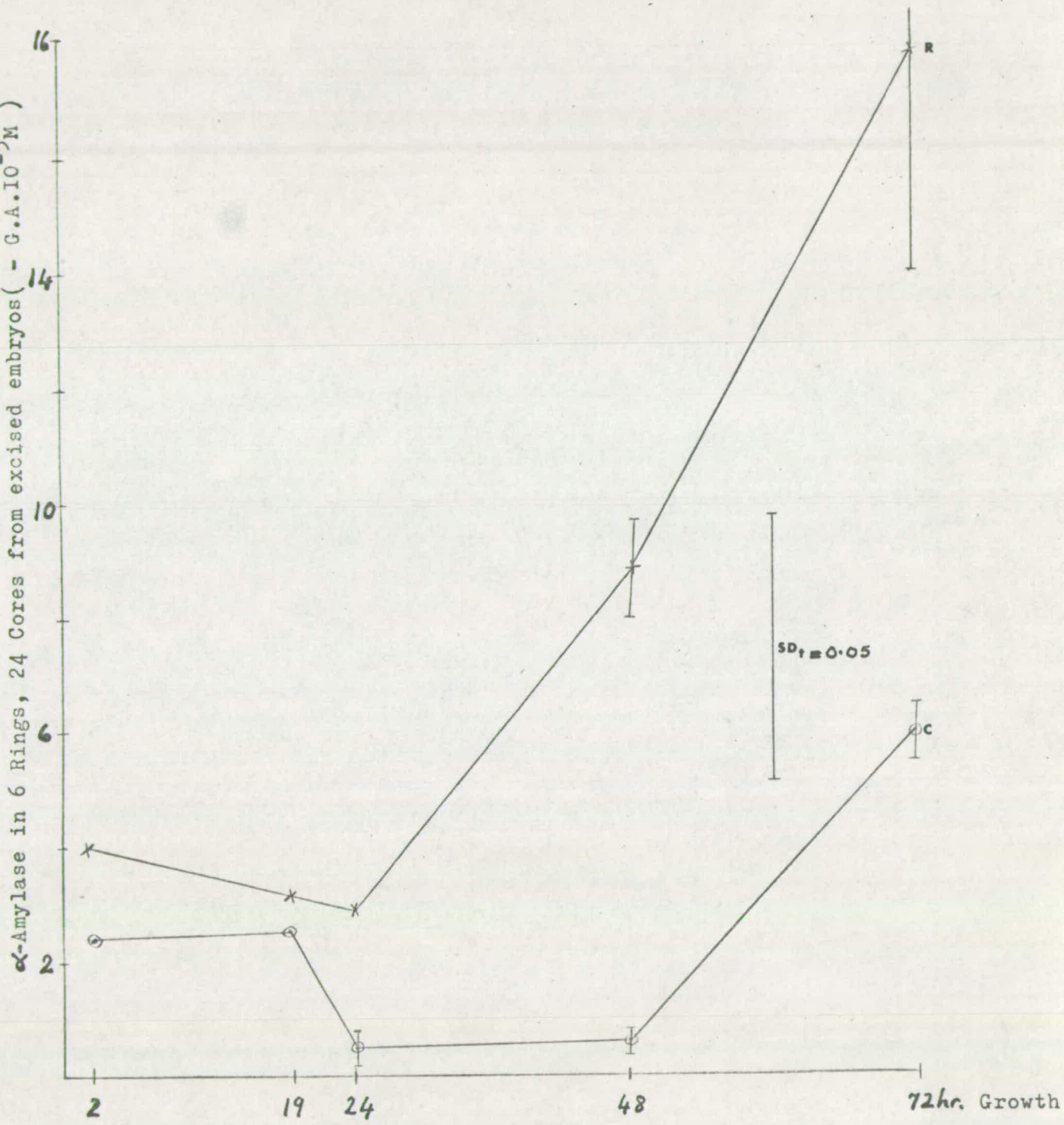


Fig. I3. Excised embryos(-G.A.). Appearance of α-amylase in the scutellum during embryo growth.Rings contaminated with aleurone cells.

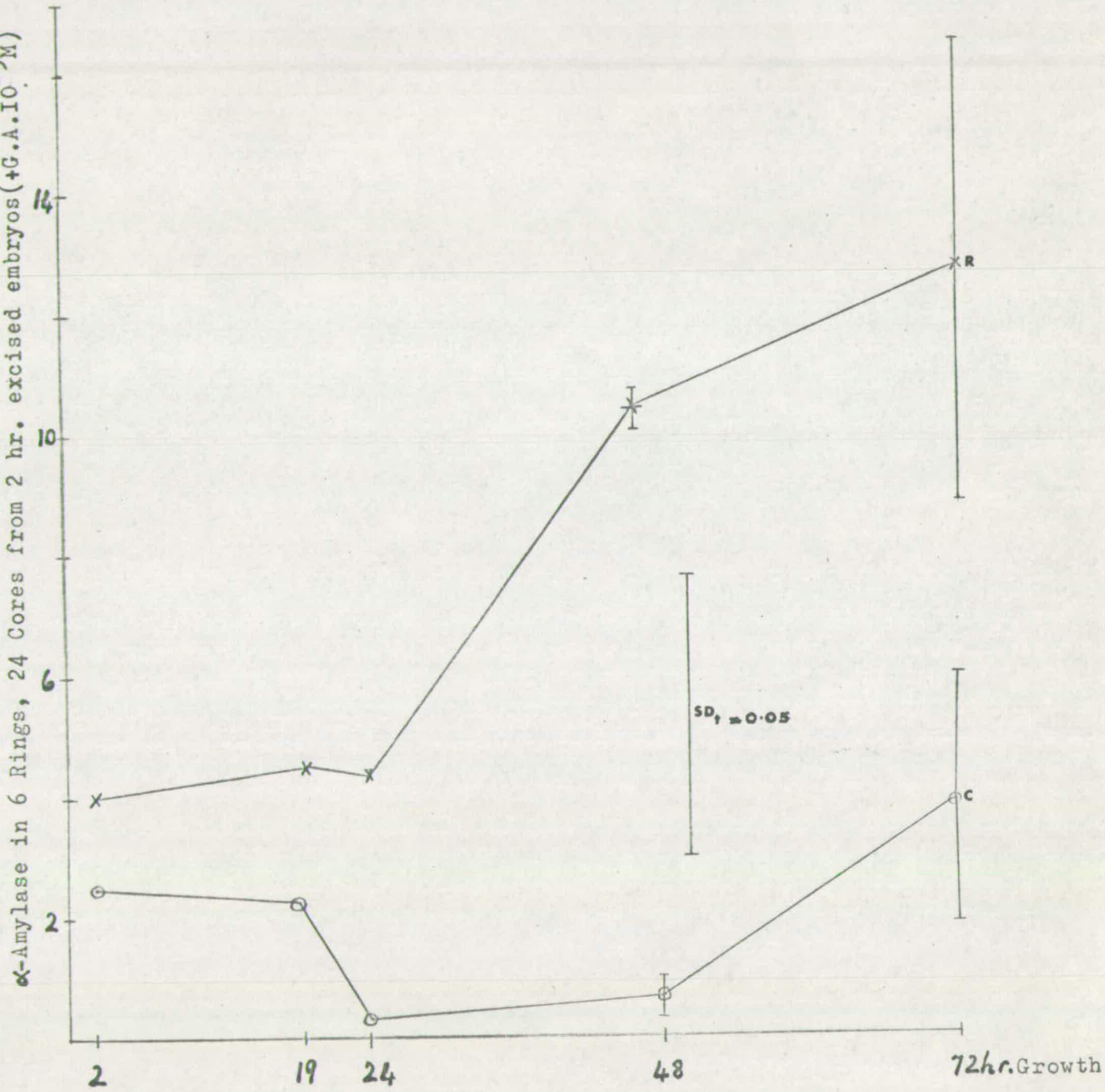


Fig. I4. Excised embryos(+G.A.). Appearance of α-amylase in the scutellum during embryo growth. Rings contaminated with aleurone cells.

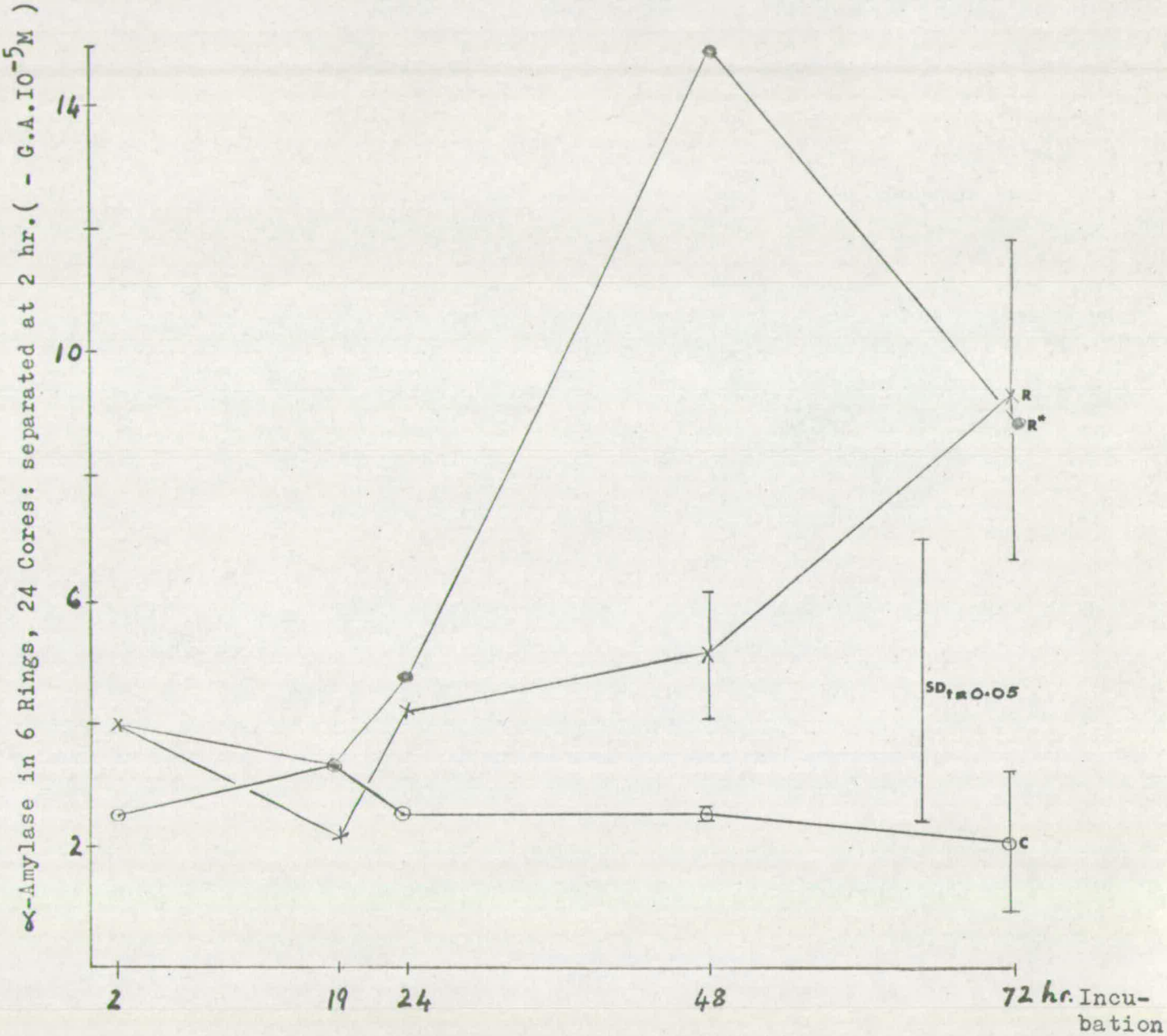


Fig. I5. 2 hr. excised Rings and Cores(- G.A.). Aleurone contamiation of scutellum(Rings) in ∝-amylase production.
R* = -G.A.IO^{-5}M + I.A.A.IO^{-II}M.

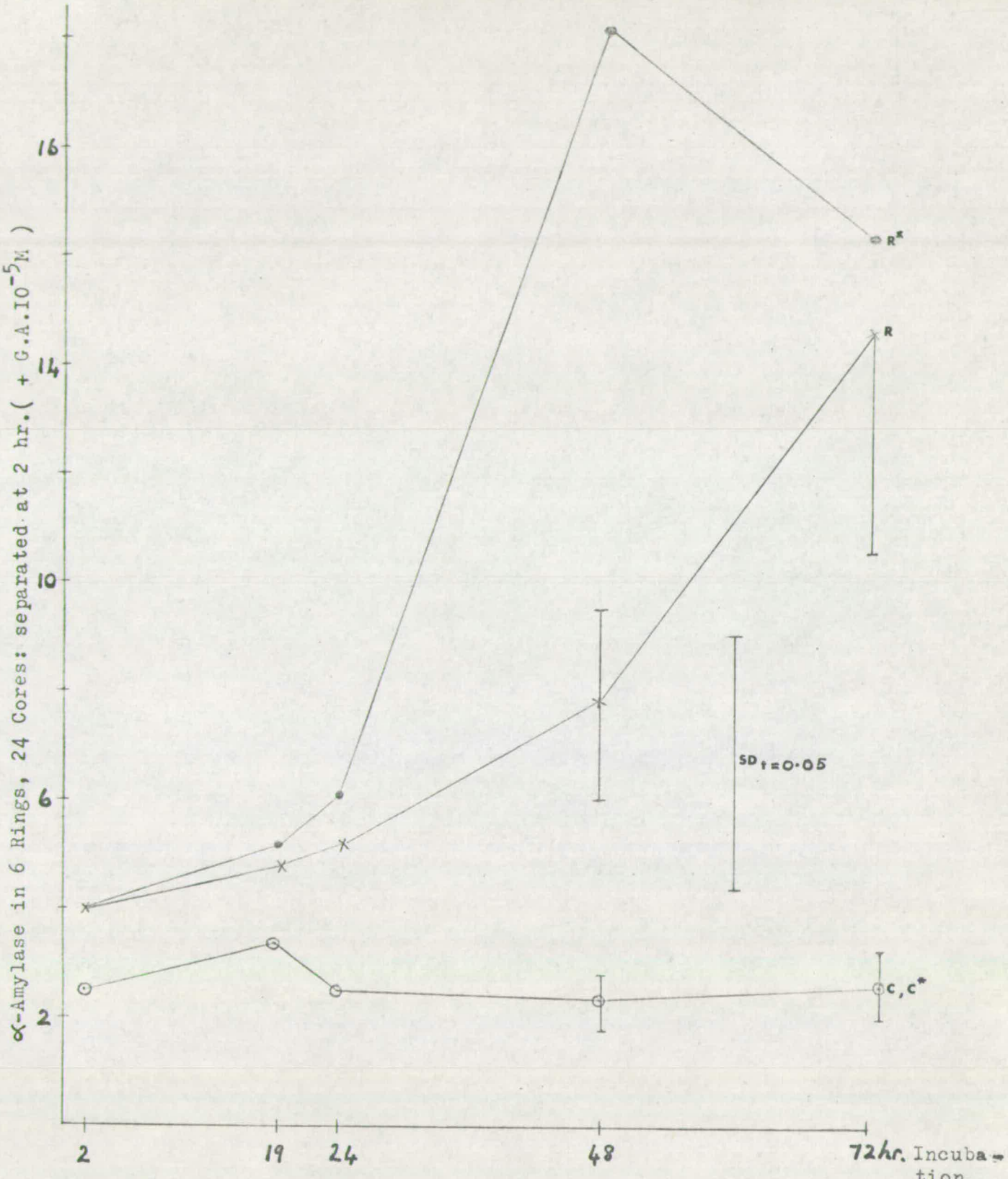
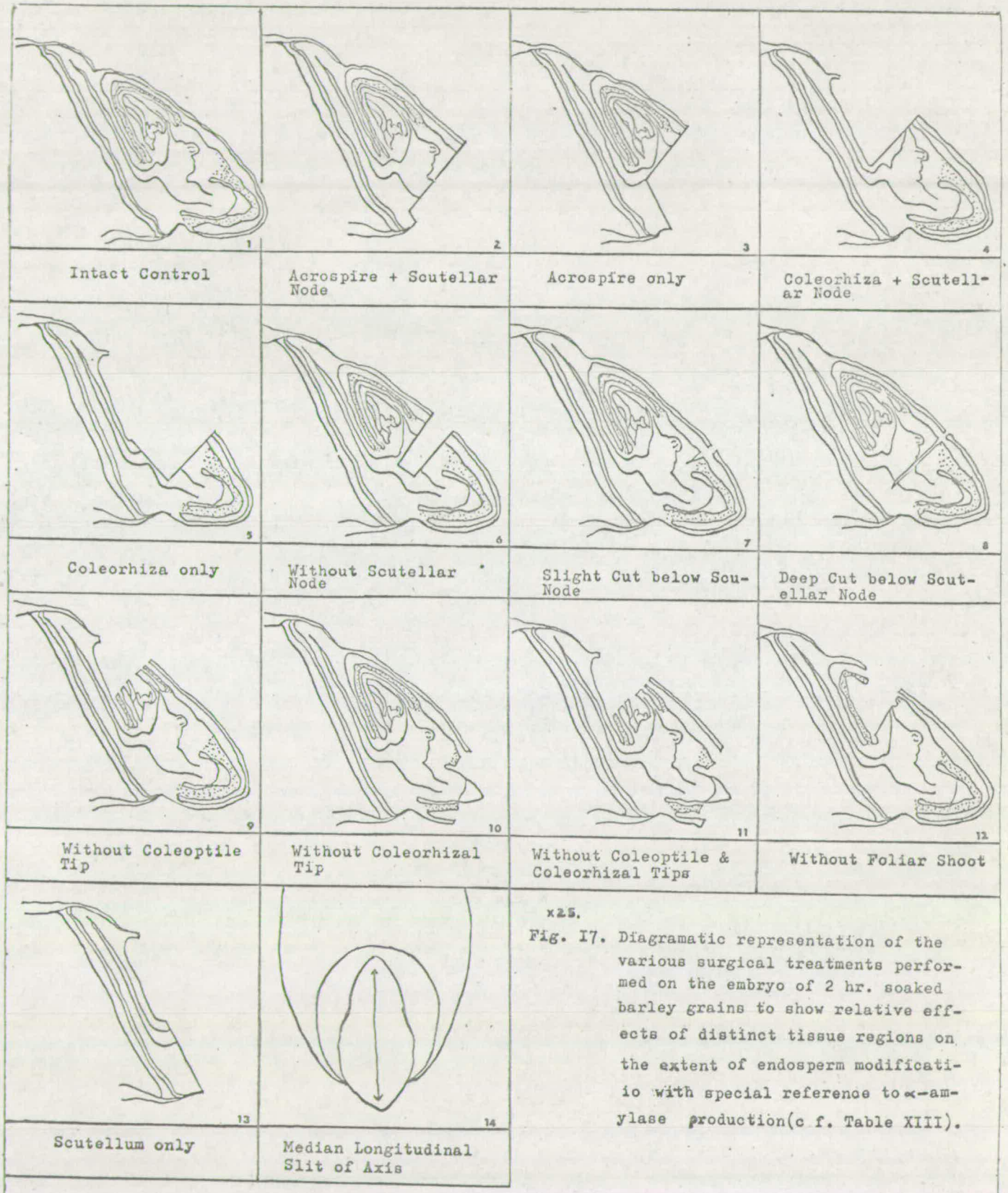


Fig. I6. 2 hr. excised Rings and Cores(+ G.A.). Aleurone contamination of scutellum(Rings) in α-amylase production.

R* and C* = G.A.IO^{-5}M + I.A.A.IO^{-II}M.



x25.

Fig. I7. Diagramatic representation of the various surgical treatments performed on the embryo of 2 hr. soaked barley grains to show relative effects of distinct tissue regions on the extent of endosperm modificatio with special reference to ∝-amylase production(c f. Table XIII).

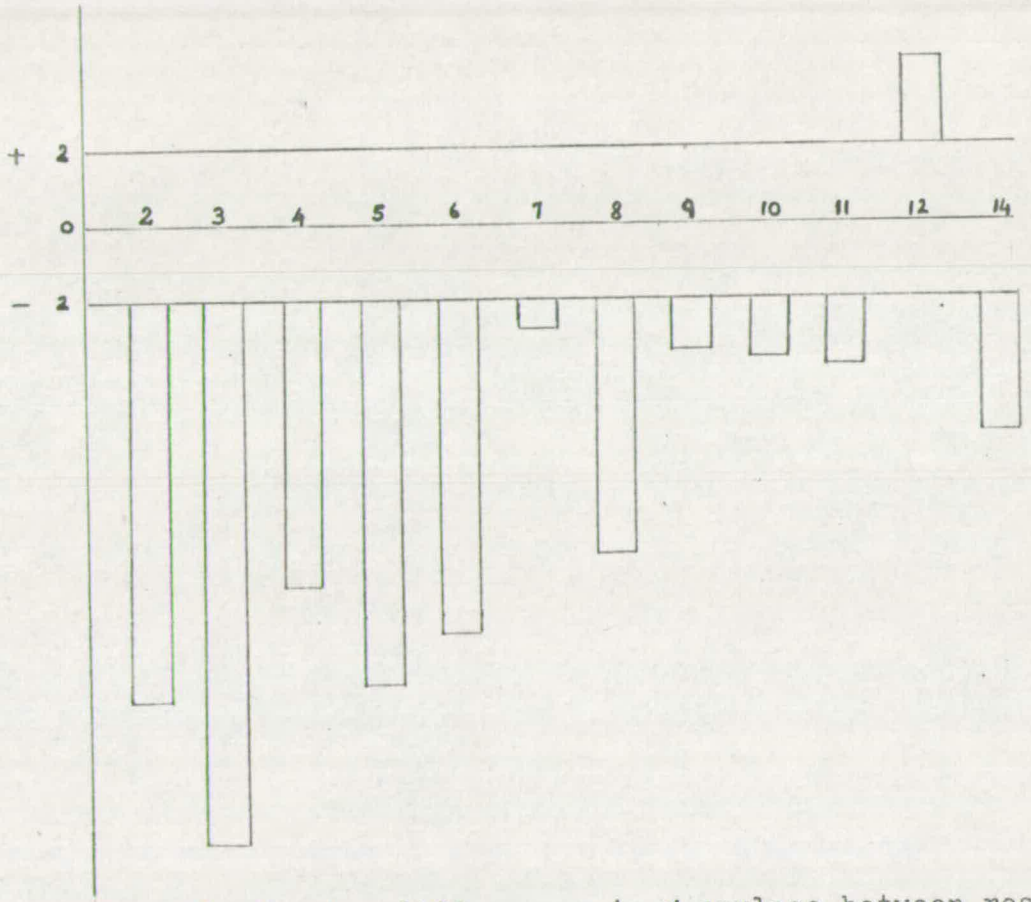


Fig. I8. Showing differences in ∝-amylase between respective surgical treatments(Table XIII) and the intact control. Standard Error limit = 2.

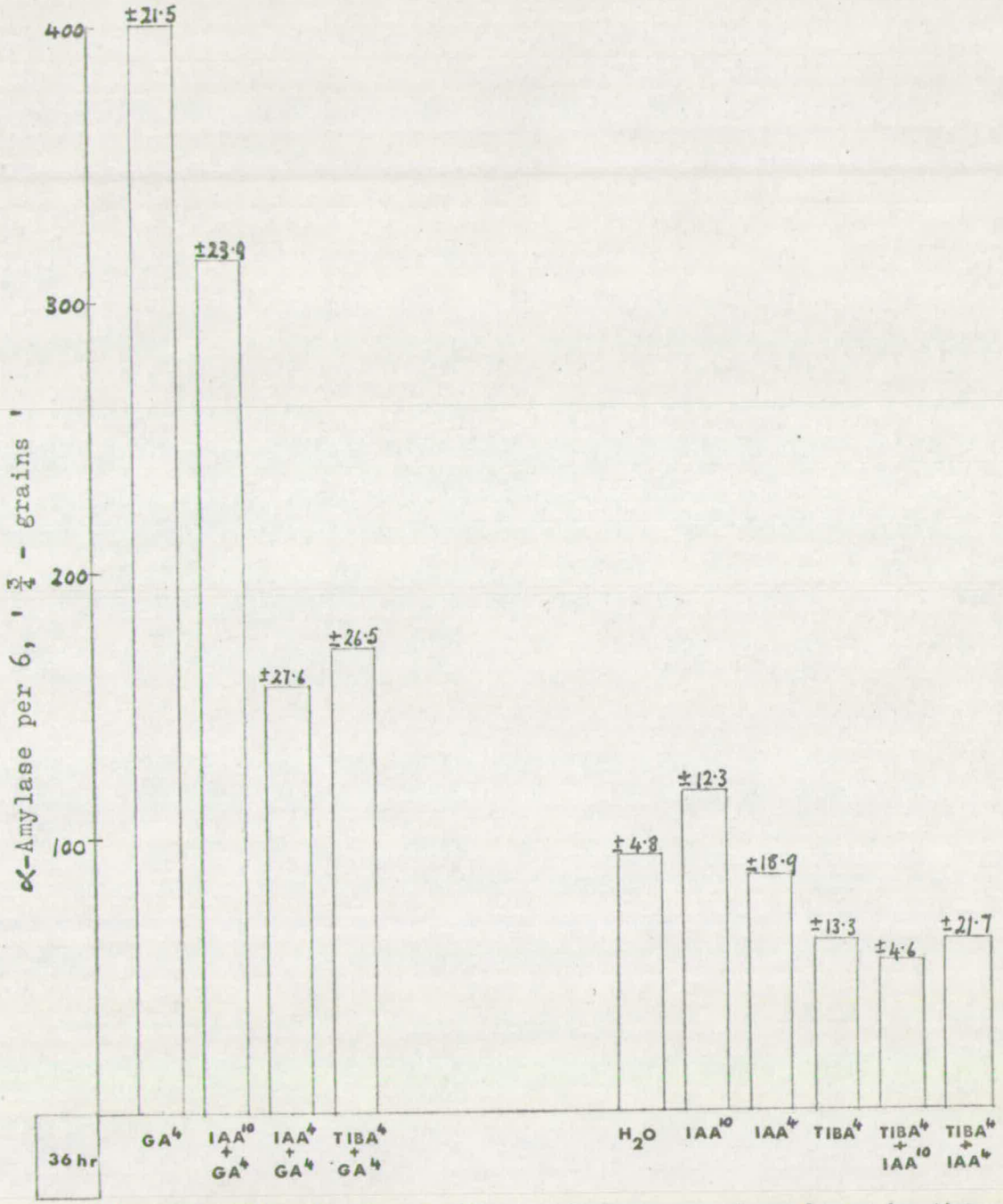


Fig. I9. Effect of growth regulators on ∝-amylase in the endosperm of barley grains.

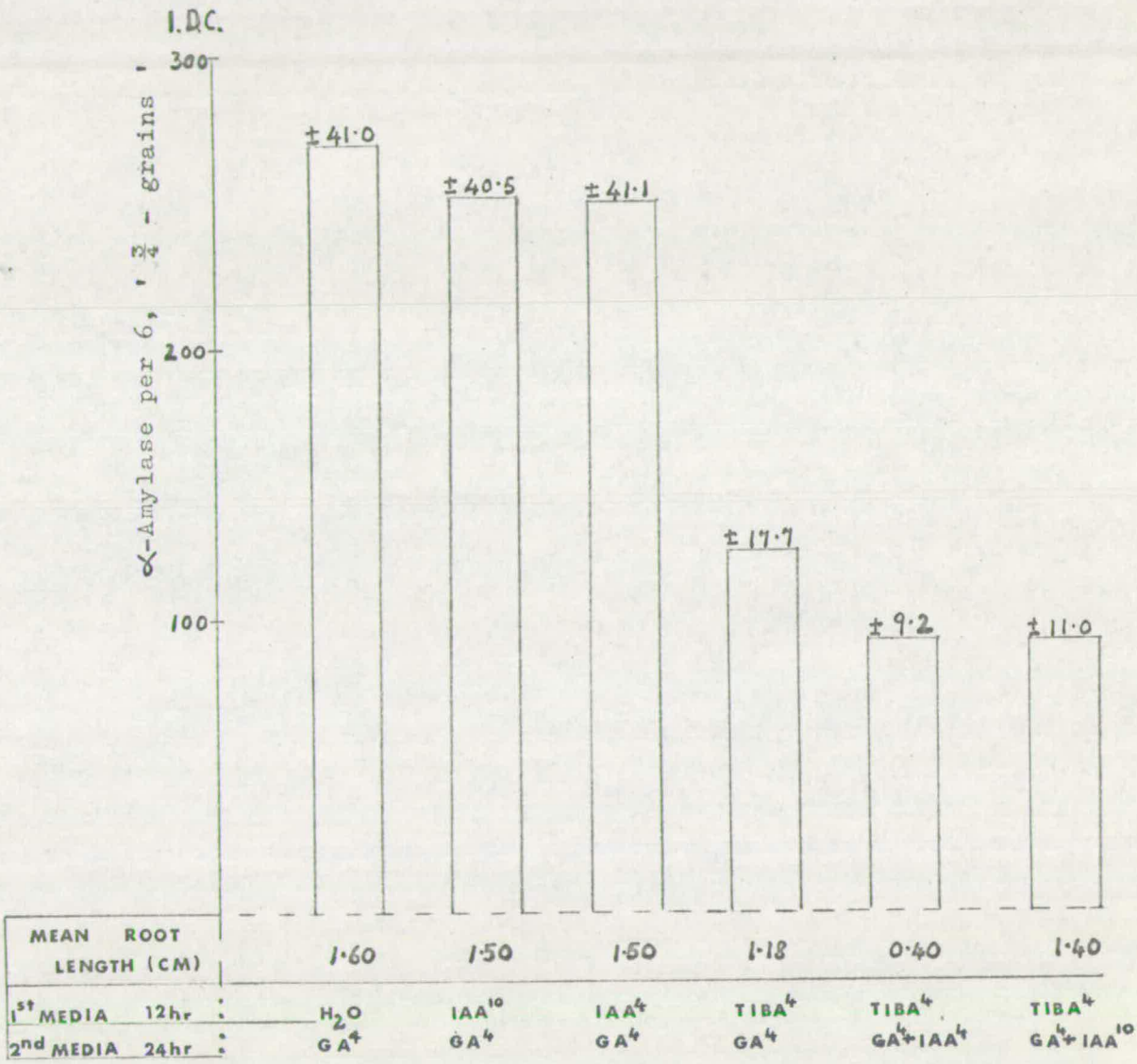


Fig. 20. Effect of growth media transfer on ∝-amylase in the endosperm of barely grains.

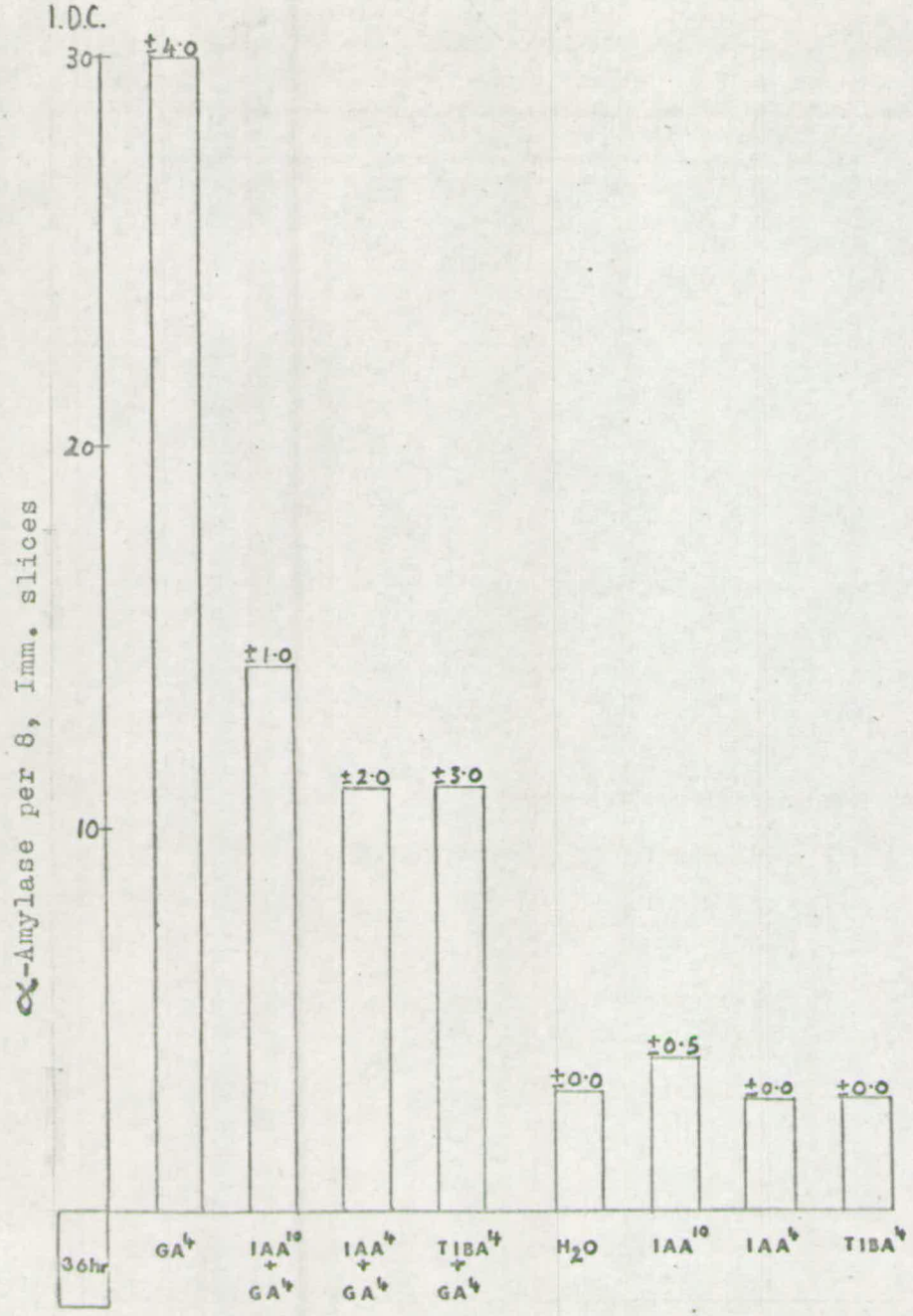


Fig. 2I. Effect of growth regulators on -amylase in endosperm slices.

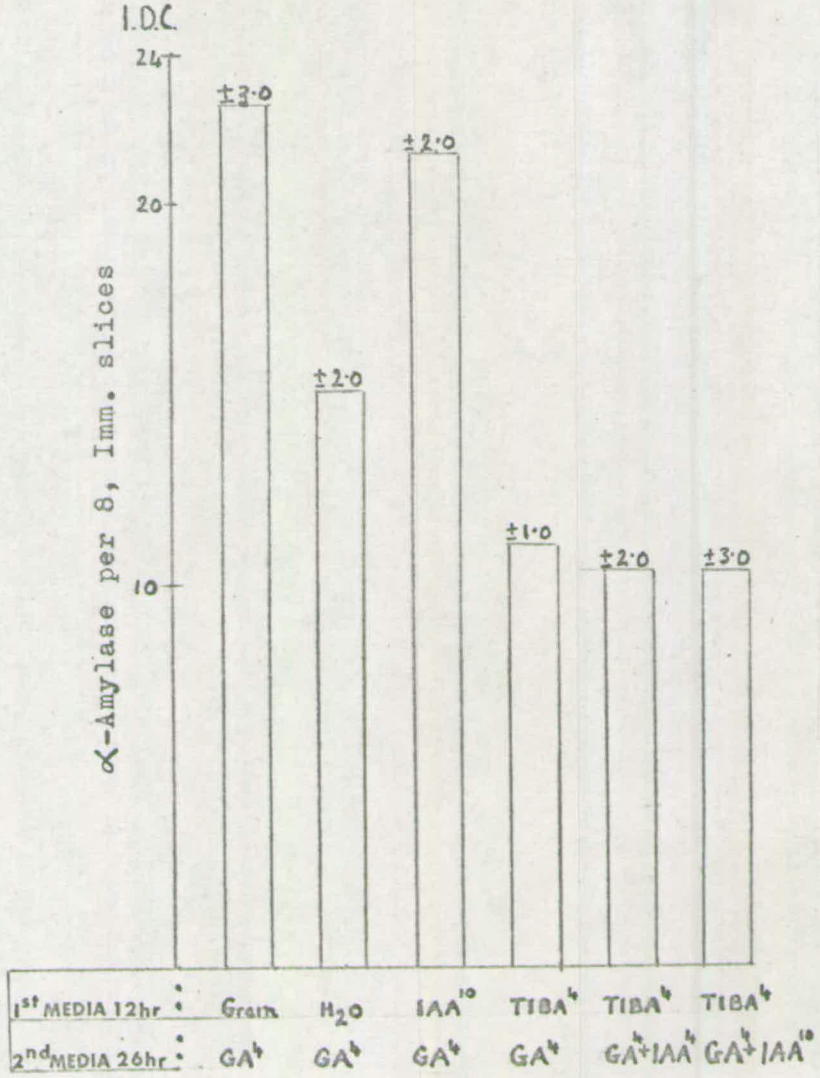


Fig. 22. Effect of growth media transfer on α-amylase in endosperm slices.

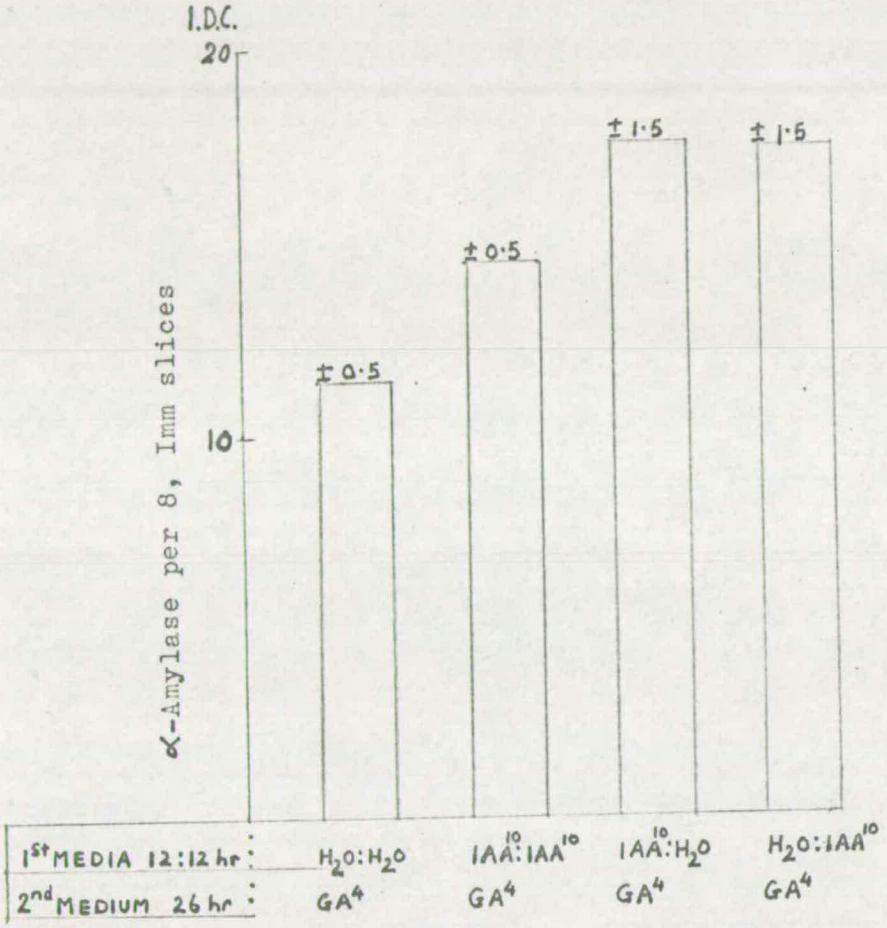


Fig. 23. Effect of duration of Indoleacetic acid pretreatment on α-amylase in endosperm slices.

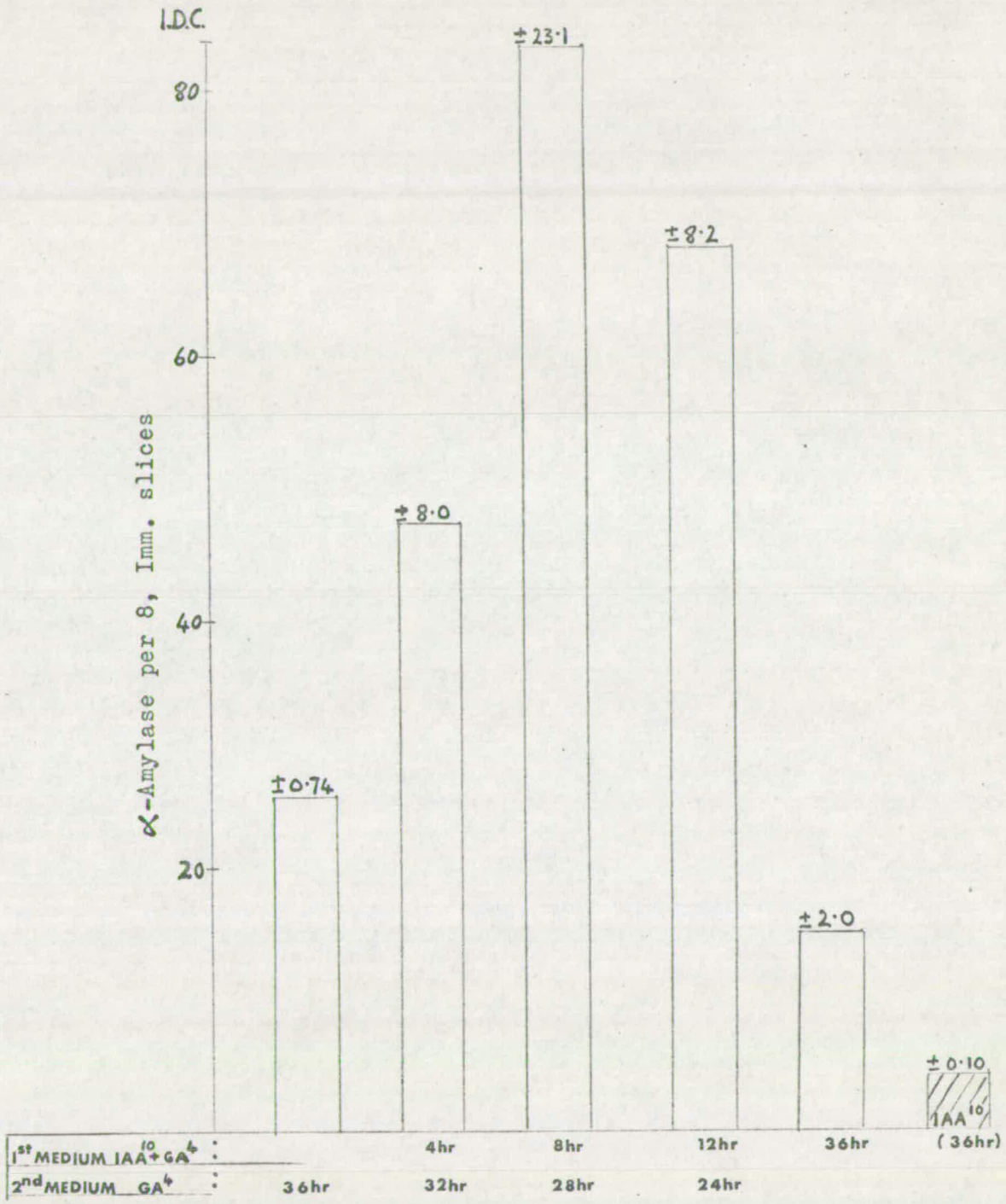


Fig. 24. Duration of pretreatment and hormonal synergism on α-amylase content of endosperm slices.

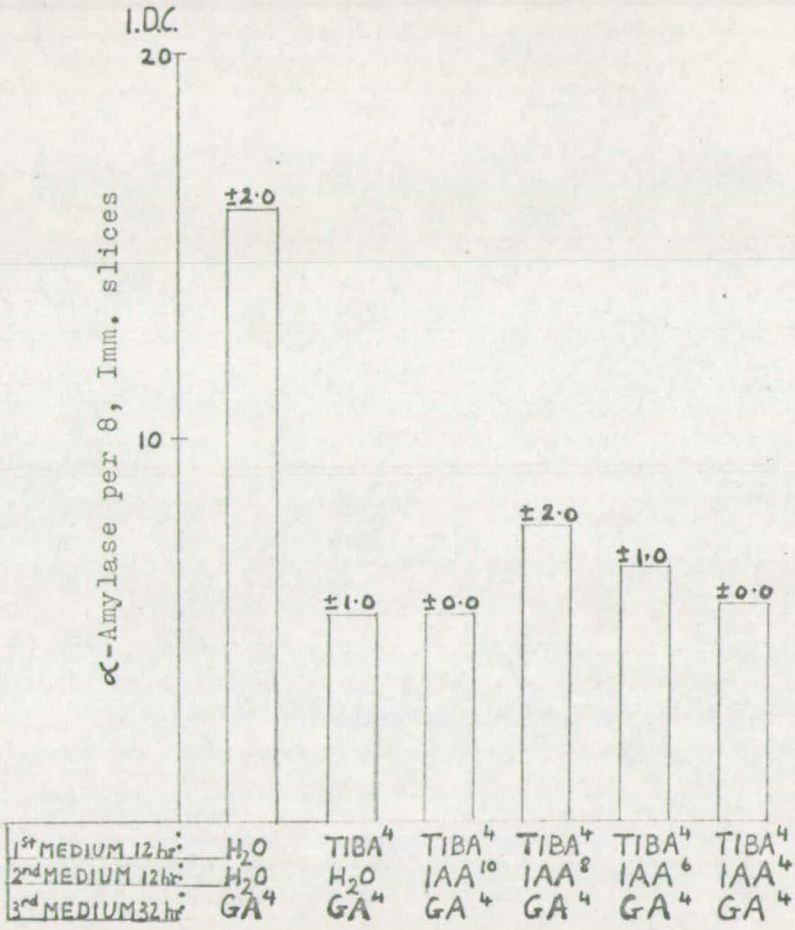


Fig. 25. Sequential transfer and resulting non-competitive action between I.A.A. and T.I.B.A. on α-amylase in endosperm slices.

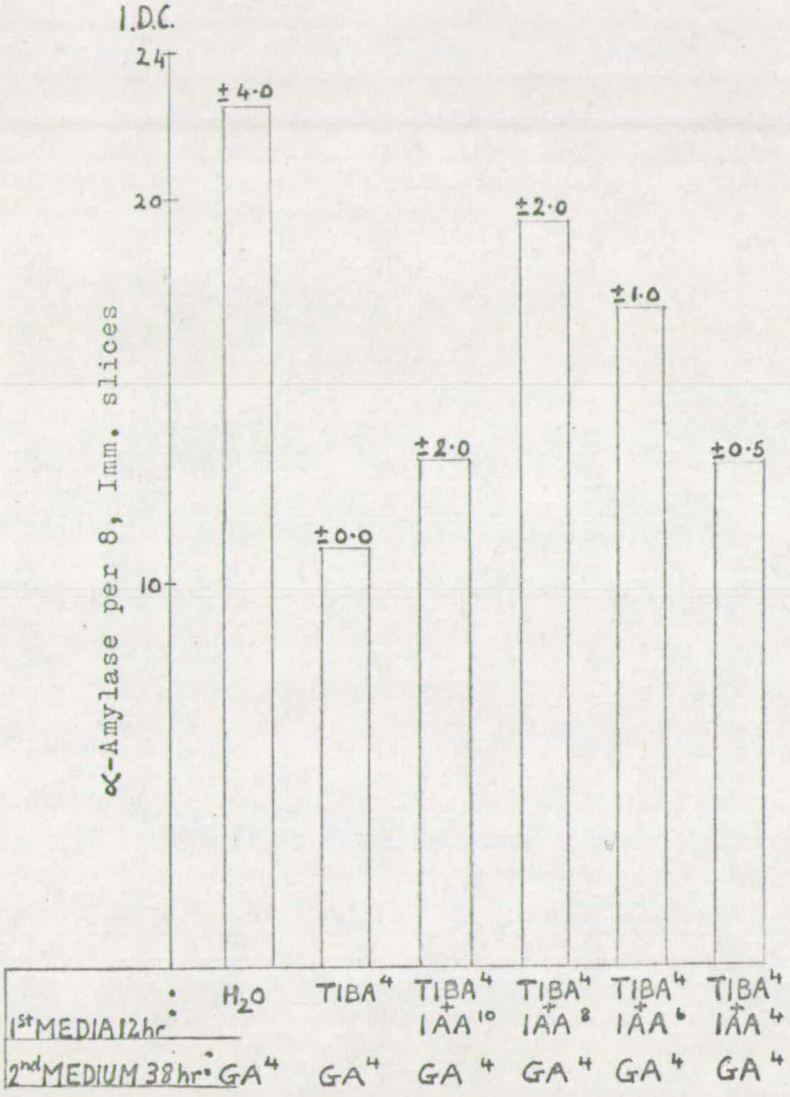


Fig. 26. Showing competitive action between I.A.A. and T.I.B.A. on ∝-amylase content of endosperm slices.

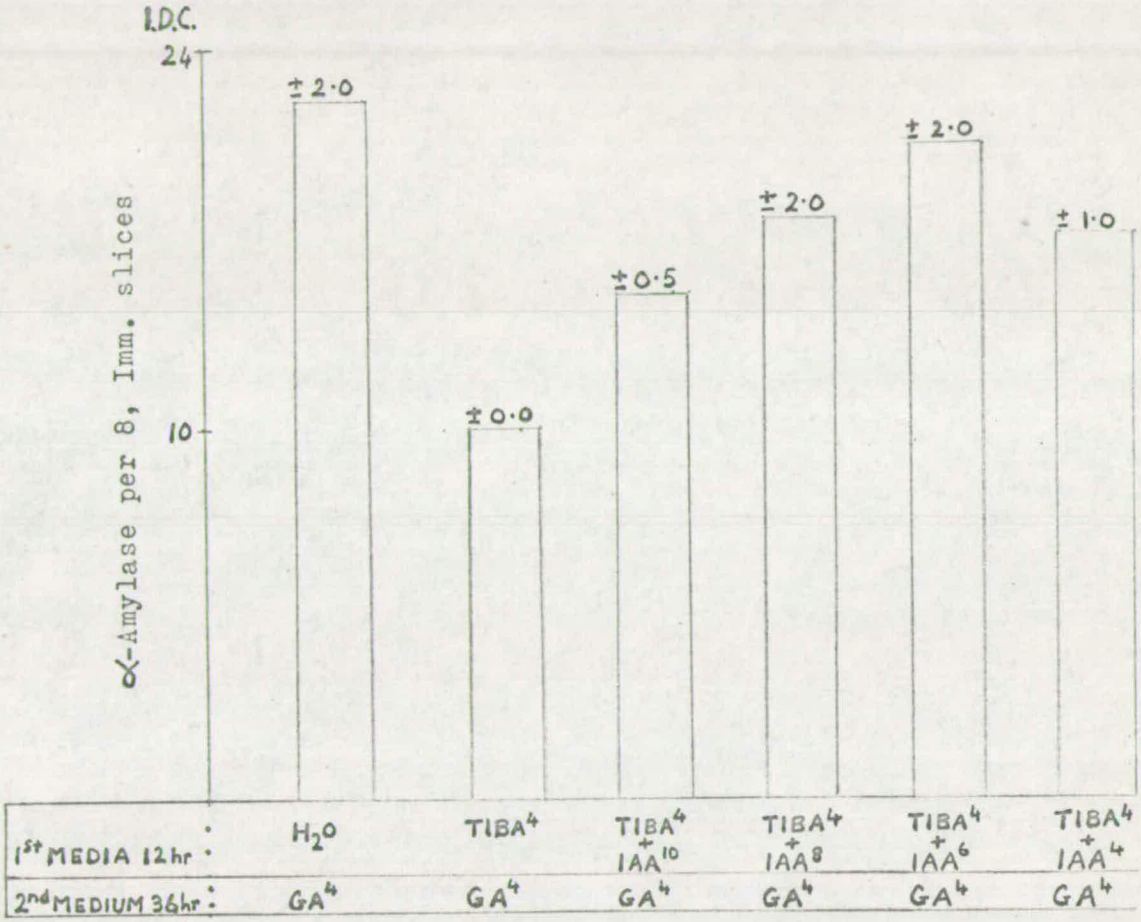


Fig. 27. Showing competitive action between I.A.A. and T.I.B.A. on α-amylase content of endosperm slices.(Aged solutions).

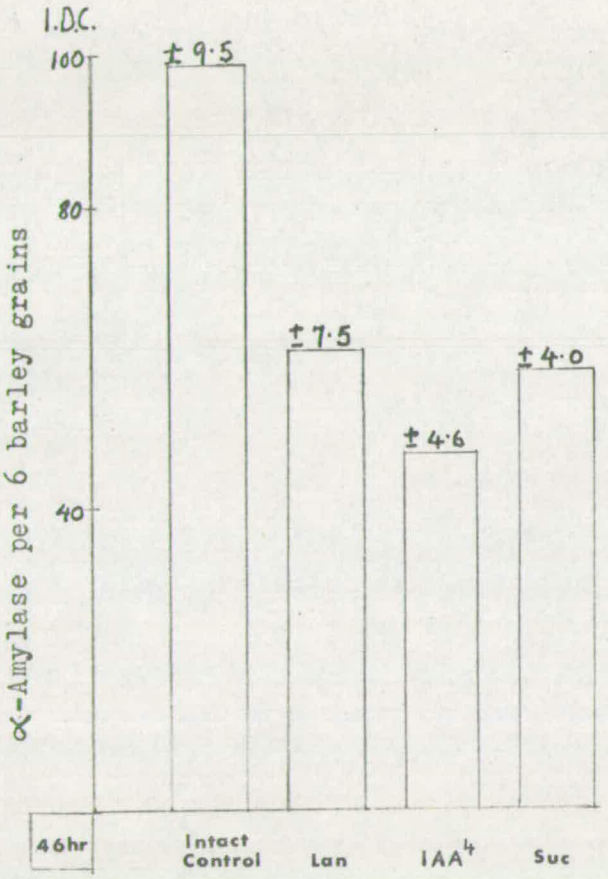


Fig. 28. Substitution of excised coleoptile tip (see Fig. 29).

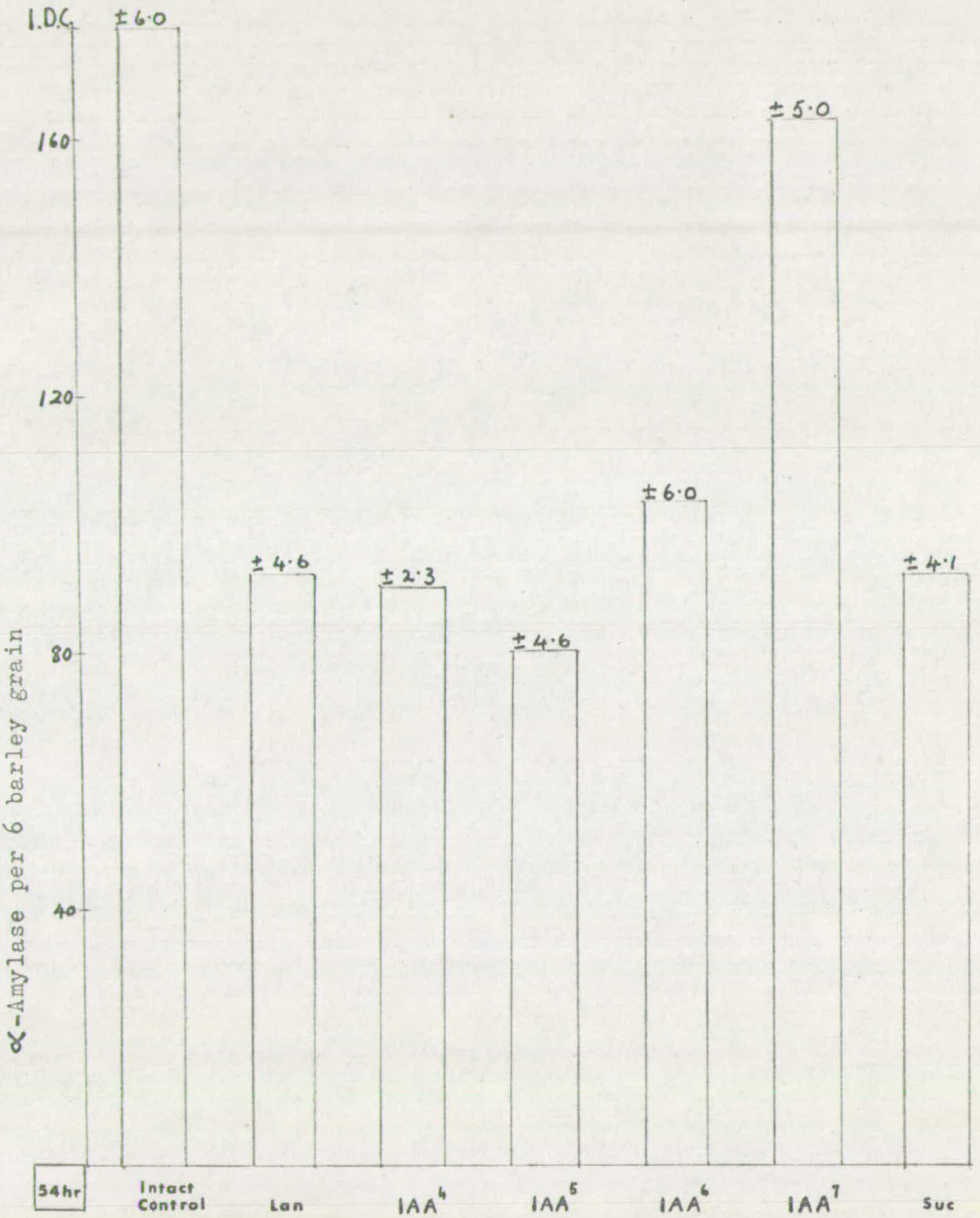


Fig. 29. Substitution of excised coleoptile tip(wider range of I.A.A.) in restoration of α-amylase content of barley grain.

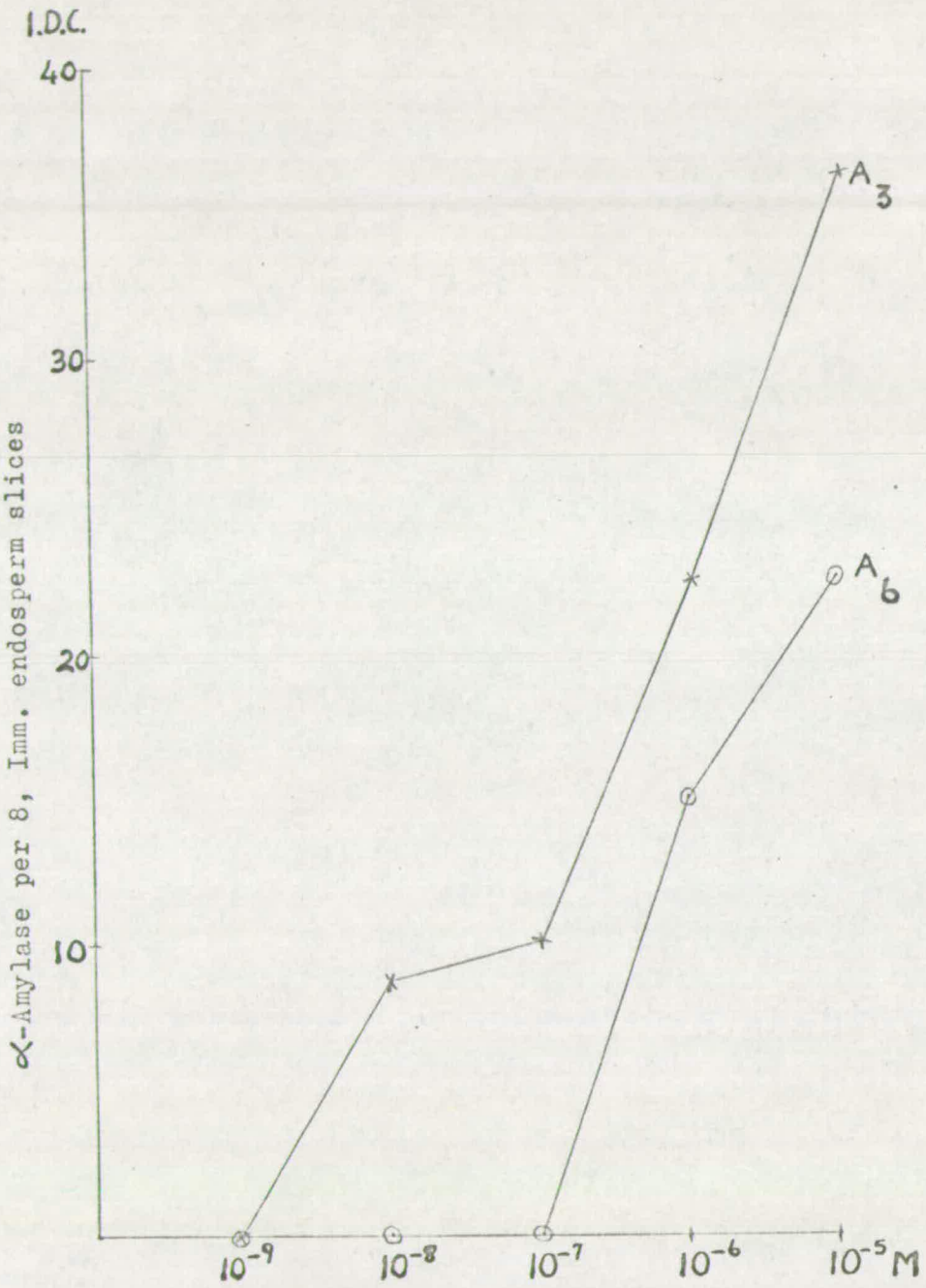


Fig. 30. Comparative effects of G.A.$_3$ and G.A.$_6$ on α-amylase production.

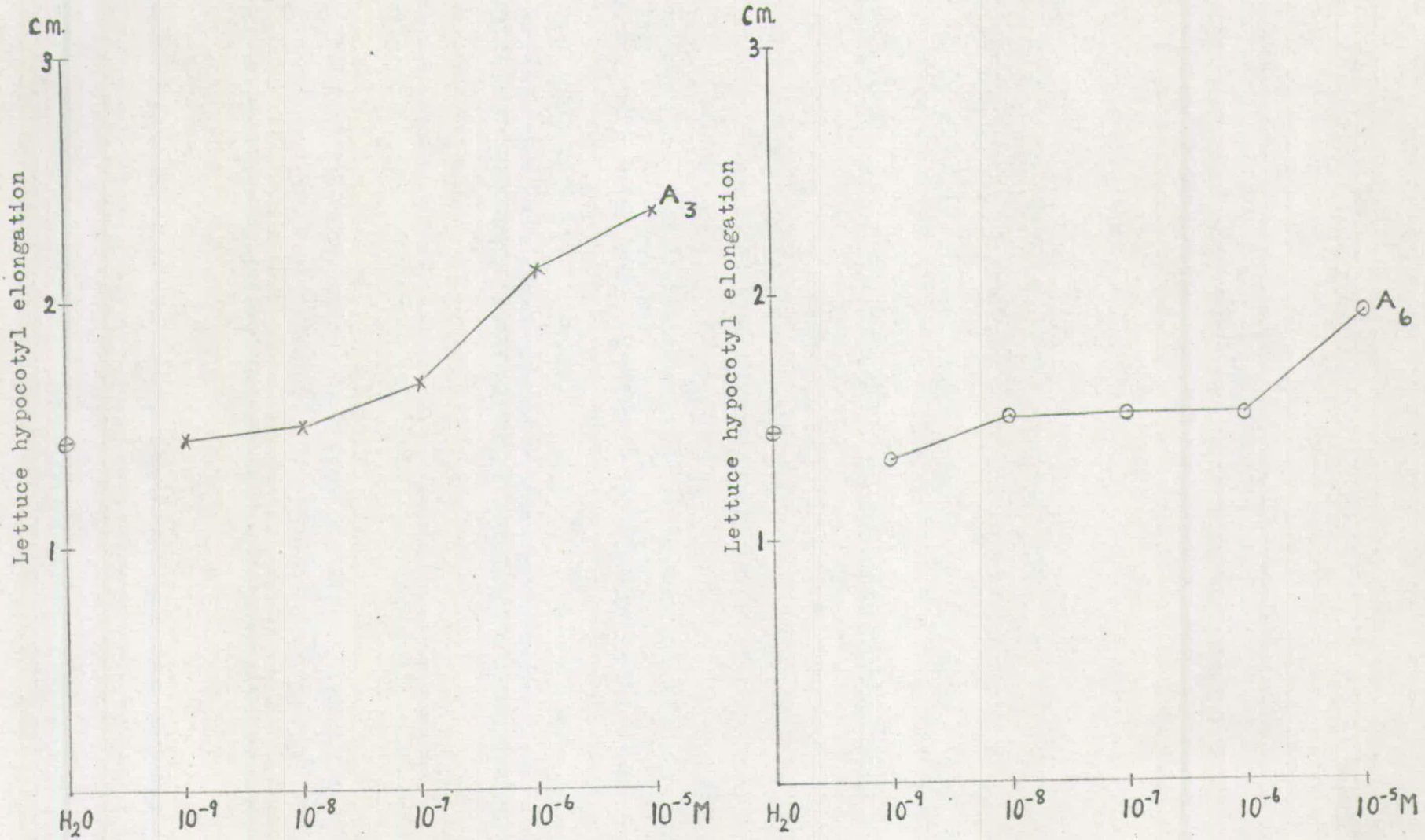


Fig. 31. Comparative growth stimulative action of $G.A._3$ and $G.A._6$.

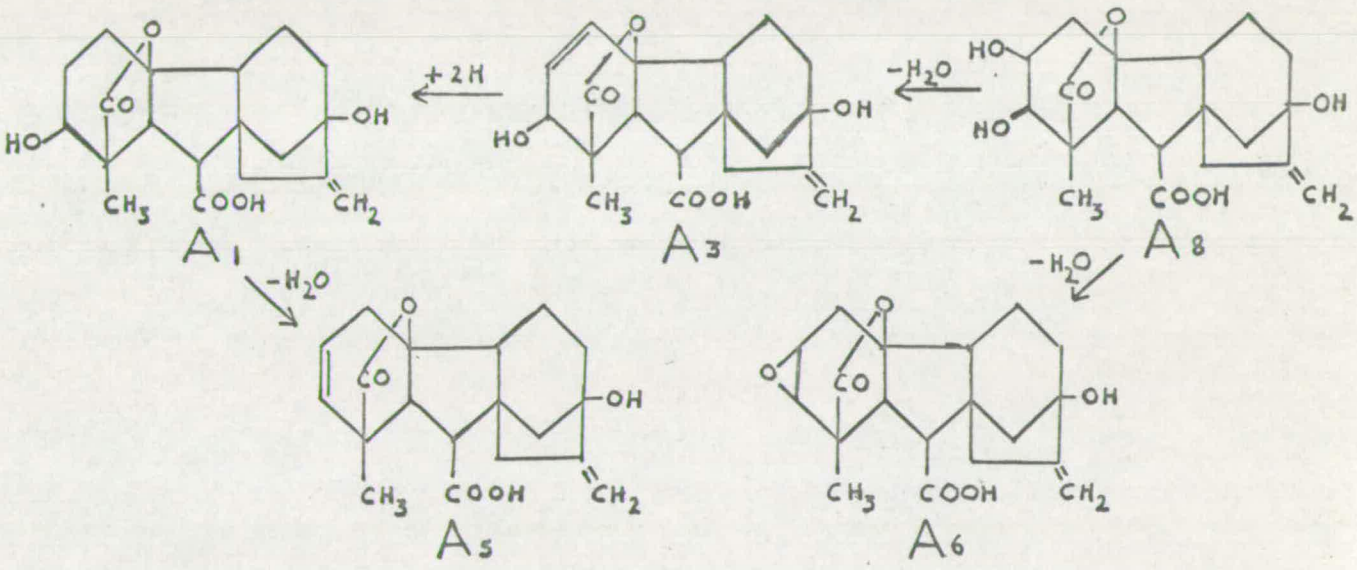


Fig. 32. Showing structural and chemical similarities between five of the known gibberellins. A_3 and A_I have greater biological activity than A_5 and A_6: A_8 appears to be inactive.

SUMMARY

1. A correlative time-course study on the structural and physiological aspects of barley germination and subsequent seedling gorwth was conducted. In toto, it was shown that the biological events during the germination (i.e. root emergence) process (0 - 17 hr.) were connected with apparently unrelated events which take place in the seedling growth phase of development.

2. Structural analyses, using light and electron microscopy, on the morphology of endosperm modification negated the unfounded belief that elongating scutellar epithelial cells secrete hydrolytic enzymes into the endosperm. Extensive observation strongly suggested that the aleurone cells, with respect to their position in the grain, was exclusively responsible for enzyme secretion. The one-sided (dorsal) pattern of modification appeared to be conditioned by a similar one-sided orientation of vascular elements in the scutellum.

3. Light microscopic studies also indicated that various subcellular changes, with special reference to storage products, take place in the scutellum.

4. Electron microscopic studies verified these preliminary findings and, as a result of greater resolution, showed that extensive subcellular changes occurred even after a 12 hr.

soaking of the grain. For example, a fat to starch conversion was strongly implied and discrete protein deposits disappeared by the completion of germination (i.e. 17 hr. after initial wetting of the grain). Lignification of provascular cells of the scutellum was also evident by 17 hr.

5. Estimations of the rate of disappearance of fat and the associated appearance of starch, and detection of the presence of isocitritase, tended to suggest further that the fat-starch parallelism in the scutellum may be a result of the inter-conversion and that the glyoxylate pathway may participate in this conversion.
6. Additional analyses of the metabolism of sucrose, protein and fat store suggested that, nutritionally, germination was an axis event. Further studies on changes in dry weight over the germination period tended to strengthen this suggestion.
7. In the ungerminated grain raffinose is mainly confined to the roots and the scutellum while sucrose is mainly confined to the acrospire (shoot) and the scutellum. Highest invertase activity was found in the roots.
8. Sucrose content of the embryo (axis and scutellum) declined during germination but synthesis occurred at the onset of endosperm modification, i.e. between 16 and 20 hr. However, the sucrose synthesising enzyme system is operative from the onset of the germination process. Sucrose

transport during early seedling growth from the scutellum to the axis was evident.

9. Raffinose degradation was more active in the axis than in the scutellum and involved the epimerisation of its galactose moiety to glucose.

10. Physiological evidence from studies on the central and peripheral regions of the scutellum tended to substantiate further the anatomical findings that scutellar epithelial cells do not contribute to the pool of hydrolytic enzymes which causes degradation of the endosperm food reserves. The ability of excised embryos to secrete hydrolytic enzymes was found to result from the contamination of the periphery of their scutella by discrete remains of aleurone cells.

11. From dissection studies it was concluded that the scutellar node region of the axis appeared to be the main embryo site of origin of the gibberellic acid-like factor which is supposed to move into the endosperm and there catalyse enzymic degradation of storage products. However, for the undamaged scutellar node to perform its secretory function in the intact grain inception of root extension seems to be necessary.

Electron microscopic studies revealed that the cells of this scutellar node region contained a large number of metabolic organelles which may be indicative of secretory abilities.

12. Evidence was obtained to suggest that a short-term Indoleacetic acid: Gibberellic acid synergistic stimulation can express itself in subsequent gibberellic acid - α-amylase response of endosperm slices. In the intact grain the coleoptile tip, with respect to its ability to secrete indoleacetic acid, may therefore directly influence endosperm modification. Indoleacetic acid at a low concentration of 10^{-10}M was found to be specially effective, not only in this synergistic response but also in epithelial elongation.
13. The secreted (gibberellic acid-like) embryo factor which must pass from the growing embryo to the endosperm to catalyse the release of hydrolytic enzymes was found to be a gibberellin. This gibberellin was however not gibberellic acid ($G.A._3$) but, possibly, $G.A._6$.
14. Gibberellic acid ($G.A._3$) was found to have greater biological activity on lettuce hypocotyl elongation and the α-amylase response of endosperm slices than commercially pure ($G.A_6$.)
15. Germination (i.e., root emergence from the coleorhiza) was found to be combined result of cell elongation and cell division. Cell elongation may be slightly more important than cell division in the escape of the roots from the coleorhiza. A sudden rise in the water content of the axis may be an important feature of root emergence.

Addendum

Margaret Radley (1967) in a very recent report concluded that the scutellum was the tissue of origin of the gibberellin-like embryo factor. However, my results in Part 3 Section III deny the scutellum of such an independent role and suggest that the axis (i.e. scutellar node in conjunction with root development) was the site of origin while subsequent transport was merely effected through the top-most half of the scutellum. Explanation for this discrepancy is quite simple. Radley's scutella were separated from their axes after 18 hr. growth while my scutellum-axis separation was performed after 2 hr. soaking.

Surely, here is justification for the structural and correlative physiological approach adopted for this study on barley germination and subsequent seedling growth, where it is observed that at 18 hr. biological events such as endosperm modification and germination are so advanced that Radley's interpretation of scutella function, after such a long initial growth period prior to tissue separation, must certainly differ for mine which is based on the knowledge that a 18 hr. growth one would certainly expect to find gibberellin-like substances in the scutellum. In fact, even a 2 hr. separation means that one-eighth of the germination-time was completed.

Since the completion of this addendum I have obtained experimental evidence, using embryo-less (endosperm only) or axes-less (endosperm + scutellum) dehusked grains, which suggest that the scutellum is not intrinsically the embryo site from which

the gibberellin-like substance originate.

Removal of the axis or whole embryo at 2-3 hr. intervals over the first 24 hr, growth period with subsequent incubation totalling 48 hr. at 25°C revealed that the ability of the scutellum, bereft of its axis, to stimulate the release of α-amylase from the aleurone of the adjoining endosperm tissue is related to the duration of its association with the growing axis.

The gibberellin-like substance, in terms of α-amylase content, reaches the aleurone cells of the endosperm between 12 - 15 hr. after the commencement of germination. Interestingly enough this was theoretically intimated in the conclusion of Parts 4A and B (Section III) of this thesis.

In toto, these findings substantiate the criticism made above concerning the possibility that the excised scutella used by Radley (1967) would surely contain a copious supply of the gibberellin-like substance which by 18 hr. growth would indubitably be en route from the axis to the aleurone cells of the endosperm. An additional point against Radley's suggestion that the scutellum is the embryo site of origin of the gibberellin-like substance is that this would imply that only the dorsal periphery of the scutellum (with reference to the one-sided pattern of endosperm modification, see Plate 2) could either synthesise or secrete this hormonal substance. My studies (Section I) revealed no structural justification for this.

In conclusion it therefore remains that for germinating barley grains the growing axis is the embryo site of origin of

the gibberellin-like substance which later enters the aleurone cells via the scutellum to catalyse the release of hydrolytic enzymes which cause endosperm modification.

BIBLIOGRAPHY

Audus, L.J. & Thresh, R. Ann.Bot., (N.S.) 1956a, 20, 79, 439

Audus, L.J. & Thresh, R. Chemistry and Mode of Action of Plant Growth Substances, Butterworths, 1956b, 1248.

Audus, L. J. Plant Growth Substances, Leonard-Hill, 1963.

Bain, J.M. & Mercer, F.V. Aust.Jour.biol.Sci., 1966, 19, 49:69

Baker, J.R. Cytological Technique, Methuen, 1963.

Beevers, H. Plant Physiol., 1956, 31, 440

Beevers, H. Nature, Lond., 1961, 191, 433

Bergal, P. & Clements, M. Barley and Malt, Academic Press, 1962. 1.

Bonner, J. & Galston, A. Principles of Plant Physiology, Freeman & Co., 1952, 343

Bonner, J. & English, J. Plant Physiol., 1938, 13, 331

Bouillenne-Walrand, M. Bull. Soc. Roy. Sci. Liege, 1959, 28, 21.

Brian, P.W. & Hemming, H.G. Ann. Appl. Biol., 1957a, 45, 489

Brian, P.W. & Hemming, H.G. Ann. Bot., 1958, 22, 1.

Brian, P.W. & Hemming, H.G. & Lowe, D. Ann. Botany, 1960, (N.S.) 24, 407.

Brian, P.W. Internat. Rev. of Cytology, 1966, 19, 229.

Briggs, D.E. J. Inst. Brew., 1961, 67, 427.

Briggs, D.E. J. Inst. Brew, 1963, 69, 13:244

Briggs, D.E. J. Inst. Brew., 1964, 70, 14

Briggs, D.E. Nature, Lond., 1966, 210, 418.

Brown, H.T. & Morris, G.H. J. chem. Soc., 1890, 67, 709.

Brown, H.T. & Edgecombe, F. Proc. Roy. Soc., 1898, 63, 3.

Brown, W.V. Phytomorphology, 1965, 15, 274

Bukovac, M.J. & Wittwer, S.H. Mich. State Univ. Agr. Expt. Sta. Quart. Bul., 1956, 39, 307.

Carpenter, W.D. & Beevers, H. Plant Physiol., 1959, 34, 403.

Cherry, J.H. Proc. Seed Protein Conference, U.S.D.A., 1963, 115.

Cleland, R. & McComb, N. Science, 1965, 150, 497

Cooke, R.C. & Pitt, D. Viewpoints in Biology, Butterworths, 3, 1964.

Coombe, B.G., Cohen, D. & Paleg, L.G. Plant Physiol., 1967, 42, 113.

DeFeketе, M. & Cardini, C.E. Arch. Biochem. Biophys., 1964, 104, 173.

Dickson, A.D. & Shands, H.L. Cereal Chem., 1941, 19, 411.

Dickson, A.D. & Buchart, B.A. Cereal Chem., 1942, 19, 251.

Duffus, J.H. J.Inst. Brew 1966, 72, 569.

Duffus, J. H. Thesis Ph.D. University of Edinburgh, 1955.

Dure, L.S. Plant Physiology, 1960, 35, 919:925

Edelman, J., Shibko S. & Keys A.J. J. Exp. Bot. 1959, 10, 178

Engel, C. Rec.Trav.Chim.Pays-Bas. 1945, 64, 318

Engel. C. & Heins, J. . Biochem. Biophys. Acta., 1947, 1, 190

Ermilov, G.G. Fiziol. Rastensii. Akad. Nauk. S.S.S.R., 1960, 7 36.

Esau, K. Plant Anatomy, 1960, 7, 36, Wiley, 1953.

French, D. Advances in Carbohydrate Chemistry, 1954,9,1

Frey-Wyssling, A. & Muhlethaler, K. Ultrastructural Plant Cytology, Elsevier, 1964.

Galston, A.W. & Dalberg, I.Y. Amer. J. Bot., 1954, 41, 373.

Griffiths, C. M., MacWilliam, I.C. & Reynolds, T. J. Inst. Brew. 1965, 71, 316.

Griffiths, C. M., MacWilliam, I.C. & Reynolds, T. Nature, Lond., 1964, 202, 1026
& J. Inst. Brew., 1967, 73, 189

Haber, H.A. & White, J.D. Plant Physiol. 1960, 35, 495

Haberlandt, G. Ber. Deut. Botan. Ges., 1890, 8, 40.

Harris, G. Barley and Malt. Academic, 1962, 583

Hayashi, T. Bull. Ag. Chem. Soc. Japan, 1940, 16, 731.

Hepler, P.K. & Newcombe, E.H. J. Exp. Bot. 1963, 14, 496.

Hillman, W.S. & Purves, W.K. 4th Internat. Conf. on Plant Growth Regulation, Iowa, 1961, 657

Horner, T.H. & Arnott, J.A. Amer. J. Bot., 1965, 52, 1027

Horner, T.H. & Arnott. J.A. Botanical Gazette, 1966, 127, 48.

Horning, E.S. & Petrie, A.H.K. Proc. Roy. Soc. 1927, 102B, 188.

Jacks, T.J., Yatsu, L.Y., & Altschul, A.M. Plant Physiol. 1967, 42, 585.

Jensen, W.A. Botanical Histochemistry, Freeman & Co., 1962.

Johnston, C.S. Thesis, Ph.D. Edinburgh University 1964.

Jones, D.F., MacMillian, J., & Radley, M. Phytochemistry, 1963, 2, 307.

Jones, D.F. Nature, 1964(a), 202, 1309

Jones, D.F. Personal Communication, 1964(b) to Duffus, J., Thesis, Ph.D. Edinburgh University, 1965.

Kefford, N.P. Plant Physiol., 1962, 37, 380

Kende, H., Ninnemann, H., & Lang, A. Naturwissenschaften, 1963, 50, 599.

Kirsop, B.H. & Pollock, J.R.A. J. Inst., Brew. 1957, 63, 383.

Kirsop, B.H. & Pollock, J.R.A. J. Inst., Brew. 1958, 64, 277.

Kornberg, H.L. & Beevers, H. Nature, Lond., 1967, 180, 35

Kurth Malting Co. U.S. Patent No. 3,085, 945: 1963.

Lang, A. Planta, 1960, 54, 498

Laufer, S. Amer. Brewer, 1964, (97(5)) 75

Lazaron, A. & Cooperstein, S.J., J. Histochem. Cytochem., 1953, 1, 234

Leloir, L.F. Biochem. J. 1964, 91, 1.

Leopold, A.C. Plant Growth & Development, McGraw-Hill, 1964.

Lockhart, J.A. Plant Physiol. 1962, 37, 759

Lubert, D.J. & Pool, N.A. J. Inst. Brew. 1964, 70, 145

Macey, A. & Stowell. K. Proc. Eur. Brew. Conv. Vienna, 1961, 85

Macey, A., Stowell, K. & White, H.B. J. Inst. Brew, 1966, 72, 29

MacLeod, A.M. New Phytologist, 1957, 56, 210

MacLeod, A.M. & Sandie, R. New Phytol. 1961, 60, 117

MacLeod, A.M. & White, H.B. J. Inst. Brew. 1961 67 182, 1962, 68, 487

MacLeod, A.M. & Miller, A.H. J. Inst. Brew, 1962, 68, 322

MacLeod, A.M. Duffus, J.H. & Johnston, C.S. J. Inst. Brew. 1964, 10, 521

MacLeod, A.M., Duffus, J.J. & Horsfall, D.J. J. Inst. Brew. 1966, 72, 36

MacLeod, A.M. J. Inst. Brew, 1967, 73, 146

MacLeod, A.M. Personal Communication 1967(a)

MacMasters, M.M. Cereal Science Today, June, 1965, 268.

MacMillian J., Seaton, J.C. & Suter, P.J. ICI. Ltd. (Pharmaceuticals Division), Technical Report, 1962, Ref. No. P/ARC/687.

MacMillian J., Seaton, J.C. & Suter, P.J. 1959, Proc. Chem. Soc. Lond., 325.

MacMillian, J., Seaton, J.C. & Suter, P.J. Advan. Chem. Ser. 1961 28, 18.

Mann, A., U.S., Dept. of Agric., Circular 16, 1908.

Mann, A. & Harlan, H.V. J. Inst. Brew. 1916, 22, 93

Marcus, A., & Velasco, J., J. Biol. Chem., 1960, 235, 563.

Mayer, A.M., & Poljakoff-Mayber, A. The Germination of Seeds, 1963, 101.

McDaniels, R.G., & Sarkissian, I.V. Physiol. Plantarum, 1966, 19, 1.

Mercer, E.H., & Birbeck, M.S.C. Electron Microscopy, Blackwell, 1961.

Millar, A. Thesis, FH-WC, Heriot-Watt University, 1962.

Murlin, J.R. J. General Physiol., 1934, 17, 283.

Ng, E.K. & Audus, L.J. J. Expt. Bot. 1965, 16(46), 107.

Nitsch, J.P. & Nitsch, C. Ann. Physiol. Veg. 1962, 4, 85.

Nieuwdrop, P.J. Acta Botanica Neerlandica, 1963, 12, 295

Nieuwdrop, P.J. & Buys, M.C. Acta Botanica Neerlandica, 1964, 13, 559.

Nooden, L.D. & Thimann, K.V. Proc. Nat. Acad. Sci. U.S., 1963, 50, 194.

Nooden, L.D. & Thimann, K.V. Plant Physiol. 1966, 40, 193.

O'Brien, J.A. Amer. J. Botany, 1942, 29, 479.

van Overbeek, J. & Dowding, L. 4th Internat. Conf. on Plant Growth Regulation, Iowa, 1961, 657.

van Overbeek, J. Science, 1966, 152, 721.

Paal, A. Jahrb. Wiss. Bot. 1919, 58, 406.

Paleg, L.G. & Hyde, B. Plant Physiol., 1964, 39, 673.

Paleg, L.G. & Plant Physiol. 1960, 35, 293:902.

Pazur, J.H., Shadaksharaswamy, M. & Meidell, G.E. Arch., Biochem. Biophys. 1962, 99, 98.

Peach, K. & Tracey, M.V. Treastise on Modern Method of Plant Analysis, Springer-Verlag, 1955, Vol. II, 317.

Phillips, I.D.J. & Jones, R.L. Nature, Lond., 1964, 204, 497.

Pilet, P.E. Physiol. Plantarum, 1965, 18, 1121.

Pollock, J.R.A., Essery, R.E. & Kirsop, B.H. Proc. Eur. Brew. Conv., Baden-Baden, 1955, 203.

Pollock, J.R.A. J. Inst. Brew., 1959, 65, 334.

Pollock, J.R.A. J. Inst. Brew, 1960, 66, 22.

Prosser, M.V., & Jackson, G.A.D. Nature, Lond., 1959, 184, 108.

Radley, M. Chem. and Ind., 1959, 877.

Radley, M. Nature, Lond., 1966, 210, 969.

Radley, M. Planta, 1967, 75, 164.

Ray, P.M. Ann. Rev. Plant Physiol., 1958, 9, 81.

Ray, P.M. 4th Internat. Conf. on Plant Growth Regulation, Iowa, 1961, 199.

Reynolds, T. & MacWilliam, I.C. J. Inst. Brew., 1966, 72, 166.

Schade, A.L. Wallerstein Labs. Commun., 1937, 1, 12

Scott, T.K. & Jacobs, W.P. 5th Internat. conf. on Plant Growth Substances - Regulateurs Naturels De la Croissance Vegetale, Paris, 1964, 457.

Sebanek, J. Sborn. Vgs. Skol Zemed Brne, 1963, 173.

Sebanek, J. Flora, 1965, 156, 303.

Sembdner, G., Gross, R. & Schreiber, K. Experientia, 1964, 20, 89.

Siegel, S.M. The Plant Cell Wall, Pergamon, 1962, 55.

Sinnott, E.W. Plant Morphogenesis, McGraw-Hill, 1966, 202.

Stowe, B.B. 4th Internat. Conf. on Plant Growth Regulation, Iowa, 1961, 419.

Street, H.E. Viewpoints in Biology, Butterworths, 1962, 1, 1.

Thimann, K.V. Amer. J. Bot. 1936, 23, 561

Thimann, K.V. Amer. J. Bot., 1937, 24, 407.

Thimann, K.V. & Lane, R.H. Amer. J. Bot., 1938, 25, 535.

Thimann, K.V. Ann. Rev. Plant Physiol., 1963, 14, 1.

Thompson, N.P. & Jacobs, W.P. Plant Physiol., 1966, 41, 673.

Van der Eb, A.A. & Nieuwdrop, P.J. Acta Botanica Neerlandica, 1967, 15, 690.

Varner, J.E. & RamChandra, G. Proc. Nat. Acad, Sci., U.S., 1964, 52, 100.

Varner, J.E., Ramchandra, G. & Chrispeels, M.J. J. Cellular & Comparative Physiology, 1965, 66, 55.

Wareing, P.E. Nature, Lond., 1958, 181, 1744.

Went, F.W. Rec. Trav. Bot. Neerl., 1928, 25, 1.

Went, F.W. & Thimann, K.V. Phytohormones, MacMillian, 1937.

Wooding, F.B.P. & Northcote, D.H. J. cell Biol., 1964, 23, 327.

Yomo, H. Hakko Kyokaishi, 1958, 16, 444.

Yomo, H. Hakko Kyokaishi, 1960, 18, 494.

Yomo, H. & Iinuma, H. Planta, 1966, 71, 113.

Zeevaart, J.A.D. & Lang, A. Planta, 1963, 59, 509.

PUBLICATIONS

1. The Embryo of Barley in Relation to Modification of the Endosperm: MacLeod, A. M. & Palmer, G. H., J. Inst. Brewing, 1966, 72, 580.

2. Experimental material from both Section III and the Addendum will be submitted for publication in the near future.

3. Much of the material from this thesis formed the basis for lectures given by the author at the 1966 Research Symposium of the Botanical Society of Edinburgh which was held in Newcastle upon Tyne; and at the 1967 Biological Conference of Scottish Universities which was held in Aberdeen.

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Plate 1. Median longitudinal section of barley embryo grown for 12 hr. Coleorhiza at limit of extension and root emergence (germination) is imminent.
Staining: Haematoxylin and Eosin.
Magnification: X 34

Plate 2. Median longitudinal section of barley embryo grown for 24 hr. Germination has taken place and endosperm modification is well advanced at the acrospire end (dorsal surface) of the embryo - endosperm junction.
Staining: Safranin and Light green.
Magnification: X 17

Plate 3. Scutellar Epithelial cells from embryo of intact grain grown for 20 hr. Cells at incipient stage of elongation and still flat-topped. Intermediate layer is still intact.
Staining: Haematoxylin and Eosin.
Magnification: X 1,300

Plate 4. Scutellar Epithelial cells from embryo of intact grain grown for 48 hr. Cells extensively elongated and their tips are approaching roundness.
Staining: Haematoxylin and Eosin.
Magnification- X 1,200

Plate 5. Scutellar Epithelial cells from embryo of intact grain grown for 72 hr. Cells at 72 hr. experimental limit of elongation; their tips are now rounded.
Staining: Haematoxylin and Eosin.
Magnification: X 1,200

Abbreviations:

P-T - Pericarp-Testa

F + C = Acrospire

Axis

C - Coleoptile
F - Foliar shoot
N - Node (scutellar node).
r - Seminal root
R - Root
Cr - Coleorhiza
Nu - Nuclear apparatus.
S - Scutellum
V - Vascular trace
E - Epithelial cells
I - Intermediate layer
A - Aleurone cell layer.
Pc - Scutellar Parenchyma cell
En - Endosperm

EM - Endosperm modification.

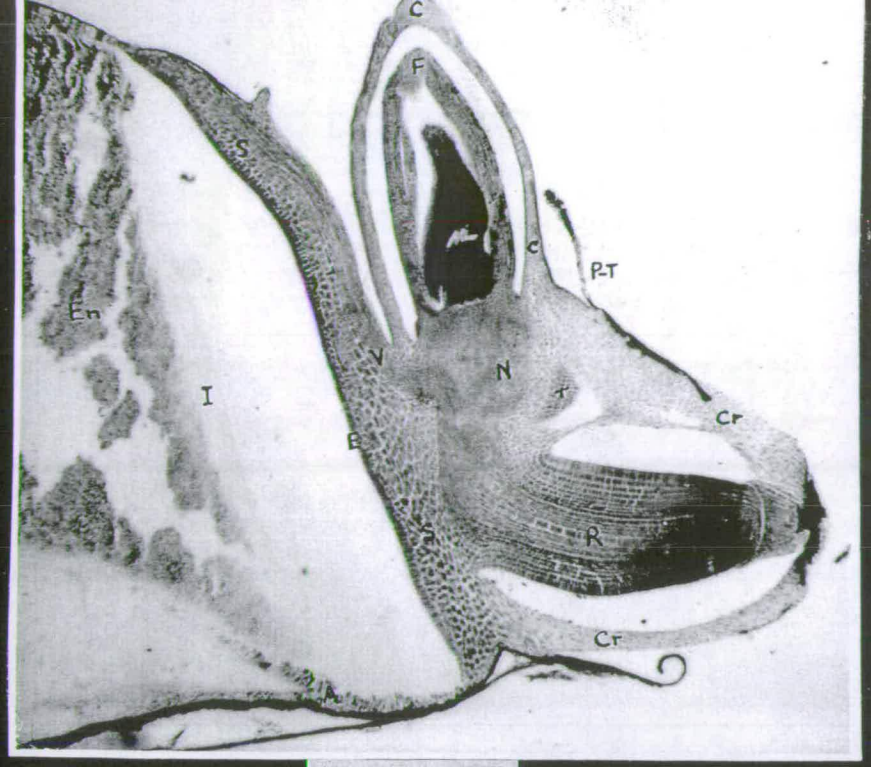

PLATE I

PLATE 2

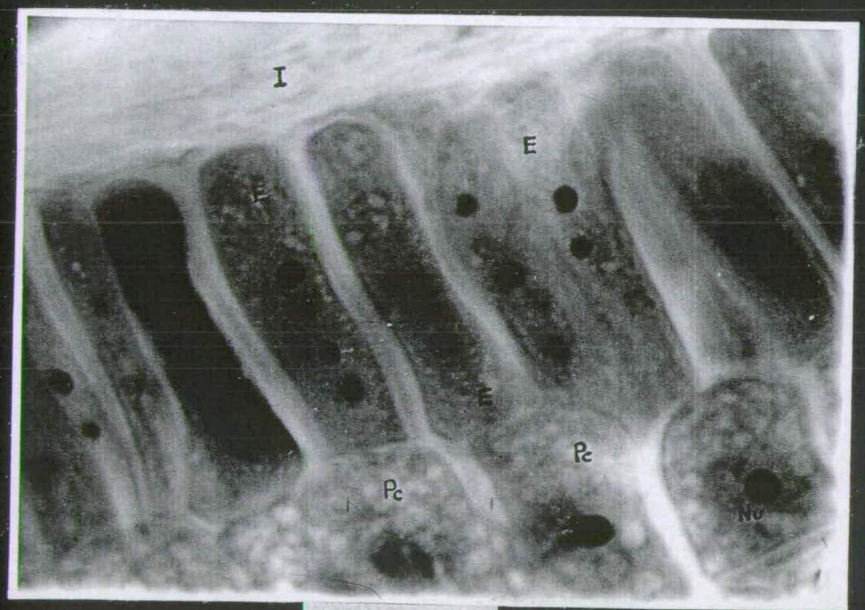

PLATE 3

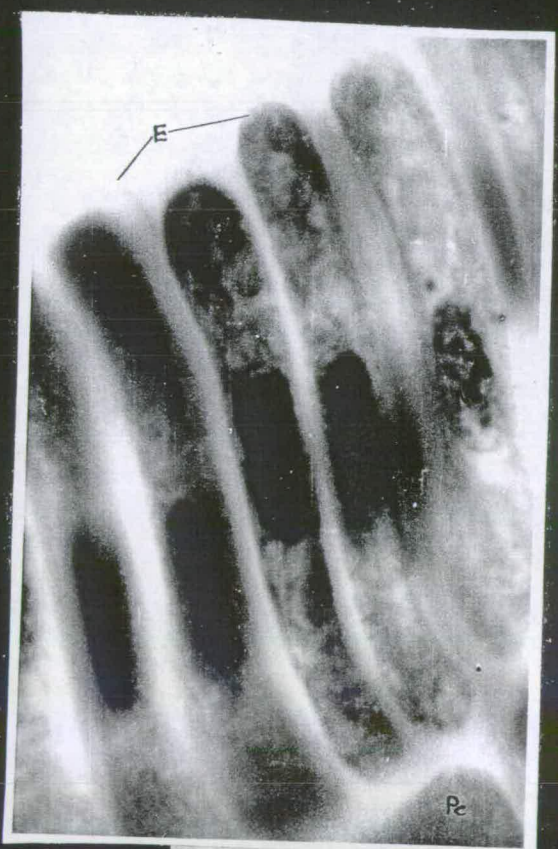

PLATE 5

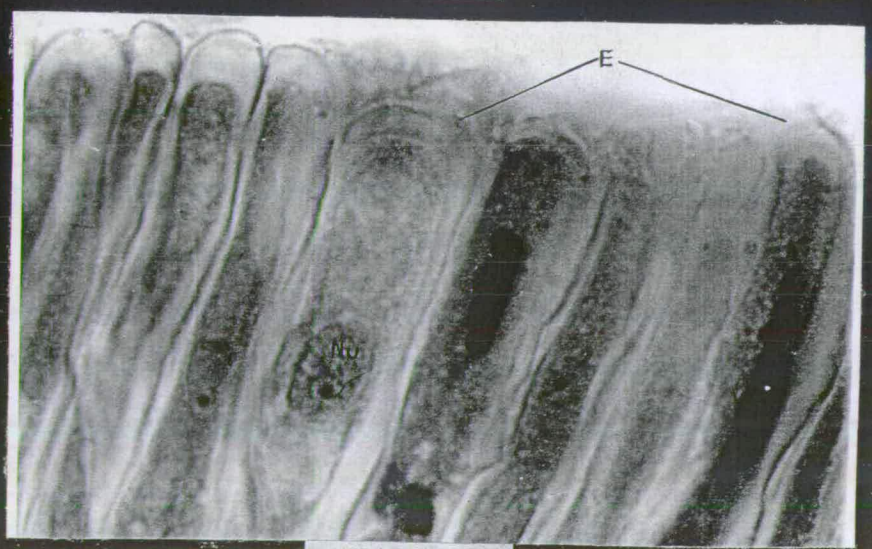

PLATE 4

Plates 6, 7, 8, 9 (for respective growth periods of: 0, 20, 24 and 48 hr.). Show important stages at the commencement and progression of Endosperm Modification. Note that modification is first apparent under the aleurone cells and that the intermediate layer is still intact - see Plate 7. Plates 8 and 9 show that the dissolution of the intermediate layer follows the general aleurone conditioned pattern of endosperm dissolution.

Plate 10. Coleorhizal end of the embryo-endosperm junction of the same 48 hr. grown embryo of Plate 9. Note the absence of any signs of modification at this surface of the grain, despite prominent aleurone layer.

Plate 6. Staining: Haematoxylin and Eosin
Magnification: X 67

Plate 7. Staining: Haematoxylin and Eosin
Magnification: X 67

Plate 8. Staining: Haematoxylin and Eosin
Magnification: X 100

Plate 9. Staining: Haematoxylin and Eosin
Magnification: X 167

Plate 10. Staining: Haematoxylin and Eosin
Magnification: X 134

Abbreviations:

A - Aleurone cells
En - Endosperm
I - Intermediate layer
E - Epithelial cells
S - Scutellum
EM - Endosperm Modification

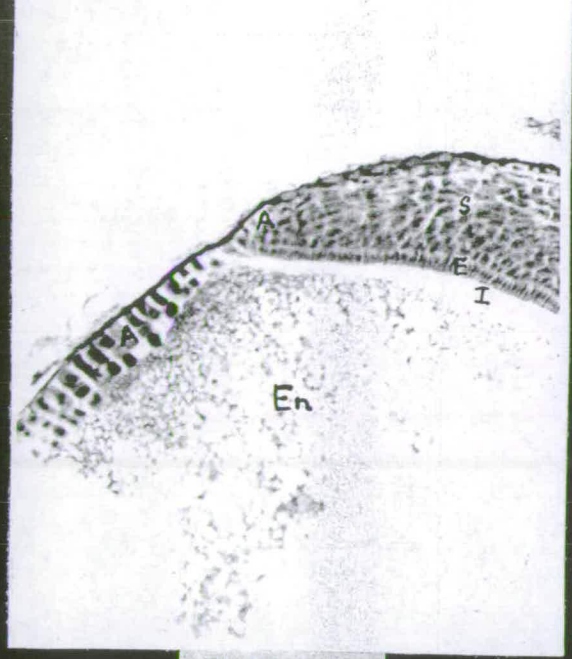

PLATE 6

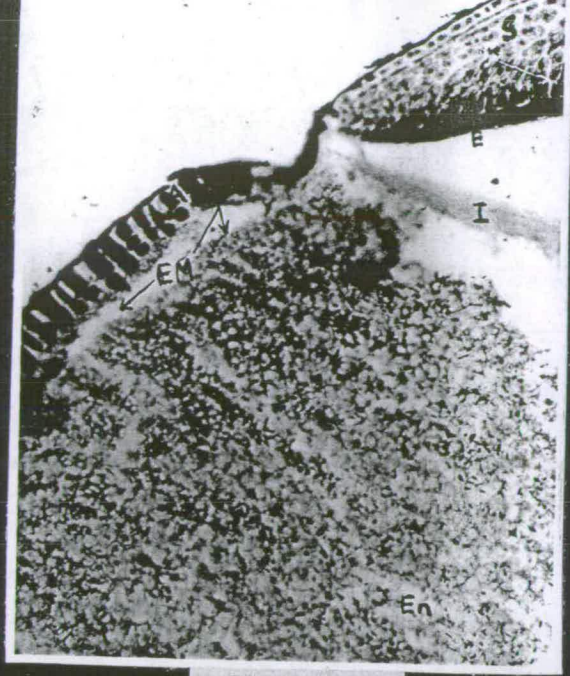

PLATE 7

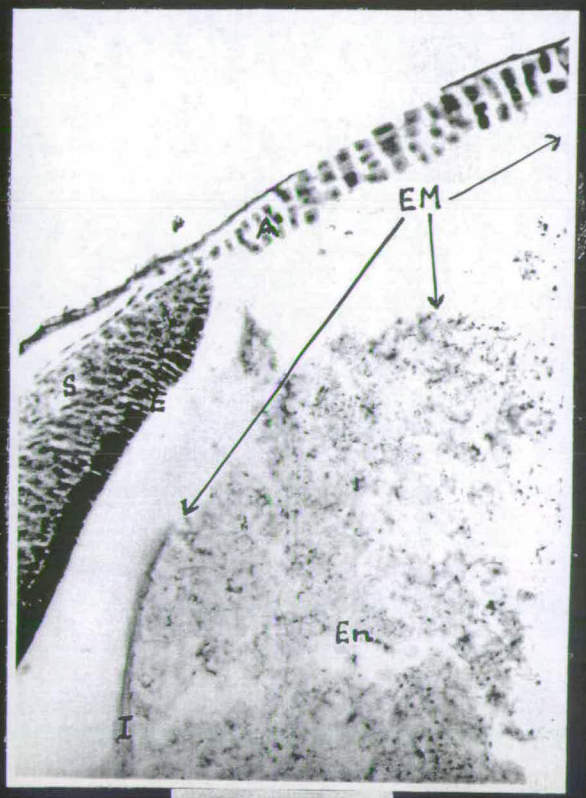

PLATE 8

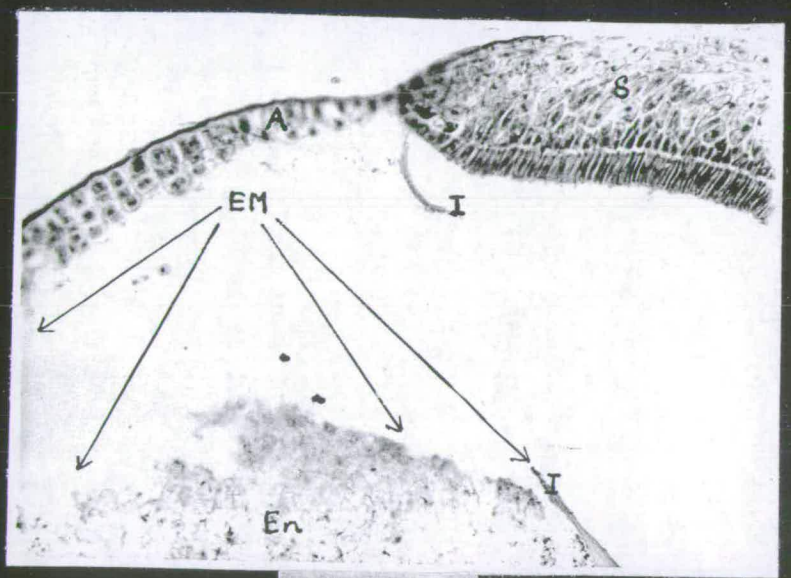

PLATE 9

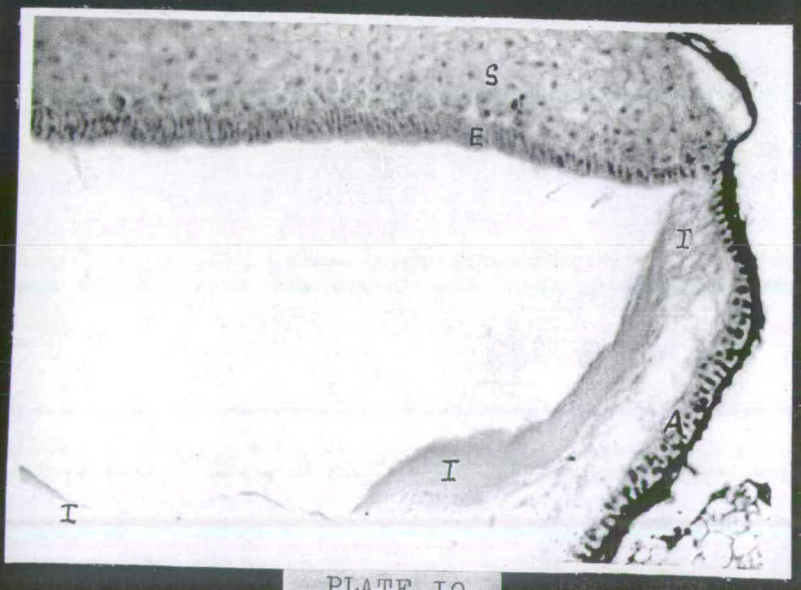

PLATE 10

Plate 11. Shows aleurone cells at 0 hr. germination. Note extensive granulation of cytoplasm and thick cell walls of these cells.
Staining: Haematoxylin and Eosin
Magnification: X 1100

Plate 12. Aleurone cellsfrom region of endosperm modification of 48 hr. grown grain. Granulation is reduced and the cytoplasm now 'tears' easily - see tr.
Staining: Haematoxylin and Eosin
Magnification: X 1100

Plate 13. Shows provascular elements of a vascular trace in the scutellum of 0 hr. grain.
Magnification: X 1200

Plate 14. Shows lignification of provascular elements (xylem) as spiral and annular wall thickening.
Staining: Haematoxylin and Eosin
Magnification: X 2000

Plate 15. To show that the aleurone layer extends into the peripheral margin of the scutellum as a single discrete layer of cells.
Staining: Haematoxylin and Eosin
Magnification: X 267

Abbreviations:

A, A^1 - Aleurone cell
Cw - Cell wall
Nu - Nuclear apparatus
tr - 'tear in cytoplasm'
Pv - Provascular cells
L - Lignification, Xylem
I - Intermediate layer
S - Scutellum
E - Epithelial cell
En - Endosperm
Pc - Scutellar Parenchyma cells

Plate 16. See overleaf.

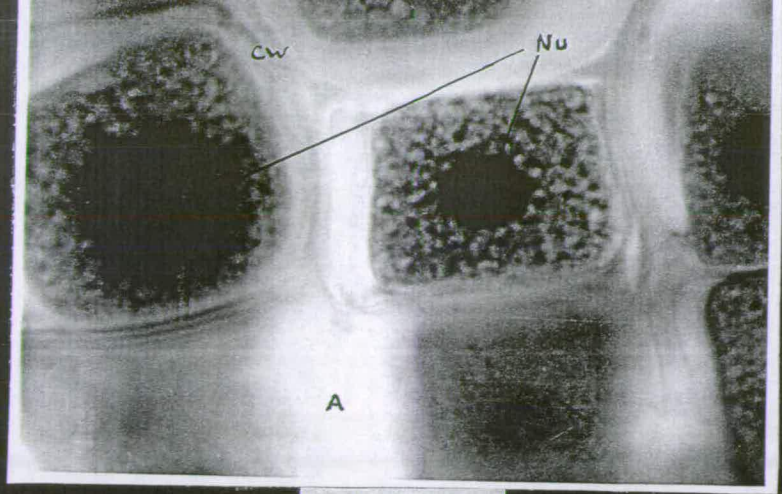

PLATE II

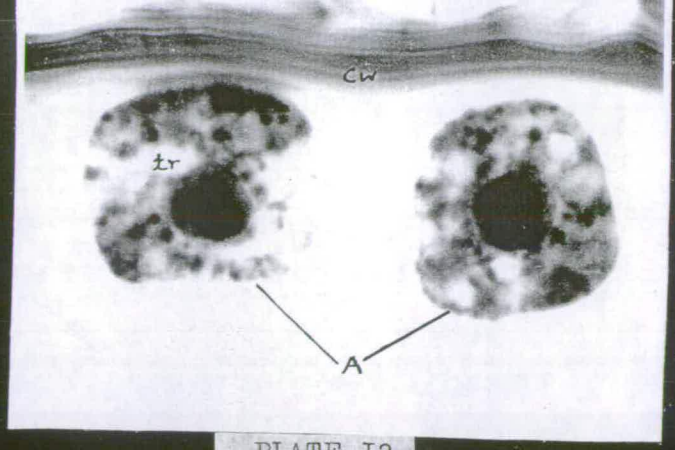

PLATE I2

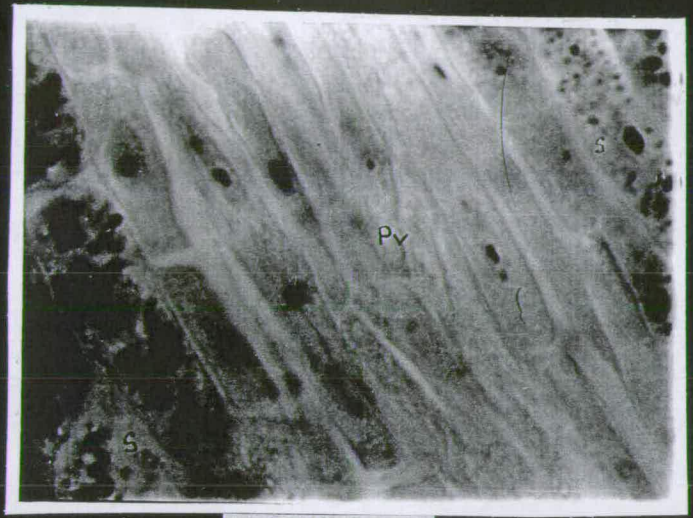

PLATE I3

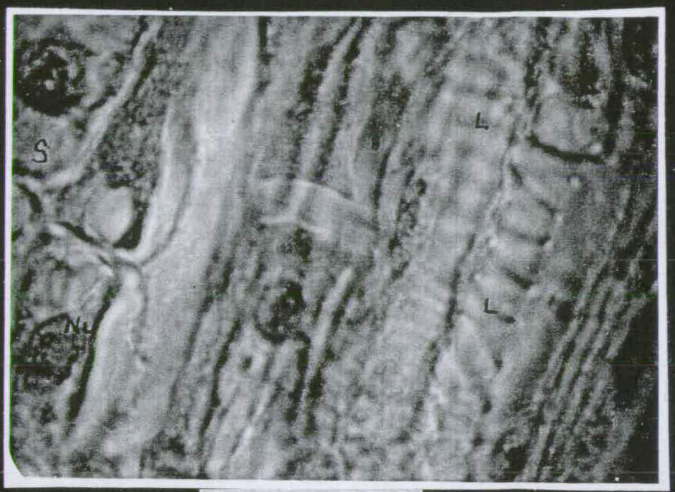

PLATE I4

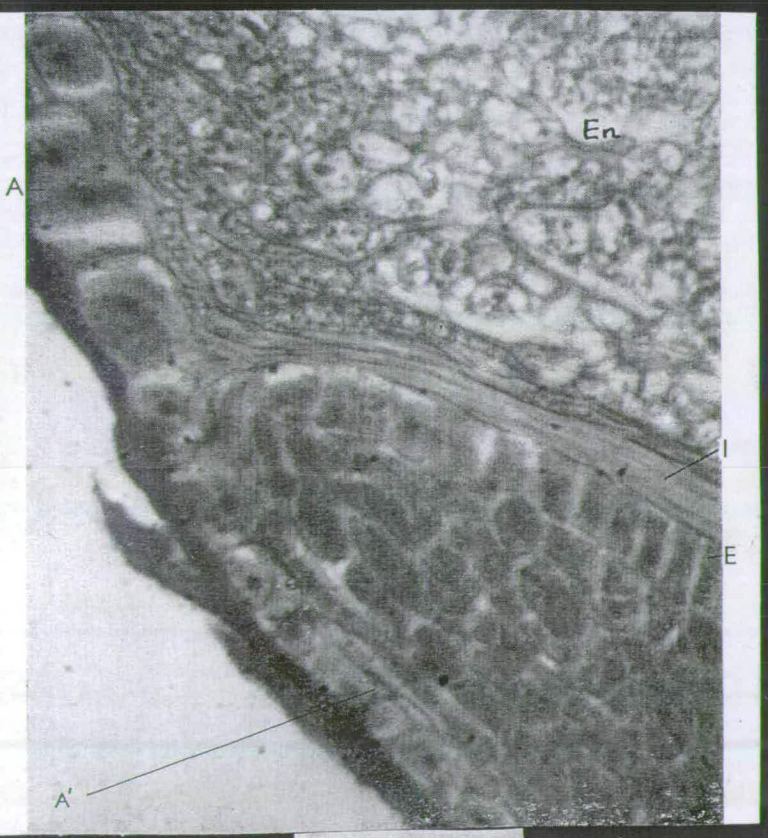

PLATE I5

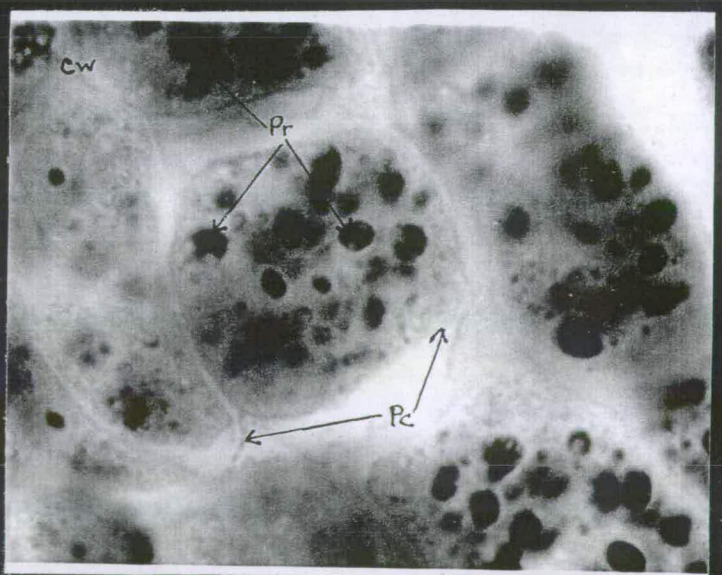

PLATE I6

Plates 16, 17, 18, 19 (for respective growth periods of 0, 20, 24 and 48 hr.). Show parenchyma cells (Pc) of the scutellum. Cells are from the central region of the scutellum. Note progressive disappearance of what may be protein-like (basophilic) bodies and the appearance of well defined bodies which have starch grain morphology.

Plate 20 (for some 48 hr. grain of Plate 19). Shows that scutellar parenchyma cells (Pc), when away from the central region of the scutellum (e.g. Coleorhizal end), rapidly loose their cytoplasmic contents.

Staining: Haematoxylin and Eosin

Magnification-

Plate 16, X 2400
Plate 17, X 2400
Plate 18, X 2400
Plate 19, X 2400
Plate 20, X 2400

Abbreviations:

Pc - Scutellar Parenchyma cell
Pr - Protein
Nu - Nuclear apparatus
St - Starch grain
Cw - Cell wall (note thinness)

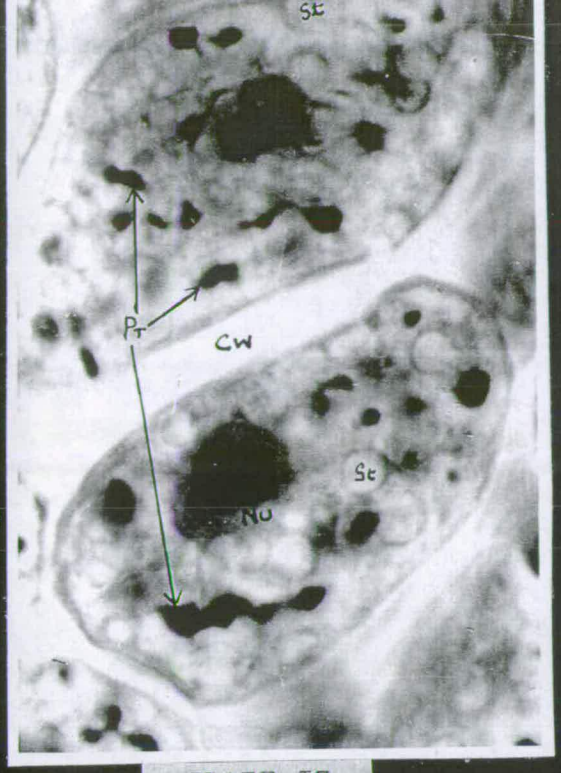

PLATE I7

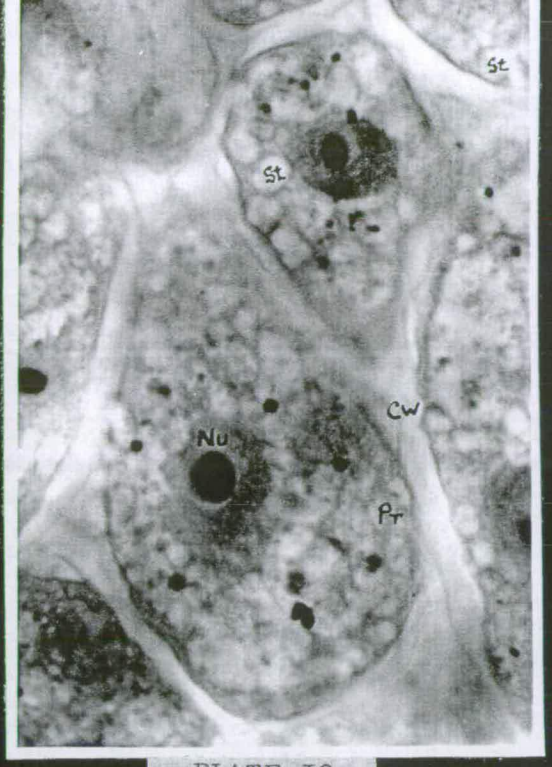

PLATE I8

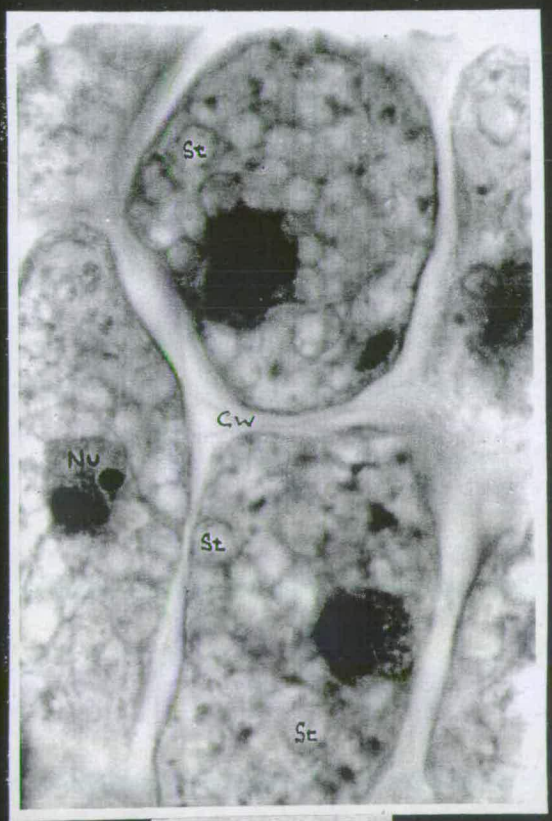

PLATE I9

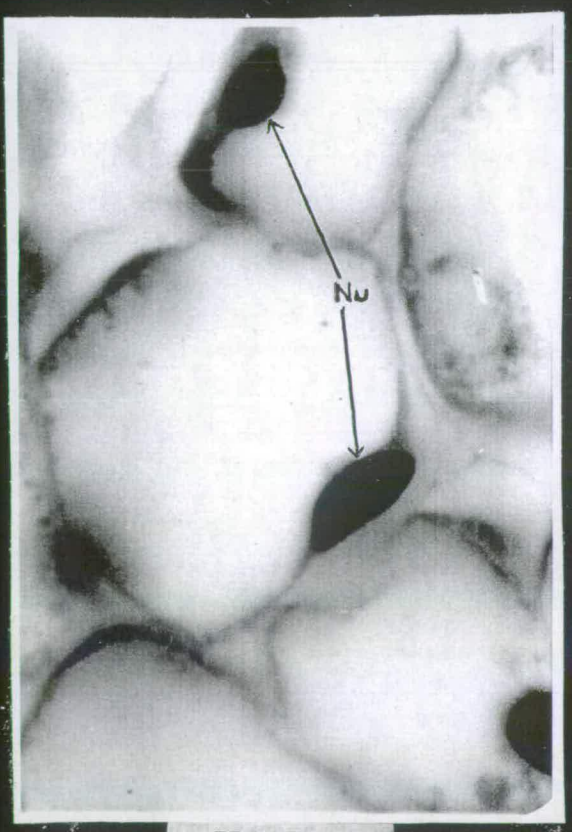

PLATE 20

Plate 21. Electron micrograph of scutellar parenchyma cell at 0 hr. germination. Electron dense material probably protein deposits termed core type (ct) or mesh-work type (mt). Electron transparent inclusions possibly fat deposits (Ft).
Fixation: Buffered Potassium Permanganate
Magnification: X 24,000

Plate 22. Shows Osmium tetroxide fixation of scutellar parenchyma cells also at 0 hr. germination. In support of labelling of Plate 21: Fat inclusions are now electron dense and large spherical transparent areas may indicate protein deposits.
Magnification: X 10,500

Plate 23. Potassium permanganate fixation of starchy endosperm of 0 hr. grain: starch grains (St) are electron transparent while protein-like matrix (Pr-m) is electron dense.
Magnification: X 13,500

Plate 24. Osmium tetroxide fixation of starchy endosperm of 0 hr. grain: starch grains remain electron transparent but the proposed protein-like matrix, as expected, is now electron transparent.
Magnification: X18000

Abbreviations:

ct - Core type protein deposit
mt - Mesh-work type protein deposit
Cw - Cell wall
Ft - Fat deposit
St - Starch
Pr-m - Protein matrix
Pr - Protein deposit
D - Deposit ?

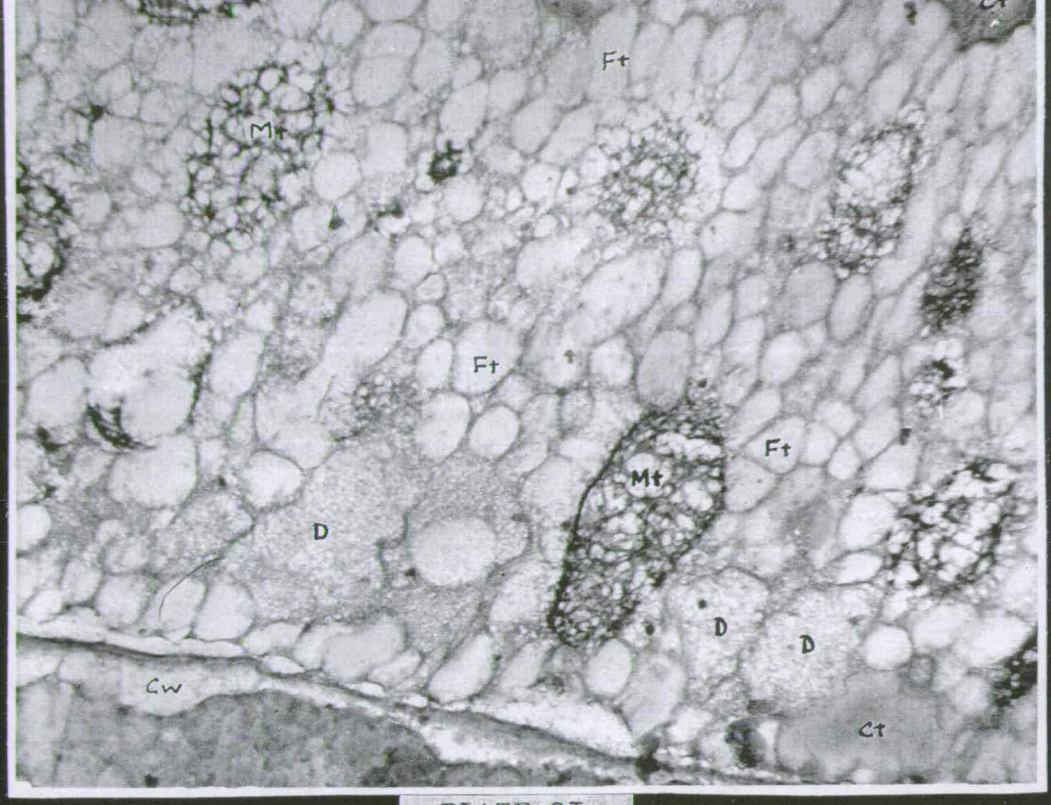

PLATE 21

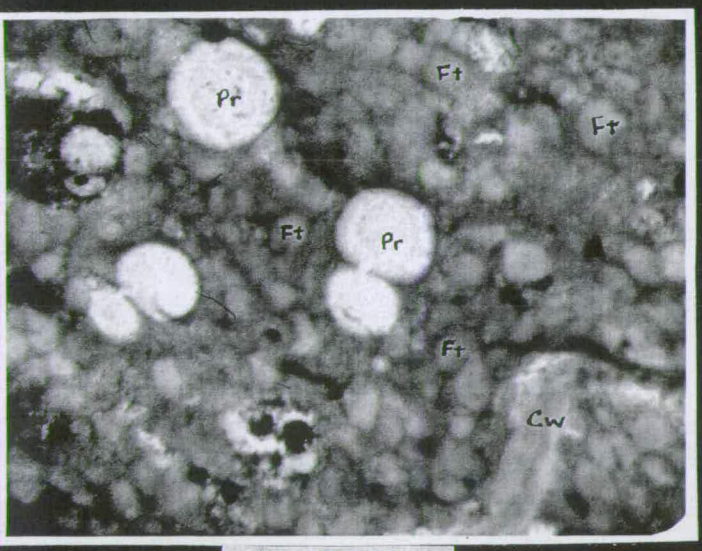

PLATE 22

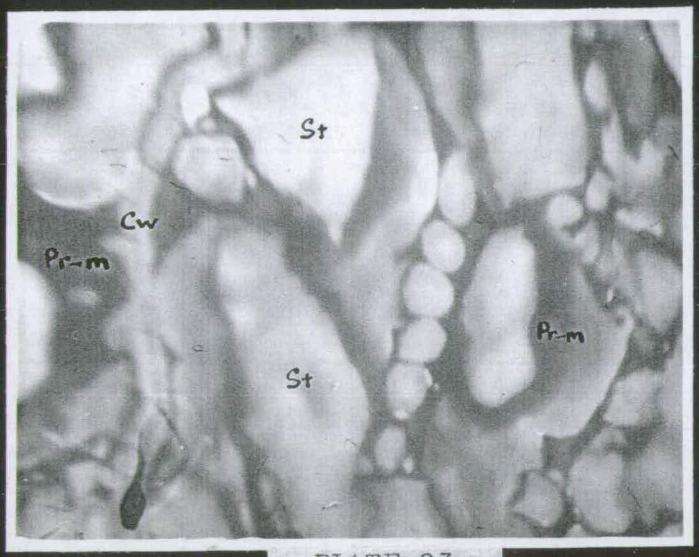

PLATE 23

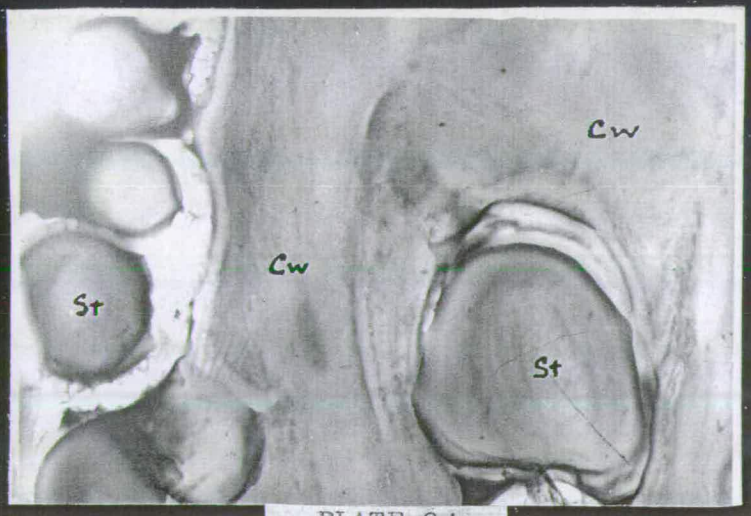

PLATE 24

Plate 25. Electron micrograph of scutellar parenchyma cell at 12 hr. germination. Note early appearance of starch grains in amyloplasts (Amp) and what could be 'shrinking' of fat inclusions. Cytoplasmic membranes are present and one large protein body (Pr) is visible.
Fixation- Buffered Potassium Permanganate
Magnification: X 13,500

Plate 26. Electron micrograph of scutellar parenchyma cell at 24 hr. germination. Amyloplasts with starch deposits are very obvious. Fat inclusions have given way to stellate shaped spherosomes (Sp). Er-membranes (er) and mitochondria (M) are now visible.
Fixation: Buffered Potassium Permanganate
Magnification: X 7,333

Abbreviations:

Amp - Amyloplast
Pr - Protein body with core type inclusion
St - Starch
Sp - Spherosomes
Ft - Fat inclusions
er - Er-membrane
M - Mitochondria
Cw - Cell wall
Nu - Nuclear apparatus

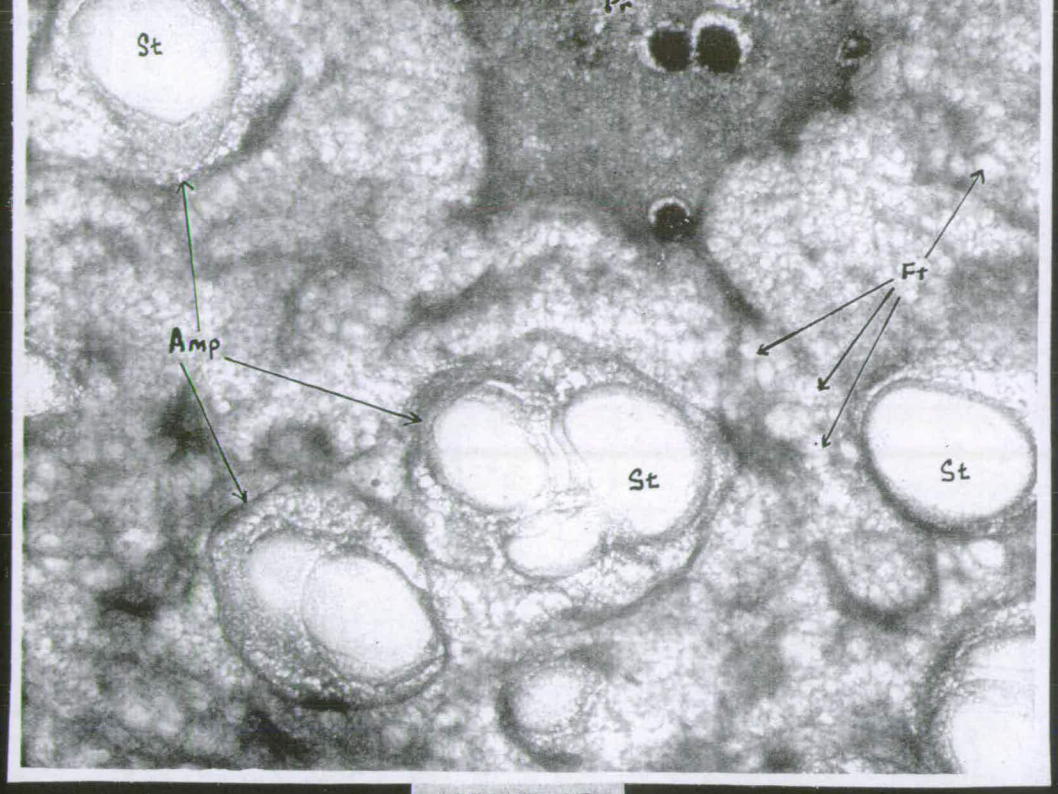

PLATE 25

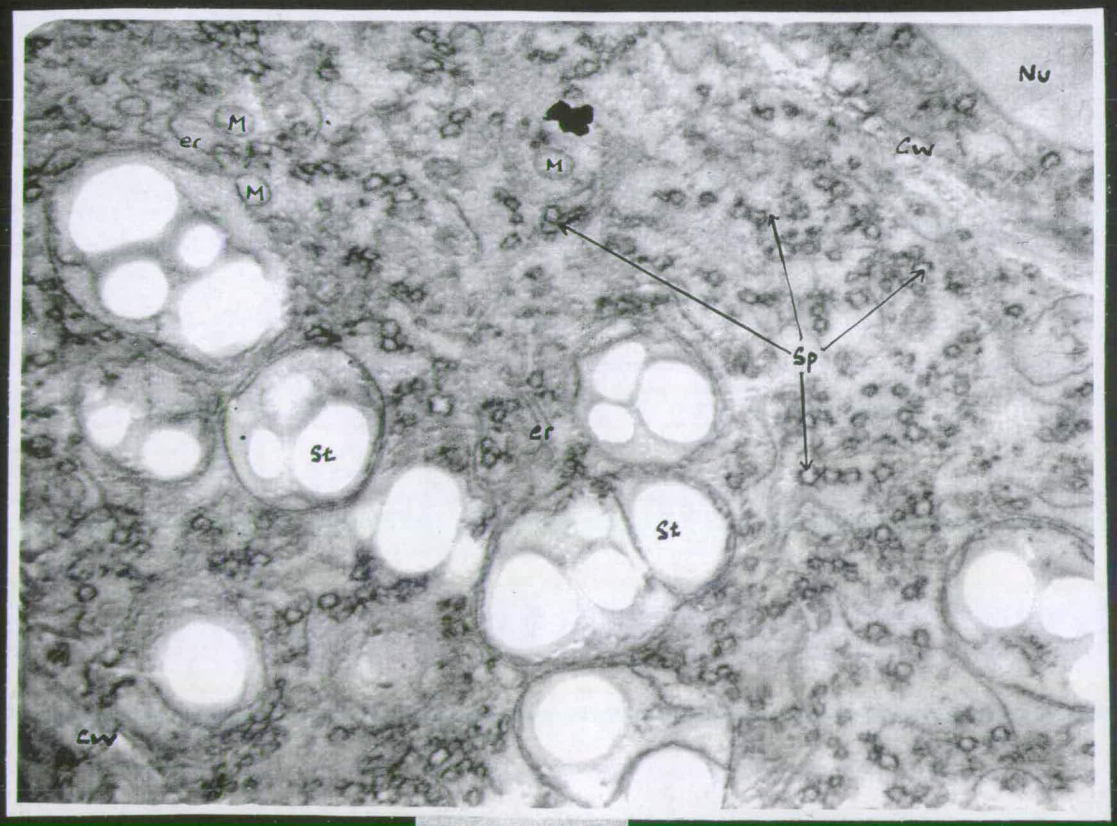

PLATE 26

Plate 27. Electron micrograph of scutellar parenchyma cells at 72 hr. growth. Starch grains (St) are opaque and their 'growth' may be completed. Spherosomes, er-membrane and mitochondria can still be seen.
Fixation: Buffered Potassium Permanganate
Magnification: X 9,000

Plate 28. Electron micrograph of 0 hr. grain to show the close tissue association between the intermediate layer (I) and epithelial cell(E). Note that I^I appears to be the cementing material between these cells and is really an extension of the material of the intermediate layer.
Fixation: Buffered Potassium Permanganate
Magnification: X 9,260

Plate 29. Electron micrograph of epithelial cells at 24 hr. growth. The intermediate layer has disappeared, starch grains in amyloplasts are present, in addition to other metabolic organelles. Plasmodesmatal-like areas (Pd) appear to be present along the lateral cell wall of these cells.
Fixation: Buffered Potassium Permanganate
Magnification: X 3,345

Abbreviations:

St - Starch
Sp - Spherosomes
er - Er-membranes
M - Mitochondria
I - Intermediate layer
I^I - Cementing material
E - Epithelial cell
W - Cell wall
Pd - Plasmodesmatal-like area

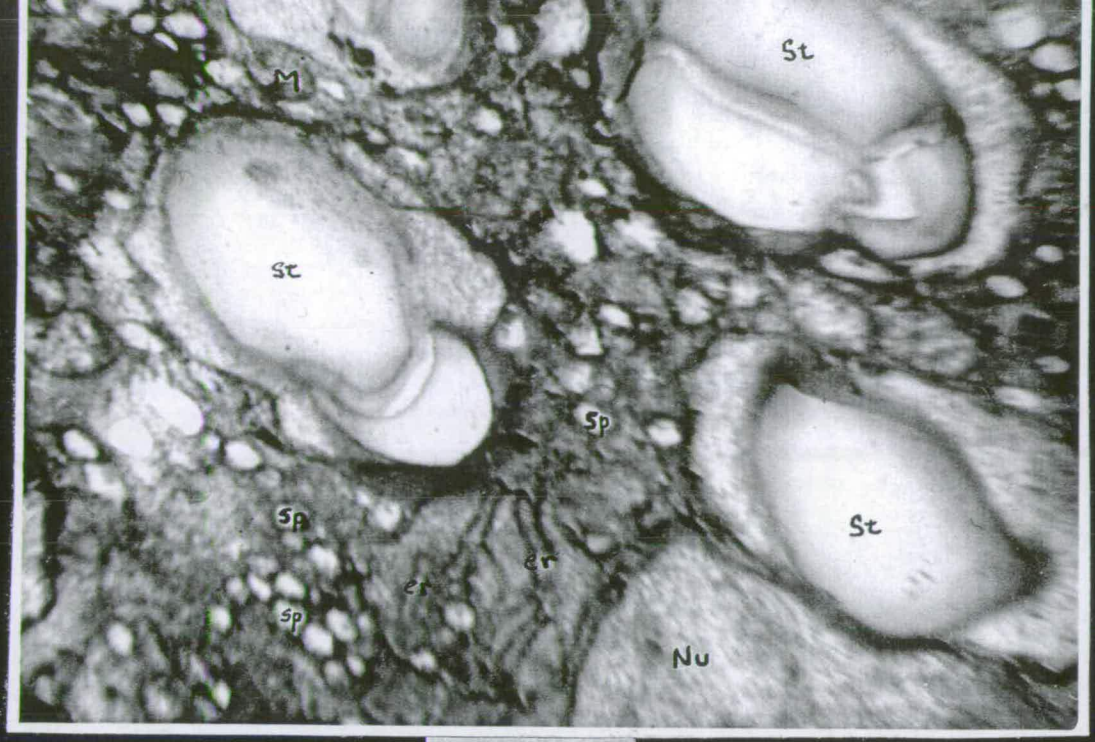

PLATE 27

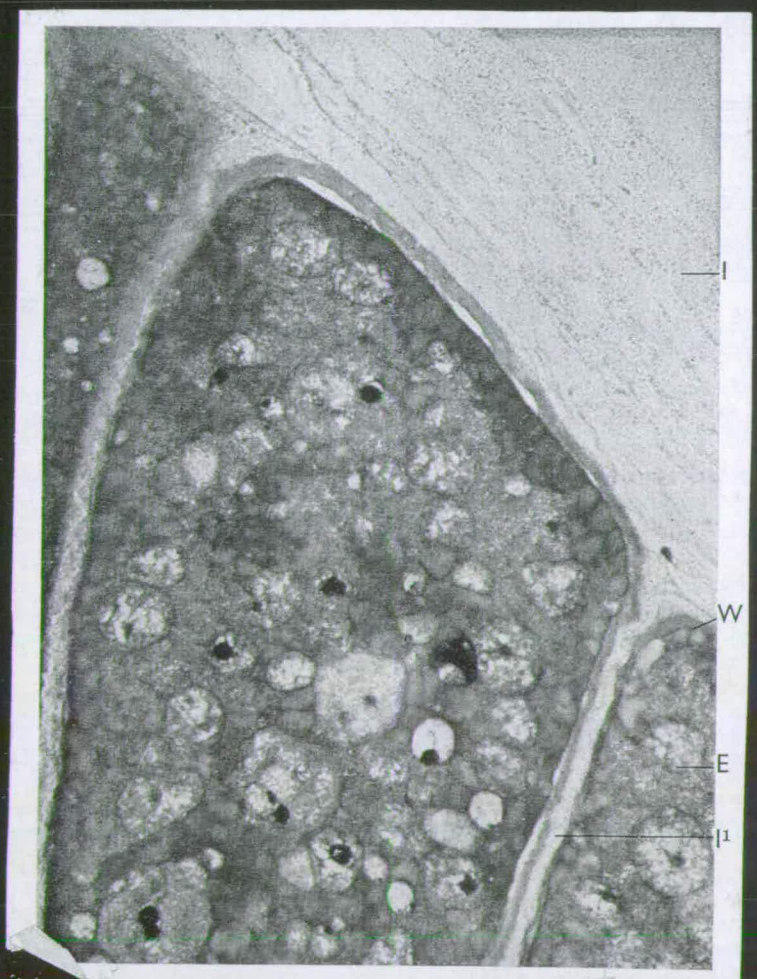

PLATE 28

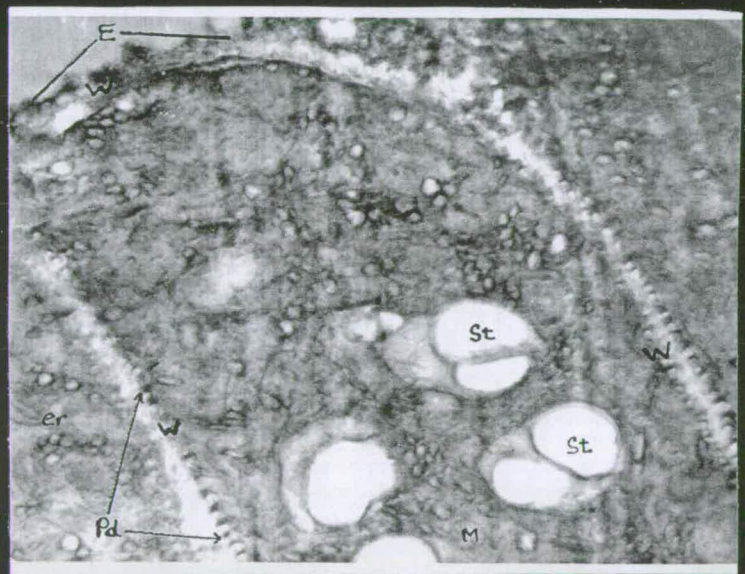

PLATE 29

Plate 30. Electron micrograph to show reticulate and annular thickening of xylem elements as evidence of lignification (L) in the scutellum of 24 hr. grown grains.
Fixation: Buffered Potassium Permanganate
Magnification: X 3,048

Plate 31. Electron micrograph to show localised vascularisation (lignification, L) of xylem cells at the scutellar node region of the axis.
Fixation: Buffered Potassium Permanganate
Magnification: X 1,800

Plate 32. Electron micrograph to show that:
(i) Advanced lignification is accompanied by cytoplasmic disintegration -see CD and L.
(ii) 'Humps' on cell wall may be presumptive sites of lignification - see h.
(iii) Large pores (P) may facilitate food transport along the vascular tract of the scutellum.
Magnification: X 4,888

Plate 32A. (iv) Shows enlargement of "ringed" end-wall of Plate 32 to show pores (P) more clearly.
Fixation: Buffered Potassium Permanganate
Magnification: X 9,776

Plate 33 Electron micrograph to show that lignin deposition starts within the middle lamella region of the cell wall (Cw) and is initially deposited onto the structuralfibres of the cell wall - see l_1, l_2, l_3 and l_4. Signs of lamination of cell wall can be seen in L of Plate 32.
Fixation: Buffered Potassium Permanganate
Magnification: X 11,200.

Abbreviations:

L - Lignin
CD - Cytoplasmic disintegration
h - 'Humps' of the cell wall - pre-linification sites.
Cw - Cell wall
P - Sieve pore
l_1 - l_4 - Stages in lignification
Nu - Nuclear apparatus

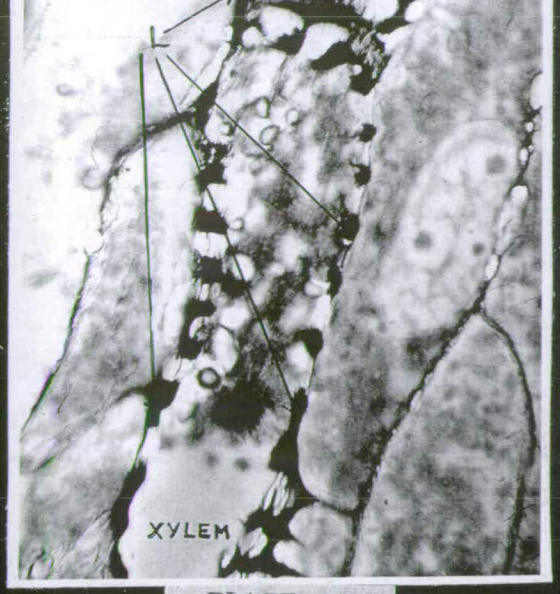

PLATE 30

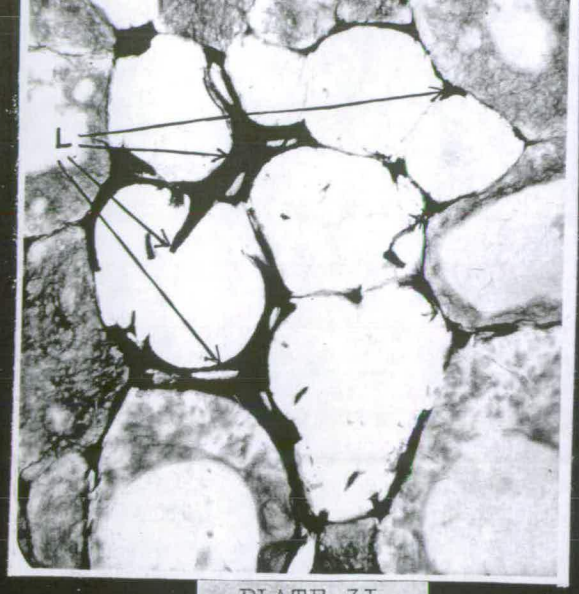

PLATE 31

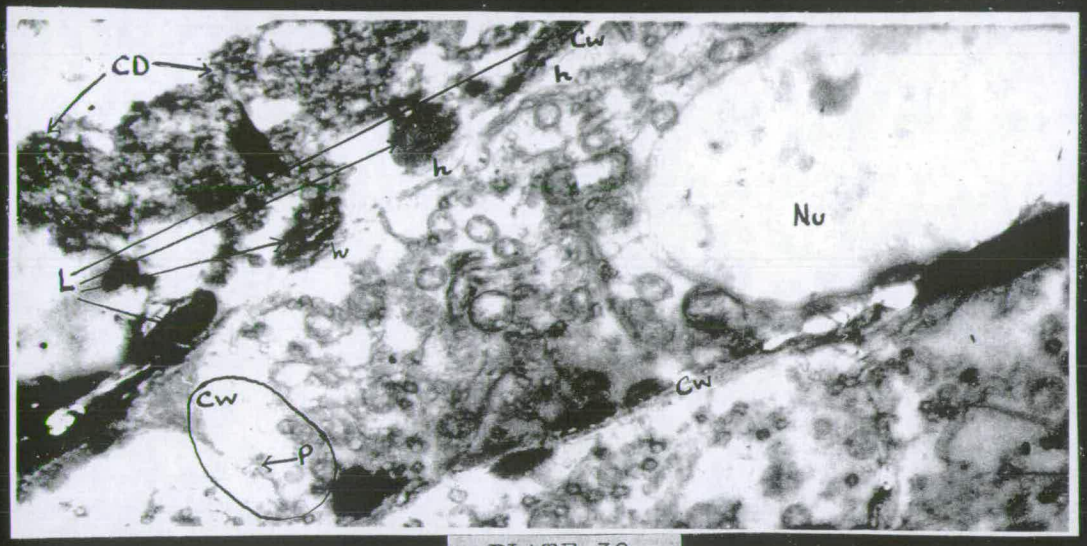

PLATE 32

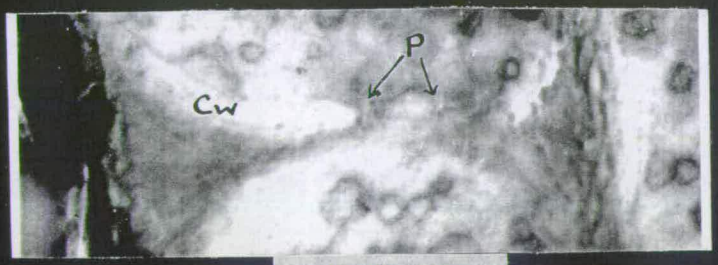

PLATE 32A

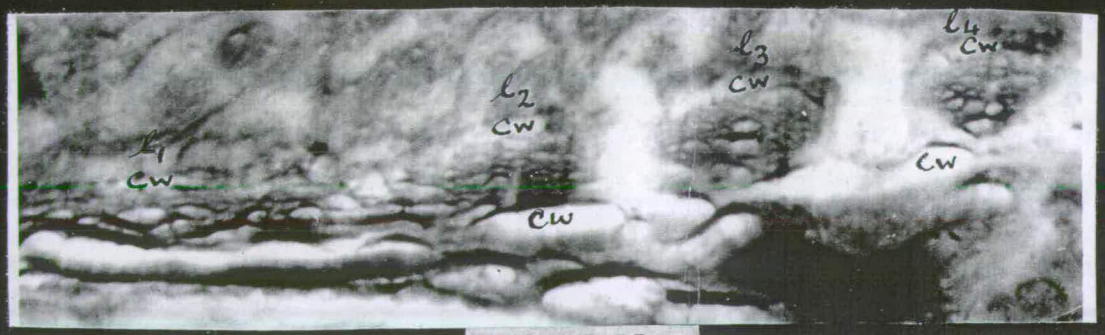

PLATE 33

Plate 34. Electron micrograph of a scutellar node cell at 0 hr. germination: cf. Plate 21, for scutellar parenchyma cell at 0 hr. germination (i.e., dry mature grain). Note that many metabolic organelles are present in the nodal cell while only storage products are present in the parenchyma cells of the scutellum.
Fixation: Buffered Potassium Permanganate
Magnification: X 20,580

Plate 35 & 36. Electron micrograph of cells of the scutellar node at 12 hr. germination. Note well developed mitochondria (M), golgi bodies (Gb), ribosomes (Rb) and er-membranes (er). At 12 hr. no such development of metabolic organelles can be seen in the scutellar parenchyma cells - see Plate 25; however, nodal cells have no amyloplasts.
Fixation: Buffered Potassium Permanganate
Magnification: Plate 35:X 12, 402. Plate 36:X 13,594.

Abbreviations

Nu - Nuclear apparatus
M - Mitochondria
Gb - Goigi bodies
Rb - Ribosomes
er - Er-membranes
Cw - Cell wall
Ps - Plasmodesmata
Ml - Middle lamella
Pr - Protein deposit?

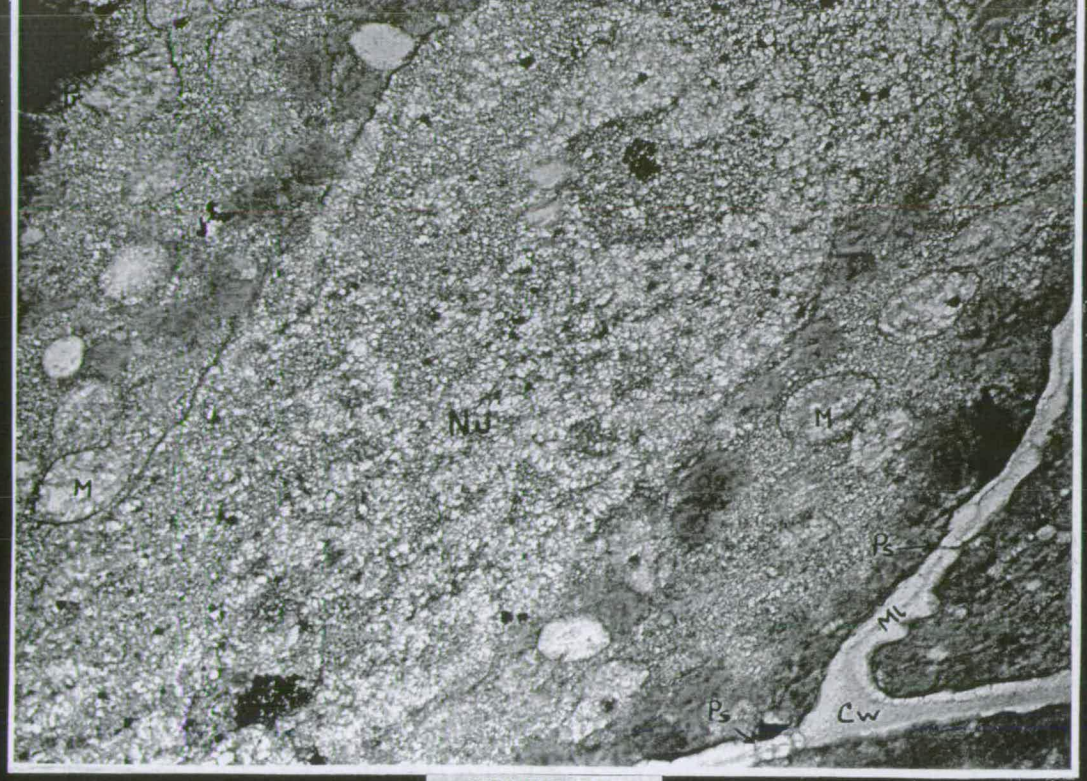

PLATE 34

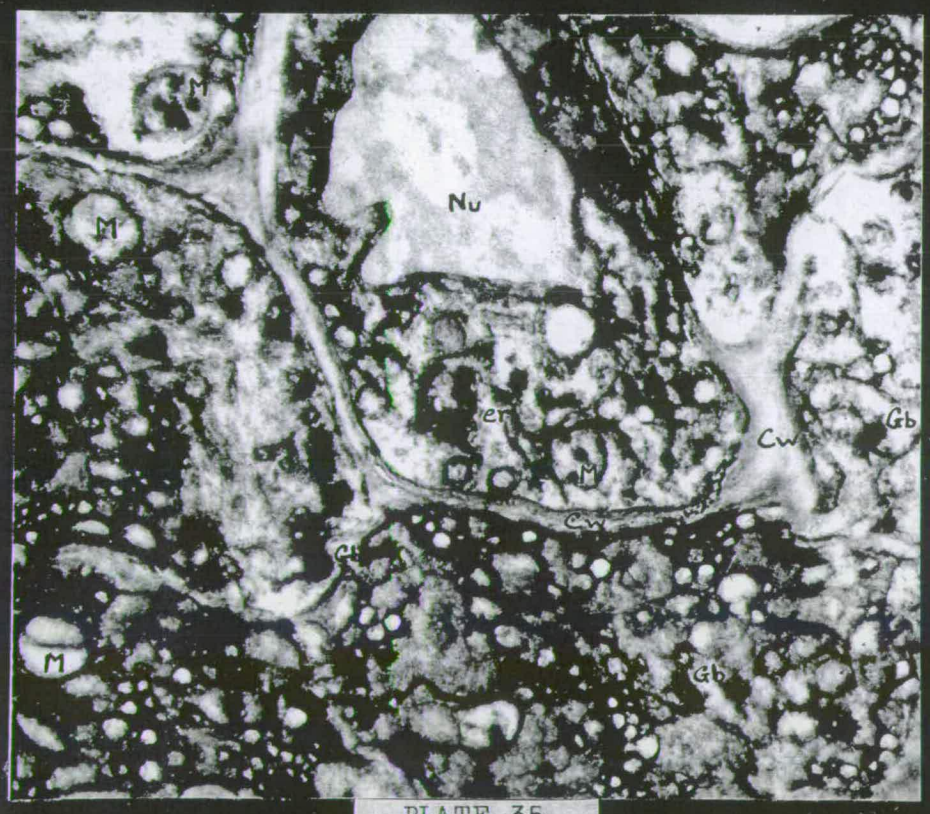

PLATE 35

PLATE 36

Plate 37. Electron micrograph of cells of the scutellar node of grains grown for 24 hr. Mitochondria (M), golgi bodies (Gb) and er-membrane (er) are still visible.
Fixation: Buffered Potassium Permanganate
Magnification: X 10,220

Plate 38. Electron micrograph of cells of the scutellar node of grains grown for 72 hr. Mitochondria (M) appear swollen and cell in general state of disintegration.
Fixation: Buffered Potassium Permanaanate
Magnification: X 15,455

Plate 39. Electron micrograph to show that at the cellular transition from the scutellum to the scutellar node regions of the embryo well developed amyloplasts (Amp_I) seem to loose their ability to synthesise starch. For 24 hr. grown grain.
Magnification: X 5.150

Plate 40. Electron micrograph to show detail of (starch-free) Amp_I type amyloplast. Also note pore (Np) in nuclear membrane.
Fixation: Buffered Potassium Permanganate
Magnification: X 13,692

Abbreviations:

M - Mitochondria
Gb - Golgi bodies
er - Er-membrane
Amp - Amyloplast
Amp_I - Amyloplast
St - Starch
Cw - Cell wall
Nu - Nuclear apparatus
Np - Nuclear pore
Nm - Nuclear membrane

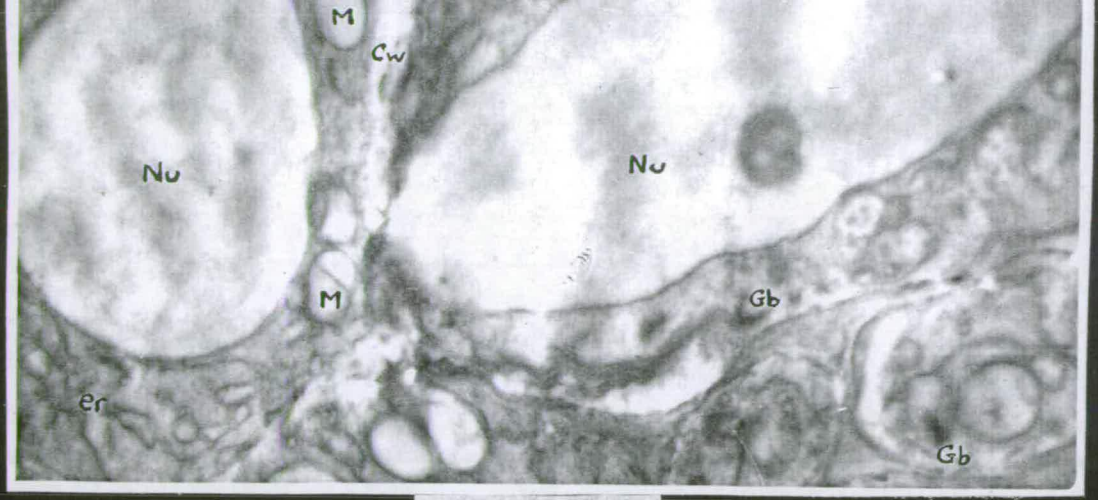

PLATE 37

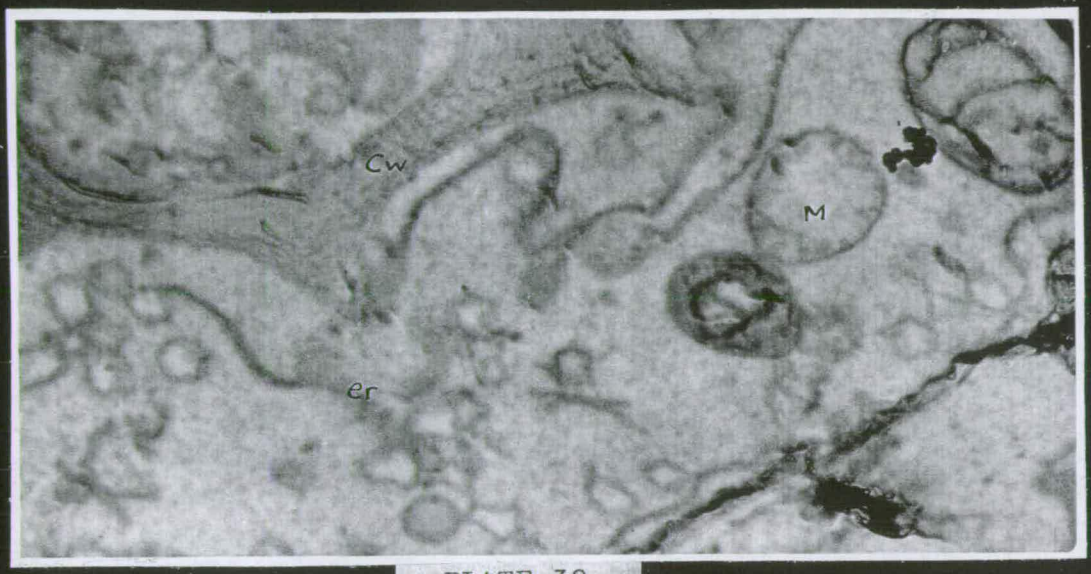

PLATE 38

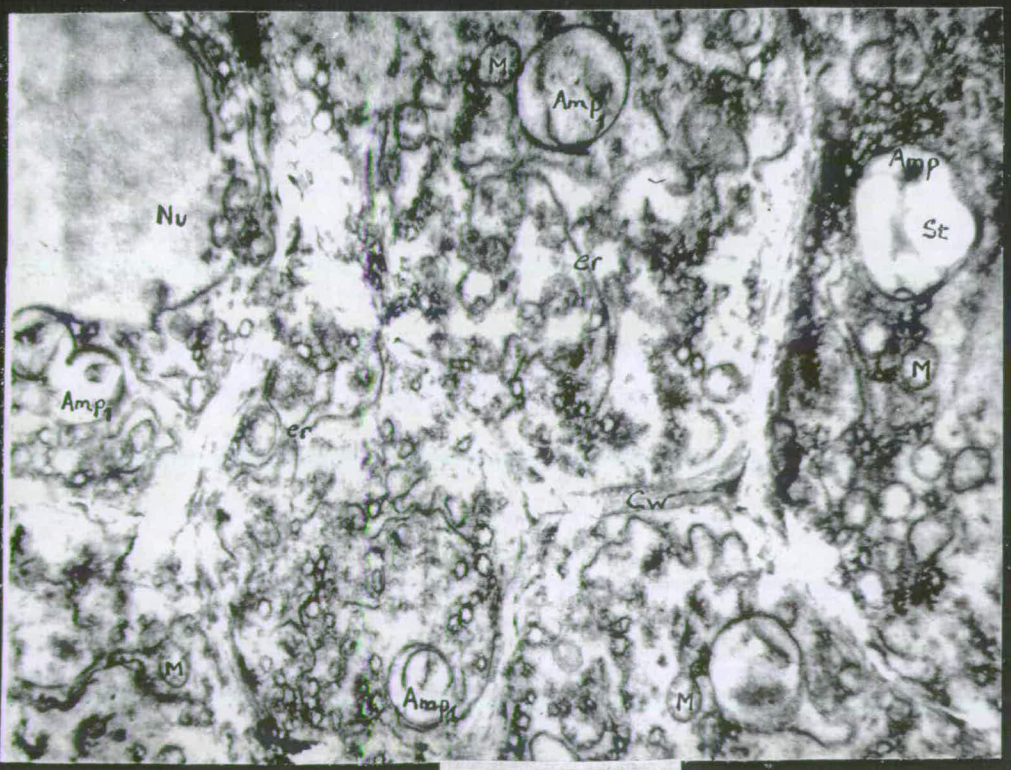

PLATE 39

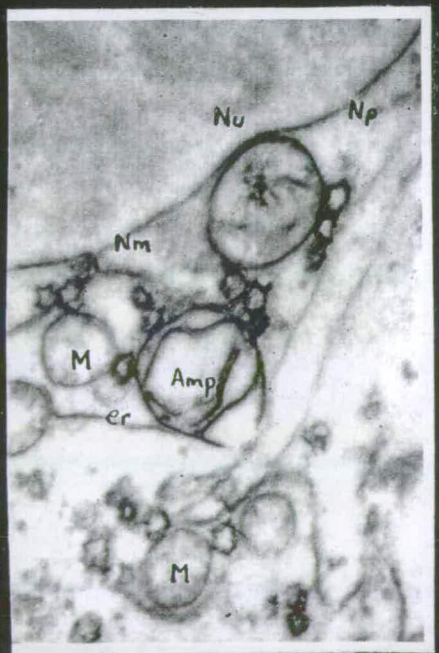

PLATE 40