W.G. Jack.

Summary.

Studies in the Physiology of Seed Germination.

Investigations have been made into the behaviour of etiolated seedlings of Pisum arvense L., cultivar. Maple, grow from seeds which were soaked anaerobically in distilled water before sowing. A high percentage of such seedlings possessed abnormal radicles when the soaking period (at 20°C) was longer than 60 hours. The abnormalities commonly found were increas diameter, excessive curling, truncated tips, and suppressed linear growth compared with the radicles of seedlings grown from seeds sown directly at the beginning of the soaking perio

The abnormalities were unlike those which would have been expected after microbial attack and, as complete death of the radicle did not occur, it was concluded that the damage must have been due to an irreversible metabolic disturbance.

It was clearly shown that raising the soaking temperature above 20°C when the seeds were soaked for 72 hours, increased the severity of the damage. When the soaking temperature was reduced to 10°C, the damage produced was negligible.

If the testas were removed from the seeds before soaking, or if complete seeds were exposed to conditions suitable for aerobic germination for about 24 hours before soaking, the damage was considerably increased. These observations indicated that, during germination, there is a certain stage a

which pea seeds are very susceptible to soaking. If the seeds were treated in such a way that the critical stage was reached earlier, damage was more severe.

An attempt was made to separate the effects of soaking and of anaerobiosis by passing certain gases through the soaking medium. It was found that, when air or argon was used the damage was reduced. On the other hand, CO_2 intensified the adverse effects and oxygen treatment produced abnormalities different from those already described. The latter effect was attributed to oxygen toxicity, and possibly was unrelated to th soaking treatment. The hypothesis was put forward that argon could reduce the adverse effects by removing respiratory CO_2 from the vicinity of the soaking seeds. Therefore, there is some evidence that the initial stimulus for the adverse effects is more likely to be the accumulation of respiratory CO_2 than the exclusion of oxygen from the seeds.

Visual assessment of the morphological damage indicated th an abnormally high auxin content in young pea seedlings could b the cause of the damage and, indeed, exogenous IAA applied to t soaking medium intensified the adverse effects.

Direct quantitative measurement of the auxin content of control seedlings and seedlings grown from soaked seeds was not practicable, but investigations were made into the ability of aqueous extracts of these seedlings to destroy synthetic IAA, presumably by IAA oxidase. Enzyme preparations of seedlings f

soaked seeds were less capable of destroying IAA *in vitro* than comparable enzyme preparations from control seedlings. It wa pointed out that, though this may signify an abnormally high a content in damaged tissues, the evidence in favour of *in vivo* enzymatic control of auxin concentration is not strong enough permit acceptance of this explanation without further evidence The application of manganous chloride to the soaking medium, however, considerably reduced the damage. Since manganous io have been shown to stimulate IAA oxidase activity *in vitro* it seems possible that this enzyme could be upset by anaerobic soaking.

When gibberellic acid was supplied to the soaking seeds, damage produced in the resulting seedlings was less than that produced by soaking the seeds in distilled water. This effec difficult to explain in so far as treatment of plant material gibberellins generally results in an increase in free endogeno auxin: however, it is not unknown for gibberellins to exert t apparently opposite effects on plant material, e.g. breaking a induction of dormancy have both been reported after treatment seeds with gibberellins. Therefore, it is inadvisable to con clude that the gibberellic acid results are necessarily eviden against the original hypothesis.

In the absence of a reliable assay technique for estimati endogenous auxin, the evidence presented in this thesis sugges that soaking disrupts *in vivo* IAA oxidase activity, thus resul

in a relatively high endogenous auxin level in seedlings grown from soaked seeds.

STUDIES IN THE PHYSIOLOGY OF SEED GERMINATION

Thesis presented by

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for the degree of

Doctor of Philosophy in the Faculty of Science

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CONTENTS.

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STUDIES IN THE PHYSIOLOGY OF SEED GERMINATION

Introduction and review of the literature

The survival of many higher plant species depends on their ability to produce seeds which will germinate successfully. Usually there must be a relatively long vegetative phase before flowers and seeds are produced but Jacques (1957) has reported flower induction in very young plants of Chenopodium polyspermum, L.

Although seeds are usually produced only after pollination and the subsequent fertilization of the ovum in the ovule by one of the male gametes, there are reports of seed production occurring without pollination and fertilization, for example in the genus, Crepis (Babcock and Stebbins, 1938). Most plants however, require the fertilization process and it is this which furnishes the initial stimulus for the transformation of the ovule and its integuments into the seed. The seed of the field pea (Pisum arvense L., cultivar. Maple) is produced in this manner and is considered representative of the normal seed: it is used for most of the work described in this investigation.

Seed and fruit development, following fertilization of the ovum, is very rapid. Carr and Skene (1961) have shown that, in French Beans (Phaseolus vulgaris), pod growth begins almost

immediately after anthesis and is completed in 16-17 days. The seeds on the other hand, do not begin growth till about 9 days after anthesis. Seed development is considered by Carr and Skene to be diauxic, there being 2 phases of rapid growth when the seeds increase their fresh and dry weights by a factor of 2 every 3 days, separated by a lag phase which lasts about 2 days.

During this period of intense activity, there is continuous production of 3 classes of material viz.

1. Structural compounds.
2. Storage compounds.
3. Functional compounds.

The structural compounds, formed as the seed develops, convert the soft fleshy tissues of the ovule and its integuments into the hard resistant seed which can withstand adverse conditions. These compounds are not of direct concern in the present investigation and are not discussed further.

The storage materials are produced by enzymes within the cells converting the soluble food materials such as sugars and amino acids, which are carried to the seed in the translocation stream, into the insoluble food reserves, starch, hemicelluloses, fats and proteins.

While the seed is being formed, the ovary or carpellary wall is stimulated to further development, possibly by growth substances secreted from the zygote (Luckwill, 1959). It is

certainly known that in the course of fruit development, functional compounds of very high physiological activity are present. The functional compounds of greatest interest in this investigation are the auxins, kinins and gibberellins.

Muir (1942) found that auxin production in the developing tobacco seed started in the ovary as soon as the pollen tube penetrated the style, and the rapidity of auxin production suggested the hydrolysis of an auxin precursor, already present in the style, by some constituent of the pollen tube. The auxin released in the ovary was found to move down the pedicel, thus preventing abscission of the developing fruit.

Avery, Berger and Shalucha (1942) found a similar situation in the developing maize fruit with the result that there was a relatively high auxin level at maturity. Hatcher (1945) summarised the investigations made up to that time on the auxin content of cereal fruits. Hamilton, Bandurski and Grigsby (1961) found that auxin could be extracted from kilogram quantities of maize fruits, using a buffered ether-water partition column technique. Although no direct quantitative measurements were made, the auxin content of the fruits was found to be much higher than that of the maize shoots. Other recent reports in the literature of growth substances in the fruit and seed are found in Nitsch and Nitsch, 1955 (Studies on auxins of bean seeds), MacMillan and Suter, 1958 (Gibberellin A_1 in Phaseolus multiflorus), Goldacre

and Bottomley, 1959 (Kinin in apple fruitlets), MacMillan, Seaton and Suter, 1959 (Gibberellin A_5 in P. multiflorus), West and Phinney, 1959 (Isolation of substances similar to gibberellins from seeds of P. vulgaris) and Skene and Carr, 1961 (Development of gibberellins in seeds of P. vulgaris).

. . .

The effects of adverse conditions on the developing seed have been investigated by Wager (1954, 1957, 1959) who subjected maturing seeds of Pisum sativum L., cultivar. Onward, to a current of air to induce accelerated wilting. It was found that the rate of loss of sugar and the rate of carbon dioxide production decreased greatly as the water content was reduced; removal of the testa led to a marked change in the physiological behaviour of the seed and the ethanol formed while the peas were wilted under anaerobic conditions was not significantly metabolised on return to air. Wager compared these results with those found when dried peas were soaked under anaerobic conditions. In the latter case he found a different response to that produced by maturing peas, when they were exposed to anaerobic conditions. This implies that the developing seed at a certain degree of dehydration was not in the same condition as a mature seed at a similar degree of hydration.

. . .

When field peas (Pisum arvense, L.) are grown for seed production, the peas are harvested at the end of the season

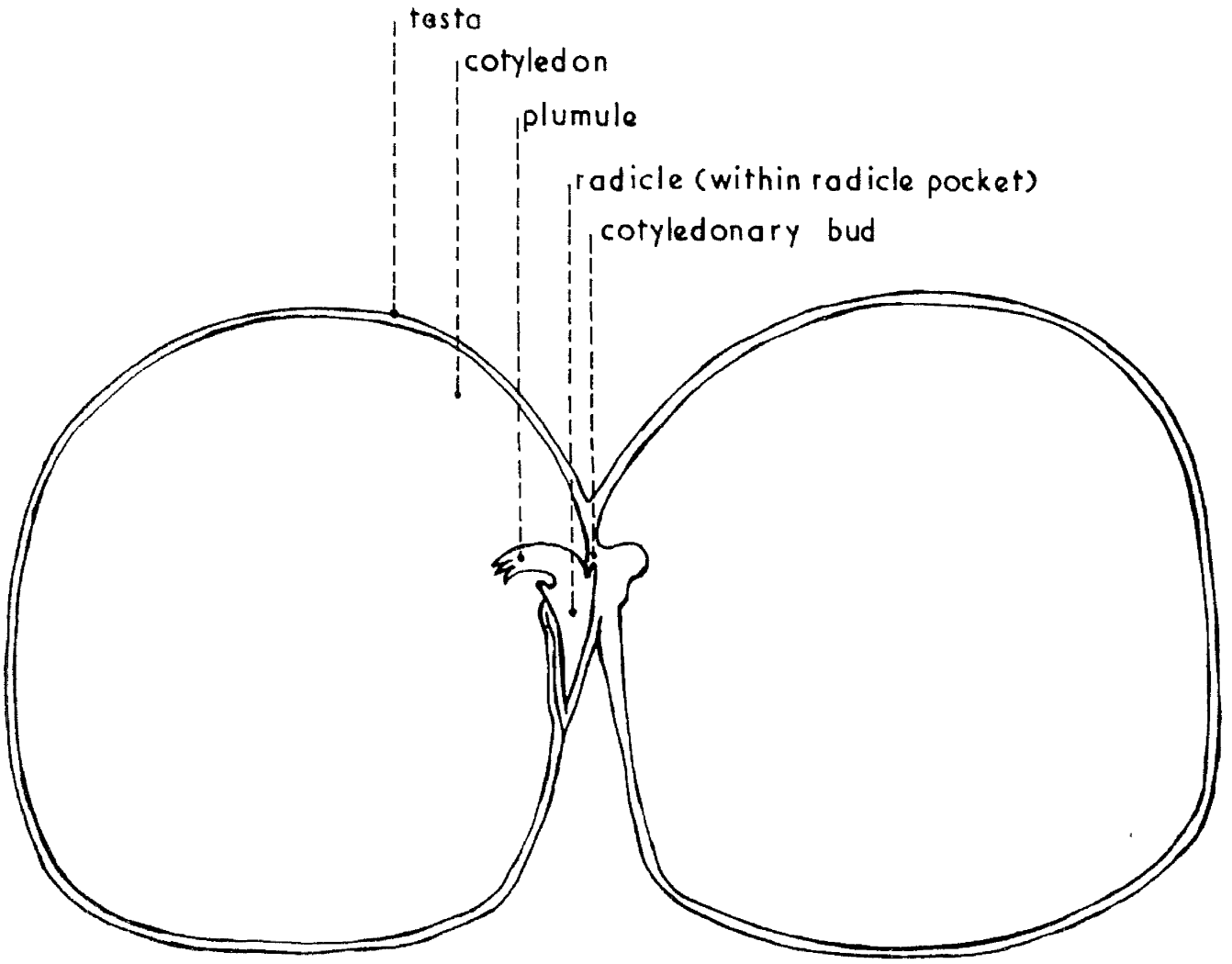


PLATE I

Diagram of a split seed of Pisum arvense L. 2 Halves of the seed are shown in surface view to demonstrate the positions of the testa, cotyledons and embryonic axis.

and stored under dry conditions till the following spring. Unlike "dormant" seeds the pea can germinate immediately after harvest and sprouting in the pod is a fairly frequent occurrence The seeds, however, are usually allowed to overwinter in a state of very much reduced metabolic activity and embryo development is suspended. The seed of the field pea is smooth and round, approximately 6 mm in diameter with a tough brown testa, enclosing the two large cotyledons which almost completely envelop the small radicle and plumule. The embryonic axis is composed of a prominent radicle lying on the periphery of the cotyledons and protected by a radicle pocket made up of part of the testa, and a plumule which is attached directly to the radicle but is turned inwards so that it lies between the cotyledons as shown in Plate 1. There are usually no lateral root primordia in the radicle but the plumule is fairly well developed, there being up to 6 nodes present at this stage. With free access to air these viable resting seeds of the field pea will germinate when they are provided with sufficient water at a suitable temperature.

Germination is best considered in the light of the definition of Toole, Hendricks, Borthwick and Toole (1956) and Evenari (1957) i.e. the period between exposure to suitable environmental conditions and the resumption of meristematic activity. Once cell division starts subsequent morphological changes are best considered as Development.

Most of the observations recorded here are concerned with the morphology of the radicle and, when this only is examined, it is obviously impossible to decide when germination ceases and development begins. The term Emergence is therefore used to indicate that the testa of the seed has burst and the radicle is beginning to protrude. Emergence follows successful germination therefore emerged seeds ipso facto have germinated.

At the beginning of germination there is rapid uptake of water, or even water vapour, against the external forces of gravity, capillarity, imbibition and hygroscopy found on soil particles (Shull, 1916). Uptake of water becomes gradually slower (Shull, 1920) and this phase is generally followed by a further steady rise which is constant throughout the remaining period of germination and development (Stanley, 1958; Drennan, 1960).

The uptake of water during germination and development is accompanied by a steady resumption of physiological activity (Stiles and Leach, 1932, 1933; Leach, 1943 and Drennan, 1960). Stiles and Leach showed that there is an increase in the rate of respiration of single seeds within the first few hours of imbibition. Thereafter the increase in respiration goes on proportionally to the water content until the seed has germinated and undergone considerable development. A similar pattern of respiration has been observed for batches of seeds

(Bailey, 1940; James and James, 1940; Ragai and Loomis, 1954; Milner and Linko, 1958; and Stanley, 1958).

Respiration is the mechanism whereby energy is made available to the seed and it was Sachs (1887) who gave the firs description of the process. He found that, in the process of respiration, oxygen was absorbed, carbon dioxide produced and the organic substances oxidised with the concomitant release of heat and other forms of energy. The chemical energy is utilised for nuclear division, the power of movement, the active absorption and transport of solutes and to produce power for the secretion of liquids (Thomas, 1957). It is also essential for the synthesis of cell constituents.

These phenomena depend directly on the presence of oxygen which is essential for this type of respiration viz. aerobic respiration. In the absence of oxygen most living plant cells do not grow and eventually die. They do respire for a time but with the release of only small quantities of energy. This is anaerobic respiration.

The most common respirable substrate in plants is hexose sugar and the two types of respiration may be illustrated thus;

Aerobic

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6H_2O + 674\ Cals.$$

Anaerobic

$$C_6H_{12}O_6 \longrightarrow 2CO_2 + 2C_2H_5OH + 24\text{-}28\ Cals.$$

The process of respiration is vitally important to the germinating seed and under normal conditions changes take place under its agency which could be likened to mirror images of the changes which occur in the developing seed. The immobile and insoluble food reserves are rapidly attacked and broken down by enzymes e.g. the amylases, invertase, proteases, peptidases, transaminases and the respiratory enzymes (see Drennan, 1960 for references). The soluble food materials are thus released for vital functions (Griffiths, 1937; Albaum and Cohen, 1943; Albaum and Eichel, 1943; Poljakoff-Mayber and Mayer, 1955 and MacLeod, 1957) and are later translocated to the sites of most intense metabolic activity (Malhotra, 1932; Hora, 1936; James, 1940; Bernstein, 1943; Folkes and Yemm, 1958). A normal germinating seed is a source of intense metabolic activity, and a supply of readily available food materials is one of its prime requirements.

When, however, seeds are subjected to certain abnormal conditions the pattern of events described above cannot proceed and damage to the seed or seedling is the eventual result. Such is the case when the seeds or seedlings undergo periods of anaerobiosis, i.e. when aerobic respiration is replaced by anaerobic respiration and its attendant characteristics. Experimentally, it is possible to create anaerobic conditions by various means involving exclusion of oxygen from the seeds. They may be placed in a vacuum, in an

atmosphere of an inert gas or under a large volume of air-free liquid. In the experiments recorded here, anaerobiosis was achieved mainly by the soaking method.

The response of seeds to such adverse conditions is a source of great interest to the plant physiologist, but there is much confusion and contradiction in reports concerning earlier work (Kidd and West, 1919 b; Crocker and Barton, 1957 p.67). This is probably because the seeds were soaked under different conditions by the various investigators.

There are several reports of the beneficial effects of soaking (Braun, 1919; Chippindale, 1934 and Novikov and Sadovskaja, 1939). However, it is the adverse effects of soaking that are of particular interest here, as they were found to provide suitable experimental material for investigating germination and development.

Kidd and West (1918 a, 1919 c, d) soaked seeds of various species under 4 cm of distilled water and their results suggested that the soaking treatment had a predetermining influence on the behaviour of the resultant plants. It was found that soaking for over 24 hours diminished the "rate of germination" of Phaseolus, Pisum and Helianthus and retarded the growth of the plants. Broad bean (Vicia faba), however, showed diametrically opposite results, there being a progressive increase in height of the seedlings produced after up to 72 hours soaking. Three main conclusions were reached as

a result of Kidd and West's work viz.

1. Soaking in distilled water previous to sowing often has a marked influence on the subsequent growth of the plants.
2. A germination test cannot be relied upon as a criterion of what this influence will be.
3. The nature of the influence is strongly specific.

Kidd and West considered that there were three possible explanations for the adverse effects they observed:-

(a) disorganised metabolism of the seed resulting from deficiency of oxygen supply and accumulation of respiratory carbon dioxide.

(b) a leaching of essential food reserves from the seed.

(c) a combination of the above two factors.

The validity of these hypotheses has since been questioned by several investigators. Tilford, Able and Hibbard (1924) soaked seeds of several species under different water regimes. Their results indicated that the adverse effects manifest in the young plants produced from the treated seeds were due to bacterial activity in conjunction with a deficiency of oxygen and an accumulation of carbon dioxide. Rhine (1924), however, found that when wheat seeds were soaked anaerobically in sterile water for a period of 22 days the frequency of germination was reduced to 2%. Soaking for a period of 29

days under the same conditions resulted in the complete failure of germination, thus indicating that bacteria played little or no part in preventing germination.

Barton (1929) found that when the seeds of 5 species were surface sterilised and placed in sterile distilled water saturated with carbon dioxide for periods of up to 3 months, 60-100% of the seeds of all 5 species tested germinated. Hence Barton concluded:-

(a) The capacity for germination is not destroyed by long continued soaking in sterile media.

(b) The destructive changes are not brought about by enzymes within the seeds, and

(c) Any change that takes place within the seed does not in any way affect the viability of the seed and is not accompanied by any visible manifestation.

Bailey (1933) found that, for surface sterilised seeds of Phaseolus soaked in sterile aerated water for only 8 hours, the percentage germination decreased from 95 to 80.3. Soaking for longer periods led to a progressive decrease in the percentage germination. It would appear therefore, that no definite conclusions have been reached regarding the detrimental effects of bacterial activity on seeds during anaerobic soaking, although the balance of evidence is that it

is of only limited importance.

Previous to the work on bacterial activity, True (1914), had found that distilled water was harmful to lupin roots and considered that the damage was attributable to the conductivity of the water. Samples of distilled water with low conductivity were found to withdraw electrolytes from the roots. The electrolytes were regarded as being essential for the maintenance of the efficient action of the protoplasmic membranes, and hence when they were removed, the cell permeability was increased. The detrimental process could be arrested by adding calcium ions to the water in sufficient quantity to make its conductivity equal to that of tap water. In this way leaching of essential material from the cell could be checked and the chemical integrity of the cell maintained. Scarth (1924) confirmed this in his investigations on Spirogyra and concluded that cations were antagonistic to the toxic action of distilled water according to their valencies but their effect was naturally limited by their own toxicity.

Pólya (1961) arrived at similar conclusions when investigating the effects of soaking on poplar (Populus alba) seeds. He considered that the damage found was due, not to anaerobiosis, but to rupture of the cell membranes. The effects could be countered by soaking in 2M sucrose for 30 minutes. Similar results were obtained by Howell (1961)

working with "Hollow Heart" of peas. The addition of sucrose during soaking (24 hours) reduced soaking damage but not the incidence of "Hollow Heart".

These reports indicate that the osmotic relations of the cell are important in determining the degree of damage caused by soaking and, as the osmotic properties of the cell are partly controlled by the nature of the membranes enclosing the seed, it is necessary to mention the various reports in the literature on the nature and importance of semi-permeable membranes in seeds viz. Brown (1907, 1909), Atkins (1909), Shull (1913), Brown and Tinker (1916), Denny (1917), Harrington and Crocker (1923), Braun (1924), Rudolfs (1925 a, b), Malhotra (1931), and Resühr (1941). Levitt (1956, 1957, 1959) records some of the more recent work on osmosis, permeability and the uptake of ions by plant cells.

Bailey (1933) in a comprehensive review of previous experiments on the effects of soaking seeds (with particular reference to beans) arrived at some very significant conclusions.

1. Soaking seeds under anaerobic conditions results in decreased germination and many of the seedlings so produced are smaller and weaker than normal seedlings and fail to reach maturity.
2. The seedlings produced from soaked seeds have a relatively high carbohydrate level and a low

nitrogenous content.

3. Bacterial activity has little influence on the adverse effects of soaking and the addition of calcium nitrate to the medium does not minimise the severity of the effects.
4. When the seeds are soaked anaerobically there is a decrease in catalase activity. When the soaking medium is aerated there is a small decrease in catalase activity soon followed by a considerable increase.

These conclusions, like those of Kidd and West (1918a, 1919b, c, d), previously mentioned (Page 10), suggest that the soaking damage is due primarily to a metabolic upset in the seed while soaking or immediately after soaking.

One aspect of anaerobic soaking so far unexamined is its effect on the auxin metabolism of the germinating seed. This has been investigated here with the dual purpose of seeking an explanation of the adverse effects of soaking and of understanding better the metabolic processes which go on in the germinating seed. In the earlier part of this introduction a description was given of the build up of auxin in the developing fruit and in the latter parts of the thesis attempts will be made to describe the fate of the auxin and its influence on the germinating seed and the developing seedling.

Berrie (1960) reported that when seeds of the garden pea (*Pisum sativum* L., cultivar. Kelvedon Wonder) were soaked anaerobically before sowing, the seedlings so produced were not only of less dry weight than the control seedlings but also showed very distinct morphological shoot aberrations.

This observation was taken up in the present investigations and originally accepted as a criterion for determining the severity of the soaking damage. The behaviour of seedlings from seeds soaked under various conditions has been studied in an attempt to discover the underlying cause of these aberrations.

The investigations described in this thesis centre round the field pea *P. arvense* L., cultivar. Maple and, for convenience, the findings are divided into 4 parts:-

Part 1. The effects of soaking pea seeds on the morphology and growth of the resulting seedlings.

Part 11. The influence of environmental conditions on the response of pea seeds to soaking.

Part 111. Some responses of pea seeds to soaking in solutions of plant growth regulatory substances.

Part 1V. The possible regulation of the endogenous auxin content in etiolated pea seedlings by *in vivo* enzymatic destruction.

PART 1.

THE EFFECTS OF SOAKING PEA SEEDS ON THE MORPHOLOGY AND GROWTH OF THE RESULTING SEEDLINGS.

When pea seeds are soaked under a large volume of water, imbibition takes place but no visible signs of germination are observed until they are removed to an aerobic atmosphere. There are several reports of the effects of soaking on seed germination (Kidd and West, 1918 a, 1919 c, d; Bailey, 1933; Berrie, 1960). Berrie soaked 200 seeds of Pisum sativum L., cultivar. Kelvedon Wonder, under 15 cm of tap water in 500 ml Erlenmeyer flasks at 20°C in darkness. It was found that, when the seeds were sown out after 48 hours treatment, the seedlings which developed from these seeds were often abnormal. The abnormalities included damage to the terminal bud (resulting in the loss of apical dominance), alterations in leaf shape, and general slight chlorosis. This report suggested that the normal course of germination and development in peas can be altered by exposing the seeds, during germination, to conditions which affect aerobic respiration. The tissues most severely affected by the treatment appeared to be the meristems

within the embryo. These observations were taken up here and the work elaborated in an attempt to discover the underlying cause of the effects. The first part of the thesis deals with the nature and scope of the problem.

MATERIALS AND METHODS.

Supplies of pea seed were obtained from McGill and Smith Ltd., Ayr. The seeds had not been treated with a commercial seed dressing but, before each experiment, all seeds used were surface sterilised for 15 minutes with 0.1% mercuric chloride then washed 3 times with sterile water. 50 seeds were placed in a 100 ml Erlenmeyer flask filled with distilled water. Each flask was sealed by pouring 2 cm of molten paraffin wax directly on the surface of the water. This wax plug kept the flasks air-tight during the soaking treatment.

The sealed flasks were placed in a thermostatically controlled electric incubator, generally at 20°C, along with control seeds sown without any soaking treatment in moist vermiculite or peralite.

Vermiculite, peralite, sand and soil were all considered as media for growing the plants. Soil was not used because of its variability and its capacity for providing the plants with a supply of nutrients. Sand was tested but it was found to be unsuitable for these experiments because of its rather poor water holding ability.

Vermiculite is a preparation from mica, composed of

hydrous silicates arranged in foliated scales. It is used extensively for insulation purposes and has many successful applications in horticulture. The vermiculite used in these experiments was generally satisfactory but latterly it was found that certain supplies were not conducive to successful plant growth: the roots of the plants were discoloured and often translucent. This may have been due to the physical properties of the vermiculite or to the presence of toxic materials.

Peralite is a siliceous material rather like sand but somewhat softer in texture and much lighter. It is commonly used in the building trade and was supplied by British Plaster and Boards Ltd., Carlisle. Peralite proved much more reliable than vermiculite. There were no harmful effects on the root system in any experiment and therefore, peralite was used exclusively in the later experiments.

The treated seeds were taken from the flasks, washed 3 times in tap water and sown out in either boxes or plastic pots (size 48, supplied by Pepgro Ltd., Emsworth, Hants.). The combination of peralite and plastic pots (25 seeds per pot) was found to be the most successful way of growing the plants. In order to make the conditions uniform in all experiments, water was added to each of the pots in such a way that each one was brought to a constant weight.

The seeds were allowed to germinate and develop for a

suitable period (usually 7 days from the start of the experiment); then appropriate observations and measurements were made.

EXPERIMENTAL RESULTS.

Preliminary experiments showed that, when seeds of the field pea, Pisum arvense L., cultivar. Maple, the garden pea, Pisum sativum L., cultivar. Kelvedon Wonder and the tomato, Lycopersicum esculentum Mill., cultivar. Ailsa Craig, were soaked anaerobically the seedlings grown from these seeds were generally not so vigorous as those grown from similar seeds given no soaking.

TABLE 1.

Mean dry weight (mg) of tomato and pea plants grown from seeds given various soaking treatments before sowing.

	Hours soaking					Age of Plants at harvest
	0	24	48	72	96	
Tomato	961	775	590	-	592	9 weeks
Garden Pea	58	49	23	-	-	25 days
Field Pea	168	135	-	117	-	25 days

The shoots of all plants in those early experiments were carefully examined for the morphological aberrations described and illustrated by Berrie (1960). No morphological aberrations were observed in tomato plants even after 7 days soaking.

Aberrant Maple pea shoots occurred only sporadically after 24 hours soaking but, when the soaking period was extended to 72 hours there was a slight increase.

TABLE 2.

The frequency of occurrence of shoot damage in seedlings of Maple pea grown from seeds soaked anaerobically before sowing.

Hrs. soaking	No. seedlings	No. abnormal shoots
0	161	3
24	163	5
48	161	7
72	164	15

Kelvedon Wonder pea seeds were much more susceptible to soaking, complete death of the seeds frequently occurring when the soaking period extended to 3 days. Damage to the shoot occurred fairly regularly after 2 days soaking but, even in this species, the criterion was not reliable enough to be used as a measure of the severity of the effects of the treatment.

If, as Berrie suggests, damage is due to an upset within the apical meristem of the embryonic axis it seemed that it would be of interest to examine other zones of meristematic activity in the young plant. The first part of the embryo to protrude from the seed is the radicle: meristematic activity

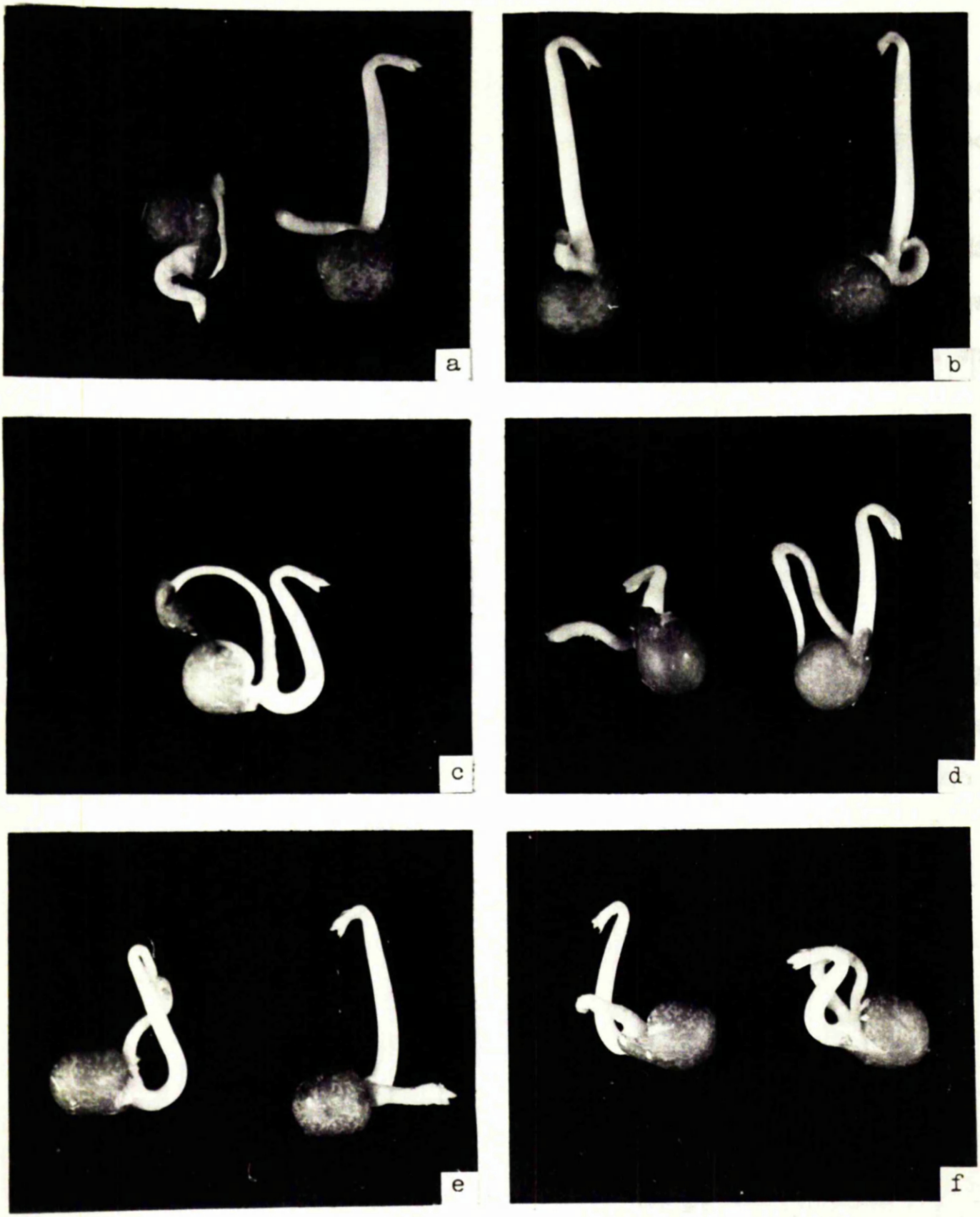


PLATE 2

7 day old Maple pea seedlings. A selection of typical seedlings from seeds subjected to 3 days soaking before sowing. The photographs show some of the abnormalities produced in the radicles.

resumes there before it does so in the shoot, hence one would expect that morphological damage might occur in the radicle first. This was found to be so and aberrations occurred with such high frequency when Maple pea seeds were soaked for upwards of 70 hours that the radicle seemed a very suitable part of the plant in which to investigate the phenomenon.

Before discussing radicle aberrations further we must be able to recognise clearly the feature or features considered to constitute damage. Plate 2 illustrates types of abnormality regularly found and such are classified as "damaged". Although this is a subjective measure of the effect of soaking, a rigorous definition of damage affords a basis for scoring seedlings and helps restrict personal choice. Damage consists of one or more of the following abnormalities:-

1. The radicle is considerably shorter than the radicle of a seedling grown from a control seed.
2. The diameter of the radicle may be substantially increased.
3. Where the radicle is longer than 5 mm it is liable to be excessively curled (Plate 2 (f)).
4. The radicle may exhibit a negative geotropic response, very often growing upwards alongside the shoot (Plate 2 (e)).
5. In almost every instance the damaged radicle is clearly distinguishable by the fact that there is apparently

no terminal growing point: the distal end of the radicle is almost always sharply truncated (Note that this is true of all radicle tips illustrated in Plate 2).

6. There may be a longitudinal depression running the entire length of the radicle. This, in extreme cases, divides the radicle into 2 giving a forked appearance.
7. If the radicle grows longer than 5 mm there is generally profuse production of lateral roots which extend right to the distal end of the radicle. (in control seedlings lateral roots are seldom present in the lower 5 cm of the radicle).

If the tip of a radicle is mechanically damaged or removed altogether, many of these abnormalities may occur. This indicates that the embryonic radicle tip is susceptible to soaking. The illustrations in Plate A (Appendix I) show that, after prolonged soaking, the meristematic tissues of the radicle are moribund.

In addition to using radicle damage as a measure of soaking effects, an objective means of assessing the severity of the treatment must be derived. The most suitable way to do this is to measure the length of the radicles. Although this procedure is reasonably convenient, it cannot be completely free from criticism. It takes considerable time, and therefore to prevent the seedlings measured last having anomalously

high values, all plants must be harvested at the same time and preserved in 70% alcohol. Accurate measurement is difficult in the case of excessively curled radicles, unravelling the contortions of the root often leading to breakage and a consequent reduction in the accuracy of the results.

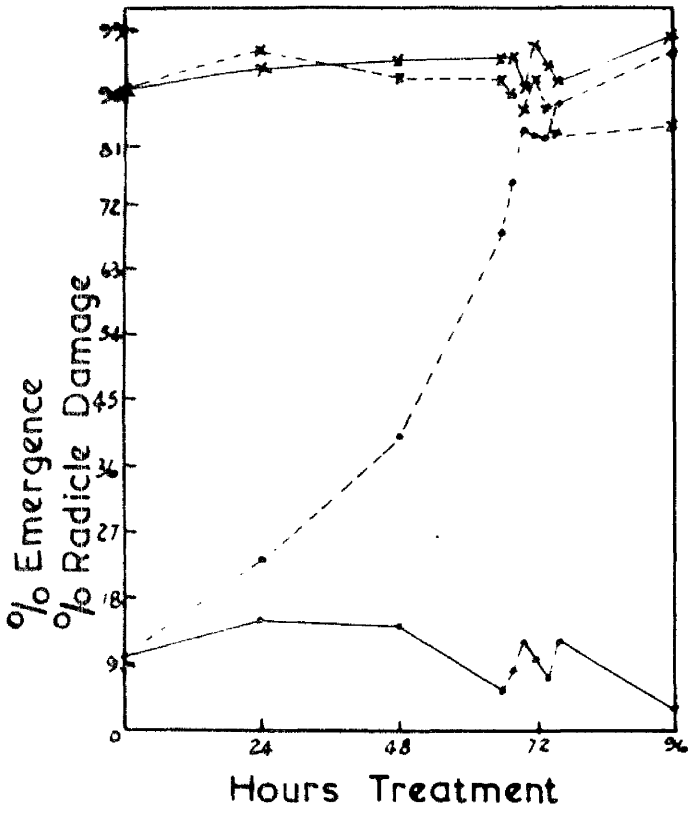
The most serious objection to using root length, however, is that often a radicle is reasonably long yet has a definite morphological aberration at its distal end or is excessively curled. Although such a radicle is abnormal, its length may be close to that of a control and thus the effects of the treatment can not be brought out clearly.

If, however, radicle length and radicle damage are recorded together, a truer picture of the effects of the treatment are displayed.

Experiment 1. The effects of various periods of soaking on the germination and development of Maple pea.

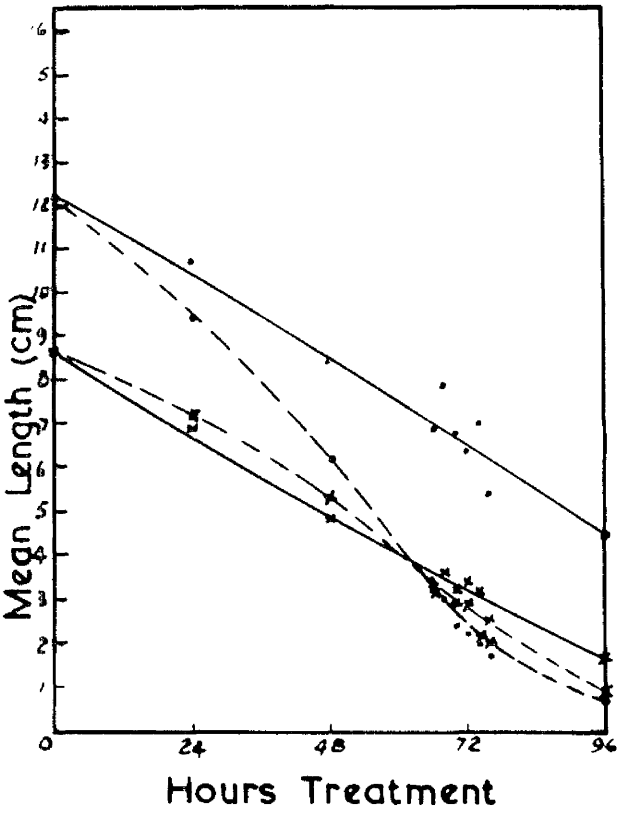
Preliminary experiments gave the impression that anaerobic soaking for up to 48 hours has little effect on the germination and development of Maple peas but, when the soaking period is extended to 72 hours the effects are quite striking. This experiment was designed to test and amplify these earlier findings.

The soaking periods selected were 24, 48, 66, 68, 70, 72, 74, 76 and 96 hours. 2 flasks, each containing 50 seeds were



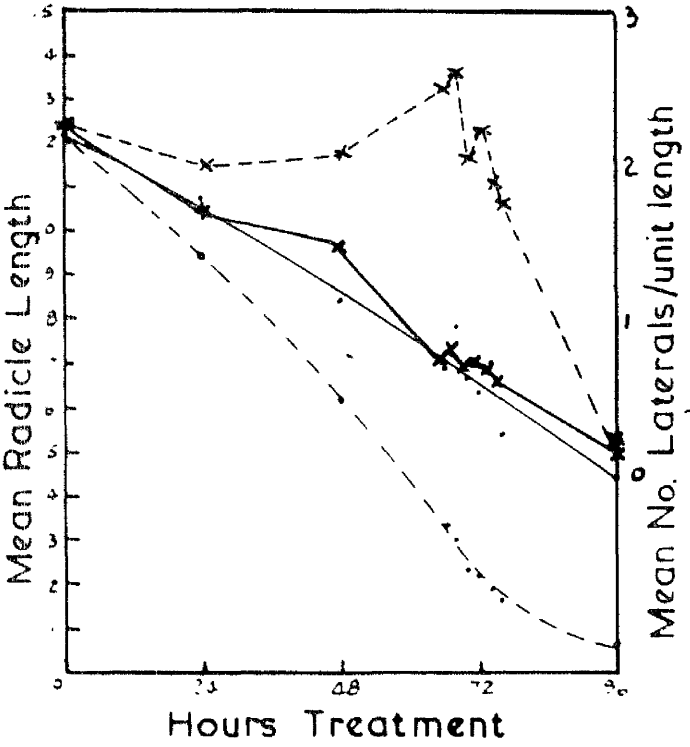
(a)

Comparison of the effects of soaking on % emergence (x) and % radicle damage (·)



(b)

Comparison of shoot length(x) and radicle length(·)



(c)

Relationship between the number of laterals per unit length(x) and mean radicle length(·)

FIGURE 1.

Some of the criteria considered for use as measures of the effects of soaking on the germination and development of Maple Pea.

Control seedlings

Seedlings from soaked seeds

set up for each soaking treatment. After soaking, the seeds in each flask were divided into samples of 25 and sown out. Control seeds were sown directly at the beginning of the experiment and also on each occasion when soaked seeds were sown out. 7 days after the start of the experiment the plants were harvested and scored for percentage emergence, shoot length, radicle length, radicle damage and also for the number of lateral roots per unit radicle length (Table 3).

Presentation of these results in graphical form (Figure 1) shows that percentage emergence, shoot length and mean number of lateral roots per unit length cannot be taken as criteria for assessing the effects of the treatment.

Percentage emergence.

A Chi squared test (X^2) shows that the difference between the number of seedlings emerged in control seeds (851) and the number emerged in treated seeds (806) is statistically significant ($X^2 = 15.38$) but obviously, particularly in the shorter soaking periods, the difference between the number of emerged seedlings from the treated and untreated seeds is too small to allow this to be used as a suitable criterion.

Shoot length.

The graphs of shoot length against treatment for seedlings from soaked and unsoaked seeds lie very close to each other. Only at the 96 hour period is there any marked divergence. Statistical analysis (analysis of variance, see page 165)

Table 3

Some effects of soaking Maple pea seeds manifest in 7 day old seedlings resulting from these seeds.

Time of Soaking (hrs.)	Emergence % S	Emergence % C	Radicle damage % S	Radicle damage % C	Mean shoot length(cm) S	Mean shoot length(cm) C	Mean radicle length (cm) S	Mean radicle length (cm) C	Mean no. laterals per unit length S	Mean no. laterals per unit length C
0	-	91	-	10	-	8.63	-	12.21	-	2.33
24	91	94	23	15	7.05	6.89	9.44	10.67	2.02	1.61
48	96	95	40	14	5.26	4.75	6.11	8.42	2.15	1.46
66	92	95	68	5	3.19	3.07	3.31	6.77	2.52	0.59
68	90	95	75	8	3.35	3.55	3.02	7.79	2.76	0.65
70	88	91	84	12	2.97	3.18	2.33	6.59	2.03	0.54
72	92	97	83	10	2.91	3.44	2.21	6.30	2.37	0.56
74	88	94	82	7	2.22	3.17	1.96	7.01	1.83	0.51
76	84	92	88	12	2.01	2.54	1.57	5.29	1.76	0.41
96	85	98	96	3	0.75	1.60	0.56	4.44	0.03	0.28
Total	806	851	639	86	29.71	32.19	30.51	63.28	17.47	6.61

S = Soaked. C = Control.

shows that in the experiment as a whole there are significant differences among the shoots but, as practically all these differences can be attributed to the 96 hour soaking period, it was decided that the differences over all were not sufficient to warrant selection of shoot length as a criterion for measuring soaking effects.

Mean number of lateral roots per unit length of radicle.

The results for mean number of lateral roots per unit length do not show a pattern definite enough to be of value in estimating the effects of the treatment. Generally, in control seedlings, the mean number of laterals per unit length is directly proportional to the length of the radicle (Figure 1 c). In seedlings from treated seeds, because of the shorter radicles and laterals extending right to the distal ends of the radicles, the number per unit length is high compared with the controls. This pattern does not, however, hold till it reaches its conclusion since at the 96 hour period the radicles are so short and poorly developed that there are very few laterals. An increased number of lateral roots per unit length cannot, therefore, be selected as a simple feature indicating damage of the seedling.

This criterion can also be criticised in that the number of lateral roots taken alone is not an accurate measure of the potential of the plant to produce a new root system. The control seedlings, for example, have 2.33 laterals per unit

length while those soaked for 72 hours have 2.37. Although these 2 figures are practically identical the root systems are vastly different. The controls have a much greater absolute number of laterals which are also longer than the laterals of seedlings from treated seeds. The figure 2.37 is in fact largely a reflection of the shorter length of the radicle itself.

From this experiment we conclude that radicle length and radicle damage are the most suitable and reliable criteria for measuring the effects of soaking (Figure 1 a and b). A difference in radicle lengths between seedlings from treated and untreated seeds is apparent at 24 hours and this difference increases with increasing length of the soaking period.

Radicle damage, bearing in mind the reservations already mentioned, appears from this experiment to be a particularly useful subjective means of assessing the effects of the treatment. There is a difference of 8% between seedlings from treated and untreated seeds at 24 hours and this increases to 73% at 72 hours and 93% at 96 hours. Such differences are obviously of considerable value in discussing the results.

If radicle length is accepted as an objective means of determining the effects the data can be used to ascertain which factors in the experiment are acting to produce the differences which occur. These quantitative data can be analysed statistically to give a concise picture of the results.

There is in this experiment, a wide variation in the lengths of individual radicles. Such variation may be due to (a) the inherent variability of the seeds, (b) the experimental methods or (c) the treatments employed. An analysis of variance table shows where this variation lies and so allows us to decide (within arbitrarily fixed limits) whether indeed it is the actual treatments which are responsible for the differences obtained. A specimen analysis of variance is shown in Appendix II.

In the analysis, all ungerminated seeds are scored as having a radicle length of 0 cm. Other values could have been given to these seeds by using one of the following methods:-

(a) The missing plant formula (Yates, 1933).

(b) Substitution of the mean value of the respective population for each missing datum.

Each of these would have avoided any zero value but, since the ultimate adverse effect of soaking is failure of the seed to germinate and since there is a higher frequency of ungerminated seeds among the treated seeds than among the controls, it is more reasonable to assign a value of 0 cm to each ungerminated seed.

TABLE 4.

Analysis of variance table: radicle lengths of seedlings grown from soaked and unsoaked Maple pea seeds.

Source of Variation	DF	Sum of Sqq.	Mean Sq.	F
Replicates	3	29.72	9.91	1.09
Times	9	16,720.19	1,857.79	205.96**
Treatments	1	5,695.99	5,695.99	631.48**
Interaction:- Times T'ments	9	1,138.96	126.55	14.03**
Residual	1977	17,827.39	9.02	
Total	1999	41,412.25		

Table 4 shows that (at P = 5% and 1%) there are significant differences among the plants due to time differences and due to the soaking treatment. The statistical interaction between these 2 sources is also significant thus indicating that the longer the seeds are soaked the greater will be the suppressing effect on the length of the radicle.

There are no significant differences among replicates which confirms that such possible sources of variation as the plastic pots, the position of the pots in the incubator, the Erlenmeyer flasks and the growing medium have no significant influence on the degree of soaking damage.

It was observed that there were considerable differences

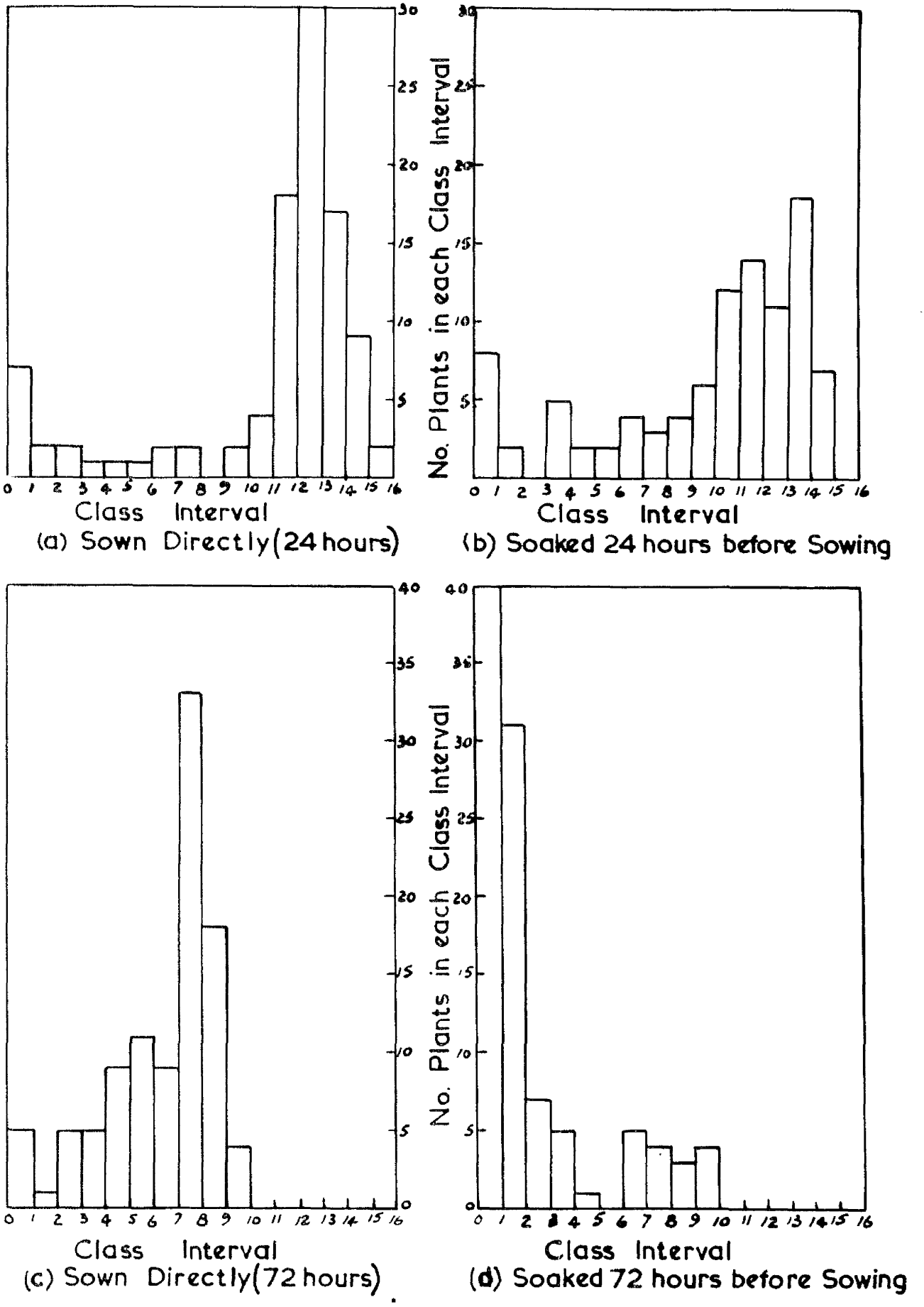


FIGURE 2

Division of radicle lengths of seedlings from soaked and unsoaked Maple peas into class intervals.(cm). Note differences between populations of seedlings from soaked and unsoaked seeds

among plants within certain treatments. This may be illustrated by selection of several of the soaking periods and the erection of suitable class intervals into which the radicle lengths are classified. All results for the 24 and 72 hour period were pooled giving 100 observations for seeds soaked for 24 hours, 100 for seeds soaked for 72 hours and 100 each for seeds sown out at the same times as these soaked seeds were sown out. Figure 2 a shows that when the seeds are sown directly and grown for 6 days there is a concentration of radicle lengths around the 11-13 cm mark and a long tail giving a negatively skew distribution. When the seeds are soaked for 24 hours before sowing, however, and grown for 6 days, there is still a concentration around the 11-13 cm mark but it is beginning to spread out and the tail becomes much more prominent. It appears in fact, that the population of seedlings is dividing into 2.

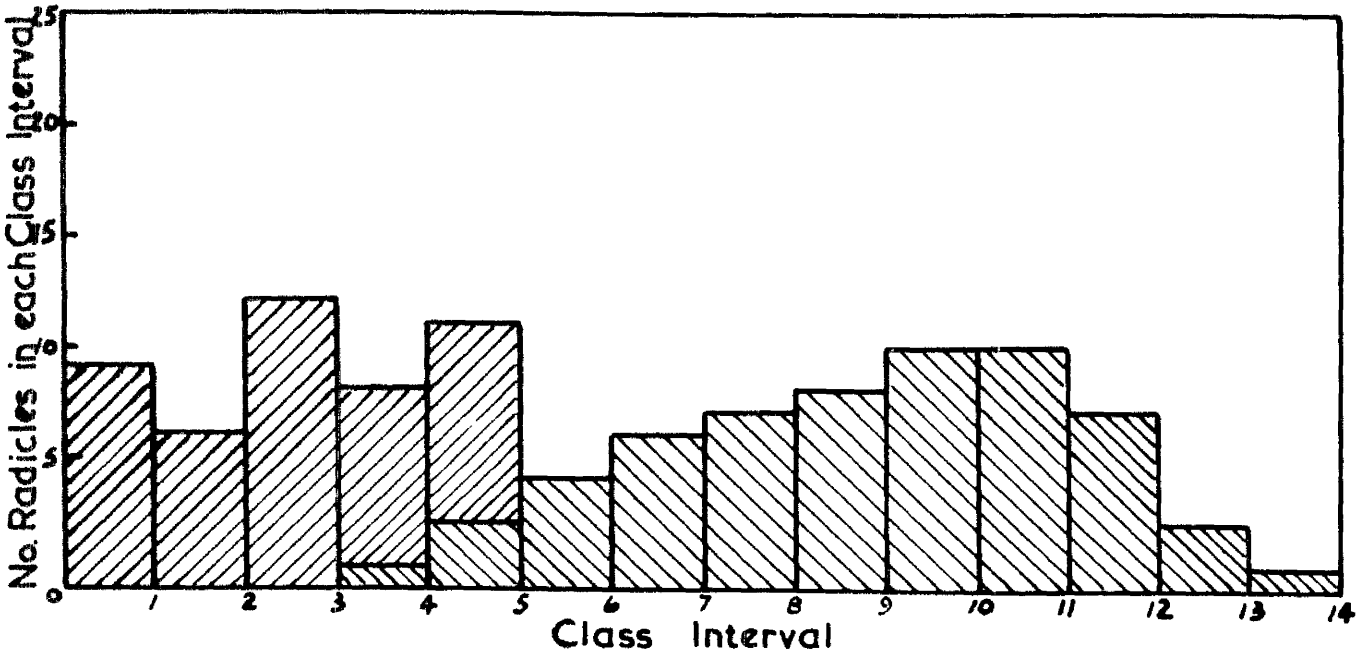
When the soaking period extends to 72 hours (Figure 2 d) the population has divided into 2. There is a large sub-population of seedlings with radicles less than 4 cm long and another smaller, but well-defined sub-population of seedlings with radicles between 6 cm and 10 cm. When the soaking period stretches to 96 hours, the small population of seedlings with longer radicles disappears almost completely and the population again becomes much more homogeneous with virtually all radicles shorter than 1 cm.

Because of this division of radicles within one treatment into 2 sub-populations of differing size, the variance of the population is high (the radicle lengths are not normally distributed); the values for 0, 24, 48, 72 and 96 hours soaking are 20.25, 20.09, 14.06, 6.89 and 0.78 respectively. Much of this variation can be attributed to the inclusion of the value 0 cm for the ungerminated seeds in the calculations.

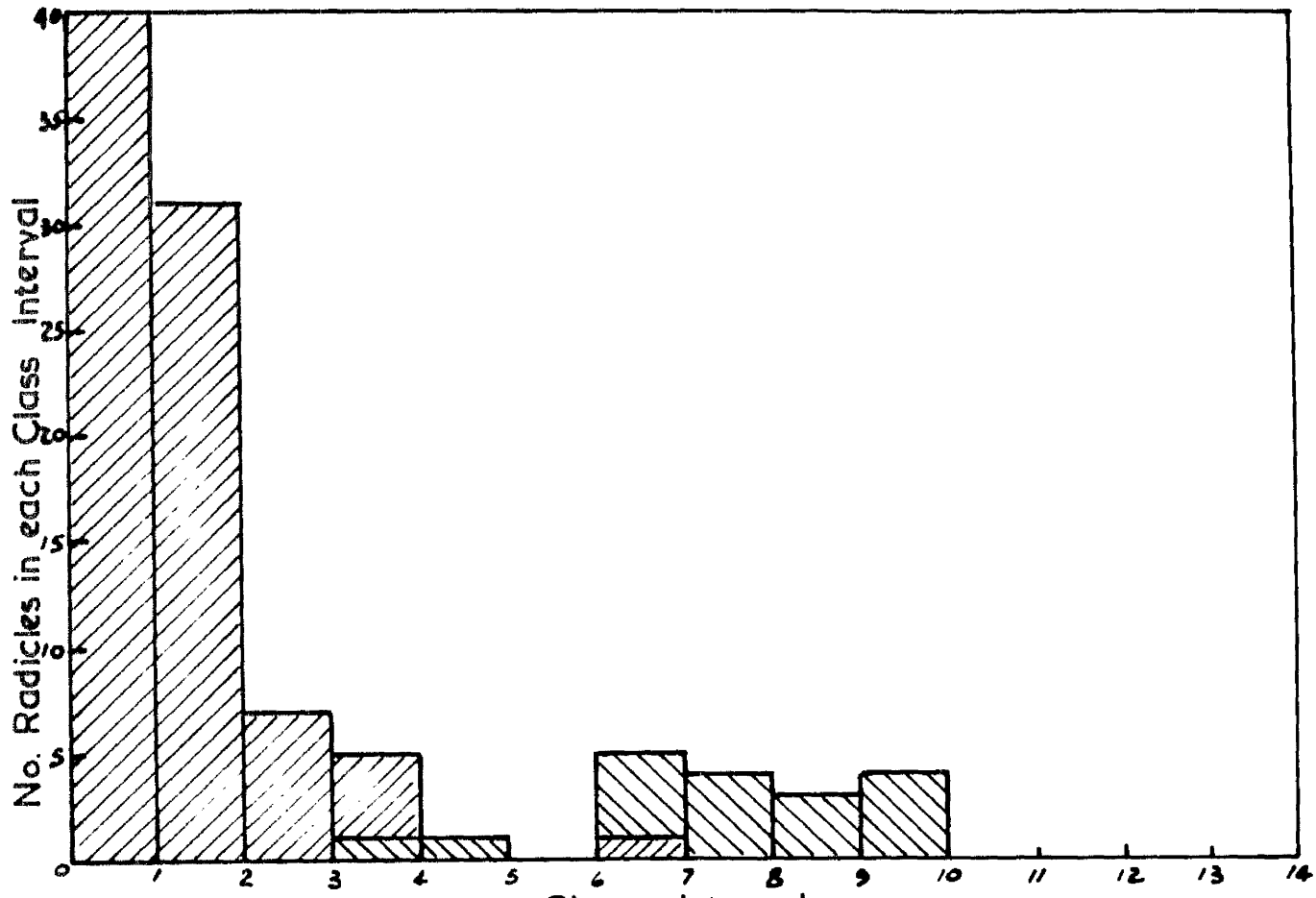
The statistical implication of this is that the wide variation within treatments may obscure real differences between treatments. Cochran (1938) has shown that this can occur when the residual mean square includes a large proportion of within treatments variation. To avoid this anomaly, the original data can sometimes be transformed in such a way that the within treatments variation is considerably reduced (see Appendix II). In this experiment, however, the differences among treatments are so large that they are significantly different from one another despite the high intra-treatment variation.

The connection between radicle damage and radicle length

If the 2 criteria selected as a means of assessing the effects of soaking, i.e. radicle length and radicle damage, are acceptable there should be a reasonable correlation between them. The 48 and 72 hour soaking periods are chosen as suitable examples in which to make this comparison. In each



(a) 48 Hours Soaking



(b) 72 Hours Soaking

FIGURE 3

The connection between radicle length and damage in Maple pea seedlings after soaking the seeds for 48 and 72 hours. The number of normal seedlings (▨) and the number of damaged seedlings (▨) in each class interval is superimposed on histograms drawn for the total number of radicles in each class interval (cm).

case the damaged radicles are considered alone and divided into categories depending on the lengths of the radicles. The numbers in each class are superimposed on a histogram drawn up as in Figure 2 for the class intervals of radicle length alone. The normal radicles are treated similarly and the numbers of these in each class interval plotted on the same histogram (Figure 3). It is quite clear from the histograms for both the 48 and 72 hour soaking periods that the 2 sub-populations are attributable to a group of seedlings with normal radicles and one of seedlings with damaged radicles. Where the 2 sub-populations meet and overlap slightly (at 3-5 cm for 48 hours and 3-7 cm for 72 hours) there may be difficulty in judging between a damaged seedling and a normal one. Apart from this, the division appears clearly discernible.

. . .

The data in Table 4 established that time and soaking treatment both have a definite influence on the development of pea seedlings. Since the seeds were all sown out at different times it is not surprising that there were differences in radicle length. When the seeds are soaked for 72 hours, for example, the radicle length is much reduced compared with the lengths of controls sown directly both at the beginning of the experiment and when the soaked seeds are sown out. It is now essential to confirm that the suppression of radicle length can be attributed not only to time differences but also to the

soaking treatment.

The suppression of radicle length may be due to one or both of 2 factors:-

(a) A simple retardation of the germination processes during soaking.

(b) A partial or complete disruption of the metabolic processes involved in germination.

If the former supplies the complete explanation, it should be possible to soak seeds for various periods, sow them all out at the same time and demonstrate that, when they are harvested together, the seedlings exhibit no differences in radicle length. This is the basis of the next experiment which involves soaking seeds for different periods, sowing them all at the same time and harvesting all seedlings together.

Experiment 2. The effects of various periods of soaking on the germination and development of Maple pea, all soaked seeds being sown out at the same time.

Initially, 4 flasks each containing 50 seeds Maple pea, were placed in an incubator at 20°C. This was repeated on each of the following 3 days and the next day all 16 flasks were removed. 4 flasks had thus received 96 hours soaking, 4 - 72 hours soaking, 4 - 48 hours soaking and 4 - 24 hours soaking. The contents of each set of 4 flasks were pooled and 6 pots were sown out, each with 25 seeds, the remainder being discarded.

Similarly 150 seeds were sown directly in 6 pots.

3 days later, half of the seedlings were harvested and scored.

TABLE 5.

Some effects of various periods of soaking on Maple peas manifest after 3 days exposure to aerobic conditions, all seeds having been sown out at the same time.

	No. of hours soaking before sowing.				
	0	24	48	72	96
% Damage	10.7	22.7	32.0	78.7	92.0
Mean radicle length (cm)	3.86	4.16	3.89	2.09	0.79
Standard error of mean	0.19	0.17	0.27	0.27	0.25

It is now possible to compare seedlings which have been exposed to aerobic conditions for the same periods of time following different soaking treatments. Obviously, under these conditions also, 72 and 96 hours soaking bring about suppression of radicle growth and a corresponding increase in the frequency of damage.

Table 6 clearly shows that the variation in this experiment is attributable to differences in duration of the soaking period. Reference to Table 5 reveals that these differences lie mainly between the 72 and 96 hour periods on the one hand, and the 0,

24 and 48 hour periods on the other.

TABLE 6.

Analysis of variance: radicle lengths of seedlings grown from seeds of Maple pea soaked for different periods and sown out at the same time.

Source	DF	Sum of Sqq.	Mean Sq.	F
Replicates	2	1.23	0.62	0.18
Treatments	4	645.39	161.35	46.10**
Residual	368	1288.73	3.50	
Total	374	1935.35		

The remaining plants were harvested 7 days after the seeds had been sown out. The results of this part of the experiment are substantially similar to the results recorded earlier (Table 5). Again the 72 and 96 hour soaking periods produced a decided suppression of radicle length and an increase in radicle damage compared with the others.

A "t" test applied to the original data pertaining to these results for radicle length reveals that, at P = 5%, there are no significant differences among the means for the 0, 24 and 48 hour soaking periods. There is, however, a significant difference between the means of the 48 and 72 hour soaking periods and between those of the 72 and 96 hour periods.

TABLE 7.

Some effects of various periods of soaking on Maple peas following 7 days exposure to aerobic conditions, all seeds having been sown together.

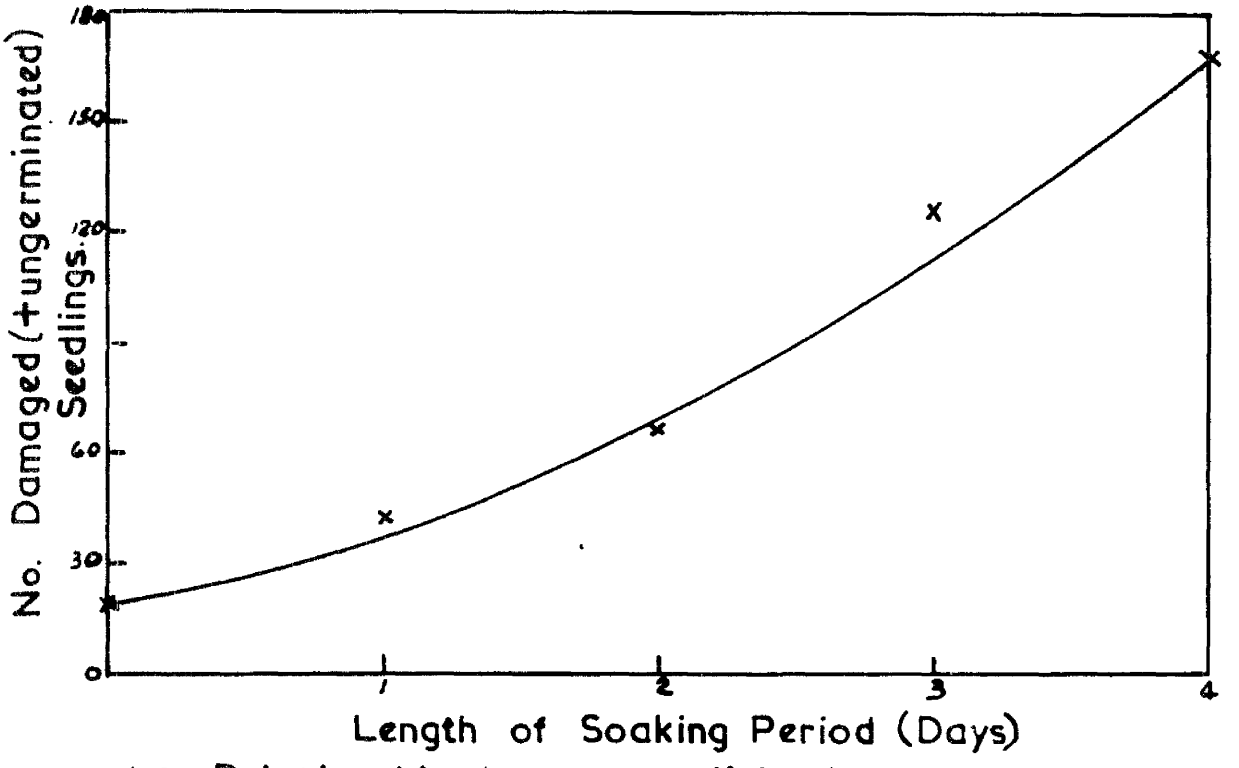
	No. of hours soaking before sowing.				
	0	24	48	72	96
% Damage	9.3	29.3	34.7	57.3	96.0
Mean radicle length (cm)	11.29	9.43	8.19	5.70	0.99
Standard error of mean	0.49	0.60	0.62	0.58	0.27

When these results are compared with those of experiment 1 it can be clearly seen that, if pea seeds are soaked anaerobically for upwards of 3 days, the seedlings produced are not so vigorous as seedlings from seeds sown directly either at the time the seeds are placed in the soaking medium or when they are sown out.

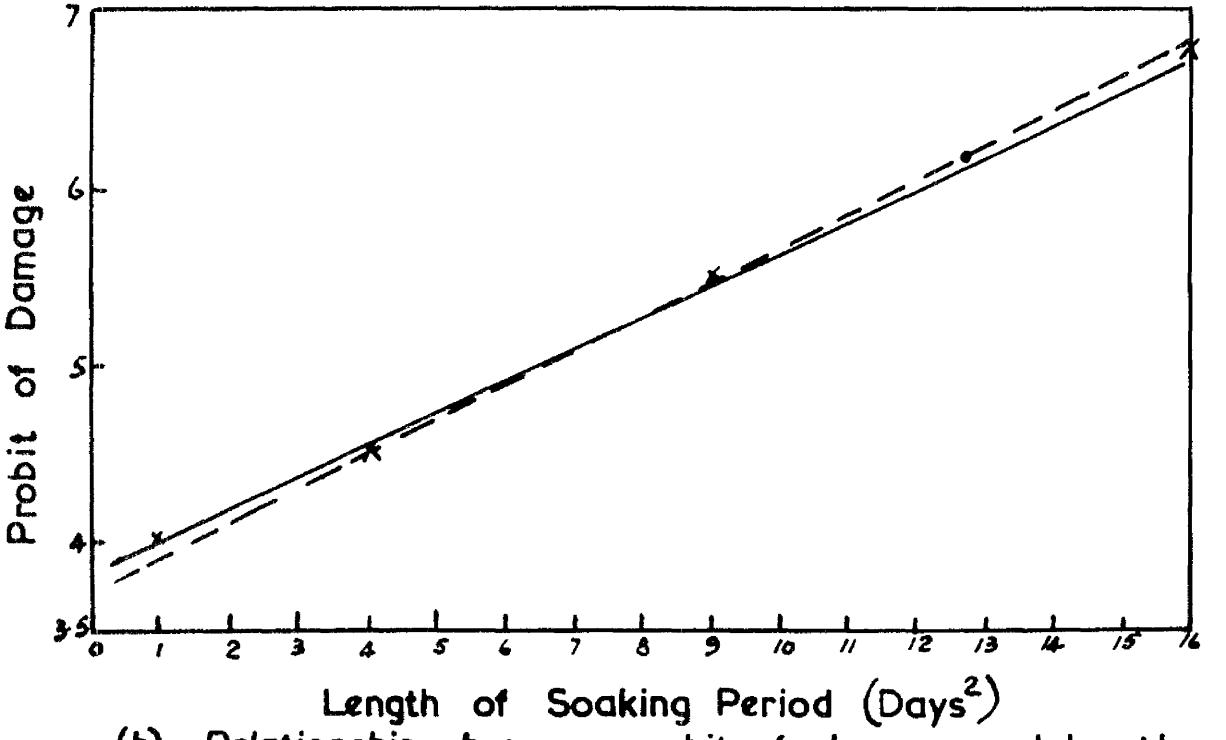
If some of the above data are pooled it is possible to record this conclusion mathematically in 2 ways based on the criteria selected to measure the soaking effects.

(a) Radicle Damage.

The data recording radicle damage can be used to carry out a probit analysis (see Appendix III). This is a statistical method used frequently to assess the value of different toxic



(a) Relationship between radicle damage and length of soaking period.



(b) Relationship between probit of damage and length of soaking period (days²)

FIGURE 4

The frequency of damage produced by soaking seeds of Maple pea for various periods before sowing. The graphs show how the raw data (a) can be transformed to give a linear relationship between the length of the soaking period and the frequency of damage (b). Note that the numbers of damaged seedlings have been converted into the appropriate percentages then into probits

substances, e.g. insecticides and fungicides or the response of individuals to new drugs, vitamins, sera and other forms of stimuli.

In the present instance, it provides a mathematical means by which quantal data can be used to record the observed effects of different soaking periods on seed germination and seedling development. The lengths of the soaking periods are considered comparable to different doses of a particular treatment.

Table 8 records the pooled data of 2 experiments in which seeds were soaked for 0, 24, 48, 72 and 96 hours before sowing. The graph in Figure 4 (a) shows the relationship which holds between the duration of the soaking period and the number of damaged seedlings produced.

It is obvious from Figure 4 (a) that a simple logarithmic transformation of the graph will not produce a straight line and it is necessary to square the values on the abscissa to achieve this. Hence in Table 8 the value of x is λ^2 (or 2 logλ). Figure 4 (b) shows that this transformation produces what is very nearly a straight line (The original data for percentage damage are converted into empirical probits.)

The equation for the probit regression line was calculated from the data in Table 8 and found to be:-

$$y = 3.794 + 0.185x$$

TABLE 8.

The effects of 0-4 days soaking of Maple pea seeds on the extent of damage produced in the seedlings: data required for probit analysis.

λ	$x(=\lambda^2)$	n	r	P'	P(C=10)	Empirical probit
4	16	175	168	96	96	6.75
3	9	175	125	71	68	5.47
2	4	175	66	38	31	4.50
1	1	175	41	23	16	4.01
0	0	175	18	10	0	-

Where λ = dose (in days), n = number of seeds tested, r = number of seeds ungerminated + number of damaged seedlings, P' = total damage %, P(C=10) = total damage %, zero treatment having been converted to 0.

The median effective dose (E.D.50) is the duration of soaking required to produce 50% damage and it is calculated from the equation by substituting 5 (the probit value equivalent to 50%) for y and solving for x. This gives the E.D.50 for the transformed data and, of course, the square root of this value gives the actual E.D.50, viz. 61.2 hours.

The E.D.50 is the value which can be most accurately assessed from the probit regression equation but it is interesting to note that the E.D.75 is 76.7 hours: similarly,

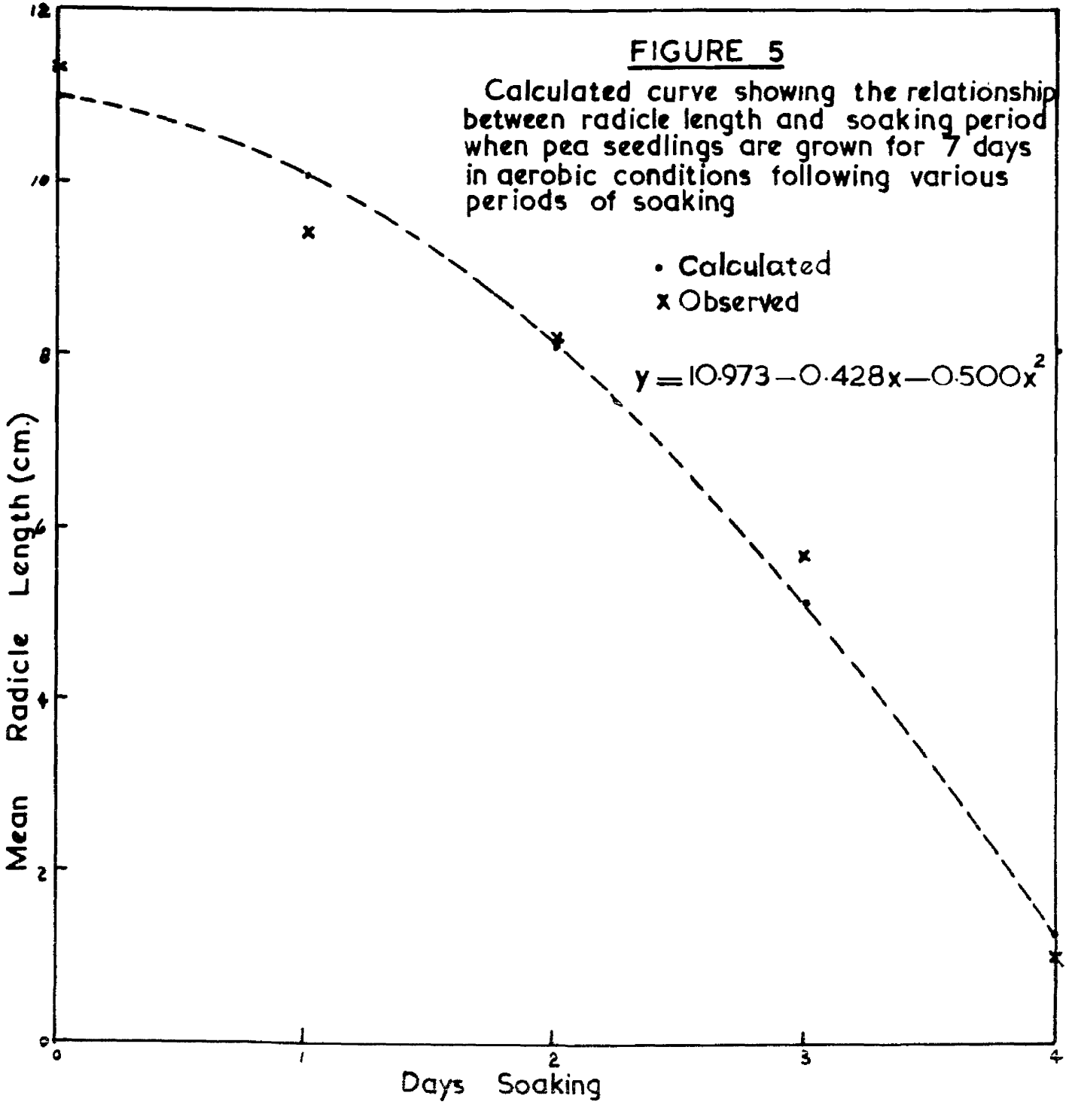


FIGURE 5
Calculated curve showing the relationship between radicle length and soaking period when pea seedlings are grown for 7 days in aerobic conditions following various periods of soaking
• Calculated
x Observed
$y = 10.973 - 0.428x - 0.500x^2$
Mean Radicle Length (cm.)
Days Soaking
0
2
4
6
8
10
12
0
1
2
3
4

the percentage damage which might be expected after 72 hours soaking is 67.4.

(b) Radicle length. Estimation of the regression line.

Regression is the statistical term used to describe the dependence of one variate on another.

The data of Experiment 2 were used to determine the form of the regression line. A detailed description of the method used is given in Appendix IV.

We must first determine whether the regression is linear or polynomial. This is done by carrying out an analysis of variance in which the variation due to the length of the soaking period is partitioned into (a) that attributable to linear regression and (b) the remainder.

It was found (see page 181) that a linear regression could not account for all the variation; hence it was necessary to partition the remainder sum of squares to determine the power of the polynomial equation required to describe the data.

The complete analysis is shown in Table 9. We can conclude from the analysis that a term in x^2 must be included in the equation derived to describe the regression line.

It is possible from the data to derive the equation which most closely fits the results of the experiment and Figure 5 shows the observed data superimposed on a curve drawn according to the calculated equation:-

$$y = 10.973 - 0.428x - 0.500x^2$$

TABLE 9.

Analysis of variance table: radicle lengths of Maple pea seedlings grown for 7 days aerobically after the seeds had received various soaking treatments.

Source	DF	Sum of Sqq.	Mean Sq.	F
Replicates	2	3.87	1.94	0.09
Treatments				
a) Linear	1	4,420.42	4,420.42	209.20**
b) Quadratic	1	262.70	262.70	12.43**
c) Cubic	1	59.58	59.58	2.82
d) Quartic	1	0.87	0.87	0.04
Residual	368	7,776.68	21.13	
Total	374	12,524.12		

This may be regarded as a true mathematical picture of the effects of soaking when the radicles are measured 7 days after exposure to aerobic conditions following the soaking treatment. The calculated curve confirms the conclusion that soaking for up to 48 hours has only a slight depressing effect on the lengths of the radicles but thereafter the effect is considerably increased.

It is interesting to compare the results of the regression with those of the probit analysis. In the latter analysis, the values on the abscissa (i.e. the soaking times) had to be squared to give a linear relationship between soaking times (x)

and the number of damaged radicles (y). An equation representing the line before transformation would therefore probably include a term in x^2, which is similar to that correlating soaking period and radicle length.

In the case of radicle damage the E.D.50 is 61.2 hours. If we turn to the line describing the relationship between duration of soaking and radicle length, the soaking period required to reduce the radicle length by 50% is found to be 68.4 hours.

The data of experiment 1 can also be used to derive regression lines. If the results for seeds sown directly at daily intervals are considered separately, an abbreviated analysis of variance table showing the relevant mean squares can be drawn up as in Table 10.

TABLE 10.

Analysis of variance table: radicle lengths of Maple pea seedlings grown from seeds sown directly at different times.

Source	DF	Mean Sq.	F
Time			
a) Linear	1	4,108.32	207.59**
b) Remainder	3	2.35	0.12
Residual	492	19.79	

Obviously the linear component of the Times sum of squares is the only significant one, therefore a straight line equation is sufficient to describe the data.

This was found to be:-

$$y = 12.494 - 2.027x$$

The results for the radicle lengths of seedlings from soaked seeds can be treated similarly.

TABLE 11.

Analysis of variance table: radicle lengths of Maple pea seedlings grown from seeds soaked for various periods before sowing.

Source	DF	Mean Sq.	F
Time			
a) Linear	1	9,538.83	767.40**
b) Quadratic	1	29.06	2.34
c) Cubic	1	69.96	5.63*
d) Quartic	1	12.68	1.02
Residual	492		

The F values can be interpreted as showing that a cubic equation will give a somewhat better fit than a straight line, the calculated equation being:-

$$y = 12.341 - 1.764x - 1.182x^2 + 0.221x^3$$

The calculated curves for these 2 equations are shown in Figure 6 which also includes the calculated curve representing the data of experiment 2.

The equations and calculated curves can be used to discriminate between the 2 possibilities put forward earlier viz.

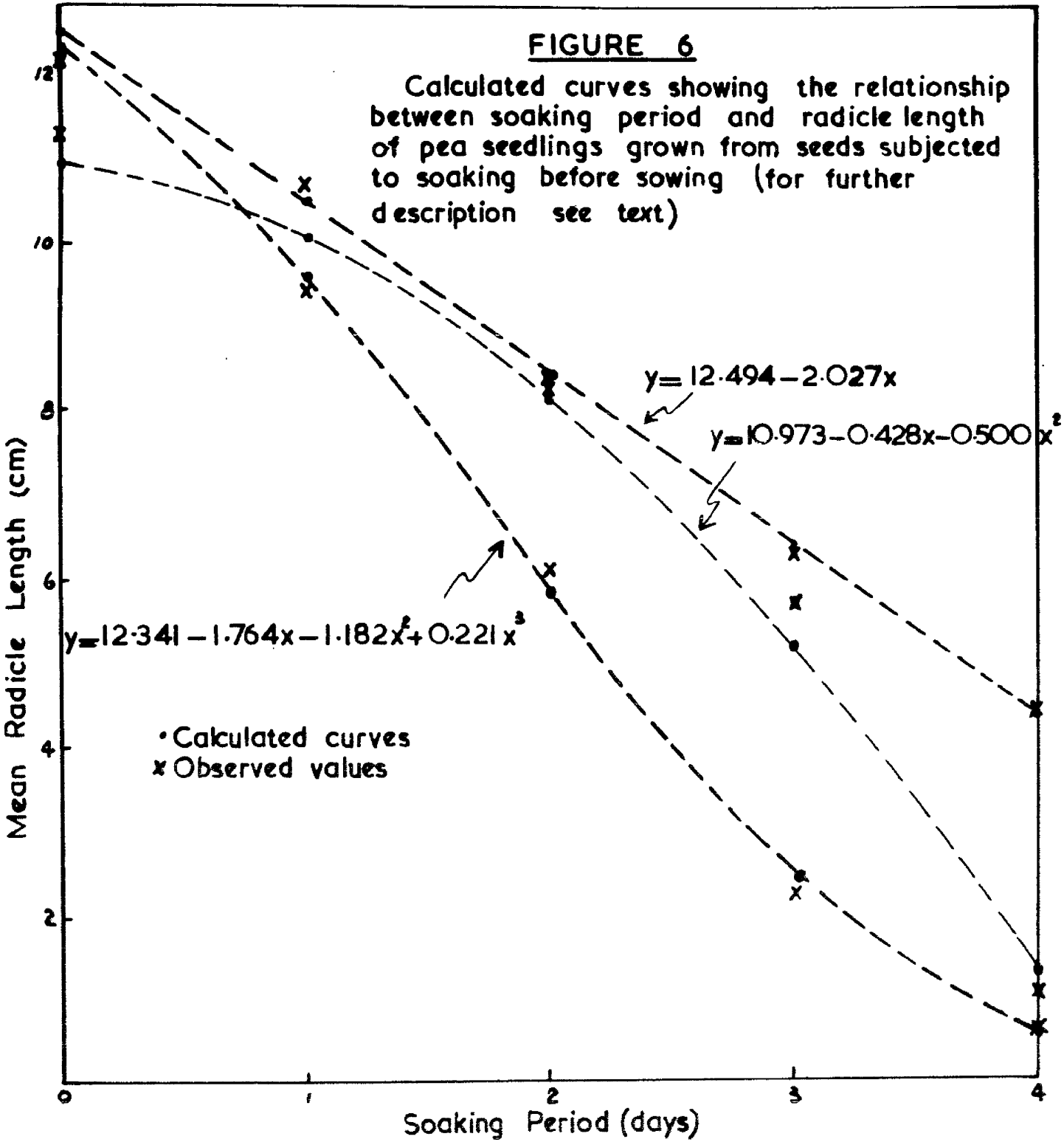


FIGURE 6
Calculated curves showing the relationship between soaking period and radicle length of pea seedlings grown from seeds subjected to soaking before sowing (for further description see text)
y= 12·494 − 2·027x
y=10·973 − 0·428x − 0·500x²
y=12·341 − 1·764x − 1·182x² + 0·221x³
• Calculated curves
x Observed values
Mean Radicle Length (cm)
Soaking Period (days)
0
1
2
3
4
2
4
6
8
10
12

(a) Soaking retards germination.

(b) Soaking upsets germination.

Let the curves be represented as follows:-

$$1.\ y = 12.494 - 2.027x$$

The points represent 7, 6, 5, 4 and 3 day old seedlings.

$$2.\ y = 10.973 - 0.428x - 0.500x^2$$

The points represent 0, 1, 2, 3 and 4 days soaking + 7 days aerobic germination.

$$3.\ y = 12.341 - 1.764x - 1.182x^2 + 0.221x^3$$

The points represent 0 days soaking + 7 days aerobic germination, 1 + 6, 2 + 5, 3 + 4 and 4 + 3.

Consider curves 1 and 3.

1. The equations are of a different form, hence the pattern of growth is not the same in seedlings from soaked and unsoaked seeds. If soaking only delayed germination, the curves would have been straight lines running on top of each other.

11. The difference between seedlings from soaked and unsoaked seeds increases with increasing length of the soaking period. This can best be shown by comparing the ratios (Table 12).

The large differences shown in Table 12 cannot be accounted for simply by assuming that soaking delays germination.

TABLE 12.

The ratio of radicle length of seedlings from unsoaked seeds to that of radicle length of seedlings from soaked seeds.

Soaking Period (Days)	Ratio (Unsoaked : Soaked)
0	1.00
1	1.09
2	1.45
3	2.78
4	8.80

Consider curve 2.

All seeds were sown out at the same time, hence if nothing happened during the soaking period all the seedlings should have possessed radicles of the same length and the curve would have been a straight line parallel to the abscissa, $y = 10.973$. This is not the case, hence the differences shown by the curve are real differences reflecting the effects of the treatment.

Clearly we cannot explain the observed effects simply by postulating that during the soaking period the processes of germination are held up until aerobic conditions are restored. We must consider the soaking period as part of germination and, obviously, this early part of germination is considerably disrupted by the soaking process.

Discussion of Part 1.

The biological implication of the results in Part 1 is that anaerobic soaking interrupts the normal germination and development of Maple pea. No attempt is made in this Part to investigate the reasons for the disruption but it is possible to draw several conclusions from the results.

Early workers in this field (Kidd and West 1918 a, b; 1919 a, b, c and d; Tilford, Able and Hibbard 1924; Rhine 1924; Barton 1929; Bailey 1933) were concerned only with either the final yield of seedlings from soaked seeds or with the germination or non-germination of seeds after various soaking treatments. There is no mention in the literature, so far as we are aware, of abnormalities in the radicle like those described here and shown in Plate 2.

A possible cause of the abnormalities which was considered was that bacteria were responsible. An experiment was carried out where the seeds were given several surface sterilization treatments then soaked under sterile conditions. It was found that even under the most carefully controlled sterile conditions, damage was just as great as in the controls. The type of damage which does occur is also unlike that which would be expected after bacterial attack. It is therefore unlikely that bacteria play any part in determining the degree of damage caused

by soaking.

The results in Part 1 give more support to the hypothesis put forward by Kidd and West (1918 a) that soaking has a physiologically predetermining influence on germination and development. Since it has been established that germination is proceeding during the soaking period it is probable that it is this biological process which is upset by the soaking. The first part of germination is simply the uptake of water by a physical process and the seeds can presumably do this equally well under aerobic and anaerobic conditions. Therefore it is to be expected that, when seeds are soaked for a period during which imbibition alone is proceeding, little damage will result. When, however, germination is completed and development begins, oxygen is required to allow the cells of the seed to respire aerobically and so provide the large amounts of energy required for metabolic reactions to proceed normally. It is possible that when the soaking period extends beyond the germination time the anaerobic conditions prevent the stimulation of these reactions, thus disrupting the normal development of the seedling. One mechanism that could be upset by the treatment is that of cell division itself and a few preliminary investigations showed that it is probably delayed by soaking. Cell division in the normal seedling begins in the radicle before it does so in the shoot. If the process is upset by the soaking it is probable that the radicle will be more adversely

affected than the shoot and this may be the reason for morphological damage occurring in the radicle with a much greater frequency than it does in the shoot.

The evidence in Part 1 does not allow us to state that cell division or any other specific process is upset by the soaking treatment but it does appear that it is disruption of one or more of the reactions involved in development that is the cause of the observed morphological aberrations.

PART 11.

THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON THE RESPONSE OF PEA SEEDS TO SOAKING.

Anaerobic soaking of Maple pea seeds at 20°C was shown in Part 1 to adversely affect the growth of the resulting plants. The data recorded in this part are results of investigations made into several aspects of the metabolism of the seeds during soaking.

The response of pea seeds to soaking at different temperatures

Most aspects of plant metabolism are temperature dependent; hence, if different responses are observed when seeds are soaked at different temperatures, it is likely that soaking upsets one or more aspects of metabolism.

Several pilot experiments revealed that plants grown from seeds soaked at temperatures less than 20°C were not so adversely affected by the treatment. Those experiments were generally carried out as long term projects, the plants being grown in the greenhouse for up to 6 weeks: the differences found were small and could not be used to demonstrate

statistically significant differences between the treatments.

A short term experiment, however, revealed that soaking temperature has an important influence on the response of the seeds to the treatment (Table 13). The data for percentage damage only are recorded but these are sufficient to justify further investigation.

TABLE 13.

The influence of soaking temperature on the frequency of radicle damage manifest in 7 day old seedlings of Maple pea, the seeds having been soaked for 3 days before sowing.

	Sown directly at 20°C	Soaking Temperature °C		
		15	20	25
% Radicle damage	0	6	93	100

The data of the following experiment confirm that soaking temperature is of real importance in determining the response of the seeds.

Experiment 3. The influence of temperature on the effects of soaking Maple pea seeds before sowing.

4 flasks each containing 50 seeds were placed in incubators at 3, 10, 20 and 25°C. After 24 and 72 hours, 2 flasks were removed from each incubator: the contents (100 seeds) were

mixed and 75 seeds were sown out at 20°C in 3 plastic pots. The seedlings grown from these seeds were harvested together 7 days from the start of the experiment.

TABLE 14.

The influence of temperature on the effects of soaking Maple pea seeds for 24 and 72 hours before sowing.

Hours Soaking	Soaking Temp.°C	% Radicle Damage	Mean Radicle Length (cm).	S.E. of Mean
0	-	6.7	12.1	0.42
24	3	34.7	5.8	0.56
	10	5.3	10.2	0.38
	20	13.3	9.8	0.45
	25	6.7	11.1	0.35
72	3	73.3	2.1	0.24
	10	14.7	5.9	0.28
	20	62.7	3.5	0.29
	25	90.7	1.3	0.22

Table 14 shows that even after only 24 hours soaking there are differences due to temperature, there being an indication that, at the higher temperatures the effects of soaking are somewhat less severe.

The results for the 72 hour period are, however, more striking. Soaking at the higher temperatures for this length

of time produces more severe damage. Table 14 shows that soaking at 20°C and 25°C, particularly the latter, results in a very high percentage of damaged radicles and the radicle lengths are considerably suppressed compared with the lengths of seedlings grown from seeds soaked at 10°C. Soaking at this temperature produces only 14.7% damage compared with 6.7% in the control and 5.3% after 24 hours soaking at the same temperature.

It appears at first sight that, for 72 hours, the lower the soaking temperature is, the less severe are the effects. This is not completely true, however, as soaking at 3°C appears to cause similar effects to those observed after soaking at 20 and 25°C. The percentage damage recorded for those seedlings includes a large proportion (> 50%) of apparently ungerminated seeds and it therefore seems possible that soaking at this low temperature results in effects which cannot be attributed solely to the soaking conditions. Some of the implications of this will be discussed in conjunction with further results of low temperature soaking.

The analysis of variance technique can be used here to summarise these observations.

Table 15 shows that the greatest differences in this experiment are, as expected, those due to the length of the soaking period. The differences due to temperature, however, are also highly significant and there is good evidence of interaction between times and temperatures.

TABLE 15.

Analysis of variance table: the effects of soaking Maple pea seeds at different temperatures before sowing on the lengths of the radicles of the resulting seedlings.

Source	DF	Sum of Sqq.	Mean Sq.	F
Replicates	2	49.01	24.50	2.46
Temperatures	3	1,290.54	430.18	43.23**
Times	1	5,403.60	5,403.60	543.08**
Interaction:- Times temp.	3	832.15	277.38	27.88**
Residual	590	5,872.40	9.95	
Total	599	13,447.70		

More specific information can be derived from the graphs in Figures 7 and 8 which show the relationship between radicle length (y) and temperature (x) for seedlings from seeds soaked for 24 and 72 hours respectively. The method of Yule and Kendall (1950) was used to determine the equations of the curves which would be the best fits for the observed data. Table 16 shows the analysis of variance table for the 24 hour soaking data, the sum of squares for temperature having been partitioned into 3 components each with one degree of freedom. Further information regarding the method used can be found in Appendix IV.

The analysis shows that the cubic component of the temperature sum of squares is significant; hence an expression

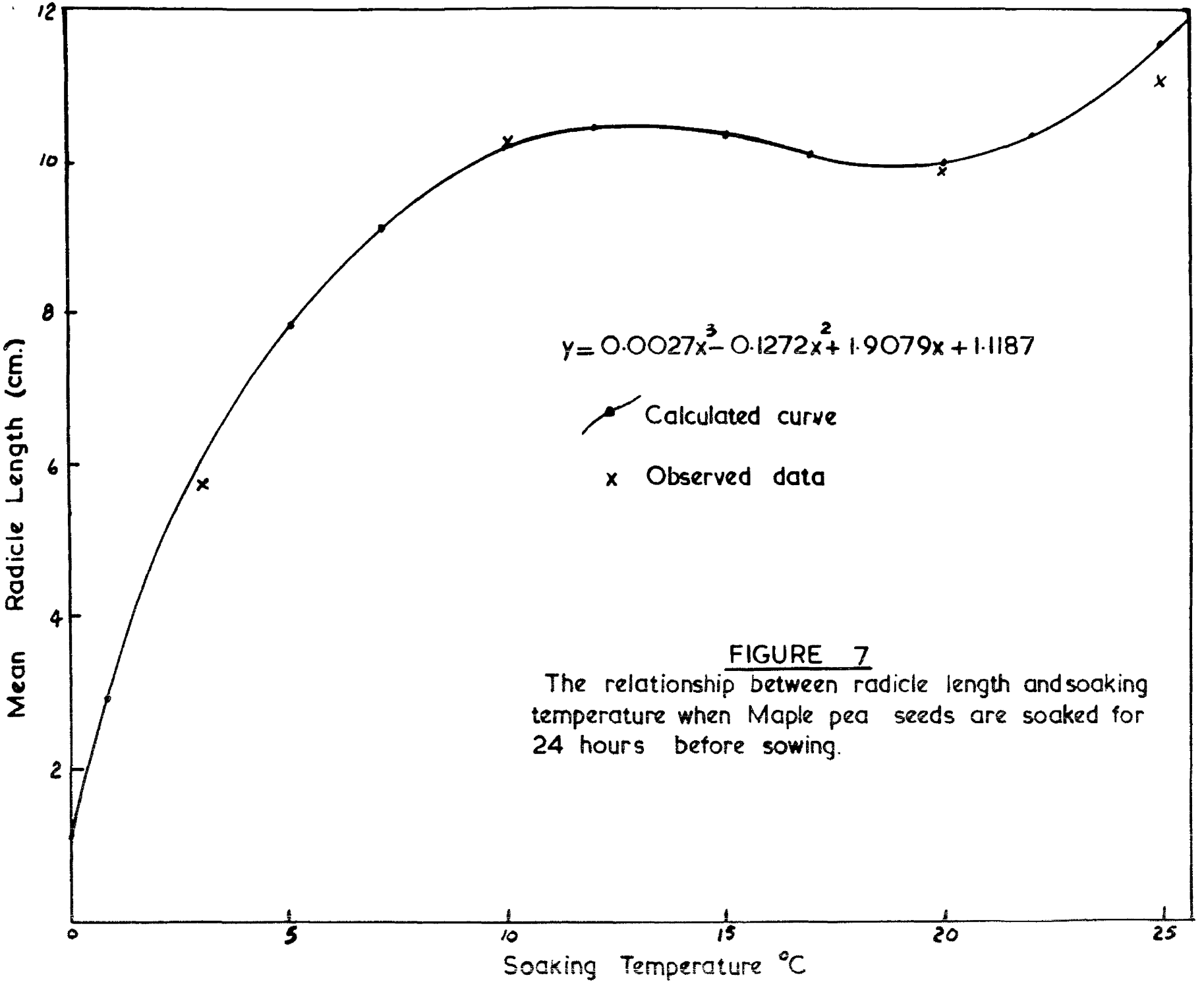


FIGURE 7

The relationship between radicle length and soaking temperature when Maple pea seeds are soaked for 24 hours before sowing.

to the power of 3 is essential to give a good fit to the data.

TABLE 16.

Analysis of variance table: radicle lengths of seedlings grown from seeds soaked at different temperatures for 24 hours before sowing.

Source	DF	Mean Sq.	F
Temperature			
a) Linear	1	856.19	57.35**
b) Quadratic	1	186.76	12.51**
c) Cubic	1	190.96	12.79**
Residual	296	14.93	
Total	299		

The calculated equation was found to be:-

$$y = 0.0027x^3 - 0.1272x^2 + 1.9079x + 1.1187$$

The graph shows that when the soaking temperature is raised to 10°C the response of the seeds (when measured as radicle length of the seedlings) alters. Instead of increasing steadily from 3°C, at 10°C the graph levels off and at temperatures between 10°C and 21°C there is no evidence of increased radicle length. The metabolism of the seeds undergoing anaerobic soaking seems to be completely different between 0-10°C and 10-20°C.

Since soaking at 10 and 20°C has only a slight suppressing effect on radicle length compared with controls (see Table 14)

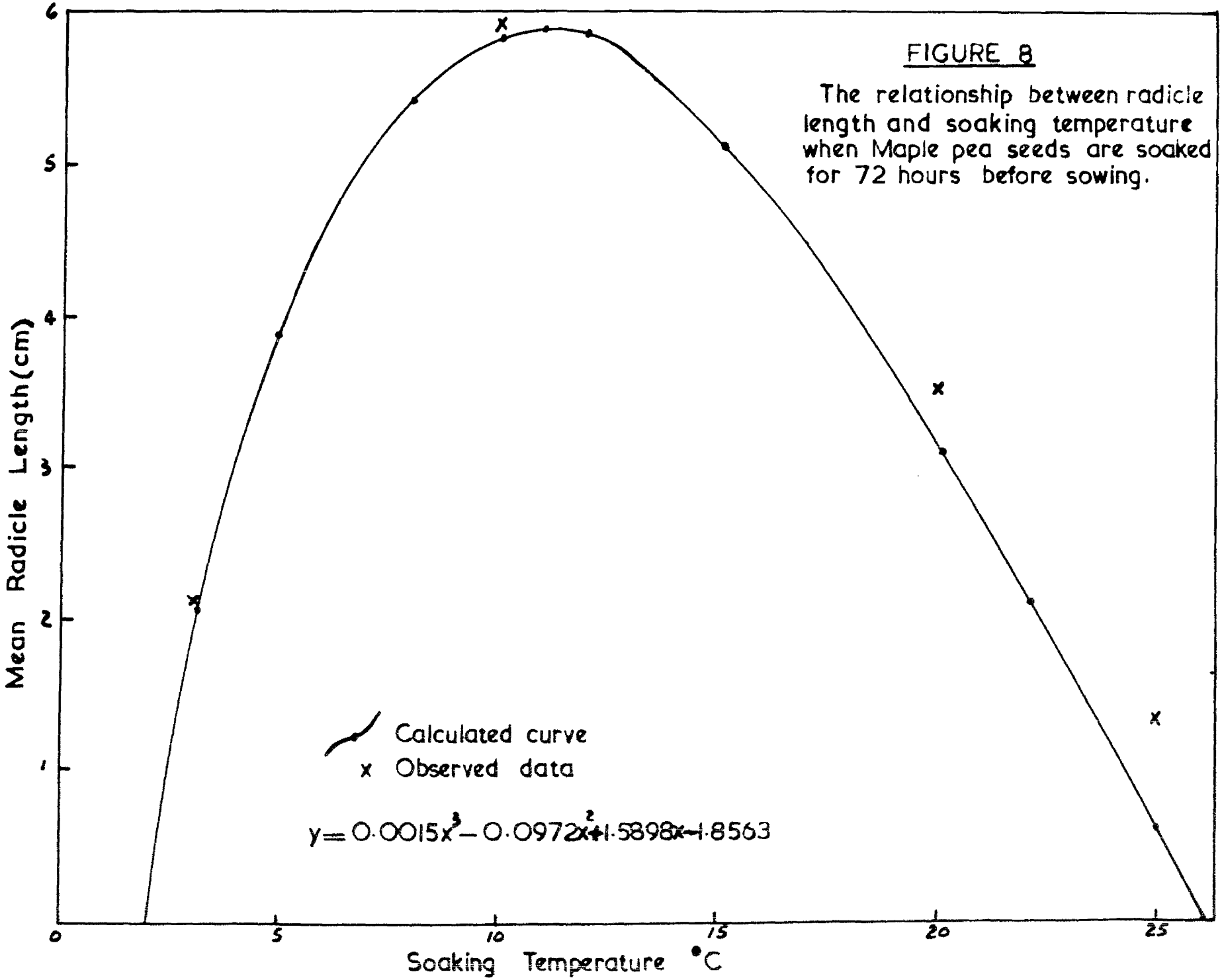


FIGURE 8

The relationship between radicle length and soaking temperature when Maple pea seeds are soaked for 72 hours before sowing.

it can be concluded that, at temperatures within this range, soaking for 24 hours has no serious detrimental effect on the subsequent growth of the plants.

Above 20°C there appears to be increased radicle length (Figure 7) and the data in this experiment show that the seedlings possess radicles very similar to those of the controls. At 25°C it is possible that the seeds complete the physical part of germination in 24 hours and the chemical reactions are already begun or are just beginning when aerobic conditions are restored. The seeds are thus prepared to resume normal germination on being restored to aerobic conditions and grow at a similar rate to the controls, the soaking period not having been long enough to interfere with the chemical reactions.

The data for 72 hours can be treated similarly and Table 17 shows the partitioning of the temperature sum of squares.

The equation which gives the best fit is:-

$$y = 0.0015x^3 - 0.0972x^2 + 1.5898x - 1.8563$$

It is at once obvious from the graph of this equation (Figure 8) that there is an entirely different pattern from that observed after 24 hours soaking. Soaking at 10°C has less depressing effect on the length of radicles than soaking at the other 3 temperatures. The expected curve shows that this is also true for the range 10-12°C. Above 12°C, however, there is a steady decrease in radicle length with increasing temperature. The percentage of damaged radicles (Table 14) at the

higher temperatures corresponds to the figures for radicle length, thus lending further weight to the argument that soaking at higher temperatures results in greater damage. If the calculated curve in Figure 8 is accurate, soaking Maple peas for 72 hours at a temperature of just over 26°C would be sufficient to completely prevent germination of the seeds.

TABLE 17.

Analysis of variance table: radicle lengths of seedlings grown from seeds soaked at different temperatures for 72 hours before sowing.

Source	DF	Mean Sq.	F
Temperature			
a) Linear	1	76.52	15.09**
b) Quadratic	1	752.06	148.34**
c) Cubic	1	64.19	12.66**
Residual	296	5.07	
Total	299		

The results of this experiment are therefore in agreement with the hypothesis that anaerobic soaking disrupts the metabolism of the seeds. At the higher temperatures metabolism would be expected to proceed faster, probably about twice as fast at 20°C as at 10°C.

If we suspect that soaking has no effect on the seeds until the metabolic processes have reached a certain critical stage,

we should expect the seeds or seedlings to attain this stage earlier at the higher temperatures and so be more severely affected.

If this reasoning is correct, when the soaking period is extended beyond 72 hours, a time should be reached when soaking at 10°C has a harmful effect on the growth of the seedlings. It was not practicable to pursue this fully but Table 18 shows the results of soaking seeds at different temperatures for 7 days before sowing then growing for 5 days before harvest.

TABLE 18.

The effects of soaking Maple pea seeds for 7 days at 4 different temperatures on the frequency of damage and radicle length of the resulting seedlings.

	Soaking Temperature °C			
	3	10	20	25
% Damage	70.7	53.3	98.7	100.0
Mean radicle length (cm)	1.8	3.3	0.3	0.04

Although soaking at 10°C again has least effect, when the results are compared with those of Table 14, it can be seen that the percentage of radicle damage has risen from 14.7 (72 hours soaking) to over 50. The mean radicle length has correspondingly decreased. It seems likely that further extension of the soaking period could result in attainment of a

stage when 100% damage would occur.

This is what has happened when the seeds were soaked for 7 days at 25°C. Emergence was reduced to 13% and those shoots and radicles which did emerge were so small that accurate measurement was practically impossible. All the radicles which did emerge (10 out of 75) were very severely damaged.

The seedlings grown from seeds soaked at 20°C were also very severely affected by the prolonged treatment. As at 25°C, the lengths of shoot and radicle were very small and only one out of the 34 radicles which emerged could be classified as morphologically normal.

Soaking at 3°C again produced enigmatic results. The data are very similar to those obtained when the seeds were soaked for 72 hours (Table 14). At low temperatures, metabolism may be proceeding so slowly (if at all) that extension of the soaking period within reasonable limits has little influence on the severity of the adverse effects of soaking.

All results for soaking at 3°C are different from the general pattern, a common feature of this group being a large proportion of ungerminated seeds present even after adequate exposure to aerobic conditions following soaking. It is probable that any metabolic reactions which do proceed at low temperature are different from those proceeding at higher temperatures. Crocker and Barton (1957), for example, have reviewed the findings of several workers on the effects of low

temperature on the enzyme reactions in seeds. The enzymes which were active generally functioned in such a way that readily available food materials were produced. It is also well known that in the potato tuber, low temperature storage results in the enzymatic production of sugars from starch.

Further investigation of this problem with the information available is impracticable: it is sufficient to state that there is evidence of an upset to the biochemical equilibrium below 10°C. The effect of soaking on the biochemical reactions is therefore likely to be different below 10°C, thus accounting for the different response of the seeds to the treatment.

Soaking temperature clearly has an important influence on the response of seeds to the treatment: the evidence provided by this experiment confirms that the metabolism of the seeds is disrupted by the treatment.

. . .

Some effects of interrupting the normal sequence of germination and development by subjecting seeds or seedlings to periods of anaerobic soaking.

If the degree of soaking damage depends on the attainment by the seed or seedling of a critical stage in its metabolism it is to be expected that, if the various processes of metabolism are speeded up, soaking would then create more severe effects. This appears to have been accomplished by raising the soaking

temperature. If seeds are allowed to germinate aerobically they will presumably reach the critical stage sooner than if they are exposed to anaerobic conditions.

Several experiments were carried out where the seeds were exposed to aerobic germination conditions for a certain time then placed under soaking conditions for a further period before being sown out. Table 19 shows some of the pooled data.

TABLE 19.

The influence of allowing seeds to germinate aerobically before being soaked on the frequency of radicle damage observed in the resulting seedlings.

Germination Conditions (Hrs.)		No. seeds sown	No. abnormal radicles
Aerobic	+ Anaerobic		
0	48	200	22
24	24	200	96
48	0	200	16

When the 48 hour period is made up of a period of 24 hours aerobic conditions followed by 24 hours anaerobiosis, the seedlings harvested 5 days later have a much larger proportion of abnormalities than when the period is made up entirely of either aerobic or anaerobic conditions. A Chi squared test confirms that the 24 + 24 hours treatment is different from the other 2.

Further experiments showed that short periods of anaerobiosis (1-8 hours) following fairly long periods of aerobic germination (e.g. 35 hours) do not produce populations containing large numbers of damaged seedlings. 2 general conclusions emerged from this earlier work.

(a) The seeds have to be exposed to at least 24 hours initial aerobic germination and

(b) The soaking period following this initial aerobic germination must be in the region of at least 20 hours before the treatment has any significant effect.

An experiment was set up to further investigate this problem.

Experiment 4. An investigation into the effects of soaking Maple pea seeds or seedlings which were previously exposed to conditions suitable for normal germination and development.

A large number of Maple pea seeds was sown out and after 24, 28 and 32 hours samples of 50 were withdrawn, washed 3 times and placed under anaerobic conditions for 24, 20 and 16 hours respectively; all seeds were thus under experimental conditions for a total of 48 hours before being removed and finally sown again under aerobic conditions. 50 seeds were also placed under anaerobic conditions at the start of the experiment and sown out at the same time as the others.

As a supplement to this experiment, 2 samples of 50 seeds were placed under anaerobic conditions at the beginning and removed after 52 and 56 hours. 50 seeds were also taken from the original lot of seeds sown aerobically after 28 hours and soaked for 24 hours (giving a total of 52 hours treatment), and 50 were taken out after 32 hours and soaked for 24 hours (giving a total of 56 hours).

TABLE 20.

Some effects of interrupting the normal course of germination and development in Maple pea by subjecting the seedlings to various periods of anaerobiosis before re-sowing.

(a) % radicle damage.

Hrs.Aer. \ Hrs.An.	0	16	20	24	48	52	56
0	-	-	-	-	8	24	24
24	-	-	-	36	-	-	-
28	-	-	72	72	-	-	-
32	-	56	-	96	-	-	-
48	10	-	-	-	-	-	-

(b) Mean radicle length (cm).

Hrs.Aer. \ Hrs.An.	0	16	20	24	48	52	56
0	-	-	-	-	8.6	7.1	6.6
24	-	-	-	6.7	-	-	-
28	-	-	3.5	3.0	-	-	-
32	-	3.5	-	1.1	-	-	-
48	11.0	-	-	-	-	-	-

The most interesting figures here are in the column below 24 where it is shown that when seeds are removed from aerobic conditions after 24 hours and then subjected to anaerobiosis, 24 hours anaerobiosis is sufficient to show increased effect (compared with 48 hours uninterrupted anaerobiosis). This effect, however, is considerably increased if the period of anaerobiosis is kept the same but the initial period of aerobiosis is increased by only 4 or 8 hours.

Differences among the treatments can be further illustrated by determining the values of "t" for various comparisons (Table 21).

TABLE 21.

The values of "t" obtained when comparing the mean radicle lengths of seedlings from seeds subjected to various soaking treatments before sowing. The first figure in each case denotes the period of aerobic germination (hours) and the second the subsequent duration of the anaerobic treatment (hours).

	24 + 24	28 + 20	32 + 16	28 + 24	32 + 24
0 + 48	2.6*	7.7**	8.7**	9.3**	17.4**
24 + 24	-	3.9**	4.3**	4.9**	8.9**
28 + 20	-	-	0	0.7	4.4**
32 + 16	-	-	-	0.8	5.4**
28 + 24	-	-	-	-	4.0**

For method of determining "t" see Goulden, 1952:Page 56.

The value of "t" for one degree of freedom is 1.98; the comparisons which show significant differences at the 1% level are denoted by 2 asterisks, at the 5% level only by one asterisk and, where no asterisk is used, it denotes that the means are not significantly different.

Obviously, when the 48 hour treatment period is broken up into an initial period of aerobiosis and a subsequent anaerobic period the adverse effects of the anaerobiosis are increased. The same is true of the 52 and 56 hour periods.

As previously shown, if the period of anaerobiosis is kept at 24 hours and the aerobic period is increased from 24 hours to 28 and 32 hours the latter 2 instances produce significantly greater adverse effects than the 24 + 24 hour treatment. Table 21 shows that, when the period of aerobiosis extends to 28 and 32 hours, further 20 hours and 16 hours anaerobiosis respectively are still sufficient to produce significantly greater adverse effects than the 24 + 24 hour treatment.

The implication of these results is that the duration of the aerobic treatment is critical in determining the response of the seeds (or seedlings) to subsequent anaerobic treatment. After 28 hours aerobiosis the seeds (or seedlings) appear to be so far advanced that a relatively short period of anaerobiosis (20 hours) is sufficient to upset further development of the seedlings. At 24 hours it is possible that the seeds have not yet reached a stage in their metabolism when they are

particularly susceptible to anaerobic conditions, unless the period of anaerobiosis is relatively long (> 24 hours).

After 28 hours in aerobic conditions the seeds are fairly well advanced: germination is likely to have been completed and cell division will be progressing. This process has a high energy requirement and it is possible that when the seedlings are subjected to the anaerobic conditions their respiration does not supply sufficient energy to enable the process to continue normally.

These results are in agreement with those describing the influence of temperature on the effects of soaking, viz. that the degree of damage depends on the stage of metabolism reached by germinating seeds under the experimental conditions.

. . .

The influence of removal of the testas on the severity of the adverse effects of soaking.

Another possible method of accelerating germination and development is to first remove or rupture the enclosing layers of the seed. The chief purpose of this is to allow the embryo more rapid access to the essential requirements for germination viz. water and oxygen.

If the conclusions reached so far are correct it is probable that, when the testas of pea seeds are chipped before sowing or removed altogether, metabolism of the seeds will start

more rapidly and so the critical period of susceptibility will be reached much earlier than in similarly treated complete seeds. It would therefore be expected that soaking seeds with testas removed for say 48 hours would have more severe effects than soaking complete seeds for the same length of time.

Experiment 5. The influence of removal of the testas of pea seeds on the frequency of radicle damage occurring in the seedlings after the seeds have been soaked for various periods.

The testas were removed from 150 Maple pea seeds and, of these, 50 were sown directly at 20°C and the remaining 100 were split into 2 samples of 50 which were soaked for 48 and 96 hours respectively, also at 20°C. 150 complete seeds were treated similarly.

7 days after the start of the experiment the seedlings were scored for radicle damage.

TABLE 22.

The influence of removal of the testas on the percentage radicle damage produced by soaking Maple peas before sowing.

Condition of seed / Hrs. soaking	Complete	Testas removed
0	4	0
48	18	64
96	88	100

A Chi squared test comparing the original data for soaking complete seeds and those with testas removed gives a value of 21.8 for the 48 hour soaking period, thus indicating that there are significant differences between the treatments. The 48 hour soaking period was selected in preference to the standard 72 hours because, at 72 hours, soaking complete seeds results in a high frequency of damage (see page 25) and, therefore, it might not have been possible to show fully the extra effects that removal of the testas appears to create.

At the 96 hour period the complete seeds produced seedlings, but almost all were damaged. Removal of the testas (plus the soaking treatment) resulted in apparent death of the seeds. There seems to be no doubt that removal of the testas increases the severity of soaking damage.

It is interesting to record at this point the results of one of the earlier long term experiments. In this, 25 seeds with testas removed were soaked for 24 hours and 25 for 48 hours: similarly 25 complete seeds were soaked for each of the 2 periods. When this experiment was carried out, most of the interest centred on shoot damage and this experiment was the only one where shoot abnormalities similar to those described by Berrie (1960) for Kelvedon Wonder peas, were found to occur with a frequency high enough to be worthy of note. The results are shown in Table 23.

TABLE 23.

The effects of removal of the testas of Maple peas on the frequency of emergence and shoot damage following soaking treatment.

	Hours soaking					
	0		24		48	
Testas	On	Off	On	Off	On	Off
No. plants emerged	24	25	24	24	24	18
No. abnormal shoots	0	0	1	4	0	18

Although a Chi squared test gives a value of 1.5 when the 2 treatments at 24 hours are compared, showing that removal of the testas produces no significant differences, when the 48 hour soaking period is considered it is clear that removal of the testas results in a much more noticeable response to soaking. In the first place, germination was inhibited and secondly, of the 18 plants produced, all 18 possessed abnormal shoots: the growing point of the main shoot appeared to have died and growth of the aerial part of the plant was being carried on by axillary shoots.

Removal of the testas before soaking, therefore, appears to have an influence on the severity of the effects of the treatment, manifest in both the radicle and the shoot of the resulting seedlings.

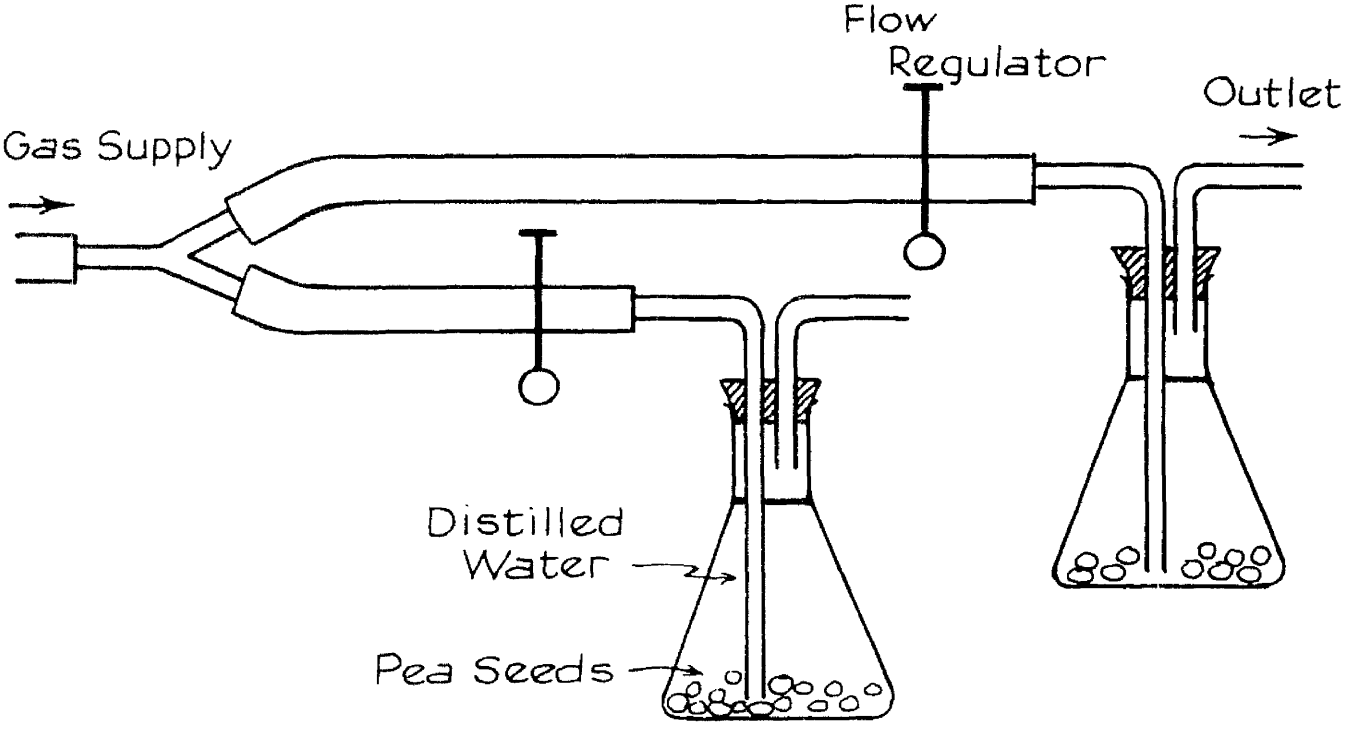


PLATE 3

Apparatus used when gases were bubbled through the medium in which pea seeds were soaked.

Investigations into the response of soaked seeds to alterations in the environment by passing gases through the soaking medium.

It is unlikely that, under soaking conditions, pea seeds can extract enough oxygen from the water for their requirements: carbon dioxide produced by anaerobic respiration is also likely to accumulate in the vicinity of the seeds. It was with this in view that several experiments were carried out where gases were passed into the environment of the seeds through the soaking medium. The gases used were air, argon, oxygen and carbon dioxide. Air was used to observe the effects of soaking under aerobic instead of anaerobic conditions. Argon was selected to enable observations to be made on the effects of passing a gas with no known effects on the metabolism of the seeds through the soaking medium. Oxygen was used because, of course, it is essential for the aerobic respiration of plant tissues and carbon dioxide was used because it is one of the end products of aerobic and anaerobic respiration.

The seeds were set up in 100 ml Erlenmeyer flasks fitted with a rubber bung and 2 glass tubes, 2 mm bore to allow of the entrance and exit of gas. Generally 2 flasks were set up in parallel for each gas (Plate 3) and the rate of flow adjusted so that 2 bubbles passed through the soaking medium every second. The air was supplied from the atmosphere via a "Hy-Flo" air pump and the other gases were supplied from commercially

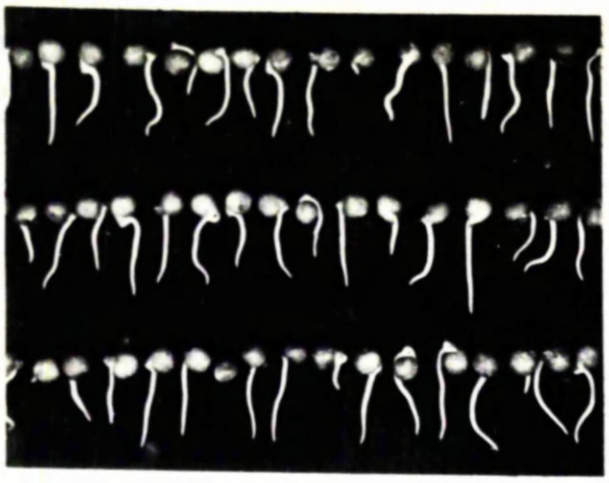
obtained cylinders.

Experiment 6. The relation of different gases to the soaking injury of Maple pea seeds.

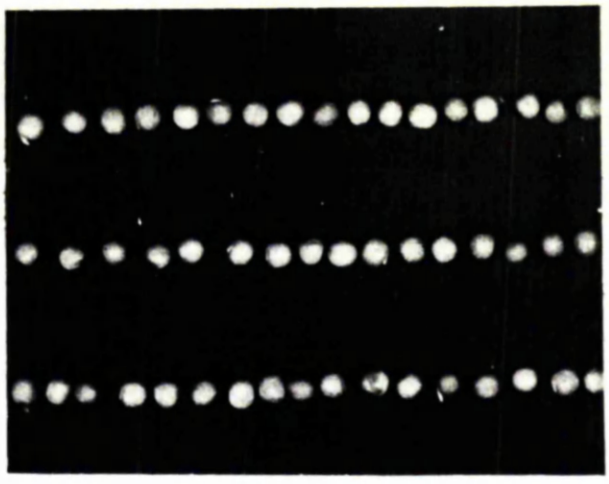
A set of flasks was set up, each with 50 Maple pea seeds and a capacity volume of distilled water. Air, argon, carbon dioxide and oxygen were bubbled through 4 pairs of flasks at a steady rate for 72 hours, during which all apparatus was kept at room temperature (a thermograph showed this to vary between 16°C and 20°C). A flask was also set up with seeds soaking in distilled water only (no gas treatment) and a further 50 seeds were sown directly.

After the soaking period the water was poured off and the lengths of the radicles, where present, were measured. The results are shown in Table 24 and a pictorial record is shown in Plate 4.

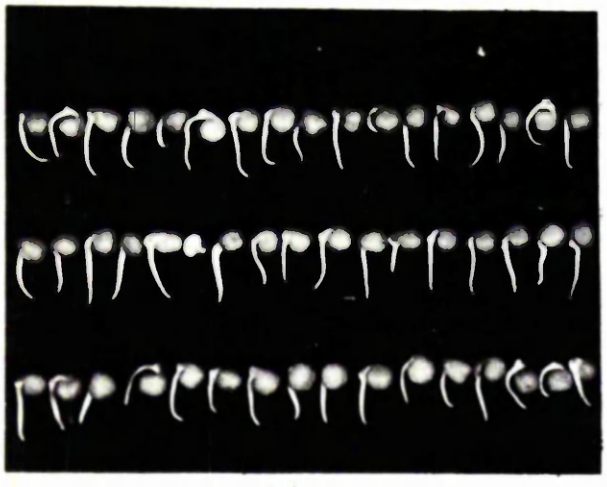
As the table shows, each treatment has produced some visible sign of germination except in the case of CO_2. The air treated seeds, as expected, made the nearest approach to the control; the higher value for mean radicle length (1.6 cm) is probably the more correct as there was a temporary blockage of the air supply in the flask containing the seeds which produced seedlings with shorter radicles. The oxygen treated seeds germinated remarkably well but the radicles were very translucent and brittle. Argon treated seeds at this stage



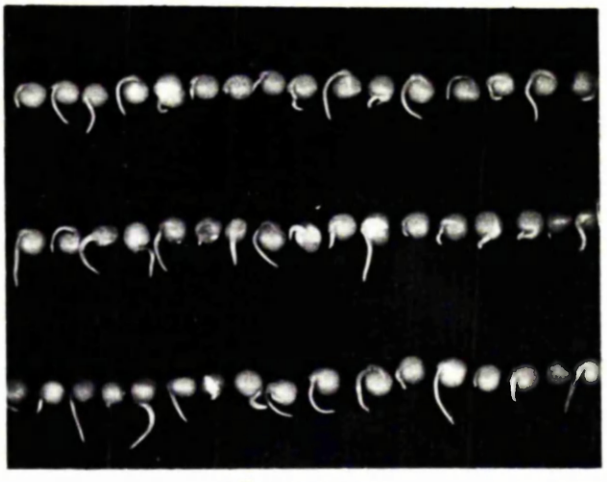
Sown directly.



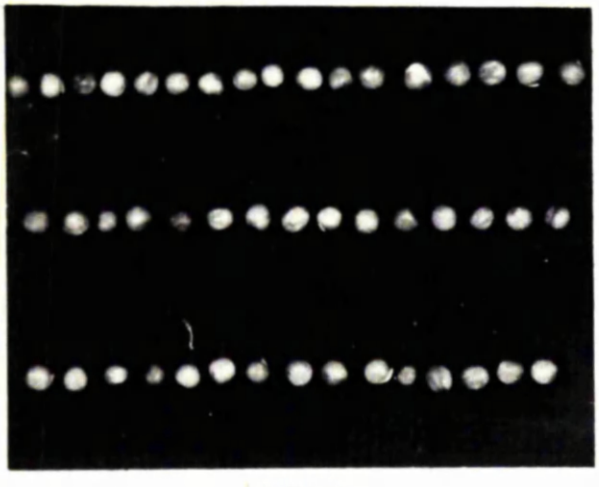
No gas.



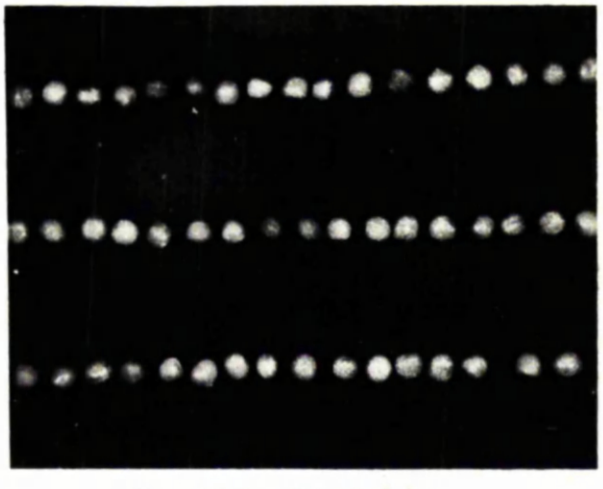
Air.



Oxygen.



Argon.



Carbon dioxide.

PLATE 4

The appearance of Maple pea seeds and seedlings at the end of the 72 hour soaking period during which gases were bubbled through the soaking medium. Each photograph is labelled with the appropriate gas.

showed little difference from the seeds soaked anaerobically.

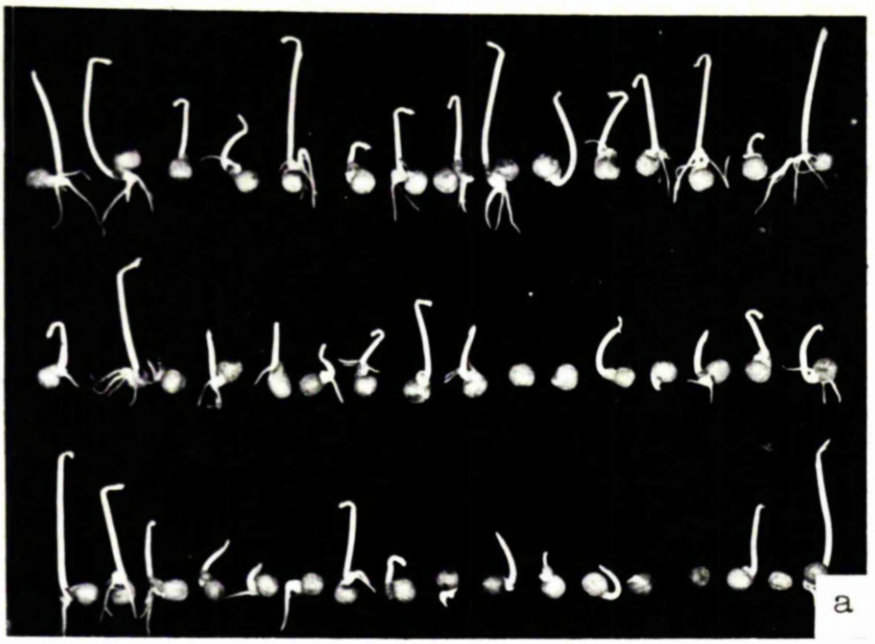
TABLE 24.

Some effects of bubbling gases through the soaking medium on the severity of damage occurring in Maple pea seedlings following soaking of the seeds. Columns a and b are replicates for each treatment.

Gas	Mean Radicle Length (cm)		No. Testas Burst	
	a	b	a	b
Oxygen	0.8	0.9	47	48
CO_2	0	0	0	0
Argon	0	0	3	8
Air	0.8	1.6	47	50
None	0	-	12	-
Control	2.1	-	49	-

All seeds and seedlings were sown out and grown for a further 4 days, then measurements were made of radicle length and frequency of damage (Table 25).

The seedlings from seeds soaked in gas-free water showed the usual signs of damage but the effect was not so great as might have been expected due probably to the soaking temperature being less than $20^{o}C$.



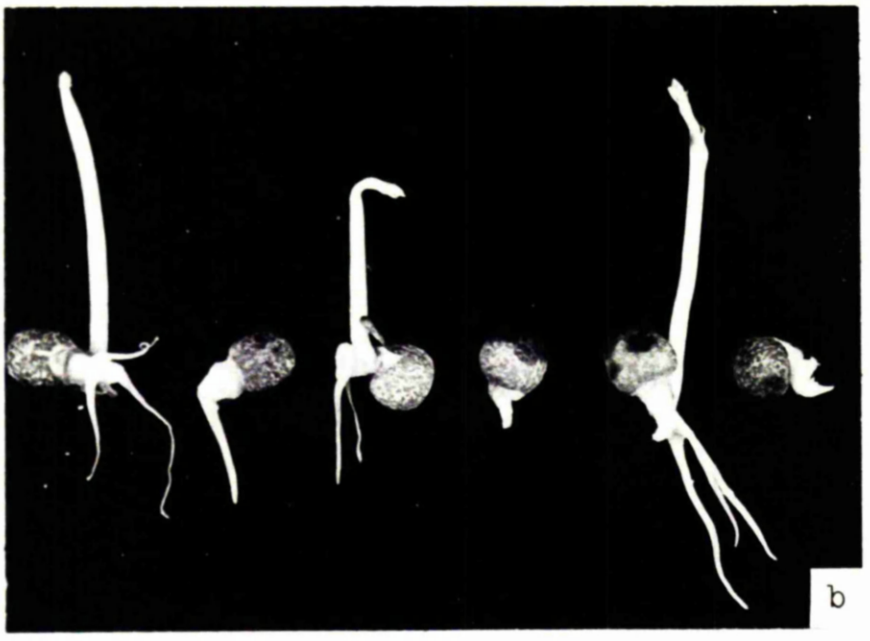


PLATE 5

Maple pea seedlings from seeds soaked for 72 hours in distilled water through which carbon dioxide was passed.

a. All the seedlings from a batch of treated seeds.

b. 6 of the more severely damaged seedlings showing very obvious swelling of the proximal ends of the radicles.

TABLE 25.

Some effects of bubbling different gases through the media in which Maple pea seeds are soaked on the germination and development of the resulting seedlings.

	Control	Air		CO_2		Oxygen		Argon		No Gas
		a	b	a	b	a	b	a	b	
% Damage	14	4	4	99	98	54	44	0	4	26
Mean rad. lgth.(cm)	10.9	8.4	8.6	+	+	3.7	3.7	9.7	8.3	8.0

++ These radicles were too short (<0.2 cm) and malformed to allow of accurate measurement.

Those seedlings grown from seeds treated with air and argon seemed to have been very similarly affected: this is surprising, especially when the air-treated seeds showed more development after the soaking period (Table 24). There is an indication here that the ameliorative effect of air may be due, not so much to the contribution of the air itself to the metabolic requirements of the seeds, but to the removal of the waste products of metabolism from the immediate vicinity of the seeds by the mechanical action of the gas flow.

CO_2 treated seeds showed very striking results (Plate 5). Damage was almost 100% and the radicles produced were so short that it was not possible to measure them accurately. It is

interesting that the effects of soaking with CO_2 treatment should be so severe particularly when the temperature was always under 20°C. The radicles seemed to develop a mass of swollen tissue just outside the seed and further development was confined to the production of lateral roots.

The results of this experiment can be compared with those of Barton (1950). In Barton's experiments, seeds of various species, including French beans (Phaseolus vulgaris), sunflower (Helianthus annuus) and the garden pea (Pisum sativum) were soaked for 24 hours in tap water through which various gases were passed. The seeds were then placed in paper towels or soil and allowed to germinate at several temperatures. The effects of the gas treatment were scored as percentage germination of the seeds but observations were also made on the vigour of the seedlings.

Barton found that, in general, air, hydrogen, nitrogen and carbon dioxide mitigated the adverse effects of soaking while oxygen increased the severity of the effects. Clearly the results of Experiment 6 are similar to those of Barton except for CO_2. Before discussing this, mention must be made of the influence of oxygen on the response of seeds to soaking.

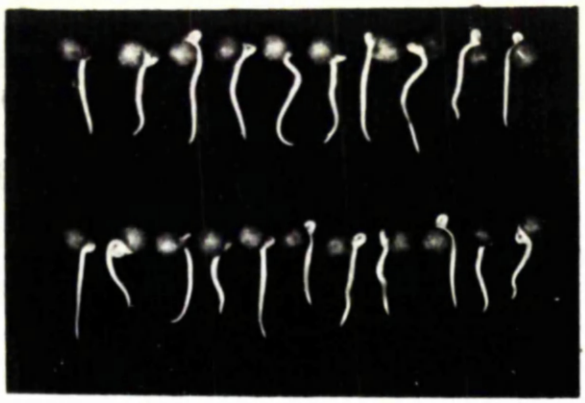
Plate 4 shows that the seedlings from oxygen treated seeds are not so vigorous as those from air-treated seeds. The radicles are very soft, translucent and brittle. This is not the same type of damage which is described in Part 1 of

this thesis: it is probably the result of prolonged exposure of the seeds to pure oxygen. This observation is not new; Albaum (1940), for example, found that the growth of oat coleoptiles became progressively poorer when gas mixtures containing increasing proportions of oxygen were supplied to the seeds during soaking. Barton found a similar response and attributed it to a possible increased moisture uptake.

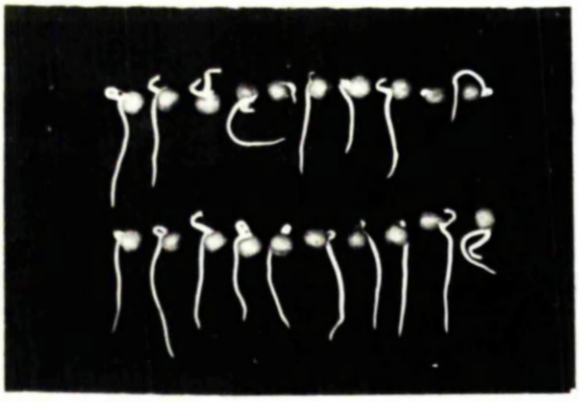
The discrepancy between the results of Experiment 6 and those of Barton for CO_2 treatment may be attributable to one or more of several factors. The most obvious one is that the seeds in Experiment 6 were soaked for 72 hours; in Barton's experiments the soaking period did not extend beyond 24 hours. This point is taken up in the next experiment.

Barton did not use Pisum arvense in any of her experiments; tap water was used instead of distilled water and there was rather wide variation in the soaking temperature in those experiments. In addition, no mention is made of the volume of water under which the seeds were soaked and there is no record of the rate at which the gases were bubbled through the soaking medium. Obviously, since there are such differences in the experimental methods it is not surprising that different results were produced.

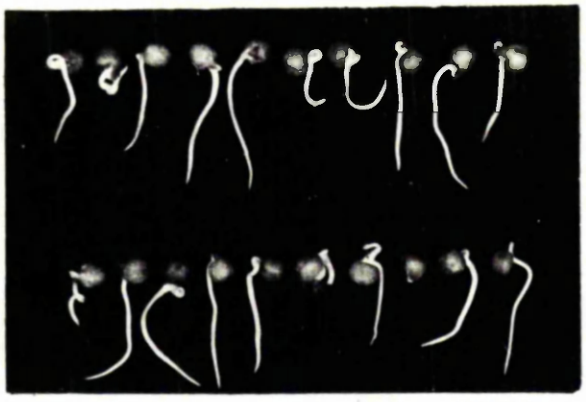
Experiment 7. A further investigation into the effects of bubbling CO_2 through the medium used for soaking Maple pea seeds.



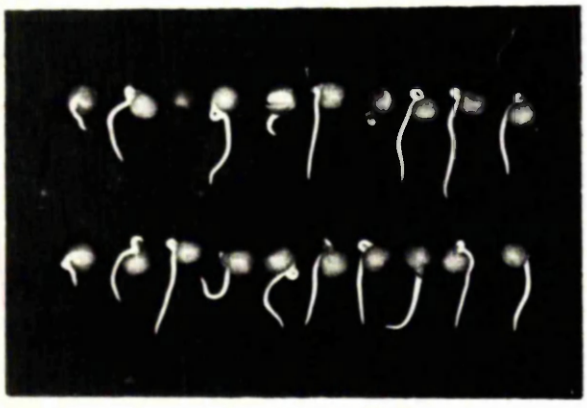
Sown directly.



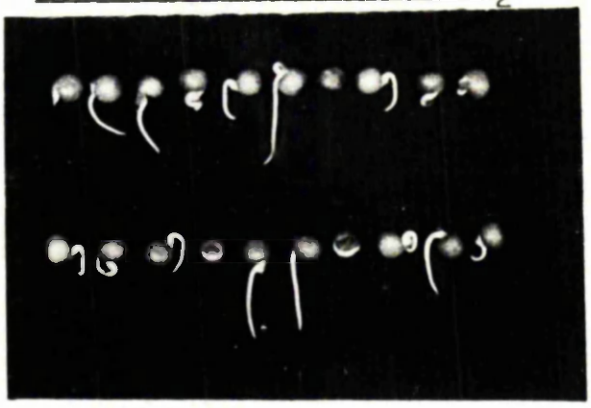
24 Hours soaking.



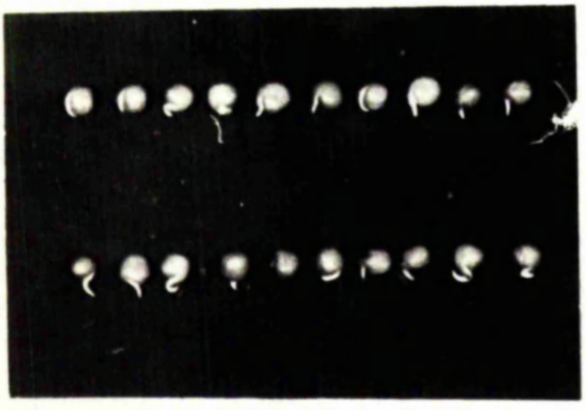
24 Hours soaking + CO_2.



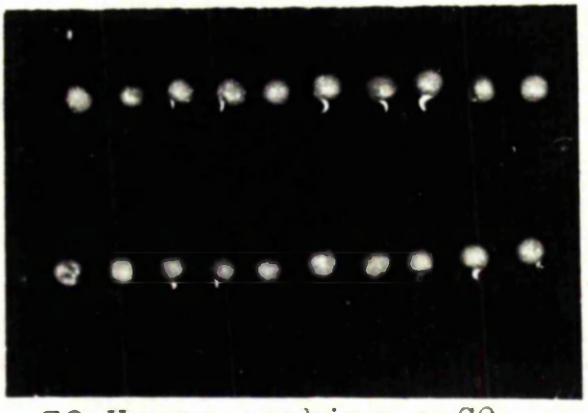
48 Hours soaking.



48 Hours soaking + CO_2.



72 Hours soaking.



72 Hours soaking + CO_2.

PLATE 6

4 day old Maple pea seedlings from seeds soaked in distilled water with and without CO_2.

6 flasks each containing distilled water and 50 Maple pea seeds were connected to a supply of CO_2 which was allowed to flow through the flasks at 2 bubbles per second. 50 seeds were also sown directly and 3 lots of 50 were soaked anaerobically.

After 24 hours, 2 of the flasks were disconnected from the system and the seeds were sown out, along with 50 from the anaerobically treated lots. This was repeated after a further 24 and 48 hours thus giving totals of 0, 24, 48 and 72 hours soaking.

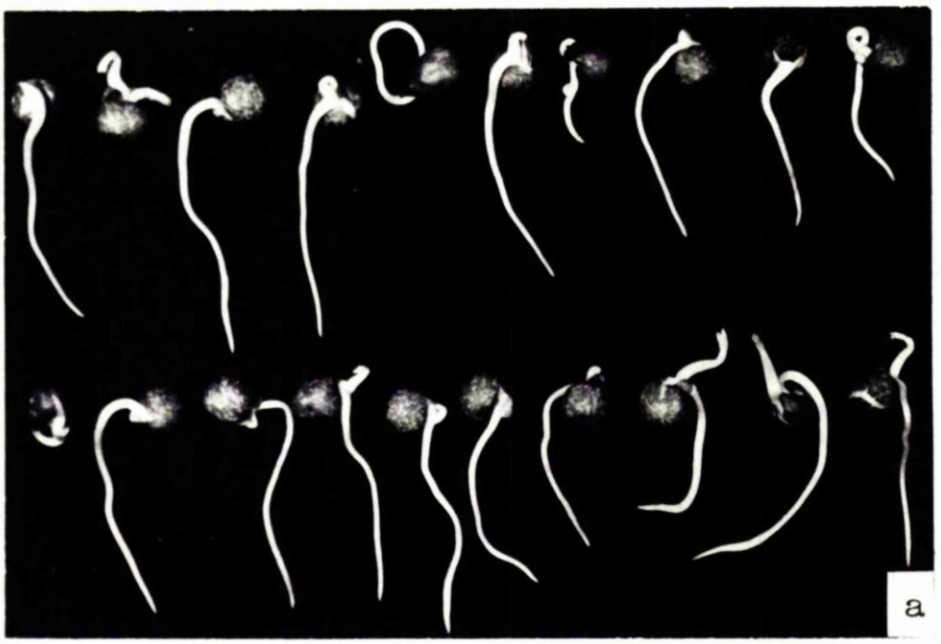
4 days after the start of the experiment, i.e. only one day after the last lot of seeds was sown out, the seedlings were scored.

TABLE 26.

The effects of passing CO_2 through the medium in which seeds are soaked on the frequency of damage and mean radicle length of the resulting seedlings.

	Duration of soaking period (Hrs.)				
	0	24		48	
			+ CO_2		+ CO_2
% Damage	8	10	28	16	72
Mean Radicle Length (cm).	2.5	2.07	2.91	1.72	1.05

(The seedlings which were removed from soaking conditions only the day previously (after 72 hours soaking) were not far enough developed to allow of accurate assessment).



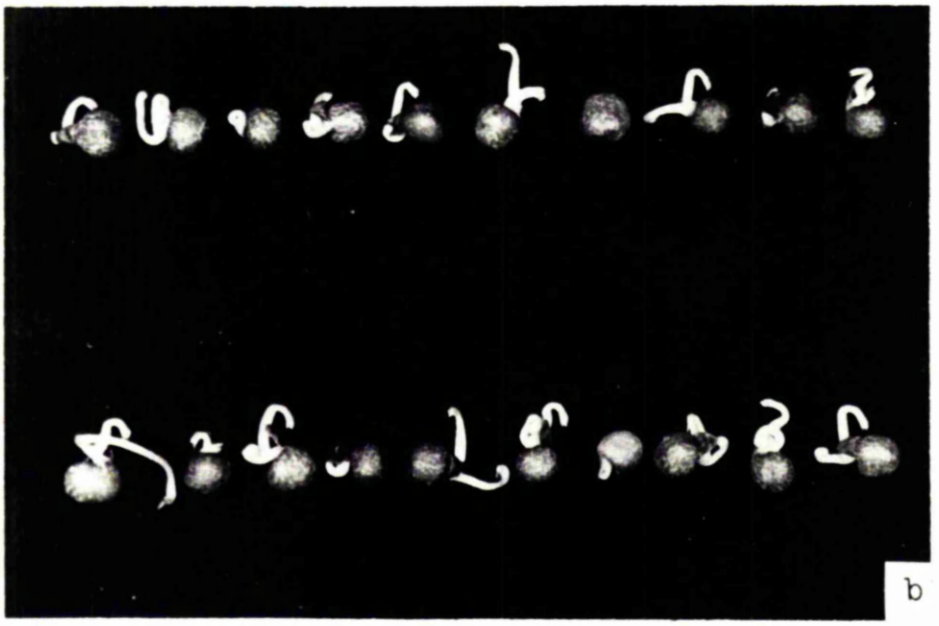


PLATE 7

7 day old Maple pea seedlings

a. Seeds soaked for 72 hours in distilled water before sowing.

b. Seeds soaked for 72 hours in distilled water with CO_2 bubbling treatment before sowing.

The data in Table 26 show that, under these conditions, CO_2 treatment during the 24 hour soaking period definitely has no beneficial effect on the growth of the radicles. The radicle lengths appear very close to one another and a X^2 test on the figures for damage reveals a value of 10.52, indicating that the populations of seedlings from seeds soaked for 24 hours are significantly different and, far from having a beneficial influence, CO_2 treatment, even after only 24 hours soaking, increases the severity of the adverse effects of soaking. At 48 hours CO_2 has a pronounced effect: there is suppression of radicle length and the X^2 for damage rises to 21.97.

The seeds which received 72 hours soaking had only one further day in which to develop and it is not surprising that the radicles were not far enough developed to allow accurate measurements to be taken.

The photographs in Plate 6 show some of the seedlings.

The seedlings from seeds soaked for 72 hours were grown on for a further 3 days then photographs were taken (Plate 7) and measurements made (Table 27) as before.

The findings obviously confirm earlier conclusions that CO_2 treatment considerably increases the damage which occurs when pea seeds are soaked for 72 hours. The same is true, but to a lesser extent, when the soaking period is 24 or 48 hours.

TABLE 27.

The influence of passing CO_2 through the medium in which Maple peas are soaked for 72 hours on the percentage damage and mean radicle length of the resulting seedlings.

	72 Hours soaking	
	No CO_2	+ CO_2
% Damage	28	93
Mean Radicle Length (cm).	3.1	0.9

Some other explanation has, therefore, to be found for the differences between these results and those of Barton. Perhaps the best line to follow in investigating this would be to carefully control the temperature at which the seeds are soaked. Reference to Figure 7 shows that soaking at 25°C for 24 hours may have a beneficial effect compared with soaking at 10°C and 20°C. CO_2 treatment at this temperature for 24 hours may have a different effect to that obtained when the soaking temperature is lower.

Discussion of Part II.

There are 2 main conclusions which emerge from the work described in Part II. Firstly, there is a critical stage in the germination of Maple pea seeds when they are very susceptible to anaerobiosis; secondly, the accumulation of carbon dioxide in the vicinity of soaking seeds increases the severity of the effects of the soaking.

When the seeds are soaked anaerobically at 20°C germination proceeds very slowly and, although there is slight suppression of radicle growth after 24 and 48 hours soaking, the seeds have to be soaked for upwards of 60 hours before the more severe effects are observed. The investigations into the influence of the soaking temperature showed that when the temperature was lowered, and presumably the rate of germination slowed down even more, 72 hours soaking was not long enough to allow the seeds to reach the stage when more severe damage occurred. Increased soaking temperature presumably leads to faster germination and more severe damage occurring in a shorter period of time.

Eyster (1936, 1939, 1940) and Kidd and West (1919 d) found that the soaking temperature was important in determining the behaviour of the resulting seedlings. Various explanations were offered, such as greater leaching of soluble materials at

certain temperatures but none was completely satisfactory.

Germination proceeds faster under aerobic conditions, therefore it is probable that under these circumstances the seeds will reach the critical stage sooner than seeds treated anaerobically. It was shown in Part II that severe damage could be produced in a shorter time by first exposing the seeds to aerobic conditions. Further evidence regarding the theory that the occurrence of severe damage depends on the attainment by the seeds of a certain stage during the period of anaerobiosis was obtained by soaking seeds with testas removed. When such seeds were allowed to germinate aerobically for 2 days their mean radicle length was 14 mm compared with only 5 mm for control seeds. Seeds without testas can therefore germinate more rapidly than controls and, since damage after even 48 hours soaking was very severe, it seems a reasonable conclusion that such seeds reach a stage in their germination when they are very susceptible to the anaerobiosis much sooner than controls.

When all these results are considered together they provide good evidence that prolonged anaerobiosis upsets some metabolic aspect of germination. The data for the gas bubbling experiments lend further weight to this and indicate that naturally produced CO_2 accumulating in the neighbourhood of the seeds may be the cause of the damage.

When the concentration of CO_2 in the vicinity of the seeds

is artificially increased there is increased severity of soaking damage. The effects of carbon dioxide on the respiration of germinating seeds have been investigated by several workers including Kidd (1915), Bailey and Gurjar (1918), Willaman and Beaumont (1928) and Bailey (1940). It was found that, in an atmosphere of CO_2, the respiration of the seeds was considerably depressed. If respiration is depressed it follows that other vital functions will be adversely affected and the seeds will not be able to germinate and develop normally.

While artificial increase in the concentration of naturally produced CO_2 gives more severe damage, a possible reduction in its concentration results in mitigation of the effects. This was done in Experiment 6 by flushing out the system with air or an inert gas. Berrie (1959) found that when seeds of Kelvedon Wonder pea were soaked in running water the damage was much less than that observed when the seeds were soaked in stagnant water for the same length of time. It was thought that the water carried away a metabolite which was responsible for the damage (This effect may have been due to the lower temperature of the running water).

The conclusion of this work is that the adverse effects of soaking may be due, not to the anaerobic conditions *per se*, but to the accumulation in the vicinity of the seeds of a product of anaerobic respiration, possibly CO_2.

PART 111.

SOME RESPONSES OF PEA SEEDS TO SOAKING IN SOLUTIONS OF PLANT GROWTH REGULATORY SUBSTANCES.

The data presented up to this stage have indicated that the aberrations caused by anaerobic soaking are due to a derangement of the metabolic processes of the seed or the young seedling. These metabolic processes encompass a very large field of biophysical and biochemical reactions. It is obviously not within the scope of this investigation to explore all the aspects of metabolism in a search for the cause of the observed effects. In this study (for reasons which will emerge later) we confine ourselves to an examination of the auxin metabolism of the germinating pea seed and an assessment of how large a part this plays in determining the intensity of the effects.

Prior to 1928, considerable scientific effort was concentrated on discovering what was the actual stimulus for growth within the plant. It was Went, however, in 1928 who finally isolated an active substance now known as an auxin, from the tip of Avena coleoptiles. He found that if

coleoptile tips were placed on blocks of agar, these blocks could be used to stimulate growth in decapitated coleoptiles. Went interpreted the normal growth of the coleoptile in terms of the action of this growth substance in conjunction with other limiting factors.

Roots were found to respond to tip removal rather differently. It was claimed that, in the root, auxins inhibited growth and that removal of the tip (and hence the source of auxin) resulted in accelerated growth. Considerable doubt now centres round these observations and Younis (1954) could not repeat Cholodny's finding (1924, 1926) that decapitation produced accelerated growth. Whether the difference in response between root and shoot depends on the concentration of endogenous auxin or a complex system of growth regulators is a matter which has yet to be settled.

Since Went's discovery, many workers have investigated the nature and properties of auxins. More recent reviews are to be found in Gordon (1954), Åberg (1957), Thimann (1957), Bentley (1958), van Overbeek (1956, 1959) and an extensive list of references is given by Audus (1959) in his monograph on Plant Growth Substances.

The auxin relations of aquatic or partly aquatic plants have received very little attention but some of the observations of Yamada (1954), Sircar (1957) and Kefford (1962) may have an important bearing on the observed results of soaking

pea seeds in water. Yamada (1954) claimed that the superior growth of rice coleoptiles (Oryza sativa L.) under water is due to the decreased capacity of the submerged coleoptile tissue for destroying endogenous auxin, compared to the extensive capacity of the tissue for destroying auxin in air.

Sircar, Das and Lahiri (1955) compared the growth of rice embryos when grown under water and in air. Some of their results are shown in Table 28.

TABLE 28.

Growth of rice embryo (var. Bahsamanik) kept under water and in air, 144 hrs. old at 25°C in darkness. (From Sircar, Das and Lahiri, 1955).

	Embryo with complete endosperm	
	Under water	In air
Mesocotyl (mm)	0	45.8
Coleoptile (mm)	34.5	7.0
Root (mm)	0.1	58.4

These results are in agreement with those of Yamada (1954) in that growth of the coleoptile is much greater under water. Of more interest to us here, possibly, is the much superior growth of the root in air to that under water. If Yamada's hypothesis is correct and if the general pattern of auxin metabolism in the root is similar to that in the stem, then it

is a reasonable contention that the roots of young rice seedlings which develop under water will also possess an abnormally high endogenous auxin content. The most common natural auxin is indole-3-acetic acid (IAA) and it is possible that there is increased supply of this auxin when plant material is allowed to grow under water (Kefford, 1962).

If this is true for semi-aquatic plants, it would seem that the conclusions should apply even more so to plants which normally require an aerobic environment for germination and development. An investigation of the auxin content of soaked seeds should therefore possibly prove a fruitful line of research.

To support the contention that this is a worthwhile investigation, a record of some of the effects of soaking pea seeds on the morphology of the resulting seedlings will be presented with comparison of some of the reported effects of auxins (chiefly IAA) on young seedlings.

1. Radicle length.

The data in experiments reported so far show that soaking pea seeds results in suppression of growth of the radicles which grow from these seeds. This effect may or may not depend on the endogenous supply of auxin but we can record a few of the reports of the effects on root length of the application of exogenous auxin. One of the bioassay techniques for determining the strength of auxin solutions is to grow cress

seedlings on filter paper moistened with the test solution (Moewus, 1948; Audus 1949, 1951). Both authors employ the observation that auxin solutions suppress the growth of the radicles of the cress seedlings by amounts depending on the concentration of the solutions.

Noirfalise (1940) showed that growth of seedling roots of Vicia faba was inhibited by growing the seedlings in solutions of IAA. Naylor and Rappaport (1950) found that IAA could increase, suppress or have no effect on the length of roots of pea depending on concentration. In 1951, Pilet found that IAA first stimulated, then suppressed the elongation of roots of Lens culinaris but Burstrom (1957) and McManus (1960) both reported initial suppression followed by adaptation, and Manos (1961) showed that IAA at a concentration of 1.2×10^{-4}M produced complete inhibition of growth of pea roots: when the concentration was reduced to 1.2×10^{-6}M, growth was initially suppressed but 6 days later stimulation was observed. This adaptation to IAA rather indicates that the exogenous IAA is being taken up by the plant and used in controlling the length of the radicle. It may not, however, be the only controlling factor (Audus and Gunning, 1958).

2. Swelling.

When seedlings are grown in media moistened with IAA solutions there is frequently an increase in the diameter of the roots (Noirfalise, 1940). Hughes and Street (1960) found

that IAA treatment of excised tomato roots can result in increased diameter (as well as a partial inhibition of linear growth). Unlike radicle length, this feature was not used to assess the effects of soaking pea seeds in water but it was frequently observed, particularly when the radicles were less than 5 mm long. Swollen radicles were often observed after the seeds had been soaked for 96 hours and Plate A shows a very distinct difference between the tip of a radicle from a seed soaked for 96 hours before sowing and a control.

3. Callus formation.

It is well known that, when IAA is applied to certain tissues, callus is produced. The formation of callus following soaking was not a frequent occurrence but it was observed on several occasions and therefore is worthy of mention.

4. "c-tumour" formation and blunt tips.

Bulbous swellings occurring just behind the root tips of seedlings are commonly referred to as "c-tumours". McManus (1960) found these occurring at the root tips of Allium cepa L., when the root tips were treated with 1 ppm IAA (It is not reported how the IAA was applied). Manos (1961) found similar aberrations when roots of pea were allowed to grow in 1.2×10^{-4}M IAA.

In the experiments on soaking pea seeds, c-tumours occurred on rare occasions but abnormalities similar to this, at probably a more advanced stage, occurred with great

regularity (see Plate 2). These we shall describe as blunt tips: instead of the root tapering at its distal end to a very fine tip it ends sharply as if truncated and is slightly swollen at its extremity. These blunt tips are in fact like c-tumours with the small tip having completely disappeared. Table 29 shows the frequency of occurrence of these aberrations.

5. Excessive curling.

Early workers in the auxin field found that they could induce roots to curl by applying agar blocks, containing auxins, eccentrically to decapitated roots. It was concluded that auxin diffused from the blocks and inhibited growth on that side of the root on which they were placed. The result was more rapid growth on the other side of the root and consequent curling. Although some doubt now surrounds these findings, the primary importance of auxin in determining the direction of growth of roots is still recognised and it seems reasonable to postulate if a treatment results in an excessively large number of curled roots, that unequal distribution of auxin plays at least some part in producing the abnormalities.

Curled radicles were observed frequently in seedlings produced from soaked seeds and Table 29 shows that when the soaking period was 72 hours, close on 40% of the radicles were abnormal in this respect.

TABLE 29.

The frequency of occurrence of radicles with blunt tips and radicles which are excessively curled among seedlings grown from soaked Maple pea seeds.

Hours soaking	No. seedlings scored	% blunt tips	% curled radicles
0	225	0.4	0
24	75	6.7	4
72	150	45.3	36.7

6. Negative geotropism.

This feature is a very striking one, with the radicles frequently appearing above the surface of the growing medium. This did not, however, occur in populations of seedlings from soaked seeds with a frequency high enough to allow the abnormality to be selected as a criterion of the effect. A record of the pooled data of several experiments is shown for interest in Table 30.

TABLE 30.

The frequency of occurrence of radicles exhibiting negative geotropism among seedlings grown from soaked Maple pea seeds.

Hours soaking	No. seedlings scored	% radicles showing negative geotropism
0	312	0
24	75	1.3
72	177	15.3
100	33	27.3 +

+ These roots were very severely damaged.

The role of auxin in the geotropic response of roots has not yet been fully explained. Until recently, the mechanism for determing geotropic response was considered to be distribution of auxin in the root in response to gravity (Went-Cholodny theory). It was thought that this auxin could possibly be IAA but considerable doubt has been cast on this simple theory. Audus and Shipton (1952), for example, erected a working hypothesis that root growth is controlled by more than one hormone. They postulated that one of these hormones might be an unidentified inhibitor whose action is opposed by IAA.

Audus and Brownbridge (1957) in the first of a series of papers on "Studies on the Geotropism of Roots" further contest the classical Went-Cholodny theory and suggest that the geotropic response is due to the production of an endogenous growth inhibitor in the extending cells of the lower side of the root. The complete independence of the growth actions of this inhibitor and of various applied auxins suggest that it is not IAA or any similar compound.

In a later communication (Lahiri and Audus, 1960) it is claimed that IAA, even if present in roots of Vicia faba, may play only a subsidiary role in the growth control of roots where many other substances with similar physiological properties are present in considerable quantities. More specific data are given later (Audus and Lahiri, 1961; Lahiri and Audus, 1961). It is claimed that the majority of the data in the literature

favour an effect of gravity on auxin metabolism as the geotropic stimulus, and not a simple transport redistribution of the auxin. It is suggested that this metabolism involves a system of growth inhibitors and stimulators viz. an IAA-like auxin AP(ii), a second auxin which inhibits root growth - AP(iii), a third auxin which accelerates root growth - AP(i) and possibly several others.

While these workers attribute little of the geotropic response to IAA itself they do not deny that the response is auxin controlled. It thus seems reasonable for us to postulate that the abnormal geotropic responses observed following soaking may be due to an upset in the auxin metabolism of the root.

7. Longitudinal split.

Frequently, several seedlings from soaked seeds were observed to possess a fine depression which ran the length of the radicle and, in extreme cases, completely divided the radicle in 2. At first this was regarded as simply a freak occurrence but it subsequently occurred with a frequency high enough to demand closer observation and it is significant to note that Manos (1961) reported gross abnormalities from day 4 to day 7 in the growth of pea roots in 1.2×10^{-4}M IAA. In many treated roots it was found that the steles split and lateral root primordia appeared.

8. Initiation of adventitious roots.

This feature was not observed till an experiment was carried out with seeds which had been soaked for 96 hours and then allowed to grow for a further 7 days. When the seedlings were scored, 32 out of 75 possessed prominent adventitious roots.

Van der Lek (1934) postulated that the formation of adventitious roots is initiated by the reaction between pericyclic cells and a hormone which travels from buds or developing shoots (auxin). Thimann and Koepfli (1935) found that synthetic IAA could be used to stimulate the initiation of adventitious roots. It is now generally accepted that auxin alone, translocated from the aerial parts of cuttings, does not induce adventitious root formation. There is considerable controversy as to what other factors are required. Went and Thimann (1937) suggested that the essentials for root formation are auxin (probably IAA), a supply of carbohydrates and the vitamin biotin. It has not yet been proved that this is applicable to all plants.

In the seedlings produced after 96 hours soaking, practically all the radicles were damaged (see experiment 2). The plants reacted to this by developing a root system at the base of the stem. Normal pea plants of this age seldom possess adventitious roots and it is possible that a high auxin status in the stem, caused by the treatment, is the initial stimulus

for their production.

9. Increased production of lateral roots.

In Experiment 1 it was shown that soaking caused an increase in the number of lateral roots per unit length of radicle. This is yet another feature of auxin metabolism. Noirfalise (1940), for example, showed that there was increased lateral root production when seedlings of Vicia faba were grown in solutions of IAA.

As with adventitious roots, auxin alone does not seem to be capable of stimulating lateral root production. Other factors such as the kinins may be involved (Skoog and Miller, 1957).

When all these comparisons between the observed effects of soaking and the known responses of young plants to auxins are considered, the similarities of the responses appear too close to allow us to neglect the hypothesis that excess auxin (possibly IAA) within the young plants is at least partly responsible for the damage.

The following experiments were carried out to investigate the response of seeds or seedlings to the application of exogenous IAA.

Experiment 8. A comparison of soaking Maple pea seeds in distilled water and in solutions of IAA.

In a preliminary experiment it was found that the effects

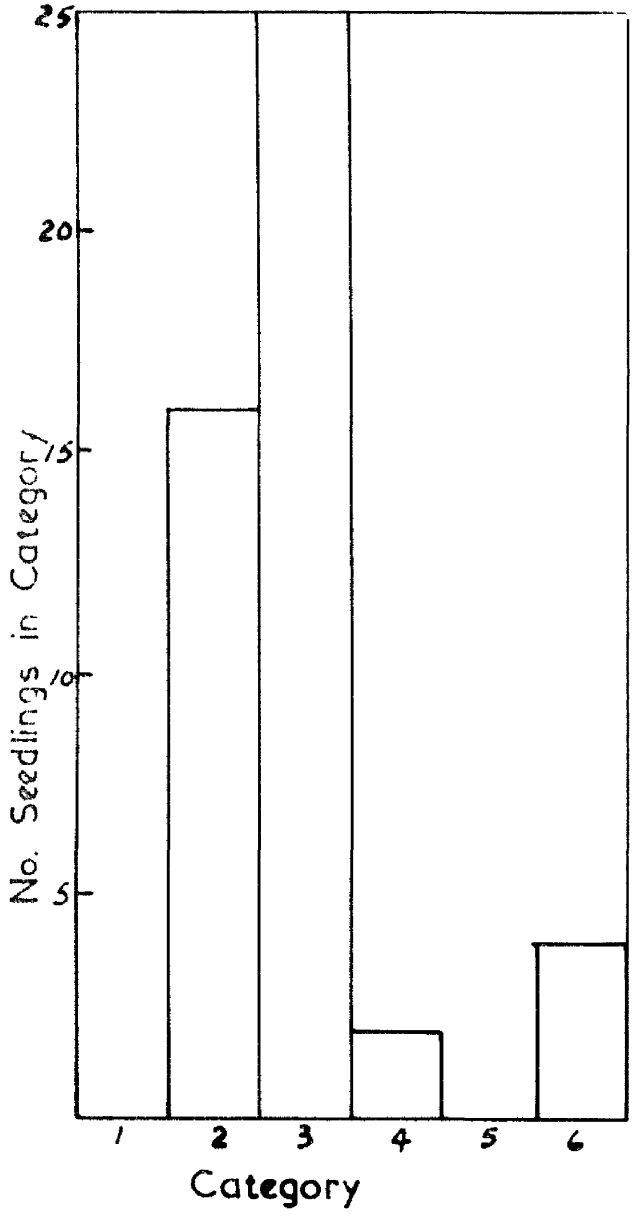
of 72 hours soaking in distilled water could be simulated by soaking seeds in solutions of IAA, at a concentration of about 10^{-5}M.

50 Maple pea seeds were sown directly and, at the same time, 3 pairs of flasks were set up with concentrations of IAA, 0, 2.5×10^{-5}M and 5×10^{-5}M respectively. After 48 hours soaking at 20°C, 3 flasks (one at each concentration) were withdrawn and the seeds were sown out. This was repeated after 72 hours. All seeds were allowed to germinate and develop till 7 days had elapsed from the beginning of the experiment.

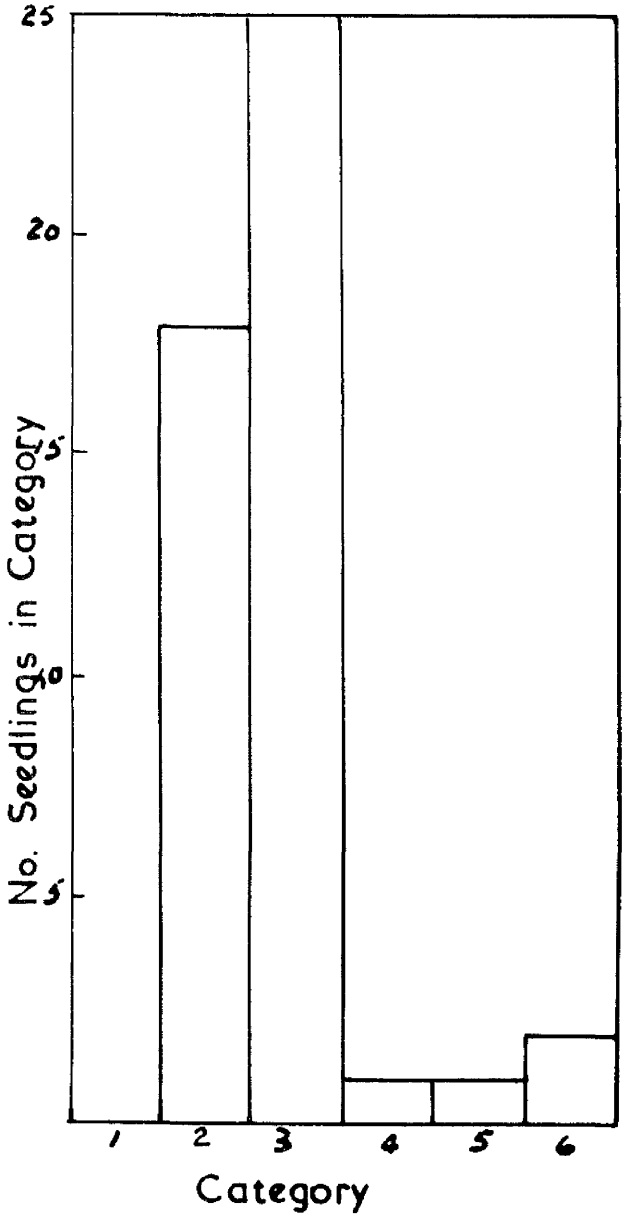
6 categories of development were erected into which all the seedlings were classified. The categories may be described as follows:-

1. Normal, radicle longer than 5 cm with many lateral roots.
2. Normal, radicle less than 5 cm with no lateral roots.
3. Radicle damaged: longer than 1 cm but either having a blunt tip or being excessively curled.
4. Radicle damaged: very short and stubby, plumule just emerging.
5. Radicle damaged: very much swollen; no plumule visible.
6. Seed ungerminated

Table 31 shows the number of seedlings in each category.



Seeds soaked for 72 hours in distilled water before sowing.



Seeds soaked for 48 hours in 2.5×10^{-5} M IAA solution before sowing.

FIGURE 9

A comparison of the effects of soaking Maple pea seeds anaerobically in distilled water and IAA solutions before sowing on the morphology of the resulting seedlings (See text for description of categories)

TABLE 31.

Classification into 6 selected categories of 7 day old Maple pea seedlings produced from seeds soaked in solutions of IAA.

Hours soaking	Conc. of IAA solution	Category					
		1	2	3	4	5	6
0	-	45	2	-	-	-	-
48	5×10^{-5}M	-	16	29	1	2	-
	2.5×10^{-5}M	-	18	25	1	1	2
	0	17	10	15	1	1	1
72	5×10^{-5}M	-	3	24	18	2	3
	2.5×10^{-5}M	-	1	40	3	3	3
	0	-	16	25	2	-	4

(Several of the seedlings in each treatment were removed before harvest for demonstration purposes)

Table 31 shows that IAA has a pronounced influence on the severity of the adverse effects of soaking. Soaking in IAA solutions brings about a decrease in the number of normal radicles (Categories 1 and 2) compared with soaking in distilled water for the same length of time.

Comparison of the 48 and 72 hour soaking periods reveals that there are similarities in the distribution of seedlings within the selected categories between the following 2

treatments, 48 hours soaking in 2.5 x 10^{-5}M IAA and 72 hours soaking in distilled water. Figure 9 shows this comparison in histogram form.

This experiment was not large enough to allow any definite conclusions to be drawn and the evidence produced is purely circumstantial. Nevertheless, there is an indication in these results that there may be an abnormally high content of IAA in soaked seeds. Another experiment gave similar results but, as will be seen later (Experiment 11) it was not possible to attribute all the soaking damage to an excess of IAA within the seed.

Experiment 10. A preliminary experiment to investigate the response of Maple pea seeds to IAA supplied to the medium in which the seeds are germinating aerobically.

25 seeds Maple pea were sown out and moistened with 200 ml 10^{-3}M IAA, 10^{-4}M IAA, 10^{-5}M IAA and distilled water. At the same time, 25 seeds were soaked anaerobically in distilled water. The latter were kept under soaking conditions for 72 hours, then sown out. 2 days later, all seedlings were harvested and scored for radicle damage: the lengths of the radicles were also measured.

The first obvious feature of this table is the high frequency of damage whenever IAA is applied to the seeds. The nature of this damage is like that found when seeds are soaked

in distilled water for upwards of 3 days but there was greater swelling at the proximal ends of the radicles. The damage cannot therefore be claimed to be exactly the same possibly because even if it were accepted that soaking in distilled water did result in an excess of auxin within the seeds, we would have to find the exact duration of the soaking period which was equivalent to an exact concentration of exogenous auxin (There is also no guarantee that all the IAA in the bathing solution is taken up by the seeds). In this case, although the damage is similar, 72 hours soaking in distilled water does not produce exactly the same type of abnormality as any of the concentrations of IAA used.

TABLE 32.

The effects of sowing seeds of Maple pea directly in media moistened with IAA solutions on the lengths of the radicles and frequency of radicle damage.

	Soaked 72 Hours Distilled Water	Conc. IAA in growing medium			
		0	10^{-5}M	10^{-4}M	10^{-3}M
% Damage	76	4	100	100	100
Mean radicle Length (cm).	1.5	4.6	2.5	1.0	0.8
S.E. of mean	0.2	0.3	0.1	0.04	0.02

The radicle lengths again show that exogenous IAA at the concentrations used has a suppressive effect on root growth. At 10^{-3}M root growth is almost completely inhibited but at 10^{-5}M

the radicle lengths are somewhat closer to those of the control seedlings and abnormalities are confined to c-tumours and excessive curling.

Experiment 11. The response of Maple peas to IAA supplied aerobically and anaerobically to the seeds for 3 days before sowing.

Since concentrations of IAA from 10^{-3}M to say 10^{-7}M are known to have different effects on the growth of peas this concentration range was selected for a more complete investigation of the response of peas to the auxin. Solutions of the following concentrations were prepared:-

$\frac{10^{-3}}{3}$ M, $\frac{10^{-3}}{3^2}$ M, $\frac{10^{-3}}{3^3}$ M, $\frac{10^{-3}}{3^4}$ M, $\frac{10^{-3}}{3^5}$ M, $\frac{10^{-3}}{3^6}$ M, $\frac{10^{-3}}{3^7}$ M and $\frac{10^{-3}}{3^8}$ M

Absolute ethanol (5% in 10^{-3}M stock solution) was used to make up the IAA solutions. Street, Griffiths, Thresher and Owens (1958) have shown that Chlorella vulgaris can utilise ethanol as a carbon source. They found that when the alga was supplied with IAA in alcoholic solution (0.4 ml ethanol/litre) there was increased dry weight and number of cells/cubic mm, compared with IAA supplied at the same concentration in aqueous solution. Cossins and Turner (1963) have shown that plants can metabolise ethanol under aerobic conditions. These authors conclude that many of the results previously attributed to IAA are possibly only a reflection of the capacity of the

plants to utilise the ethanol, used to prepare the IAA solution, as a source of carbon. Because of this it is perhaps inadvisable to recommend this method of dissolving IAA for any further investigations.

Nevertheless, when this experiment was repeated using IAA made up only in aqueous solution it was shown statistically that there were no significant differences between the experiments. The results of this experiment are therefore acceptable as being attributable to differences in IAA concentration and not to small differences in ethanol concentration.

2 flasks at each of the above concentrations were set up with 50 seeds in each; 2 flasks were also set up containing 50 seeds and distilled water. The flasks were kept at 20°C for 72 hours then the seeds were removed and washed: 75 seeds from each solution were then sown out in peralite moistened with tap water.

For the aerobic treatment, samples of 25 seeds were placed in pots containing dry peralite. The peralite was then moistened with equal amounts of each of the above solutions (3 samples of 25 seeds at each concentration). After 72 hours, these seeds were removed, washed and resown in peralite moistened with tap water. At this stage, several of the seeds had already germinated.

4 days later all seedlings were harvested; the lengths

of the radicles were measured and a count made of the number of damaged radicles.

TABLE 33.

The effects of 72 hours aerobic and anaerobic exposure of Maple pea seeds to IAA before sowing on the lengths of the radicles of the seedlings and the frequency of radicle damage.

(a) Mean radicle length (cm).

	Concentration of IAA								
	1	2	3	4	5	6	7	8	0
Aerobic	5.8	7.3	7.9	8.4	8.1	8.1	8.2	8.3	8.6
Anaerobic	1.8	3.3	3.8	3.9	3.4	3.5	3.9	4.5	4.1

(b) Radicle damage (%).

	Concentration of IAA								
	1	2	3	4	5	6	7	8	0
Aerobic	35	33	35	45	30	23	15	16	16
Anaerobic	80	65	64	60	71	68	57	39	57

Where 1 = $\frac{10^{-3}}{3^1}$ M 2 = $\frac{10^{-3}}{3^2}$ M etc.

A striking feature of this experiment was the way in which the radicles of the aerobically treated seedlings had apparently recovered from the IAA treatment. On removal from the IAA the radicles were very short and rather swollen (compared with the controls) but they appeared to be able to adapt themselves to IAA taken up and recover from the treatment. The result of

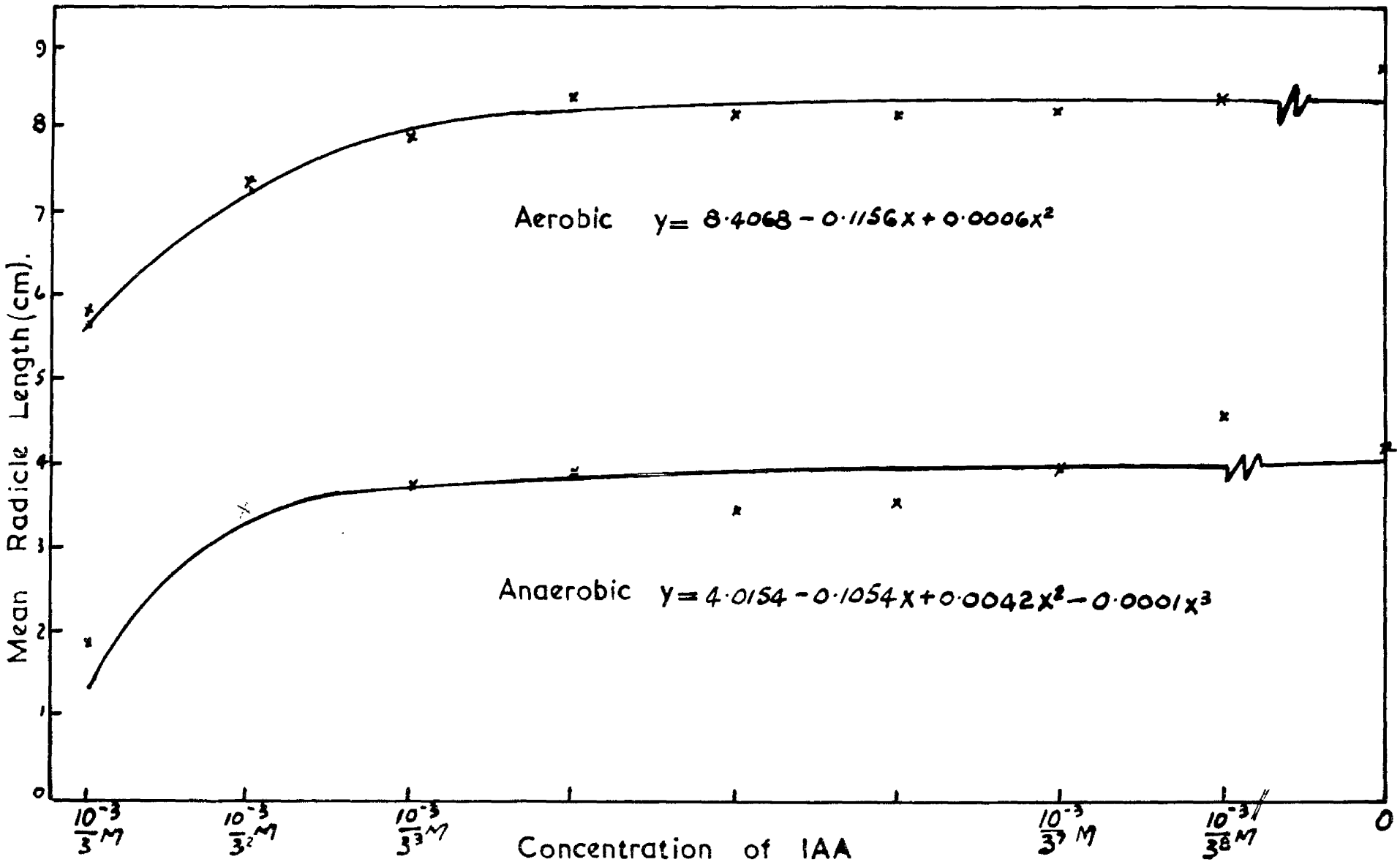


FIGURE 10

A comparison between the effects of supplying Maple pea seeds with exogenous IAA anaerobically and aerobically for 72 hours before sowing on the radicle length of the resulting seedlings.

this was the absence of severe damage in seedlings from seeds which had been exposed to IAA aerobically for the first 3 days of germination and development.

An analysis of the variation found in root length shows that there are significant differences attributable to IAA treatment. It can be seen from Table 33 (a) that most of this effect is confined to the 2 highest concentrations.

TABLE 34.

Analysis of variance: the radicle lengths of Maple pea seedlings after 72 hours aerobic and anaerobic exposure of the seeds to IAA before sowing.

Source	DF	Sum of Sqq.	Mean Sq.	F
Replicates	2	13.41	6.70	0.61
Soaking	1	5,234.87	5,234.87	477.19**
IAA concs.	8	917.65	114.71	10.46**
Interaction	8	343.22	42.90	3.91**
Residual	1330	14,593.90	10.97	
Total	1349	21,103.05		

Figure 10 shows the relationship between radicle length and IAA concentration. The first striking feature of the 2 curves is their similar pattern and similar equations, except of course for the constant.

$$\text{Aerobic:-}\quad y = 8.4068 - 0.1156x + 0.0006x^2$$

$$\text{Anaerobic:-}\quad y = 4.0154 - 0.1054x + 0.0042x^2 - 0.0001x^3$$

The term in x^3 in the second equation, though very small, is required to give the best agreement with the data.

It can be seen from the 2 curves that concentrations of IAA below $\frac{10^{-3}}{3^4}$ M (*ca.* 10^{-5}M) have little effect on radicle length whether applied aerobically or anaerobically. Above this concentration the suppressive effect of IAA increases steadily with increasing concentration.

Since the response of pea seeds to exogenous IAA supplied under aerobic conditions is so similar to that of seeds to exogenous IAA supplied anaerobically it is not possible in this experiment to demonstrate any additive or synergistic effect of endogenous auxin. If this were the case one would have expected that under anaerobic conditions, at certain concentrations there would have been a greater suppressing effect of IAA on radicle length than was achieved by exposing the seeds to IAA under aerobic conditions at these same concentrations.

The design of this experiment and the nature of indole acetic acid, however, make it possible that such an effect may have been obscured. The uptake of IAA under aerobic and anaerobic conditions is not the same: under anaerobic conditions the seeds are surrounded by the solution whereas under aerobic conditions the solution is confined to the surface of the peralite particles surrounding the seeds. IAA is also very liable to oxidation and to destruction by micro-organisms so there is no guarantee that all the IAA supplied to the seeds

is taken up.

Finally, the response of seeds immediately on the restoration of normal germination conditions may be different. After 3 days exposure to IAA under aerobic conditions, the seedlings appear able to accomodate themselves to any excess IAA taken up and subsequent growth is very like that of the controls. After anaerobic exposure of the seeds to IAA, some are so badly damaged that recovery is impossible.

Although this experiment does not shed any light on the activity of the endogenous auxin it does provide further evidence that, when soaking seeds are supplied with exogenous IAA, the adverse effects of the treatment are considerably intensified.

. . .

If the auxin metabolism of the seeds is upset by soaking, one would expect that other growth regulatory substances, e.g. the Gibberellins and the Kinins could influence the intensity of the observed effects. This statement is based on recent work which points to the existence of an interaction between auxins, gibberellins and kinins. Recent reviews on the gibberellins include those by Stowe and Yamaki (1957), Brian (1959 a, b), Phinney and West (1960) and Brian, Grove and MacMillan (1960). Kinins have been considered by Miller, Skoog, von Saltza and Strong (1955); Miller, Skoog, Okumura, von Saltza and Strong (1955, 1956); Strong (1958) and Miller (1958, 1960, 1961).

Brian and Hemming (1957, 1958) were first to demonstrate that, in light grown pea stem sections, growth responses to gibberellic acid depend on the presence of auxin. The mechanism of the synergistic response obtained has not yet been elucidated but Galston and Warburg (1959) have put forward evidence in favour of Brian and Hemming's hypothesis (1958) that stem extension is controlled by 3 factors - auxins, gibberellins and a third factor, possibly a fatty acid ester (Stowe, 1958).

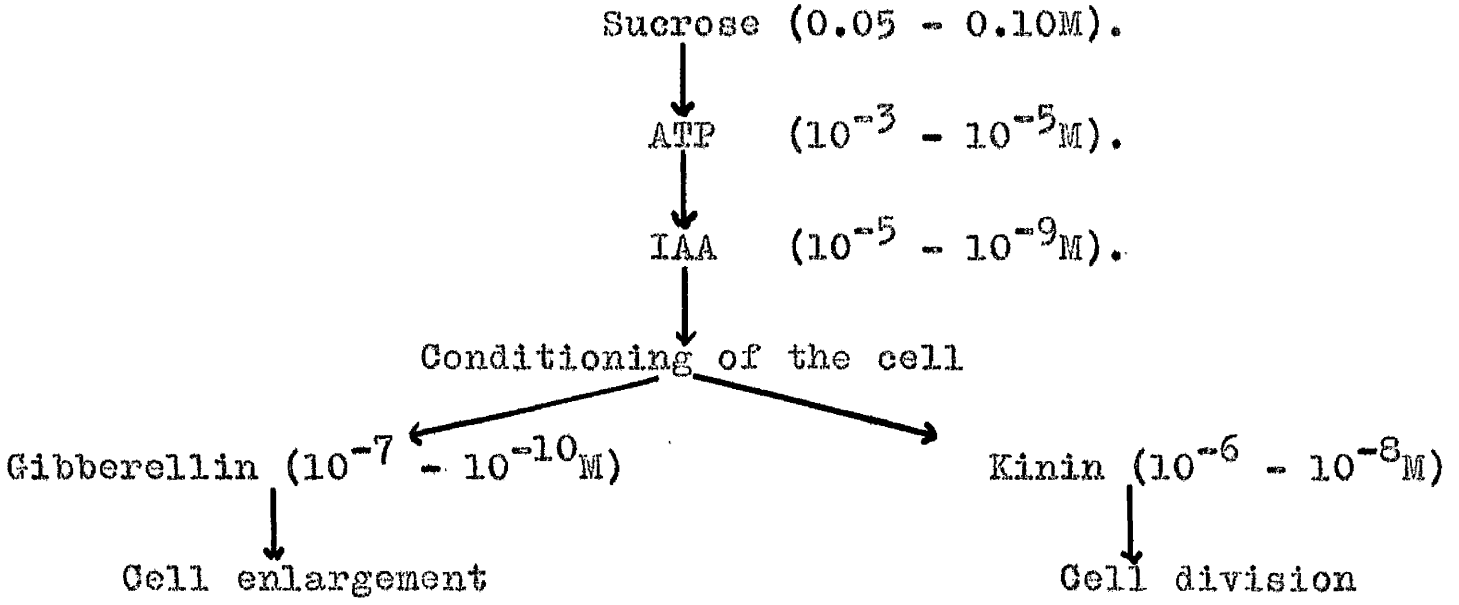
Brian and Hemming (1958) postulated that the gibberellin blocked the action of some inhibitory influence in the plant, thus releasing the full growth promoting potential of the endogenous auxins.

Gibberellin has also been claimed to reduce the enzymatic destruction of IAA (Pilet, 1957; Pilet and Collet, 1959) and Galston (1957) has put forward evidence for the existence of an inhibitor of IAA oxidase in plant tissues, which increases in concentration after treatment of plants with gibberellin. There is much conflicting evidence surrounding these last 2 claims and further reference will be made to this later.

Interaction between the kinins and auxins has not been extensively studied but Katsumi (1962) has shown that sections of pea stem placed in solutions of kinetin show lateral swelling, presumably due to kinetin induced cell division. The effect was enhanced when the auxins IAA, NAA or 2:4 D were added.

The evidence is thus indicative of the growth responses of

both the gibberellins and the kinins being dependent on the presence of an available supply of endogenous auxin. Kefford and Goldacre (1961) attempted to summarise the evidence by putting forward a scheme whereby the endogenous auxin (probably IAA) predisposes the cells to be activated into either enlarging or dividing. If gibberellin is supplied, growth occurs by means of cell enlargement; kinetin brings about growth by cell division.



(From Kefford and Goldacre, 1961)

This scheme cannot possibly explain all the responses, however, because, for example, gibberellins can stimulate cell division (Yabuta and Hayashi, 1939; Griffith, 1957). There is also a gap in the centre of the scheme as there is no real evidence of IAA creating a certain condition within the cell.

Obviously, at present, the ideas on auxin, kinin and gibberellin interaction are in a state of flux. For our purpose, it is sufficient at present, to know that there is interaction:

it is hence possible that, if soaking results in the accumulation of auxin in the seed, the other growth regulatory substances will have an effect on the response of the seeds to the treatment.

Most of the work carried out, involved gibberellic acid (gibberellin A_3) but a small supply of kinetin was available and the results of one experiment using kinetin are recorded.

Experiment 12. The influence of gibberellic acid on the response of Maple pea seeds to anaerobic soaking.

8 pairs of flasks were set up, each pair containing capacity volumes of the following solutions:- distilled water and gibberellic acid, concentrations 10^{-8}M, 5 x 10^{-8}M, 10^{-7}M, 5 x 10^{-7}M, 10^{-6}M, 5 x 10^{-6}M and 10^{-5}M. 50 seeds were placed in each flask which was then sealed and kept at 20°C for 3 days. 75 seeds from each pair of flasks were then sown out and left to grow for a further 4 days, then the radicles of the seedlings were measured and scored for damage.

Table 35 indicates that, in the concentration range 10^{-7} - 10^{-6}M, gibberellic acid has a mitigating influence on the adverse effects of soaking. The differences among the treatments, however, are small and an analysis of variance (Table 36) shows that they are not large enough to be statistically significant.

TABLE 35.

The effects of soaking Maple pea seeds for 72 hours in solutions of gibberellic acid (GA) on the frequency of damage and mean radicle length of the resulting seedlings.

Concentration GA	Mean rad. lgth. (cm)	% Damage
0	3.4	69
10^{-8}M	3.9	55
5 x 10^{-8}M	3.6	57
10^{-7}M	3.9	48
5 x 10^{-7}M	3.9	48
10^{-6}M	4.1	45
5 x 10^{-6}M	3.6	64
10^{-5}M	3.4	57

TABLE 36.

Analysis of variance: radicle lengths of pea seedlings after soaking the seeds in solutions of gibberellic acid.

Source	DF	Sum of Sqq.	Mean Sq.	F
Replicates	2	0.63	0.32	0.05
GA concs.	7	31.23	4.46	0.67
Residual	590	3,927.29	6.66	
Total	599	3,959.15		

A contingency table, drawn up to determine if there is a connection between gibberellic acid treatment and frequency of damage, is shown in Table 37. The value of Chi squared is 6.64 which is slightly higher than the expected value for one degree of freedom at the 5% probability level (3.84). This is evidence that there is a connection between gibberellic acid treatment and the frequency of radicle damage.

TABLE 37.

Contingency table showing observed and expected relationship between gibberellic acid and the frequency of radicle damage in seedlings of Maple pea produced from seeds soaked in solutions of gibberellic acid.

Observed / Expected	GA	No GA
No. normal radicles	244 / 233.6	23 / 33.4
No. damaged radicles	281 / 291.4	52 / 41.6

The evidence put forward so far in favour of there being a connection between soaking damage and gibberellic acid treatment is not strong but is sufficient to justify further investigation.

Experiment 13. The influence of gibberellic acid and kinetin on the severity of the adverse effects of soaking Maple pea seeds.

A factorial experiment was designed to investigate the response of pea seeds to soaking in solutions of gibberellic acid and kinetin. The concentrations of gibberellic acid were 10^{-7}M and 10^{-6}M and those of kinetin 10^{-7}M, 10^{-6}M and 10^{-5}M. The gibberellic acid solutions were made up from a 10^{-3}M stock solution containing 5% ethanol. Difficulty was experienced when making up a stock solution of 10^{-4}M kinetin. Heat was required to ensure that the solid material dissolved in aqueous solution.

50 Maple pea seeds were sealed in each of the solutions for 3 days then sown out and allowed to grow for a further 4 days. The seedlings were harvested and scored for radicle length and damage (Table 38).

An analysis of variance (Table 39) shows that significant differences within the experiment can be attributed to gibberellic acid treatment. Kinetin effects are only just below the level of significance (5%) and there is significant interaction between the 2 growth regulatory substances.

The evidence now points rather more strongly towards gibberellic acid having a moderating influence on the adverse effects of soaking, a maximum influence being exerted at a concentration of 10^{-6}M.

TABLE 38.

Some effects of soaking Maple pea seeds anaerobically in solutions of kinetin and gibberellic acid on the growth of the resulting seedlings.

(a) Mean radicle length (cm).

Conc. GA / Conc. Kinetin	0	10^{-7}M	10^{-6}M
0	2.58	3.29	4.05
10^{-7}M	3.43	2.98	4.32
10^{-6}M	2.54	2.67	4.14
10^{-5}M	3.29	2.55	3.17

(b) % Radicle damage.

Conc. GA / Conc. Kinetin	0	10^{-7}M	10^{-6}M
0	74	46	38
10^{-7}M	58	46	26
10^{-6}M	68	62	30
10^{-5}M	58	58	50

Table 38 (a and b) shows that gibberellic acid at this concentration has an influence on both root length and percentage damage: this influence is exerted independent of kinetin except when the latter is present in high concentration (10^{-5}M). Under these circumstances the apparent moderating influence of

the gibberellic acid is not shown. Since no further work was done with kinetin, it is inadvisable to draw any further conclusions on its effects from this one experiment.

TABLE 39.

Analysis of variance: radicle lengths of pea seedlings after soaking of the seeds in solutions of gibberellic acid and kinetin.

Source	DF	Sum of Sqq.	Mean Sq.	F
Kinetin	3	29.88	9.96	2.49
GA	2	135.88	67.94	17.03**
Interaction	6	70.84	11.81	2.96**
Residual	588	2,347.30	3.99	
Total	599	2,583.90		

When the data of Experiments 12 and 13 are compared, a striking feature is the discrepancy between the mean lengths of the radicles of the controls. A possible explanation for this is that different supplies of seeds were used: In Experiment 13 the seeds were approximately 12 months old while in Experiment 12 they were only about 6 months old. The mean lengths of seedlings soaked in 10^{-6}M gibberellic acid are identical; therefore, there is a considerably greater moderating influence shown in Experiment 13.

The question now arises as to whether or not these results should be accepted as proof that gibberellic acid has an

influence on the response of seeds to soaking. In the light of present knowledge it is difficult to accept that gibberellic acid can have any moderating influence. In the first place there is no guarantee that much of the gibberellic acid penetrates the seeds. Brian and Elson (1963) have shown that barley grains under anaerobic conditions take up very little gibberellic acid from solution. Secondly, it is difficult to conceive how the gibberellic acid can have an effect on root length. It has been reported that crude gibberellins have an inhibitory effect on root growth (Yabuta and Hayashi, 1939; Yabuta, Sumiki, Fukunaga and Horiuchi, 1951): this has been confirmed by more recent work (Brian, Elson, Hemming and Radley, 1954). Reports of gibberellins stimulating root growth are infrequent but Whaley and Kephart (1957) have shown that excised roots of certain strains of maize respond to gibberellins by increased growth and Butcher and Street (1960) have shown that at suitable concentrations (in the region of 0.01 mg/l., ca. 3×10^{-8}M) and under suitable nutritional conditions gibberellic acid can stimulate growth of excised tomato roots. Butcher (1963) has provided evidence that gibberellin biosynthesis (probably gibberellin A_1) proceeds in cultured root tissues and increased growth may be attributable to this natural gibberellin.

It does seem possible then, that the gibberellins can have a stimulatory effect on root growth under suitable conditions.

Where seedlings from seeds soaked in gibberellic acid are concerned, increased growth may be produced simply by the gibberellic acid exerting a direct influence on the young radicle when aerobic conditions are restored to the seeds. A more attractive hypothesis is that there is interaction between the supplied gibberellic acid and the endogenous auxin either during soaking or immediately on removal of the seeds to aerobic conditions.

Street (1958) has postulated that if endogenous auxin levels in the root are supra-optimal, gibberellin accelerates the normal loss of meristematic activity (ageing) thus inhibiting growth of the root. Is it possible that this effect is due simply to the high auxin content <u>per se</u>? If gibberellin is supplied - at the appropriate concentration - it is possible that it could interact with the excess endogenous auxin, thus directing it towards the control of essential plant functions such as cell extension and preventing it from exerting its ageing effect on the meristematic tissues.

Discussion of Part 111.

The hypothesis put forward in this Part is that anaerobic soaking of pea seeds results in an abnormally high endogenous auxin content. To test this, the obvious experiment is to compare directly the auxin content of seedlings given the soaking treatment and control seedlings of the same age. Unfortunately, it has not been possible to make accurate quantitative measurements of the auxin contents of plants (Larsen, 1955). The difficulties involved are that the various forms of auxin in the plant may undergo changes during extraction, e.g. auxin may be liberated from a bound form, precursors may be converted into auxins and most important from our point of view, auxin may be inactivated. The latter process may go on enzymatically even in the presence of solvents like ether and chloroform. Because of the difficulties involved and the strong possibility of obtaining erroneous results, the problem was not approached from this angle.

The line of approach pursued was to supply the seeds with recognised plant growth regulatory substances and note the response of the plants to various concentrations of these substances. Bennet-Clark and Kefford (1953) and Housley and Griffiths (1962) have claimed that IAA is present in pea seedlings. Therefore, it is to be expected, that if the

adverse effects of soaking are caused by an abnormally high endogenous auxin content, these adverse effects could be simulated by artificially raising the IAA content of the seeds during soaking. It is possible that if IAA is supplied exogenously that it is not taken up by the seeds and, since extraction of IAA is difficult, it is not possible to measure directly the amount of IAA in the seeds before and after application of the exogenous IAA. It is possible, however, to measure the IAA content of the bathing solution before and after the pea seeds have been soaked. When this was done, it was found that, after 72 hours soaking, most of the exogenous IAA had disappeared. Whether it had been taken up or not was still difficult to determine because of the possibility of microbial or chemical destruction. Neither of these possibilities seem likely because, in a control flask without seeds, the IAA content was similar at the end of the 72 hour period to what it was at the beginning of the experiment. Surface sterilisation of the seeds probably removed most of the bacteria before soaking: therefore the evidence points to the IAA being taken up by the seeds. Probably a more convincing argument that the seeds have taken up the IAA is that definite effects are manifest in the young seedlings. This could, of course, have been due to the response of certain endogenous growth substances to the applied IAA but it seems unlikely.

The crux of the argument is whether or not the damage

produced by the exogenous IAA resembles that produced by the soaking treatment. Although IAA treatment generally resulted in the occurrence of abnormalities similar to those found when the seeds were soaked for long periods in distilled water, several well defined differences in response were observed. The principal differences were abnormally large swelling in the hypocotyl zone (immediately outside the seed) and profuse callus formation. Both of these were noticed when seeds were soaked only in distilled water but the frequency of occurrence and degree of severity were not so great as in the IAA treated seedlings. Apart from this, the other characters, such as swelling of the tip, excessive curling and even the negative geotropic response on a few occasions were similar to those found when seeds are soaked in distilled water. Experiment 11 shows that it is very difficult to prove by this method that these abnormalities are caused by excessively high contents of endogenous auxin but, all the evidence considered together, does indicate that it plays at least some part in causing the damage.

The results of the experiments with gibberellic acid have also to be treated with considerable caution. As in the case of IAA there is no guarantee that the gibberellic acid presented to the seeds is taken up. The most striking results for gibberellic acid treatment are those obtained when the seeds were sown in November (Experiment 13). It is possible that these seeds, being at least 12 months old, had a depleted

content of natural gibberellins and so responded more vigorously to the exogenous supply than seeds sown in May which were probably no more than 8 months old.

The increased root length in Experiments 12 and 13 may be due simply to the seed taking up some of the exogenous gibberellic acid during soaking and using this for increased root growth on the restoration of aerobic conditions. The influence of gibberellic acid on the frequency of damage, however, indicates that its effect may be further reaching than simply bringing about slight cell extension in the seedling roots.

Brian and Hemming (1958) postulate that gibberellin acts by inhibiting an endogenous inhibitor of auxin induced growth. If this hypothesis is correct it is not possible to interpret our results on the basis that soaking results in an abnormally high level of endogenous auxin. If this is so, according to Brian and Hemming it would be expected that the gibberellic acid would inhibit an inhibitor of auxin induced growth thus allowing the latter to exert more influence on growth processes i.e. the effects of soaking would be expected to be more severe when the seeds are presented with exogenous gibberellic acid than when they are not. Although the beneficial effects of gibberellic acid treatment are only slight there was certainly no indication that soaking in gibberellic acid increased the intensity of the damage.

If the results given in Experiments 12 and 13 are accepted it is essential to look further for an explanation. Apart from the possibility of simple root cell extension the only other explanation is that the gibberellic acid can augment the endogenous gibberellin supply and so direct some of the excess auxin into activities which can be considered normal in the young seedling. In this way there would be less endogenous free auxin available to disrupt the growth of the seedlings. Whether or not this is a feasible explanation depends on the auxin content of the soaked seed. If it can be proved that it is abnormally high then the gibberellic acid results can be more seriously considered. In the meantime, it is probably sufficient to note that gibberellic acid mitigates the adverse effects of soaking: because of this and the known interaction between auxin and gibberellin it is possible that endogenous auxin is involved in soaking damage.

PART IV.

THE POSSIBLE REGULATION OF THE ENDOGENOUS AUXIN CONTENT IN ETIOLATED PEA SEEDLINGS BY *IN VIVO* ENZYMATIC DESTRUCTION.

The experiments in Part III of this thesis do not allow us to state conclusively that soaking upsets the auxin metabolism of the seeds. Another approach to the problem is to investigate the fate of the endogenous auxin. It is probable that plants are capable of producing more auxin than they require, and that this excess is controlled in such a way that only the correct amount of auxin is made available for the requirements of the plant. It is not known how the plant controls the amount of available auxin but there are 2 current hypotheses, either of which (or both) may be operative.

The auxin may be bound in an inactive form in the plant cell. Siegel and Galston (1953) postulated that the auxin could be bound to a protein but Andreae and Good (1955) found that this was unlikely to occur *in vivo* and showed that the auxin could conjugate with aspartic acid to produce indole-acetylaspartic acid. In subsequent papers, (Good, Andreae and van Ysselstein, 1956; Andreae and van Ysselstein, 1956,

1960 a, b; Andreae and Good, 1957; Andreae, Robinson and van Ysselstein, 1961) accumulated evidence has been put forward in support of this and there is little doubt that exogenous IAA can be converted to indoleacetylaspartic acid when taken up by plants. The formation of indoleacetylaspartic acid has not yet been demonstrated *in vitro*.

The other hypothesis is that the level of auxin is controlled by the enzymatic degradation of any excess produced: the enzyme system responsible is rather loosely referred to as IAA oxidase. Galston (1956) postulated that IAA oxidase not only metabolises exogenous IAA but also controls the endogenous auxin level in untreated tissues. Briggs, Steeves, Sussex and Wetmore (1955), on the other hand, consider that IAA oxidase is only operative at cut surfaces but Andreae and van Ysselstein (1956) and Reinhold (1954) were unable to find any relationship between the area of cut surface and IAA oxidase activity. Andreae and van Ysselstein (1960 b) consider that enzymatic degradation can occur in tissues known to possess peroxidase activity. They conclude however, that the disappearance of IAA, when taken up by pea root tissue during the first 6 hours of incubation, could be almost entirely attributed to conjugation of the exogenous auxin with endogenous aspartic acid. Any enzymatic destruction was considered to be confined to a small area of tissue, probably the root cap and the epidermis.

The part played by these 2 systems in the control of auxin

within the plant has been the subject of considerable discussion but some measure of agreement does seem to have been achieved. Fang, Theisen and Butts (1959), using radio-active (carboxyl-^{14}C- labelled) IAA have shown that, when sections of pea root tips are supplied with exogenous IAA over a period of 19 hours the major portion of the absorbed IAA (80 - 92%) is metabolised via oxidative decarboxylation, the remainder being changed into indoleacetylaspartic acid: very little free IAA is left in the tissues after 24 hours incubation. These are substantially the same results as those found by Andreae and van Ysselstein (1956).

It is clearly impracticable to examine here all the aspects of the fate of either endogenous or exogenous auxin but several experiments were carried out to investigate the possible role of IAA oxidase in pea seedlings. Two lines of investigation were pursued (a) the influence of supplying soaking seeds with manganous ions and (b) the estimation of the in vitro IAA oxidase activity of aqueous extracts of pea seedlings.

The manganous ion is known to affect several biochemical reactions e.g. it stimulates the activity of arginase, enolase and peptidases in animals (Fruton and Simmonds, 1958). Ochoa and Weisz-Tabori (1948) have shown that the decarboxylation of oxalosuccinic acid to α-keto-glutaric acid by decarboxylase in animal tissues requires a supply of manganous ions. Malic

enzyme activity has been found in plants and bacteria (Kraemer, Conn and Vennesland, 1951) and Ochoa, Mehler and Kornberg (1948) have shown that manganous ions are required for the malic enzyme to break down malic acid to pyruvic acid and CO_2.

Wagenknecht and Burris (1950), Gortner and Kent (1953), Siegel and Galston (1955), Galston and Dalberg (1954), McLachlan and Waygood (1956), Hillman and Galston (1956), Mudd, Johnson, Burris and Buchholtz (1959), Furuya and Galston (1961) and Sacher (1962) have all claimed that IAA oxidase activity is stimulated *in vitro* by manganous and/or manganic ions.

Kenten and Mann (1949, 1950) postulated that the manganous ion acts in a redox capacity and, though this is probably true, the exact role of manganese in connection with IAA oxidase has still not been determined. Kenten (1955) in further investigation of the role of manganese showed that the influence of manganese on the activity of a preparation of horse radish peroxidase (which can oxidise IAA *in vitro*) depended on the concentration of the manganous ion. More recent work (Furuya and Galston, 1961) indicates that manganese stimulates IAA oxidase activity by the destruction of a natural inhibitor which is claimed to be a glucoside of quercetin (Furuya, Galston and Stowe, 1962). The manganous ion had little effect on an inhibitor-free system and it was shown that its effect could not be simulated by other metallic ions such as Ca^{++},

Ce^{+++}, Co^{++}, Cu^{+}, Fe^{++}, Mg^{++}, Ni^{++} and Zn^{++}.

In the following experiments the manganous ion was supplied to soaking Maple pea seeds in the form of manganous chloride (other salts such as the sulphate gave similar results). The ion was supplied to the seeds either aerobically before soaking or during the soaking treatment.

Drennan, Berrie and Armstrong (1961) showed that oat grains could take up enough manganese during 8 hours soaking in 2% manganese chloride to offset later signs of manganese deficiency in the seedlings. It is, therefore, reasonable to expect that pea seeds are capable of taking up fairly large quantities of the ion from solution.

Experiment 14. An investigation into the influence of manganous chloride pretreatment of Maple pea seeds on the adverse effects of soaking the seeds in distilled water.

25 Maple pea seeds were placed in each of 3 pairs of flasks containing 10 ml respectively of distilled water, 1% $MnCl_2.4H_2O$ and 2% $MnCl_2.4H_2O$. All 6 flasks were shaken steadily at 20°C for 5 hours, after which time the seeds were nearly fully imbibed. The solutions were poured off and, after washing, the seeds were placed under anaerobic soaking conditions at 20°C for 72 hours. The seeds were then sown out and harvested 4 days later. Table 40 shows the influence of the manganese pretreatment on the frequency of radicle damage.

TABLE 40.

The influence of 5 hours pretreatment of Maple pea seeds with manganous chloride on the severity of the effects of subsequent soaking on the frequency of damage found in the seedlings.

Concentration $MnCl_2$	-	0	1%	2%
Hours soaking	0	72	72	72
% Damage	2	92	48	54

There are obviously significant differences in frequency of damage between the seedlings from seeds pretreated with manganous chloride and those pretreated with only distilled water. A repeat experiment, using only 1% $MnCl_2$ gave substantially similar results, thus confirming that 5 hours pretreatment of pea seeds can moderate considerably the adverse effects of 72 hours subsequent anaerobic soaking.

Experiment 15. An investigation into the moderating influence of the manganous ion on the adverse effects of soaking Maple pea seeds when the ion is supplied during the soaking period.

Several experiments were set up in which Maple pea seeds were soaked for 72 hours in solutions of manganous chloride at

various concentrations. Control seeds were sown directly at the beginning of the experiment and seeds were also soaked for 72 hours in distilled water for comparison. The seedlings were scored for damage 7 days after the start of the experiment. The pooled results of all the experiments are shown in Table 41.

TABLE 41.

The influence of adding the manganous ion to the soaking medium on the severity of radicle damage produced by soaking Maple pea seeds for 72 hours before sowing.

Concentration $MnCl_2.4H_2O$	-	0	10^{-5}M	10^{-4}M	10^{-3}M	10^{-2}M
Soaking period (Hrs.)	0	72	72	72	72	72
% Damage	4	79	72	66	30	26

Manganous ions, supplied in the soaking medium at the appropriate concentration can obviously minimise the adverse effects of soaking Maple pea seeds. These results clearly support the findings in Experiment 14. Table 42 shows that this moderating influence can also express itself in greater radicle length of seedlings grown from treated seeds.

The increased radicle length due to manganese treatment was a consistent feature of all experiments, and it is possible to demonstrate that the original results from which

the data of Table 42 were compiled show significant differences attributable to manganese at the 1% probability level.

TABLE 42.

The effects of soaking Maple pea seeds for 72 hours in solutions of manganous chloride on the radicle length of the resulting seedlings.

	Soaking treatment (Hrs.)			
	0	72		
Concentration $MnCl_2$	-	0	10^{-3}M	10^{-2}M
Mean radicle length (cm).	11.01	2.98	3.40	3.34
S.E. of mean	0.38	0.27	0.21	0.36

Although the results of manganese experiments provide good evidence that this element can in some way mitigate the adverse effects of soaking Maple pea, no data have so far been presented to show that the mitigating effect is confined to the manganous ion. It is possible that manganese acts in a nutritional capacity or, even more simply, that at concentrations of 10^{-2}M - 10^{-3}M it alters the osmotic pressure of the solution to a value which moderates the adverse effects of the treatment. To investigate these possibilities, observations were made on the influence of various solutions on the adverse effects of soaking Maple peas. Ferrous sulphate

($FeSO_4.7H_2O$), cupric sulphate ($CuSO_4$), sodium borate ($Na_2B_4O_7.10H_2O$) and zinc sulphate ($ZnSO_4.7H_2O$) were investigated over a wide concentration range (10^{-1}M to 10^{-9}M) and it was found that only 2 treatments (10^{-3}M zinc sulphate and sodium borate) gave any moderating effect. This could not be successfully repeated.

Experiment 16. An investigation into the influence of various ions on the adverse effects of soaking Maple pea seeds.

50 Maple peas were subjected to anaerobic soaking conditions in 9 flasks containing the following solutions, all at 10^{-3}M concentration - ferrous sulphate, manganous chloride, zinc sulphate, sodium borate, sodium chloride, calcium chloride, cupric sulphate, sucrose and also distilled water. In addition, 50 seeds were sown directly at the beginning of the experiment.

The seeds were kept under soaking conditions at 20°C for 72 hours, then sown out and grown for a further 4 days. At the end of this period the seedlings were harvested and scored for damage.

The results, shown in Table 43, indicate that any ameliorative influence on the adverse effects of soaking is confined to the manganous ion. The only group of seedlings which approached those grown from seeds soaked in manganous chloride in health and vigour were those from seeds soaked in

calcium chloride but, as Table 43 shows, the frequency of damage with the latter ion was considerably greater. The results of anaerobic soaking in sucrose do not support those of Pólya (1961) and Howell (1961) who found that the adverse effects of soaking certain seeds aerobically in distilled water could be offset by adding sucrose to the medium.

TABLE 43.

The influence of soaking Maple pea seeds for 72 hours in various solutions on the frequency of damage observed in the resulting seedlings.

Solution	% Damage
Distilled water.	76
$FeSO_4$.	76
$MnCl_2$.	30
$ZnSO_4$.	58
$Na_2B_4O_7$.	64
NaCl.	66
$CaCl_2$.	54
$CuSO_4$.	60
Sucrose.	76
Sown directly.	6

With the reservation that zinc sulphate, sodium borate and calcium chloride may have a slight mitigating influence, it is possible to conclude from these experiments that any pronounced moderating influence is confined to the manganous ion.

It is not known how the uptake of manganese by pea seeds moderates soaking damage. The explanation is possibly a simple one e.g. the supplying of a certain nutritional requirement, alteration of the oxido-reduction status of the ions within the plant or the stimulation of certain enzyme systems in its capacity as a co-factor. It is likely that the manganese has no effect until the seeds are removed from the soaking medium and restored to aerobic conditions. Investigation of all the possible roles of manganese in the plant immediately on restoration of aerobic conditions is obviously impracticable here. Investigations were made, however, into the *in vitro* activity of the enzyme system, IAA oxidase, which has been shown to be stimulated by manganous ions.

. . .

Larsen (1936) and (1940) described a substance obtained from the pressed juice of *Phaseolus* seedlings which could destroy auxin. He claimed that the "substance" possessed the ability to destroy both the auxin extracted from maize (*Zea mays*) and indole acetic acid. Tang and Bonner (1947, 1948) took up the study and gave the auxin inactivating substance (extracted from pea epicotyls) the name - indole acetic acid oxidase. In their 1948 paper, Tang and Bonner claimed that the enzymatic inactivation of IAA can take place, not only *in vitro* but also *in vivo*. Since then, however, it has not been found possible to establish the *in vivo* activity of the enzyme

but the evidence of Fang, Theisen and Butts (1959) and Kefford (1962) points rather more strongly to the importance of IAA oxidase in whole plants.

No attempt was made here to demonstrate the in vivo activity of IAA oxidase but several in vitro investigations were pursued. The in vitro activity of IAA oxidase appears to be controlled by 2 sets of factors, one which gives enhancement to activity (Wagenknecht and Burris, 1950; Goldacre, Galston and Weintraub, 1953; Siegel and Galston, 1955; Hillman and Galston, 1956) and one which gives inhibition (Tang and Bonner, 1948; Hillman and Galston, 1957; Ray, 1958; Gortner and Kent, 1958; Galston, 1959; Sondheimer and Griffin, 1960; Sacher, 1961, 1962; Furuya, Galston and Stowe, 1962). These systems are investigated briefly in aqueous extracts from seedlings grown normally and in seedlings grown from seeds exposed to soaking conditions before sowing.

MATERIALS AND METHODS.

(a) Preparation of the crude enzyme.

The enzyme was prepared from dark grown seedlings of Maple pea. Control seedlings were grown at 20°C and, to investigate the activity of the enzyme under abnormal conditions, the seeds were soaked for the appropriate length of time, then sown out under the same conditions as the controls.

When required for experiment, the seedlings were harvested, washed and ground in a small M.S.E. blendor for

20 seconds with one crushed distilled water ice cube and 75 ml ice cold distilled water. The homogenate was then squeezed through butter muslin and the juice centrifuged in an M.S.E. minor centrifuge at 3,000 R.P.M. for 6 minutes. The supernatant liquid was then poured off and made up to 100 ml with distilled water.

(b) Estimation of indole acetic acid.

The activity of the enzyme was measured by adding known volumes of the crude enzyme preparation to a standard solution of IAA and determining the amount of IAA subsequently destroyed. A stock solution of 10^{-2}M IAA was made up and from this a solution of IAA containing 100 µg/ml was prepared by adding 6 ml of the stock solution to 99 ml distilled water.

A colorimetric method based on that of Tang and Bonner (1947) was used for the determination of IAA in solution. 1 ml of IAA solution containing 5-100 µg/ml was pipetted into one of a set of optically matched test tubes containing 4 ml of Salkowski reagent (Salkowski, 1885).

Salkowski reagent:-

0.5 M $FeCl_3$: Dist. H_2O: H_2SO_4(S.G. 1.84) = 3:100:60 by volume.

A pink colour develops when this reagent is added to IAA solutions. Usually the colour develops to a maximum then fades: most accurate results are obtained by adding the reagent and leaving the colour to develop for 30 minutes. The intensity of colour developed was determined colorimetrically

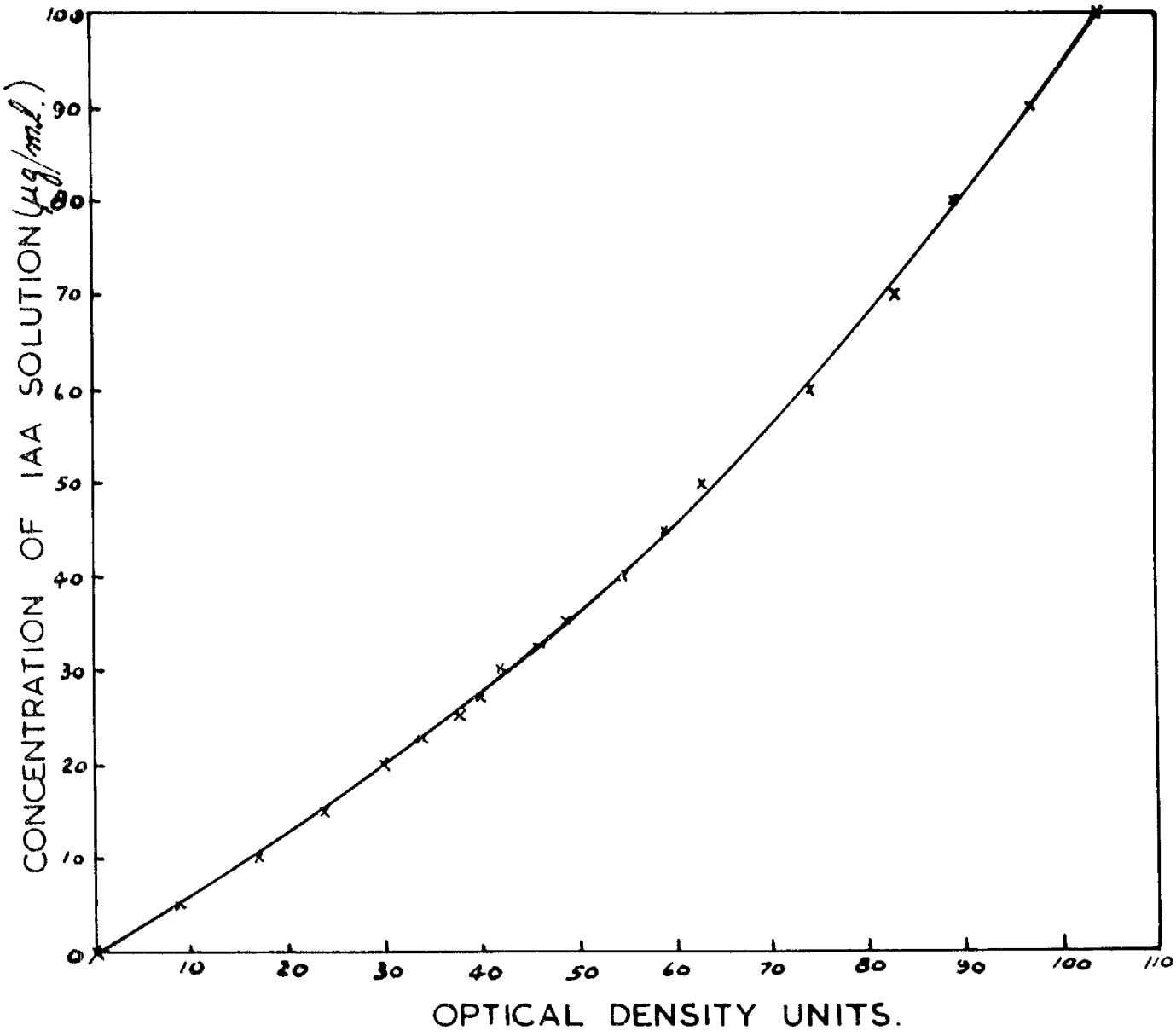


FIGURE 11

The number of optical density units equivalent to a known concentration of IAA. Note that a straight line relationship holds between 20 µg/ml and 60 µg/ml

in a Unicam S.P. 600, set at 534 mμ.

It was necessary to calibrate the spectrophotometer so that the optical density could be readily converted to μg IAA. The results of an experiment to determine this are shown in Figure 11.

From Figure 11, it can be seen that an almost linear relationship holds between the optical density and amount of IAA per ml in the concentration range 20-60 μg/ml. This range was selected for all IAA determinations: it can be calculated that in this range, 1 optical density unit equals 0.82 μg IAA.

Other substances such as tryptophan, indole propionic acid, indole butyric acid, indole pyruvic acid and indole can give colour reactions with Salkowski reagent (Larsen, 1955). Tang and Bonner, in their 1948 paper, however, claim that none of these gives a colour reaction in the concentration range used here. Further accuracy was achieved by measuring the optical density of the pink solutions at 534 mμ, the wavelength giving maximum absorption of the IAA-Salkowski colour.

Platt and Thimann (1956) however, showed that the Salkowski reaction is subject to interference from various sources. Development of the colour is delayed by exposure of the reagent to light, the presence of ferrous ions and the presence of other reductants such as ascorbic acid or cysteine. Various polyphenols, which are very widespread in plants, inhibit development of the pink colour.

Great care must therefore be exercised when using the Salkowski reagent to estimate IAA in the presence of plant extracts.

(c) Buffer solution.

All investigations were carried out at pH 6.6 which was found by Tang and Bonner to be the optimum pH for IAA oxidase activity. Sörensen's phosphate buffer (M/15 KH_2PO_4 + M/15 $Na_2HPO_4.12H_2O$) was used in all experiments (Documenta Geigy, P. 105).

(d) Reaction mixture.

The reaction mixture was usually set up in 100 ml conical flasks and contained 20 ml substrate (i.e. 100 µg/ml IAA solution), 20 ml crude enzyme preparation and 10 ml buffer solution. This gave a final concentration of 40 µg IAA per ml in the reaction mixture.

The flasks were placed in a metabolic shaking incubator maintained at 25°C and the amount of IAA in 1 ml samples of the reaction mixture was measured colorimetrically at zero time and at suitable intervals thereafter.

Several preliminary experiments were carried out to develop the technique finally used for the estimation of IAA oxidase activity. One of these was to investigate the activity in crude enzyme preparations of 1-9 day old seedlings grown from seeds sown directly. For the first 3 days there was no degradation of IAA and, in fact, the results showed a

nett gain of Salkowski staining material in the reaction mixture during this period. After the 3 day period the activity increased steadily till the 7th day then levelled off.

Experiment 17. Preliminary investigation of the behaviour of IAA oxidase in aqueous extracts from aerobically grown Maple pea seedlings.

6 day old Maple pea seedlings were used to prepare the crude enzyme (see Page 128). 2 aliquots of 20 ml were placed in 2 flasks containing 10 ml buffer and 20 ml IAA thus giving complete reaction mixtures. A further 2 aliquots of 20 ml were boiled for 3 minutes, then added to flasks containing buffer and IAA. A third pair of aliquots was added to 2 flasks containing buffer and distilled water instead of IAA. Finally, 2 flasks were set up containing buffer, IAA and 20 ml each distilled water in place of the enzyme.

The amount of IAA present per ml in each flask was measured colorimetrically at zero time and after 3 hours (Table 44).

There is good agreement between the replicates in Table 44 and several conclusions can obviously be drawn from the results. Where the reaction mixture is complete a considerable amount of IAA disappears during the 3 hour period. It may be degraded or it may be converted into another substance which gives no colour with Salkowski reagent. The latter

cannot be conversion into indoleacetylaspartic acid which does give a pink colour with Salkowski reagent.

TABLE 44.

Changes in IAA content during 3 hours digestion of reaction mixtures containing buffer solution, synthetic IAA and enzyme preparations from 6 day old etiolated Maple pea seedlings.

Contents of Reaction Mixture	µg IAA/ml in reaction mixture	
	0 Hrs.	3 Hrs.
1. Complete	43	31
2. Complete	44	31
3. Complete (Enzyme boiled)	44	44
4. Complete (Enzyme boiled)	44	45
5. Minus IAA	1	0
6. Minus IAA	2	0
7. Minus enzyme	43	43
8. Minus enzyme	44	44

Complete medium:- 20 ml enzyme
20 ml IAA (100 µg/ml)
10 ml buffer

When the enzyme is boiled (3, 4) or is absent (7, 8) none of the IAA is destroyed. The third set of readings in Table 44 shows that there is very little Salkowski staining material in the enzyme preparation itself which could interfere with the values obtained.

The evidence presented in this experiment therefore

indicates that the aqueous extract from 6 day old Maple pea seedlings is capable of enzymatically degrading synthetic IAA.

It is now possible to compare the in vitro IAA oxidase activity of enzyme preparations from the seedlings produced from soaked and unsoaked seeds. Because of the difficulties involved in working with this enzyme, care was taken to ensure that identical treatment was always given to both enzyme preparations.

Experiment 18. A preliminary investigation into the in vitro IAA oxidase activity of seedlings grown from soaked and unsoaked Maple pea seeds.

3 samples of Maple pea seeds were treated as follows:-

(a) Sown directly.

(b) Soaked anaerobically for 48 hours before sowing.

(c) Soaked anaerobically for 72 hours before sowing.

When the control seedlings were 7 days old, the seedlings were harvested and enzyme breis were prepared as described on Page 128. Reaction mixtures were set up and the amount of IAA destroyed determined. In this experiment (and all further ones on IAA oxidase activity) 3 reaction mixtures were set up for each treatment. One of these contained 10 ml buffer, 20 ml crude enzyme and 20 ml distilled water; the other 2 contained IAA instead of distilled water. The former

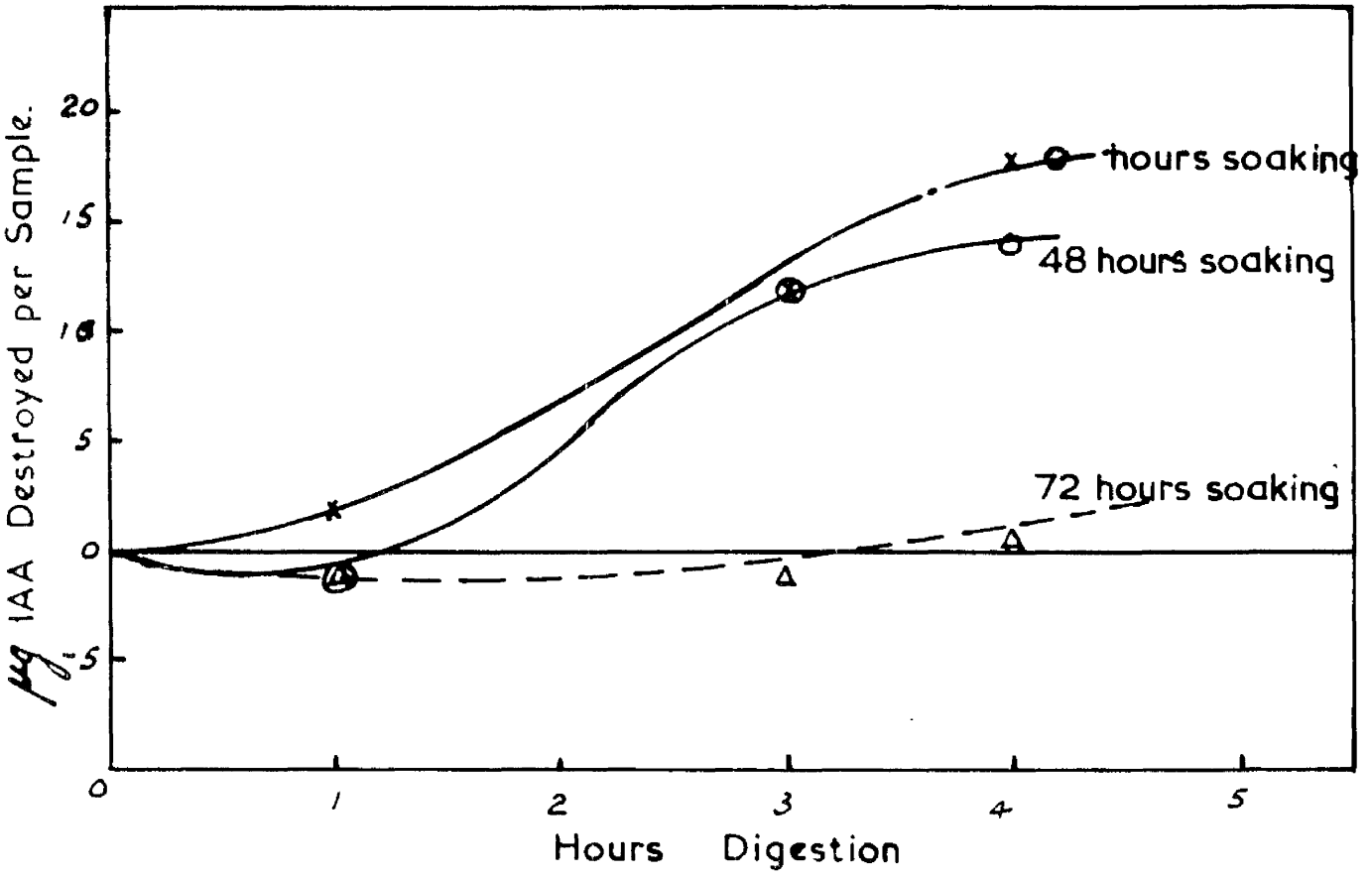


FIGURE 12

The IAA oxidase activity of seedlings from variously treated seeds of Maple pea.

was used as the "blank", thus any changes in the IAA content in the other 2 (as determined by the Salkowski test) were measures of the destruction of the synthetic IAA and not of other Salkowski staining material possibly contributed by the enzyme preparation itself.

Table 45 shows the amount of IAA destroyed by the enzyme preparation and the results are shown in graphical form in Figure 12.

TABLE 45.

The amount of IAA (µg)/ml destroyed by enzyme preparations from the seedlings produced by Maple pea seeds which had been subjected to various periods of soaking before sowing.

Hours digestion	Hours soaking		
	0	48	72
0	0	0	0
1	2	-1	-1
3	12	12	-1
4	18	14	1

A feature of this experiment, shown clearly in Figure 12, is the rather long lag phase which occurs before any significant amount of IAA is destroyed by the plant extracts. This has been reported by several workers, for example, Galston, Bonner and Baker (1953).

The most interesting feature of this experiment, however, is the difference which exists in the IAA oxidase activity of

seedlings from seeds given various soaking treatments.

The enzyme preparation from control seedlings shows considerable activity, approximately half the IAA being destroyed after 4 hours digestion. The enzyme preparation from seedlings grown from seeds soaked for 48 hours before sowing also has considerable activity but is somewhat less than the control. The third enzyme preparation, however, shows practically no IAA oxidase activity, even after 4 hours. It therefore appears that soaking of the seeds before sowing has an effect on the in vitro capacity of enzyme preparations from the seedlings to destroy synthetic IAA. An obvious explanation of this could be that the ability of Maple pea seedlings to destroy IAA develops steadily as the seedling grows. It could be argued, in spite of the evidence put forward in Part 1, that we are dealing here with seedlings of different ages. Further inspection of Figure 12, however, reveals that, while this argument may be acceptable as an explanation of why the IAA oxidase activity of the enzyme preparation from the seedlings grown from seeds soaked for 48 hours is less than the controls, it cannot explain why the IAA oxidase activity of the enzyme preparation from seedlings grown from seeds soaked for 72 hours is so low. Its activity would have been expected to be shown as a curve following the same pattern as the 48 hour curve, but displaced 24 hours in time. This is obviously far from true in this experiment. It

is possibly worth noting that these differences in IAA oxidase activity can be compared with the percentage damage occurring after the respective treatments.

Experiment 19. A daily investigation of the behaviour of IAA oxidase in seedlings from soaked and unsoaked Maple pea seeds.

A large batch of Maple pea seeds was sown directly and, at the same time, 20 flasks were set up with peas under soaking conditions. Each day for the next 3 days, 25 g batches of seeds or seedlings were removed from the incubator and tested for IAA oxidase activity. 2 25 g samples were withdrawn from each of the 2 sets of treatments so that duplicate readings could be made (see Table 46). The enzyme brei was made from a batch of soaked seeds first, followed by a batch of normal seedlings, the other batch of soaked seeds and finally the second batch of seedlings from seeds sown directly.

After the 72 hour period the soaked seeds were sown out and, from then on, seedlings from these seeds were compared with the controls for IAA oxidase activity at daily intervals.

The reaction mixtures were set up in 100 ml Erlenmeyer flasks which were shaken steadily at 25°C. Duplicate 1 ml samples were withdrawn after 0, 1, 2 and 3 hours and tested for residual IAA in the Unicam S.P. 600.

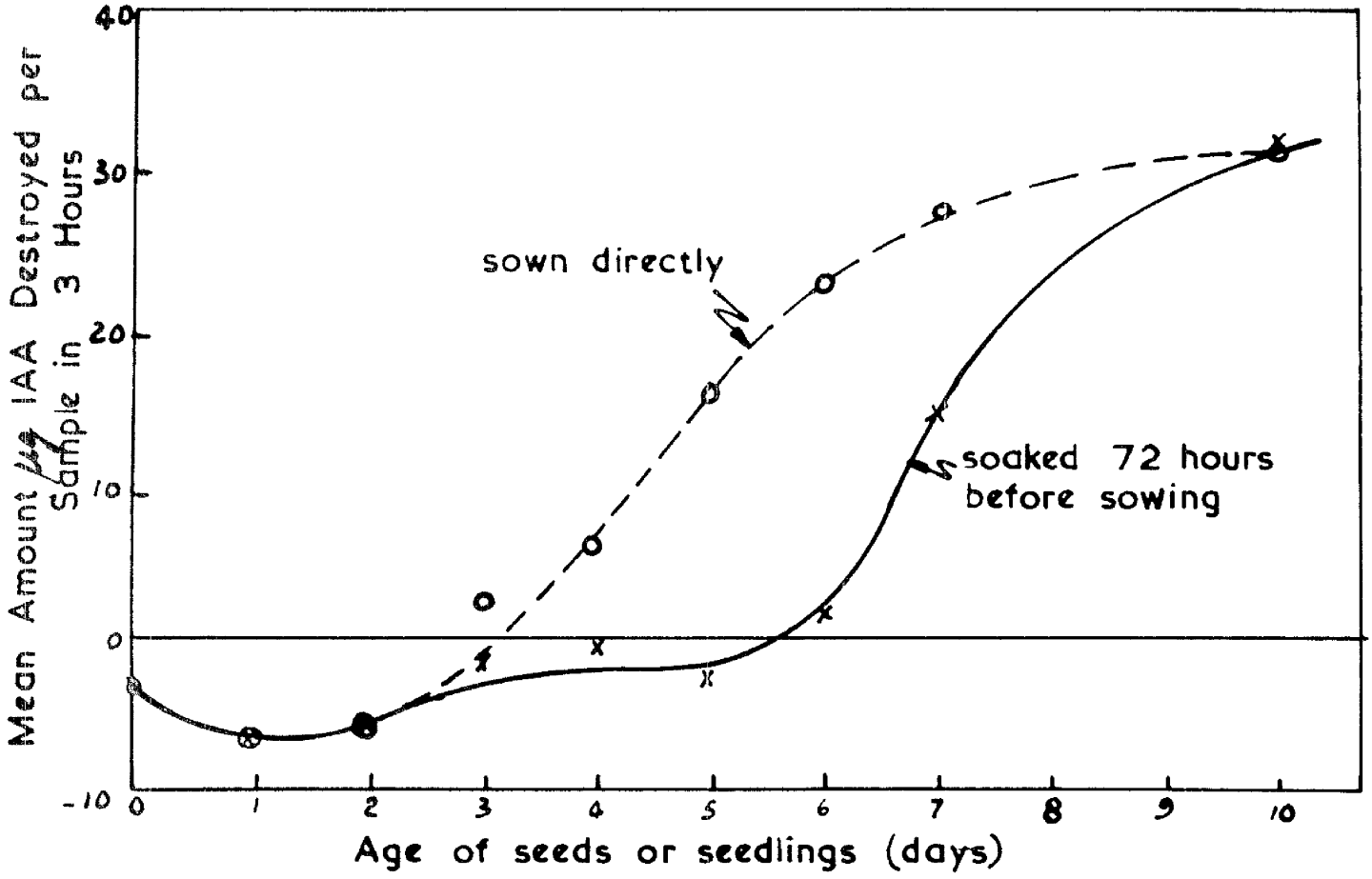


FIGURE 13

The IAA oxidase activity of seedlings from soaked and unsoaked seeds of Maple pea

The destruction of IAA followed the general pattern shown in Experiment 17 and so the results shown in Table 46 are only for the amount of IAA destroyed per ml after 3 hours digestion. All the differences in the activity of the enzyme can be seen in the table and also in Figure 13.

TABLE 46.

The amount of IAA (µg) destroyed per ml of reaction mixture by enzyme preparations of seedlings from soaked and unsoaked seeds during 3 hours digestion. The results in columns a and b are for the 2 batches of seedlings (replicates) used to prepare the enzymes at each daily interval.

Age of seeds or seedlings (days)	Soaking treatment			
	72 Hrs.		0 Hrs.	
	a	b	a	b
0	-	-	-1.6	-2.8
1	-5.2	-6.8	-6.0	-6.0
2	-5.6	-5.2	-5.5	-6.4
3	-1.2	-1.6	0.4	3.6
4	1.2	0.4	6.6	5.2
5	-3.8	-2.0	15.6	14.2
6	0.2	3.2	22.8	22.0
7	15.2	13.6	27.2	25.2
10	30.4	31.2	29.2	31.5

A striking feature of this experiment is that, in reaction mixtures containing enzyme preparations from 0-3 day old seedlings (unsoaked) and 0-5 day old seedlings (soaked), there was

an increase in the amount of Salkowski staining material at the end of the 3 hour digestion period. This could have been due to a synthesis of IAA but this is unlikely. It is more probable that other substances from the plants (e.g. polyphenols) are interfering with the colour development at certain stages.

It is obvious from the results illustrated in Figure 13 that we must reconsider the possibility that soaking merely delays IAA oxidase activity. At first sight it seems possible to move the curve for IAA oxidase activity in the preparations from seedlings grown from soaked seeds to the left so that it closely fits the control curve. This may be true but there is rather strong evidence against it. In the first place, the 3 day soaking period must be considered as part of germination (Part 1). During this time the behaviour of enzyme preparations from seedlings from soaked seeds and controls is practically the same so auxin metabolism of the seeds, if it is proceeding, is the same in both cases. If the hypothesis that soaking only delays germination is to be accepted, then it is essential that the soaked seeds are considered not to have germinated or developed during the soaking period. This is certainly not the case as is shown, for example, by the fact that imbibition has already taken place in these seeds.

The evidence in this experiment points to enzyme

preparations from seedlings from soaked seeds being unable to destroy IAA for 3 days after removal of the seeds from soaking conditions. It is during this time that abnormalities become evident in the radicles of the young seedlings and it is possible that these are the result, at least partially, of the suppression of in vivo IAA oxidase activity.

The suppression of IAA oxidase activity in seedlings from soaked seeds may be due to one or more of several factors and the following experiment was designed to test if there is an inhibitor of the enzyme present.

Tang and Bonner (1948) claimed that the in vivo activity of the IAA oxidase system is controlled by an inhibitor. Numerous workers since then have produced evidence of the existence of in vitro inhibitors of IAA oxidase (Galston, Bonner and Baker, 1953; Kenten, 1955; Stutz, 1957; Ray, 1960; Sacher, 1961, 1962). It has been suggested that the inhibitor is a polyphenol (Kenten, 1955; Ray, 1958) and, as previously mentioned, Furuya, Galston and Stowe (1962) have evidence that a glucoside of quercetin is responsible for the inhibition.

Experiment 20. An investigation to determine if anaerobic soaking results in the formation or persistence of a substance which inhibits IAA oxidase activity.

In this experiment, a large batch of Maple pea seeds was

sown directly and 3 batches of 50 were soaked for 72 hours before sowing. The seedlings were harvested 5 days after the start of the experiment. 25 g samples were selected for the enzyme preparations which are referred to as E (S.D.) for the enzyme from control seedlings and E (72) for the enzyme prepared from seedlings grown from seeds soaked for 72 hours before sowing. The reaction mixtures were set up by pipetting 10 ml buffer into each of 10 100 ml conical flasks and adding solutions as follows:-

1. 20 ml IAA (100 µg/ml) + 20 ml E (S.D.).
2. 20 ml dist. water + 20 ml E (S.D.).
3. 20 ml IAA + 20 ml E (72).
4. 20 ml dist. water + 20 ml E (72).
5. 20 ml IAA + 10 ml E (S.D.) + 10 ml E (72).
6. 20 ml dist. water + 10 ml E (S.D.) + 10 ml E (72).
7. 20 ml IAA + 10 ml E (S.D.) + 10 ml dist. water.
8. 20 ml dist. water + 10 ml E (S.D.) + 10 ml dist. water.
9. 20 ml IAA + 10 ml E (72) + 10 ml dist. water.
10. 20 ml dist. water + 10 ml E (72) + 10 ml dist. water.

The reaction mixtures denoted by even numbers were used as blanks for the corresponding IAA solution when readings were taken in the spectrophotometer. Samples were taken after 0, 1, 2 and 3 hours digestion and the results are shown in Table 47.

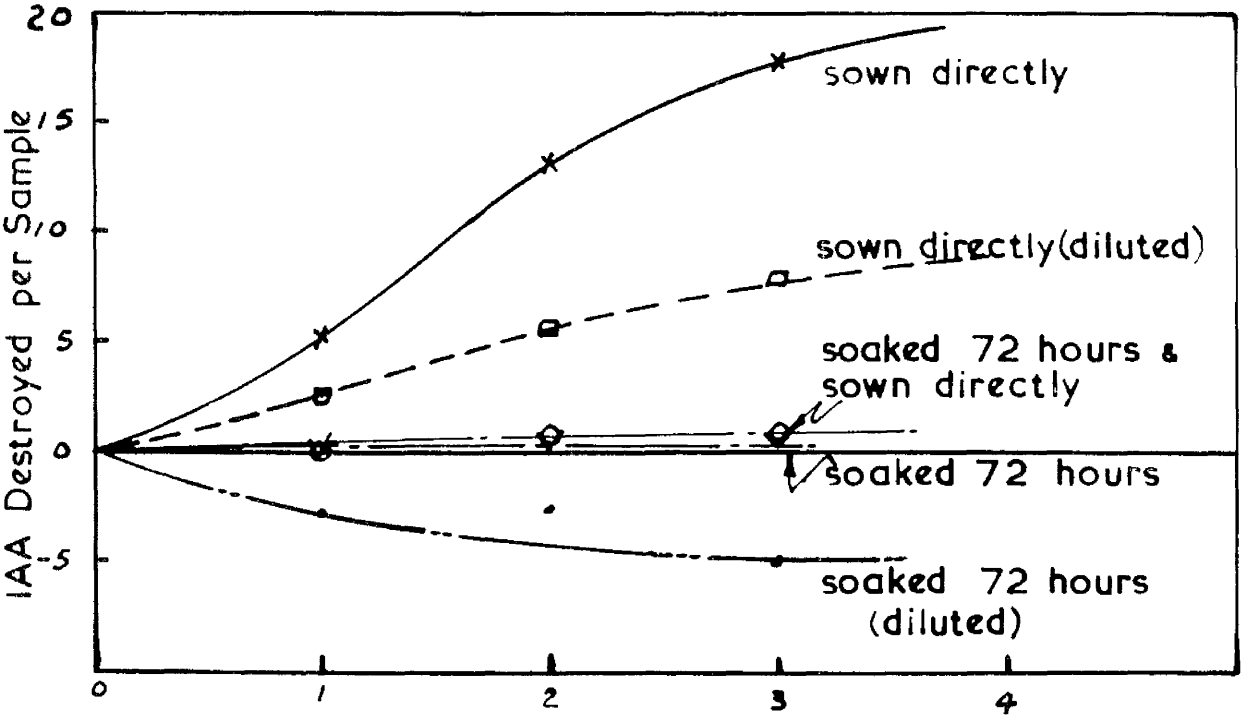


FIGURE 14

The IAA oxidase activity of enzyme preparations of Maple pea seedlings from variously treated seeds. The graphs show the inhibitory effects of the preparation from the seedlings of soaked seeds on a preparation from normal seedlings.

TABLE 47.

The amount of IAA (µg)/ml destroyed by various enzyme preparations obtained from Maple pea seedlings after several hours digestion.

Hours digestion	Enzyme constitution of reaction mixture				
	20 ml E(SD)	20 ml E(72)	10 ml E(SD) 10 ml E(72)	10 ml E(SD) 10 ml D.W.	10 ml E(72) 10 ml D.W.
0	0	0	0	0	0
1	5.7	0	0	2.9	-2.5
2	13.1	0	0.8	5.2	-2.5
3	18.0	0	0.8	7.8	-5.0

Figure 14 shows that when the enzyme preparation of seedlings from seeds soaked for 72 hours before sowing was added to the enzyme preparation from control seedlings, the IAA oxidase activity of the latter was inhibited. This effect is clearly not simply due to dilution (Figure 14). A repeat experiment gave similar results and an obvious possible conclusion is that the enzyme preparation from seedlings grown from soaked seeds contains a substance which inhibits the activity of the enzyme. If this is true, the presence of the inhibitor may be explained in at least 2 ways, viz.

(a) It may be produced during the soaking treatment.

(b) It may be present in all young seedlings and is usually broken down, probably aerobically in the course of normal germination and development. The soaking treatment may prevent the breakdown.

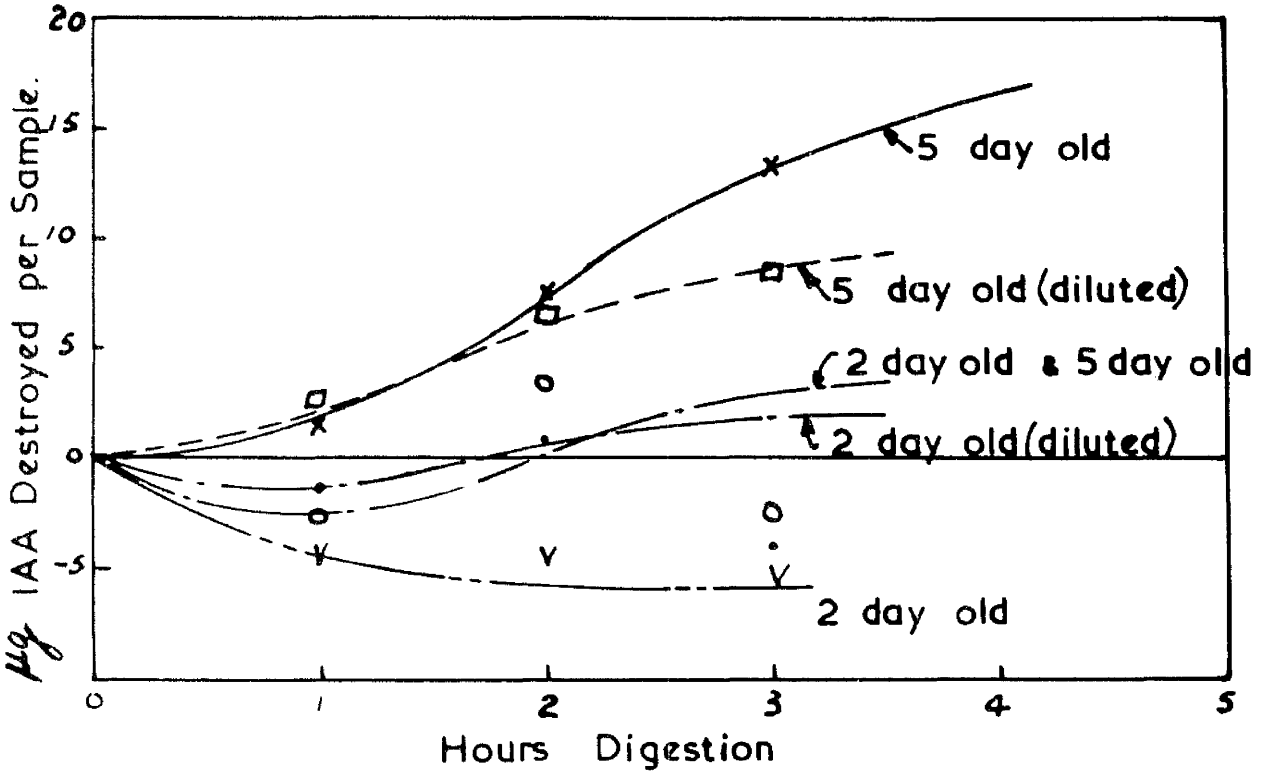


FIGURE 15

The IAA oxidase activity of enzyme preparations from two day and five day old Maple pea seedlings and the interaction between them.

Experiment 21. An investigation to determine if there is an inhibitor of IAA oxidase present in very young Maple pea seedlings.

100 Maple pea seeds were sown directly at 20°C and 3 days later another batch of 100 seeds was sown out. After a further 2 days a 25 g sample of seedlings was harvested from each batch and used for the preparation of the respective enzymes.

The experiment was then set up as in Experiment 20 except that the enzyme from 2 day old normal seedlings was substituted for the enzyme from seedlings produced from seeds soaked for 72 hours before sowing.

The results are recorded in Table 48 and Figure 15.

TABLE 48.

The mean amount of IAA (µg)/ml destroyed by enzyme preparations from 2 and 5 day old Maple pea seedlings in each reaction mixture after several hours digestion.

Hours digestion	Enzyme constitution of reaction mixture				
	20 ml E(5)	20 ml E(2)	10 ml E(5) 10 ml E(2)	10 ml E(5) 10 ml D.W.	10 ml E(2) 10 ml D.W.
0	0	0	0	0	0
1	1.6	-4.1	-2.5	2.5	-1.6
2	7.4	-4.1	+3.3	6.6	+0.8
3	13.4	-5.3	-2.5	8.2	-4.1

There is a firm indication that, when the enzyme preparation from 2 day old seedlings is added to that of the 5 day old seedlings, an inhibitory effect, not attributable to dilution, is exerted.

These 2 experiments investigating the inhibitory effect of certain enzyme preparations on IAA oxidase activity are obviously very much of an exploratory nature but there is considerable evidence that anaerobic soaking may result in failure of the seed or seedling to break down a naturally occurring substance which inhibits the in vitro activity of IAA oxidase.

Discussion of Part IV.

If the work described in this part is a true representation of the physiological processes going on in the young etiolated pea plant then we have gone a long way to explaining the morphological abnormalities of the seedlings described in Part 1. Critical inspection of the results, however, reveals that it is not possible to draw any such firm conclusion.

Most of the difficulties are concerned with the IAA oxidase work. All the studies in Part IV were into the *in vitro* behaviour of the enzyme and, of course, it is dangerous to interpret *in vivo* reactions from the results of *in vitro* experiments unless there is other strong evidence that the reactions examined *in vitro* do proceed in the plant.

The first difficulty encountered was the specificity of the Salkowski reagent which is claimed to give a pink colour only with IAA at the concentrations used. In spite of this claim, however, there is no guarantee that the seedlings did not provide other Salkowski staining materials at a concentration which would give a pink colour with the reagent. Further interference could be expected from polyphenols extracted from the seeds: these inhibit development of the pink colour. Although care was taken to standardise procedure, it is possible that these 2 factors could have upset some of the

results.

Nevertheless, it was generally found that the measures of IAA content at zero time were reasonably close to the expected value of 40 µg/ml (see Table 44). There can also be no doubt that, particularly in the controls, the pink colour produced by reaction of IAA and Salkowski reagent varied with the length of the digestion time and it was clearly acceptable to say that the progressive decrease in colour produced was a measure of the destruction of IAA. It is not, however, possible to state categorically that the IAA is oxidatively decarboxylated by IAA oxidase. Although this seems a probable explanation, the important point is that the IAA disappears in the controls but does not do so when aqueous extracts of seedlings from soaked seeds are presented with the IAA, unless these seedlings are a total of at least 7 days old. It is, perhaps, significant to note that, by this time, the damage in which we are interested is already manifest in the seedlings and they are beginning to recover by the production of lateral roots.

Another difficulty encountered in this work was the influence of light on <u>in vitro</u> IAA oxidase activity. It has been shown that there is greater IAA oxidase activity in enzyme preparations from light grown seedlings than from dark grown seedlings (Galston and Hand, 1949) and it is thought that, in the light, there is a photoreceptor which stimulates the IAA oxidase system. Galston and Baker (1949, 1951) and

Galston and Hand (1949) in a series of papers on "The physiology of light action", concluded that light activated a flavoprotein enzyme to produce hydrogen peroxide which was considered to overcome a natural inhibition of the IAA oxidase system. In all the experiments carried out in Part IV of this thesis, etiolated seedlings were used: the reactions were allowed to proceed in the light but it was found that this did not interfere with any of the results. As a precaution however, all enzyme preparations were always exposed to the same light conditions.

The present state of knowledge with respect to IAA oxidase is that evidence for its in vivo activity is rather slim. Galston (1956) produced circumstantial evidence that IAA oxidase in certain plant cells can destroy IAA and reference has already been made to some of the evidence put forward in favour of the hypothesis that the endogenous auxin content of plants is enzymatically controlled (Tang and Bonner, 1948; Fang, Theisen and Butts, 1959; Kefford, 1962). Against these claims are the findings of Briggs, Steeves, Sussex and Wetmore (1955) who could find no evidence of the in vivo enzymatic destruction of IAA.

Although the enzyme has not been proved to play an active part in controlling the amount of available auxin in the plant the evidence against such a contention is also not strong enough to allow us to neglect the possibility. Further

discussion of the results obtained *in vitro* together with the results of the manganese work is therefore desirable.

One interesting experiment which was carried out but not reported here concerned the IAA oxidase activity of 5 day old seedlings grown from pea seeds suffering from "Marsh Spot" (Piper, 1941). The IAA oxidase activity of enzyme preparations from these seedlings was found to be considerably less than that of control seedlings of the same age. The seeds suffering from "Marsh Spot" contained only 9 ppm. manganese in the dry matter while the controls contained 15 ppm. Further supplies of pea seed with "Marsh Spot" were not available to allow this experiment to be repeated but the results of the one experiment are indicative of a possible connection between the manganese content of pea seeds and *in vivo* IAA oxidase activity.

Considerable evidence has now accumulated that *in vitro* IAA oxidase activity can be stimulated by the substituted phenol - 2:4 Dichlorophenol (DCP) - as well as manganous ions. Goldacre (1949) claimed that 2:4 dichlorophenoxyacetic acid could stimulate IAA oxidase activity but Goldacre, Galston and Weintraub (1953) showed that the effect observed was attributable to contamination of the 2:4D solution by DCP. The stimulatory effect of DCP has been confirmed by subsequent work (Siegel and Galston, 1955; Hillman and Galston, 1956; Furuya and Galston, 1961; Sacher, 1962).

Sacher (1962) suggests that DCP and the manganous ion act together to control the *in vitro* activity of IAA oxidase. It is thought that the DCP is used to terminate the lag phase which occurs after supplying the enzyme with its substrate (IAA) and the manganous ion presumably operates by destroying the natural inhibitor of the enzyme.

Hillman and Galston (1956) also found that DCP and manganous ions together, could control *in vitro* IAA oxidase activity. It was found that IAA degradation was inhibited by 10^{-5}M to 10^{-3}M Mn^{++} at low concentrations of DCP (10^{-6}M). The same concentrations of manganous ions greatly enhanced IAA destruction at higher levels of DCP (10^{-5}M to 10^{-4}M). It was suggested that DCP supplemented or replaced a naturally occurring phenolic cofactor and the concentration of this cofactor in plant breis determined the response of the enzyme to added manganous ions or DCP.

When Maple pea seeds were soaked for 72 hours in solutions of DCP, it was found that a moderating influence on the adverse of soaking could be achieved when the concentration was 10^{-5}M or 5×10^{-6}M. Further work would, however, be required to establish a definite connection between DCP and the moderation of the adverse effects.

The existence in the plant of an inhibitor of IAA oxidase seems to be fairly widely accepted. Hillman and Galston (1957) showed that it occurred as a dialysable substance

produced in etiolated pea shoots after several hours exposure to red light and it was claimed that the same substance is found in various parts of light grown pea plants (Tang and Bonner, 1948; Galston, 1959).

In Experiment 20 it was shown that, in seedlings from soaked seeds, there is inhibition of IAA oxidase activity. Presumably this is caused by a substance or substances within the seeds or young seedlings which blocks one or more of the enzymes in the oxidase system. Experiment 21 shows that, what is possibly the same substance, is present in very young (2 day old) seedlings and this can inhibit IAA oxidase activity. In the aerobically growing seedling there may be destruction of this inhibitor (possibly involving manganous ions) around the fourth day thus allowing enzymatic destruction of the auxin to take place. The identity and mode of action of the inhibitor is outside the scope of this discussion but its effect, when extracted from the seedlings grown from soaked seeds, indicates that further investigation of this problem might prove a fruitful line of research. First of all, however, it would be necessary to carry out further experiments to definitely establish that the inhibitor is present. The effect of the inhibitor on IAA oxidase when supplied with manganous ions and DCP in vitro would also prove an interesting investigation.

If soaking results in the accumulation of IAA in pea

seeds, it is possible that manganous ions can moderate the adverse effects by stimulating the in vivo activity of IAA oxidase, thus allowing the seeds or seedlings to degrade excess IAA, probably on the restoration of aerobic conditions. Confirmation of this hypothesis depends on the demonstration of definite in vivo IAA oxidase activity in etiolated pea seedlings.

General Discussion.

The experiments reported in Part 1 of this thesis established that anaerobic soaking of Maple pea seeds has a pronounced predetermining influence on the growth of the plants produced from these seeds. The results of this predetermining influence are manifest as a reduction in radicle length of the young plants and/or the occurrence of morphological abnormalities in the radicle. Further work was concentrated on an examination of the possible causes of these abnormalities.

Good evidence was produced in Part 11 that the cause could be attributed to a derangement of the metabolic processes of the seeds, either during soaking or immediately on the restoration of aerobic conditions. Investigation of the metabolic behaviour of the seeds led to the conclusion that respiratory CO_2 accumulating in the neighbourhood of the seeds could possibly be responsible for the blockage of certain biochemical reactions within the seed or young seedling, thus being indirectly responsible for the observed effects of soaking.

The remainder of the work consisted of an investigation into the auxin metabolism of the seeds and young seedlings. Critical appraisal of the results obtained, considering all the aspects together, is necessary to decide whether soaking does

have any effect on the auxin metabolism at any of the stages investigated. The initial stimulus for the investigation into auxin metabolism depended on the accumulation of a mass of subjective evidence which showed that the seedlings obtained from soaked seeds were similar in appearance to seedlings which had been supplied with exogenous auxin. The fact that the roots were more severely affected than the shoots seems to favour this conclusion since it is established that roots respond to much smaller auxin concentrations than shoots (Audus, 1959; p. 37). In spite of very striking similarities between seedlings from soaked seeds and seedlings exposed to exogenous auxin it was not possible to find 2 populations of seedlings from the 2 treatments which could be classed as identical. It is unlikely, under the conditions of the experiments involving soaking in exogenous IAA (see Part III), that it would be possible to determine exactly the conditions which would be required to produce seedlings similar to those grown from seeds soaked for say 72 hours in distilled water. Although none of the objective means of comparing soaking treatment with the application of exogenous IAA could produce any definite results, the subjective evidence can be accepted, not as proof that soaking results in excess endogenous auxin, but as information that this may be true and is certainly worthy of further approach from other angles.

The other main line of approach was the investigation of

the *in vitro* IAA oxidase activity of enzyme preparations from seedlings produced from soaked and unsoaked seeds, and the possible connection between this enzyme and certain materials (claimed to affect its activity *in vitro*) which were supplied to the soaking seeds. If it could be shown that seedlings from soaked seeds have less capacity to destroy auxin *in vivo* than controls, then it could more readily be accepted that excess auxin is the cause of the damage. This, however, is not possible with our present state of knowledge of the enzyme system.

The best evidence that soaking upsets *in vivo* IAA oxidase activity can be obtained from the manganese work (Part IV). Clearly, since manganous ions can mitigate the adverse effects of soaking and also stimulate *in vitro* IAA oxidase activity it is possible that manganous ions in the plant can stimulate *in vivo* IAA oxidase activity. It was pointed out, however, in Part IV that the manganous ion could have an influence on more aspects of metabolism than IAA oxidase. Further evidence regarding the connection between the manganous ion and IAA oxidase activity could be obtained by soaking seeds in manganese solutions, then measuring their IAA oxidase activity. It was decided that, in view of the difficulties involved in measuring IAA oxidase activity, that this work should not be included here. Purification of the enzyme and measurement of IAA content by a fluorimetric method are considered essential

before proceeding further with this work.

It is difficult to relate the gibberellic acid results to the IAA oxidase work. It has been reasonably well established that, when gibberellic acid is applied to plant tissues, there is an increase in the amount of free endogenous IAA (Kuraishi and Muir, 1962, 1963; Bouillenne-Walrand and Leyh, 1963). The mechanism responsible for the increased free IAA content is, however, still obscure. Pilet (1957) and Pilet and Collet (1959) claim that gibberellic acid can inhibit in vitro IAA oxidase activity and Pilet and Wurgler (1958) go even further and claim that gibberellic acid can inhibit IAA oxidase activity in intact plants of Trifolium ochroleucum. Watanabe and Stutz (1960) found that there was a suppression of IAA oxidase activity when gibberellic acid was applied to the terminal buds of lupin plants and the suppression was attributed to the gibberellic acid enhancing the level of IAA oxidase inhibitors. Ságí and Garay (1961), however, found that there was no inhibition of IAA oxidase activity when gibberellic acid was applied in various ways to lupin plants. Kato and Katsumi (1958) also found no evidence of gibberellic acid affecting IAA oxidase activity in pea seedlings.

If gibberellic acid does inhibit IAA oxidase activity one would expect that, when seeds are soaked in gibberellic acid, there would be an increase in the amount of endogenous free auxin. According to our hypothesis that soaking in distilled

water results in excess auxin within the seeds we should therefore expect soaking in gibberellic acid to increase the damage. This certainly does not occur.

It is possible to erect several hypotheses regarding the role of gibberellic acid in the light of these results but no definite conclusion can be put forward. Obviously further work is required (a) to decide if IAA oxidase is indeed present in vivo, (b) to assess how large a part it plays (if any) in controlling the endogenous auxin supply and (c) to determine if gibberellic acid has any influence on the activity of the enzyme.

The importance of this preliminary work on the auxin relations of pea seeds is obvious from the stand-point of general plant growth regulation. Its possibilities may, however, reach further than this. Various authors (Moulton, 1942; Wolfe, 1952) have suggested that auxin produced by parasitic fungi (or by the host in response to the fungal attack) is responsible for the hyperplasia and hypertrophy in infected tissues. Pilet (1952) and Gruen (1959) have shown that there is an abnormally high auxin level in gall tissues and Bitancourt (1954) claims that the excess auxin found in infected tissues is due to the fungus suppressing the activity of IAA oxidase thereby allowing IAA to accumulate and cause the damage. The information provided in this thesis indicates that, in peas, under anaerobic conditions, the IAA oxidase

system may be inhibited thereby allowing IAA to accumulate.

In animal tissues, if the cells of the embryo are deprived of oxygen the orderly processes by which the tissues unfold and the organs develop are disrupted; malformations and other abnormalities then occur. The nett result of the treatment can be transformation of the normal cells into cancer cells. Warburg (1930) postulated that cancer cells occur when normal cells adopt an anerobic metabolism as a means of survival after injury to the respiratory mechanisms. Much controversy surrounds this work and Skipper and Bennett (1958) review some of the recent thoughts on the subject

Although tumour formation and hypertrophied pea seedling tissue seem far removed from each other, the similarities in the responses of both tissues to the same treatment are close enough to indicate that anaerobiosis in both the plant and animal world is a subject which requires much closer study.

General Summary.

Part 1.

1. Anaerobic soaking of Maple pea seeds has a pronounced predetermining influence on the growth of seedlings produced from such seeds.
2. Peculiar abnormalities occur in the radicles after prolonged anaerobic treatment of the seeds.
3. The radicles of the seedlings from soaked seeds are more severely affected than the shoots: when the soaking period is longer than 60 hours, at least 50% of the radicles are generally damaged and the mean radicle length is much less than that of seedlings from seeds sown directly at the beginning of the soaking period.

Part 11.

4. The temperature at which the seeds are soaked has an influence on the severity of the adverse effects of soaking. Least damage occurs when the soaking temperature is about 10^{o}C.
5. The damage is increased by removing the testas from the seeds, or by exposing whole seeds to aerobic germination conditions for about 24 hours, before soaking.
6. Air and argon passed through the soaking medium mitigate the adverse effects: oxygen treatment results in a

different type of damage (due probably to oxygen toxicity) and CO_2 intensifies the severity of soaking damage.

7. There are many similarities between soaking damage and the response of roots to the application of exogenous IAA.
8. Soaking seeds in solutions of IAA produces more severe damage in the seedling than soaking in water but it was not possible to relate the increased damage to a high endogenous auxin content when seeds are soaked only in distilled water.
9. Gibberellic acid has a slight mitigating influence on the adverse effects. It is possible that kinetin also exerts a moderating influence.

Part IV.

10. The adverse effects are moderated by supplying the soaking seeds with manganous ions.
11. The in vitro capacity of aqueous extracts of seedlings from soaked seeds to destroy IAA is less than that of aqueous extracts of control seedlings.
12. The failure of aqueous extracts of seedlings from soaked seeds to break down IAA may be due to the presence of an inhibitor of the enzyme IAA oxidase.

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13. The implications of all these findings are discussed and it is tentatively suggested that the stimulus for damage may be the accumulation of metabolic CO_2 in the vicinity of the seeds. The CO_2 possibly blocks the IAA oxidase system for

a period and could therefore, indirectly result in the accumulation of IAA in the seedlings. It is possible that the plants respond to this excess IAA by the production of abnormalities in the radicles. Some of the objections to this hypothesis are pointed out and recommendations are made for further work to test some of the ideas put forward.

APPENDICES.

I. Some observations on the anatomy of the radicles of seedlings grown from soaked pea seeds.

II. The Analysis of Variance.

III. The Probit Analysis.

IV. Methods of fitting curves to observed data:-

(a) When the intervals separating the values of one variate are equal.

(b) When the intervals are unequal.

Appendix I.

Some observations on the anatomy of the radicles of seedlings grown from soaked pea seeds.

50 Maple pea seeds were soaked at 20°C for 96 hours, then sown out and allowed to grow for 3 days. After this period, the terminal 5 mm of several radicles were removed and fixed in Formalin Acetic Alcohol (Formalin:45% Acetic Acid:70% Ethanol = 1:1:1 by volume). This process was repeated on each of the next 2 days and, on each day, control radicles were treated similarly for comparison.

The excised radicle tips were embedded in paraffin wax by the chloroform method (Johansen, 1940) and longitudinal sections 12 μ thick were cut using a Leitz rotary microtome. The sections were then fixed on a slide with Haupt's adhesive and the cell walls and nuclei were stained following the procedure of Sharman (1943).

Some of the more interesting abnormalities observed in the damaged radicles are shown in Plate A.

A prominent feature of the radicles of seedlings grown from treated seeds is that the diameter is greatly increased compared with the control.

In Plate A (b) the dark region represents an area of moribund tissue which extends in a "sleeve" 4-6 cells wide encircling the root. The "sleeve" of dead cells stops short

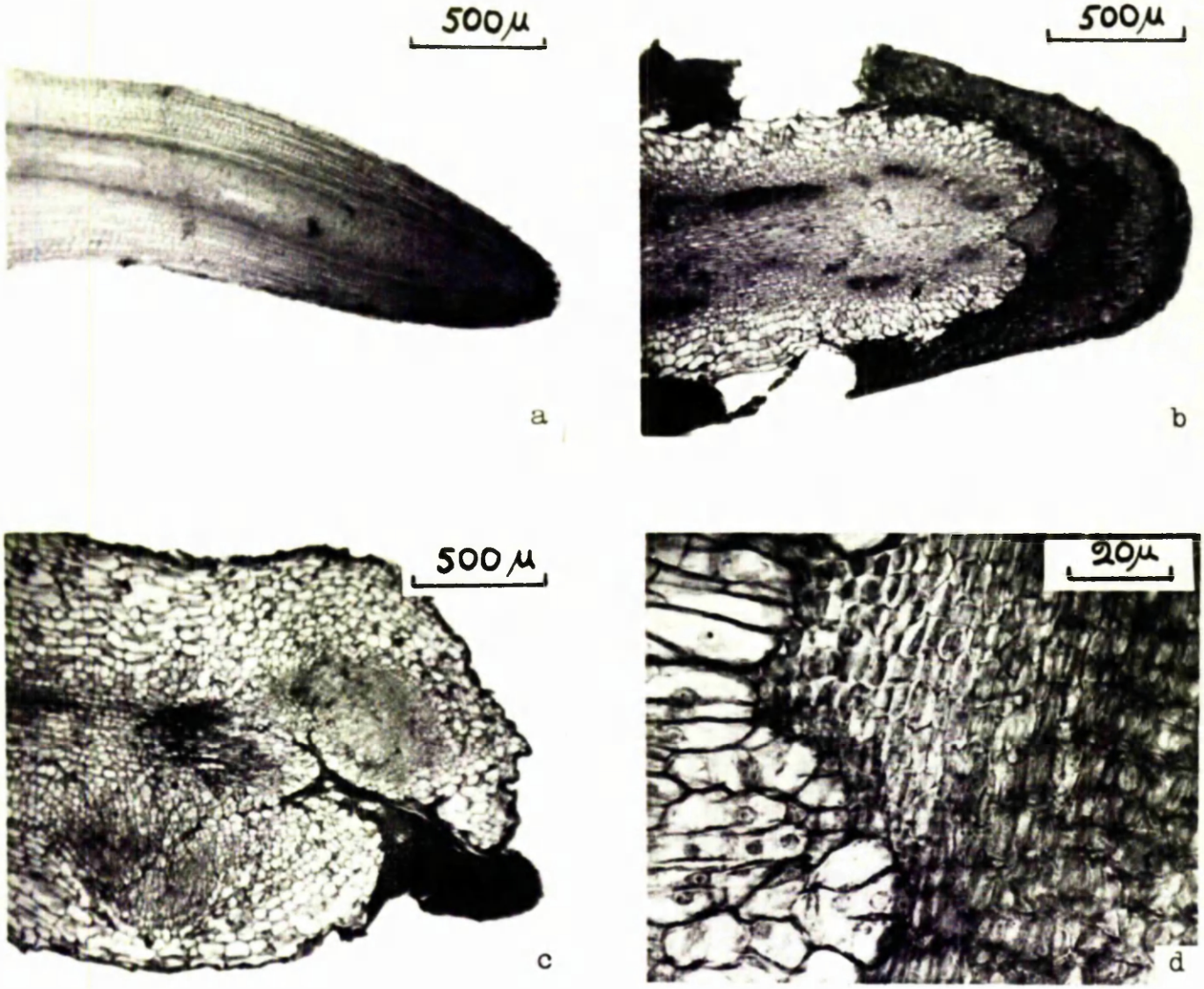

PLATE A

Longitudinal sections of radicle tips of Maple pea seedlings.

a. Normal, 6 days old.

b. Radicle of seedling from seed soaked for 4 days and grown for a further 2 days. Note large mass of dead cells in place of root cap and meristem, also ruptured "sleeve" of dead cells around the cortex.

c. Radicle of seedling from seed soaked for 4 days but grown for a further 3 days. Note envelopment of dead cells by cortical cells, also initiation of lateral roots.

d. High power of region including dead cells and those immediately behind.

(for fuller description, see text)

of the shoot which is apparently not affected in this way. At the distal end of the radicle, however, the region of dead cells extends to more than 4-6 cells wide. All the cells of the meristem are apparently dead, as well as those of the surrounding tissues. Plate A (c) shows this region in greater detail. (It appears in this section that the dead cells on the sides of the radicle have been sloughed off).

Although the meristematic cells of the single histogen are dead, the radicle still seems to be capable of growth. This is obvious from the way the "sleeve" of dead cells has already ruptured (Plate A (b)). The unaffected cells of the cortex immediately behind the dead meristem form a sheath which can grow round the dead meristematic cells and completely envelop them (Plate A (c)). This seems a reasonable explanation of how the tip of a damaged radicle very often appears truncated (see page 22).

In the region surrounding the dead meristem the cells are relatively large and appear to divide rather irregularly thus giving a hyperplasia which remains unorganised. This type of tissue is reminiscent of callus formation.

Although the distal end of the radicle is completely disorganised and also the outer layers of the cortex, the central part of the radicle appears to be normal. Plate A (c) shows lateral root primordia arising normally from the pericyclic region and other sections (not illustrated here) show

that the vascular tissue resembles that of the controls very closely.

The anatomy of damaged radicles confirms that soaking produces a syndrome which is a direct result of death of the meristematic tissues. Although initial damage may be very severe the radicle is still capable of partial recovery and the seedling may revert to a normal mode of life as a result of the radicle producing lateral roots which can take over, at least in part, the function of the damaged main root.

Appendix II.

The Analysis of Variance.

In the work described in this thesis and indeed, in any biological experiment, it is important to distinguish variation resulting from any treatment applied from that due to individual differences or other uncontrolled variables. This has been done here by the Analysis of Variance, using the methods of calculation given by Snedecor (1946).

In Experiment 1 the variation can be partitioned as follows:-

Source	Degrees of freedom	
Between treatments	1	(Soaked versus non-soaked)
Between times	9	(10 different soaking periods)
Between replicates	3	(4 replicates in each treatment)
Interactions:-		
Treatments x Times	9	
Treatments x Replicates	3	
Times x Replicates	27	
Times x Reps. x T'ments.	27	
Residual	1,920	
Total	1,999	(2,000 observations - 1)

The raw data of Experiment 1 are given in Table A and the sums of squares can be calculated as follows:-

TABLE A.

Raw data of Experiment 1 required to derive sums of squares for analysis of variance table.

Hours	Radicle length (cm)				
	Soaked		Total at each Time interval	Not soaked	
	Total of 25 observations	Total of 100 observations		Total of 25 observations	Total of 100 observations
0	299.1 303.1 303.6 297.2	1,203.0	2,441.4	330.5 326.0 265.2 316.7	1,238.4
24	249.7 221.3 232.4 241.0	944.4	2,011.8	279.1 253.8 252.9 281.6	1,067.4
48	168.3 126.8 184.1 131.6	610.8	1,452.9	215.0 227.8 196.2 203.1	842.1
66	83.0 72.2 103.2 72.1	330.5	1,007.5	161.0 168.9 162.0 185.1	677.0
68	65.7 77.5 95.6 62.9	301.7	1,081.4	185.4 174.4 210.9 209.0	779.7
70	57.8 54.8 53.5 66.4	232.5	952.1	181.3 163.9 189.1 185.3	719.6
72	76.8 59.6 39.0 45.5	220.9	851.0	167.4 173.4 137.5 151.8	630.1
74	45.1 42.9 58.2 49.7	195.9	897.3	203.8 157.2 161.2 179.2	701.4
76	31.8 43.3 24.6 57.3	157.0	685.5	147.8 169.3 131.3 80.1	528.5
96	18.5 11.4 10.0 16.0	55.9	499.5	106.7 107.3 109.2 120.4	443.6
Totals 4,252.6		4,252.6	11,880.4	7,627.8	7,627.8

$$\text{Correction} = \frac{T^2}{n} = \frac{11,880.4^2}{2,000} = 70,571.95$$

1. Total sum of squares.

$$\text{Total sum of squares} = Sx^2 - \frac{T^2}{n}$$

(where x is an individual observation)

$$= 111,984.20 - 70,571.95$$

$$= 41,412.25$$

2. Replicates.

$$\text{Sum of squares} = \frac{(299.1 + 249.7 \ldots 106.7)^2 + (303.1 + 221.3 \ldots 107.3)^2 \text{ etc.}}{500} - 70,571.95$$

$$= 29.72$$

(where 500 is the number of observations going to make up the totals to be squared in the numerator).

3. Times.

$$\text{Sum of squares} = \frac{2441.4^2 + 2011.8^2 \ldots 499.5^2}{200} - 70,571.95$$

$$= 16,720.19$$

4. Soaking treatments.

$$\text{Sum of squares} = \frac{4252.6^2 + 7627.8^2}{1000} - 70,571.95$$

$$= 5,695.99$$

The remaining sums of squares are for interactions, some of which are only of theoretical interest but are included at this point to give a complete picture.

5. Times x Soaking Treatment.

$$\text{Sum of squares} = \frac{1203.0^2 + 944.4^2 \ldots 443.6^2}{100} - 70,571.95 - 16,720.19 - 5,695.99$$

$$= 1,138.96.$$

6. Replicates x Times.

$$\text{Sum of squares} = \frac{(299.1 + 330.5)^2 + (303.1 + 326.0)^2 \ldots (16.0 + 120.4)^2}{50}$$

$$- 70,571.95 - 29.72 - 16,720.19$$

$$= 304.75$$

7. Replicates x Soaking Treatments.

$$\text{Sum of squares} = \frac{(299.1 + 249.7 \ldots 18.5)^2 + (303.1 \ldots 11.4)^2 \ldots + (316.7 \ldots 120.4)^2}{250}$$

$$- 70,571.95 - 29.72 - 5,695.99$$

$$= 48.24$$

8. Replicates x Soaking Treatments x Time.

$$\text{Sum of squares} = \frac{299.1^2 + 303.1^2 + 303.6^2 \ldots 120.4^2}{25} - 70,571.95 - 29.72$$

$$- 5,695.99 - 16,720.19 - 1,138.96 - 304.75 - 48.24$$

$$= 345.52$$

The sums of squares for the theoretical interactions (which are not significant and are of no value in explaining the results) can be included in the Residual sum of squares. It is quite clear that inclusion of these in the Residual makes little difference to the analysis of variance table. (Column

Table B

Analysis of variance : radicle lengths of seedlings from seeds exposed to various periods of soaking.

Source	DF	Sum of Sqq.	Mean Sq.	F	F_1
Replicates	3	29.72	9.91	1.11	1.09
Times	9	16,720.19	1857.79	208.27**	205.96**
Treatments	1	5,695.99	5695.99	638.56**	631.48**
Interactions:-					
(a) Replicates Times	27	304.75	11.29	1.27	-
(b) Replicates Treatments	3	48.24	16.08	1.80	-
(c) Times Treatments	9	1,138.96	126.55	14.19**	14.03**
(d) Replicates Times Treatments	27	345.52	12.79	1.43	-
Residual	1920	17,128.88	8.92		
Total	1999	41,412.25			
Residual (when interactions a, b and d are included)	1977	17,827.39	9.02		

F_1, Table B gives F values when the theoretical interactions are included in the Residual).

The completed Analysis of Variance table is then as shown in Table B.

If the F value for a source of variation is more than that given in the tables for the 5% level we can say that that item is contributing significant differences in the experiment; if it is more than the value at the 1% level the differences are highly significant. The former is denoted by one asterisk; the latter by 2 asterisks.

The F values are obtained by comparing the mean squares (estimated variances) of the various items with the residual mean square which is taken here as the error variance. This is a measure of the variation among the individual plants and it is therefore valid to compare the estimated variances of the other sources with this.

Another value with which the estimated variances of the other items can be compared is the Times, Treatment interaction. This is a measure of the dissimilarities among the differences between each of the 2 values for the total of 100 observations at the various times, i.e. 1,238.4 - 1,203.0 = 35.4; 1,067.4 - 944.4 = 123.0; 842.1 - 610.8 = 231.3 etc. Since this interaction is significant when compared with the residual variance it is necessary to test the Times and Treatment mean squares against the Interaction mean square. This

is necessary because, unless the mean square for these 2 items is significantly greater than the interaction mean square, it might only represent differences attributable to the same causes as those which result in the significant interaction (see Snedecor, 1946; P. 277-9). When the Times and Treatment mean squares are tested against the Interaction mean square, values of 14.7 and 45.0 respectively are obtained. Both are significant at the 1% level thus confirming that significant differences in the experiment can be attributed to both Times and Treatment in their own rights.

It is now possible to draw conclusions from the experiment.

(a) There are no significant differences among the replicates, hence factors such as position of the pots in the incubator contribute little to the variation observed.

(b) Soaking has an effect on the variation.

(c) Some of the variation in the experiment is attributable to time differences.

(d) There is interaction between times and soaking treatment: this probably means that the longer the seeds are soaked the greater is the effect of the soaking.

All analysis of variance tables shown here are derived in a similar fashion to that described.

Transforming the original data.

In some experiments, inspection of the original data reveals that there are rather wide differences between certain treatments but an analysis of variance shows that these differences are not significant. Cochran (1938) has shown that the reason for this may be that there is wide variation within one or more of the treatments, thus giving an abnormally high residual mean square. This can sometimes be reduced by a transformation, which makes the distribution of individuals within different treatments more homogeneous (This is, of course, a theoretical requirement for any Analysis of Variance).

In the example of analysis of variance shown there are large differences within treatments as shown by the variances of x (Table C). The differences between treatments are, however, large enough to show significant differences and transformation of the data in this particular experiment is therefore not essential.

One transformation was carried out, however, in an attempt to reduce the within treatment variation. 10 was added to each original value and then the logarithm of this sum was taken. The variances of each population using the transformed data were calculated from the formula:-

$$\text{Variance} = \frac{1}{n-1}\left[Sx^2 - \frac{(Sx)^2}{n}\right]$$

TABLE C.

Comparison of the variances within certain treatments using the raw data and transformed data for calculation.

Hours soaking	Variance of x	Variance of log (x + 10)
0	20.25	0.0305
24	20.09	0.1044
48	14.06	0.0238
72	6.84	0.0067
96	0.78	0.0009

Where x = length (cm) of individual radicles.

Obviously this transformation is of no value in reducing the within treatment variation and, if it were necessary, another system would have to be found.

The major difficulty in effecting a transformation for the data of this experiment is that the distribution of radicle lengths is not normal in any treatment. All the distributions are bimodal (see Figure 2), thus making it very difficult to find a standard transformation that will reduce the variation in all the treatments.

Appendix III.

The Probit Analysis.

The probit analysis, developed by Finney (1947), is a particularly useful statistical technique in the measurement of a quantal response by a subject to a certain stimulus. In the experiments recorded here the subject is the pea seed, the stimulus can be regarded (Experiments 1 and 2) as the soaking treatment, the length of the soaking period being the dose and the response being the damage manifest in the radicle of the seedling or non-germination of the seed.

The data obtained must first be arranged such that a linear relationship holds between the number of damaged seedlings (+ ungerminated seeds) and the amount of the dose. This is generally done by effecting a logarithmic transformation but it can be seen from Figure 4 that it may be achieved in this example by squaring the values on the abscissa. (Note that this is the same as converting λ into $2 \log \lambda$). The data in Table D are obtained as follows:-

1. Enter values of λ (where λ = length of soaking period in days).
2. $x = \lambda^2$.
3. n = number of treated seeds.
4. r = number of seeds ungerminated + number of badly damaged seedlings.

Table D

The effects of soaking seeds of Maple pea : computations required for the fitting of a Probit Regression equation

λ	$x=\lambda^2$	n	r	P'	P(C=10)	Empirical Probit	Expected Probit (Y)	w	nw	Working Probit (y)	nwx	nwy
4	16	175	168	96	96	6.75	6.8	0.16135	28.2	6.748	451.2	190.2936
3	9	175	125	71	68	5.47	5.5	0.50056	87.6	5.467	788.4	478.9092
2	4	175	66	38	31	4.50	4.5	0.42716	74.8	4.504	299.2	336.8992
1	1	175	41	23	16	4.01	3.9	0.22250	38.9	4.012	38.9	156.0668
0	0	175	18	10	0	-	-	-	-	-	-	-
									229.5 Snw		1577.7 Snwx	1162.1688 Snwy

$\bar{x}$	$\frac{Snwx}{Snw}$	6.8745
$\bar{y}$	$\frac{Snwy}{Snw}$	5.0639
$(Snwx)^2$		2,489,137.29
$(Snwx) \times (Snwy)$		1,833,533.71576
$(Snwy)^2$		1,350,636.3197
$\frac{(Snwx)^2}{Snw}$		10,845.914
$\frac{(Snwx) \times (Snwy)}{Snw}$		7,989.340
$\frac{(Snwy)^2}{Snw}$		5,885.125
$Snwx^2$		15,550.5
$Snwxy$		8,858.5440
$Snwy^2$		6,045.8318
Sxy	$Snwxy - \frac{Snwx \times Snwy}{Snw}$	869.204
Sxx	$Snwx^2 - \frac{(Snwx)^2}{Snw}$	4,704.586
Syy	$Snwy^2 - \frac{(Snwy)^2}{Snw}$	160.7068
$\frac{1}{Snw}$		0.004,357.3

5. $P' = \frac{100r}{n}$, finding P' to the nearest whole number.
6. P = adjusted percentage damage, found as $\frac{P'-10}{90} \times 100$ where 10 is P' for x = 0 (i.e. the controls).
7. Find empirical probit for each adjusted percentage. (Fisher and Yates, 1948; P. 50).
8. Plot empirical probits against x (see Figure 4) and draw provisional straight line to fit the points.
9. Read off values of Y (expected probit) for each value of x.
10. Find value of weighting coefficient (w) corresponding to each value of Y, C = 10 (Finney, 1947; P. 226).
11. Calculate nw to 1 decimal place, then find Snw.
12. Find working probit (y) for each value of P and Y (Finney, 1947; P. 239).
13. Calculate values of nwx, then find Snwx.
14. $\bar{x} = \frac{Snwx}{Snw.}$
15. Calculate nwy, and find Snwy.
16. $\bar{y} = \frac{Snwy}{Snw.}$
17. Determine reciprocal of Snw to at least 7 decimal places.
18. Calculate $(Snwx)^2$, $(Snwy)^2$, Snwx x Snwy and divide each by Snw.
19. Calculate $Snwx^2$, $Snwy^2$ and Snwxy.
20. Sxx, Syy and Sxy are calculated as shown in Table D.
21. Find b $(= \frac{Sxy}{Sxx})$.
22. The equation for the probit regression line is then as follows:-

$$Y = \bar{y} + b(x-\bar{x})$$

In this example $Y = 3.7935 + 0.185x$

The straight line represented by this equation is shown in Figure 4.

23. The value of $X^2_{(3)}$ is calculated from the expression

$$Syy - \frac{(Sxy)^2}{Sxx}$$

If the value of X^2 is not significant then there is no significant heterogeneity in the populations hence the variances may be derived from true weights without any heterogeneity factor. In this case X^2 is 0.1155 which is not significant.

24. $$\text{Variance of } b = \frac{1}{Sxx}$$

$$\text{Standard error of } b = \sqrt{\frac{1}{Sxx}}$$

In this example $b = 0.1848 \pm 0.0146$.

25. The square of the median effective dose (E.D. 50) can be calculated from the equation for the regression line, i.e. the value of x which gives $Y = 5$. This is m which, in this example, equals 6.522 days. Obviously, the square root of this gives the estimated value of the median effective dose, viz. 2.55 days or 61.2 hours.

26. An estimate of the reliability of this value is obtained by calculating its variance and standard deviation.

$$V_m = \frac{1}{b^2}\left\{\frac{1}{Snw} + \frac{(m-\bar{x})^2}{Snw(x-\bar{x})^2}\right\}$$

$$= \frac{1}{b^2}\left\{\frac{1}{Snw} + \frac{(m-\bar{x})^2}{Sxx}\right\}$$

$$= 0.1280759$$

(Where V_m is the variance of m for the transformed data).

$\sqrt{V_m}$ = standard deviation of m (transformed data) = 0.36

Standard deviation of m (original data) = $\sqrt{0.36}$

= 0.6 days.

It is hence possible to conclude from these data that the median effective period of soaking for Maple pea seeds has a maximum likelihood estimate of 61.2 hours. The most extreme values of E.D. 50 likely to be encountered are 46.8 and 75.6 hours.

Thus it is possible to treat quantal data in such a fashion that definite conclusions may be drawn and compared with those of other quantitative observations.

Appendix IV.

Calculation of a Polynomial Regression Equation.

There are 2 methods of calculating polynomial regression equations depending on the distribution of the values of x. When the values of x are arranged at evenly spaced intervals the method used is based on those described by Mather, 1949 (Page 129-146) and Goulden, 1952 (Page 166-176). When the values of x are not equally spaced the method employed is based on those of Yule and Kendall, 1950 (Page 340-357) and Goulden, 1952 (Page 177-181).

In both cases it is essential to draw up an analysis of variance table as described in Appendix II.

(a) The data of Experiment 2 depend on values of x which are evenly spaced and these data are used to illustrate the former method.

The analysis of variance for the data of Experiment 2 is shown in Table E.

There are differences in the lengths of the radicles due to differences in the length of the soaking period and, to determine the order of the polynomial regression equation required, it is necessary to partition the Times sum of squares. This may be achieved from the data in Table F.

TABLE E.

Analysis of variance: root lengths of Maple pea seedlings grown from seeds exposed to various soaking periods then sown together and grown for 7 days.

Source	DF	Sum of Squares	Mean Sq.	F
Replicates	2	3.87	1.94	0.09
Times	4	4,743.54	1,185.89	56.12**
Residual	368	7,776.71	21.13	
Total	374	12,524.12		

TABLE F.

Original data required to partition Times sum of squares.

x	0	1	2	3	4
y	845.0	707.3	614.5	427.7	74.4

Where x = soaking period in days and y = total radicle length (cm) of 75 Maple pea seedlings.

From Table F the following can be calculated:-

$$S(x) = 75(0 + 1 + 2 + 3 + 4) = 750$$

$$S(x^2) = 75(0 + 1 + 4 + 9 + 16) = 2,250$$

$$S(x-\bar{x})^2 = S(x^2) - \frac{(S(x))^2}{n}$$

$$= 2,250 - \frac{750^2}{375} = 750$$

$$S(xy) = (0 \times 845.0) + (1 \times 707.3) + (2 \times 614.5) + (3 \times 427.7) + (4 \times 77.4)$$

$$= 3,517.0$$

Correction necessary to give $S[y(x-\bar{x})]$

$$= \frac{Sy \times S(x)}{n} = \frac{2,668.9 \times 750}{375} = 5,337.8$$

$$S[y(x-\bar{x})] = 3,517.0 - 5,337.8$$

$$= -1,820.8$$

Sum of squares for which this regression accounts is:-

$$\frac{S[y(x-\bar{x})]^2}{S(x-\bar{x})^2} = \frac{(-1,820.8)^2}{750} = 4,420.42$$

This represents the sum of squares which can be accounted for by a linear regression equation and, to ascertain if this is sufficient to account for all the data, we must consider again the analysis of variance table.

TABLE G.

Analysis of variance: partitioning of Times sum of squares into linear component and remainder.

Source	DF	Sum of Sqq.	Mean Sq.	F
Replicates	2	3.87	1.94	0.09
Times:-				
(a) Linear	1	4,420.42	4,420.42	209.20**
(b) Rem'der	3	323.12	107.71	5.09**
Residual	368	7,776.71	21.13	
Total	374	12,524.12		

Table G shows that although a linear regression describes the data reasonably well there is still a portion of the sum of squares giving significant results, i.e. it is possible to obtain a better fit by raising the equation from the linear one to a higher power of x.

Reference is now made to the Tables of ξ' compiled by Fisher and Yates, 1948 (Page 70). In this example, n = 5 and so Table H can be constructed giving values of ξ' 1 to 4.

TABLE H.

Values of ξ' corresponding to values of x.

x	y	ξ'_1	ξ'_2	ξ'_3	ξ'_4
0	845.0	-2	+2	-1	+1
1	707.3	-1	-1	+2	-4
2	614.5	0	-2	0	+6
3	427.7	+1	-1	-2	-4
4	74.4	+2	+2	+1	+1
10	2,668.9				
	$S(\xi'^2)$	10	14	10	70
	λ	1	1	5/6	35/12

The sums of squares for components up to the 4th power can be calculated from this table. For the linear sum of squares the coefficients of y are ξ'_1, for the quadratic ξ'_2 etc.

(a) Linear.

$$\frac{(-2 \times 845.0 - 1 \times 707.3 + 0 \times 614.5 + 1 \times 427.7 + 2 \times 74.4)^2}{10 \times 75}$$

$$= 4,420.42$$ (This confirms previous result).

(Where 10 is the value of $S(\ell_1'^2)$ and 75 is the number of individuals going to make up the y values).

(b) Quadratic.

$$\frac{(2 \times 845.0 - 1 \times 707.3 - 2 \times 614.5 - 1 \times 427.7 + 2 \times 74.4)^2}{14 \times 75}$$

$$= 262.70$$

(c) Cubic.

$$\frac{(-1 \times 845.0 + 2 \times 707.3 - 2 \times 427.7 + 1 \times 74.4)^2}{10 \times 75}$$

$$= 59.58$$

(d) Quartic.

$$\frac{(1 \times 845.0 - 4 \times 707.3 + 6 \times 614.5 - 4 \times 427.7 + 1 \times 74.4)^2}{70 \times 75}$$

$$= 0.87$$

The complete analysis of variance table can then be constructed (Table I).

This shows that the data can be suitably represented by a quadratic equation, i.e. of the form

$$y = a + bx + cx^2$$

TABLE I.

Analysis of variance table: radicle lengths of Maple pea seedlings grown for 7 days aerobically after the seeds had received various soaking treatments. Division of Times sum of squares into 4 components.

Source	DF	Sum of Sqq.	Mean Sq.	F
Replicates	2	3.87	1.94	0.09
Times:-				
a) Linear	1	4,420.42	4,420.42	209.20**
b) Quadratic	1	262.70	262.70	12.43**
c) Cubic	1	59.58	59.58	2.82
d) Quartic	1	0.87	0.87	0.04
Total Times	4	4,743.57		
Residual	368	7,776.68	21.13	
Total	374	12,524.12		

Determination of the coefficients in the equation.

The value of the constant (a) is obtained simply by determining the mean of all observations in the experiment.

$$a = \frac{S(y)}{n} = \frac{2,668.9}{375} = 7.117$$

Let equation be:-

$$y = a + b^{1}\mathcal{E}'_{1} + c^{1}\mathcal{E}'_{2}$$

Then:-

$$b^1 = \frac{S(\xi'_1 y)}{S\xi'^2_1}$$

$$= \frac{(-2 \times 845)+(-1 \times 707.3)+(0 \times 614.5)+(1 \times 427.7)+(2 \times 74.4)}{10 \times 75}$$

(from Table H)

$$= -2.428$$

$$c^1 = \frac{S(\xi'_2 y)}{S\xi'^2_2}$$

$$= \frac{(2 \times 845)+(-1 \times 707.3)+(-2 \times 614.5)+(-1 \times 427.7)+(2 \times 74.4)}{14 \times 75}$$

$$= -0.500$$

$$\therefore \quad y = 7.117 - 2.428\xi'_1 - 0.500\xi'_2$$

This equation is then converted to the more general formula by using the following identities (see Goulden, 1952; Page 187).

$$\xi'_1 = \lambda\xi_1 = \lambda x \qquad \text{where } x = (X-\overline{X})$$

and $\xi'_2 = \lambda\xi_2 = \lambda(x^2 - \frac{n^2-1}{12})$

In this illustration n = 5. The values of λ may be obtained from Table H.

$$\therefore \quad \xi'_1 = \lambda x = 1x$$

$$\xi'_2 = \lambda(x^2 - \frac{25-1}{12}) = 1(x^2-2)$$

$$\therefore \quad y = 7.117 - 2.428x - 0.500(x^2-2)$$

$$= 8.117 - 2.428x - 0.500x^2$$

But this value of x represents deviation from the mean

which in this example is 2. Hence, to complete the generalisation, it is necessary to substitute X-2 for x.

$$y = 8.117 - 2.428(X-2) - 0.500(X-2)^2$$

$$= 10.973 - 0.428X - 0.500X^2$$

The calculated curve is then drawn through points determined from this equation. It is shown in Figure 5 (facing page 39).

(b) Calculation of a polynomial regression equation when the values of x are not evenly spaced.

As an example of this type of calculation we can select some of the data from Experiment 3. Table J shows the raw data required.

TABLE J.

Mean radicle length of seedlings of Maple pea after exposure of the seeds to 24 hours soaking at 4 different temperatures before sowing.

x(°C)	3	10	20	25
y(cm)	5.77	10.15	9.77	11.06

The analysis of variance for these figures is included in Table 15 (Page 52) and it is clear that there are significant

differences among the radicle lengths attributable to differences in soaking temperatures. This analysis includes the 24 and 72 hour periods, however, and it is necessary to separate the 2. Table 16 (Page 53) shows an analysis of variance for the 24 hour period only and the Temperature sum of squares is partitioned into 3 components. This cannot, however, be done till a suitable equation for the regression line has been calculated.

Obviously a straight line does not represent the data in Table J so it is essential to find a polynomial of a higher degree. The method of finding the degree of the polynomial which gives the best fit is based on that described by Yule and Kendall (1950; Page 352-357).

For the purposes of these data it is assumed that an equation including a power of x^3 at least will be required.

The raw data required for this are shown in Table K.

If we consider the general form of a cubic equation $y = a + bx + cx^2 + dx^3$, we can calculate the values of a, b, c and d from the following 4 simultaneous equations.

$$Sy = an + bSx + bS(x^2) + cS(x^3).$$

$$Sxy = aSx + bS(x^2) + cS(x^3) + dS(x^4).$$

$$Sx^2y = aS(x^2) + bS(x^3) + cS(x^4) + dS(x^5).$$

$$Sx^3y = aS(x^3) + bS(x^4) + cS(x^5) + dS(x^6).$$

Table K

Data required to obtain curve which will express relationship between soaking temperature and length of radicle of seedling after pea seeds have been soaked for 24 hours.

y	x	x^2	x^3	x^4	x^5	x^6	xy	x^2y	x^3y
5.77	3	9	27	81	243	729	17.31	51.93	155.79
10.15	10	100	1,000	10,000	100,000	1,000,000	101.50	1,015.00	10,150.00
9.77	20	400	8,000	160,000	3,200,000	64,000,000	195.40	3,908.00	78,160.00
11.06	25	625	15,625	390,625	9.765,625	244,140,625	276.50	6,912.50	172,812.50
36.75	58	1,134	24,652	560,706	13,065,868	309,141,354	590.71	11,887.43	261,278.29

Where y = mean radicle length (cm) and x = temperature ^{o}C.

The last row represents totals for each column.

By substitution we obtain:-

$$36.75 = 4a + 58b + 1{,}134c + 24{,}652d$$

$$590.71 = 58a + 1{,}134b + 24{,}652c + 560{,}706d$$

$$11{,}887.43 = 1{,}134a + 24{,}652b + 560{,}706c + 13{,}065{,}868d$$

$$261{,}278.29 = 24{,}652a + 560{,}706b + 13{,}065{,}868c + 309{,}141{,}354d$$

The abbreviated Doolittle method (see Goulden, 1952; page 180) may be used to calculate the values of a, b, c and d (Table L). In this example, the values are a = 1.118,712, b = 1.907,995, c = -0.127,202 and d = 0.002,672. Hence equation is:-

$$y = 1.1187 + 1.9079x - 0.1272x^2 + 0.0027x^3$$

and the calculated line can be drawn in as shown in Figure 7 (facing page 53). It will be seen that a cubic equation is necessary to describe the data and it is possible to test whether the term in x^3 is significant. This may be done by returning to the analysis of variance table.

The components of the Temperature sum of squares are readily derived from the Doolittle table drawn up to evaluate a, b, c and d.

The sum of squares accounted for by a linear regression is given by:-

$$75\left[(5,2) \times (6,5)^2\right]$$

where the numbers within the brackets refer to row and column numbers respectively in Table L, and 75 is the number of observations contributing to the mean value of y.

Table I.

Solution of simultaneous equations by the Doolittle method.
(Numbers in brackets refer to rows and columns respectively.)

	1	2	3	4	5
1	n	Σx	Σx^2	Σx^3	Σy
2	$\frac{n}{n}$	$\frac{\Sigma x}{n}$	$\frac{\Sigma x^2}{n}$	$\frac{\Sigma x^3}{n}$	$\frac{\Sigma y}{n}$
3		Σx^2	Σx^3	Σx^4	Σxy
4		(2,2).(1,2)	(2,2).(1,3)	(2,2).(1,4)	(2,2).(1,5)
5		(3,2) - (4,2)	(3,3) - (4,3)	(3,4) - (4,4)	(3,5) - (4,5)
6		$\frac{(5,2)}{(5,2)}$	$\frac{(5,3)}{(5,2)}$	$\frac{(5,4)}{(5,2)}$	$\frac{(5,5)}{(5,2)}$
7			Σx^4	Σx^5	$\Sigma x^2 y$
8			(2,3).(1,3)	(2,3).(1,4)	(2,3).(1,5)
9			(6,3).(5,3)	(6,3).(5,4)	(6,3).(5,5)
10	Subtract rows 8 and 9 from 7.				
11			$\frac{(10,3)}{(10,3)}$	$\frac{(10,4)}{(10,3)}$	$\frac{(10,5)}{(10,3)}$
12				Σx^6	$\Sigma x^3 y$
13				(2,4).(1,4)	(2,4).(1,5)
14				(6,4).(5,4)	(6,4).(5,5)
15				(11,4).(10,4)	(11,4).(10,5)
16	Subtract rows 13, 14 and 15 from 12.				
17				$\frac{(16,4)}{(16,4)}$	$\frac{(16,5)}{(16,4)}$
18				a	(17,5)
19			c	- (17,5).(11,4)	(11,5)
20		b	- (19,3).(6,3)	- (17,5).(6,4)	(6,5)
21	a	- (20,2).(2,2)	- (19,3).(2,3)	- (17,5).(2,4)	(2,5)

Where a = (21,2) + (21,3) + (21,4) + (21,5), b = (20,3) + (20,4) + (20,5), c = (19,4) + (19,5).

The quadratic sum of squares is given by:-

$$75\left[(10,3) \times (11,5)^2\right]$$

The cubic sum of squares is given by:-

$$75\left[(16,4) \times (17,5)^2\right]$$

The number of degrees of freedom for each component is 1 and the complete analysis is shown in Table 16 (Page 53).

It is quite clear from this that both the quadratic and the cubic components are significant and, since the latter is significant, we must conclude that a term in x^3 should be included in the equation.

BIBLIOGRAPHY.

Åberg, B., 1957. Auxin relations in roots. (Ann. Rev. Plant Physiol., 8, 153 - 180).

Albaum, H.G., 1940. The growth of oat coleoptiles after seed exposure to different oxygen concentrations. (Biol. Bull., 79, 360 - 361).

Albaum, H.G., and P.P. Cohen, 1943. Transamination and protein synthesis in germinating oat. (J. biol. Chem., 149, 19 - 27).

Albaum, H.G., and B. Eichel, 1943. Relationship between growth and metabolism in the oat seedling. (Amer. J. Bot., 30, 18 - 22).

Andreae, W.A., and N.E. Good, 1955. The formation of indoleacetylaspartic acid in pea seedlings. (Plant Physiol., 30, 380 - 382).

Andreae, W.A., and N.E. Good, 1957. Studies on 3-indoleacetic acid metabolism. IV. Conjugation with aspartic acid and ammonia as processes in the metabolism of carboxylic acids. (Plant Physiol., 32, 566 - 572).

Andreae, W.A., J.R. Robinson and M.W.H. van Ysselstein, 1961. Studies on 3- indoleacetic acid metabolism. VII.

Metabolism of radioactive 3-indoleacetic acid by pea roots. (Plant Physiol., 36, 783 - 787).

Andreae, W.A., and M.W.H. van Ysselstein, 1956. Studies on 3-indoleacetic acid metabolism. III. The uptake of 3-indoleacetic acid by pea epicotyls and its conversion to 3-indoleacetylaspartic acid. (Plant Physiol., 31, 235 -240).

Andreae, W.A., and M.W.H. van Ysselstein, 1960, a. Studies on 3-indoleacetic acid metabolism. V. Effect of calcium ions on 3-indoleacetic acid uptake and metabolism by pea roots. (Plant Physiol., 35, 220 - 224).

Andreae, W.A., and M.W.H. van Ysselstein, 1960, b. Studies on 3-indoleacetic acid metabolism. VI. 3-indoleacetic acid uptake and metabolism by pea roots and epicotyls. (Plant Physiol., 35, 225 - 232).

Atkins, W.R.G., 1909. The absorption of water by seeds. (Royal Dublin Soc. Sci. Proc. n.s., 12, 35 - 46: abstr. in Exp. Sta. Rec., 21, 725 - 726).

Audus, L.J., 1949. The biological detoxication of 2:4 dichlorophenoxyacetic acid in soil. (Plant and Soil, 2, 31 - 36).

Audus, L.J., 1951. The biological detoxication of hormone herbicides in soil. (Plant and Soil, 3, 170 - 192).

Audus, L.J., 1959. Plant Growth Substances. Leonard Hill (Books) Ltd., London.

Audus, L.J., and M.E. Brownbridge, 1957. Studies on the geotropism of roots. I. Growth rate distribution during response and the effects of applied auxin. (J. exp. Bot., 8, 105 - 124).

Audus, L.J., and B.E.S. Gunning, 1958. Growth substances in the roots of Pisum sativum. (Physiol. Plant., 11, 685 - 697).

Audus, L.J., and A.N. Lahiri, 1961. Studies on the geotropism of roots. III. Effects of geotropic stimulation on growth-substance concentration in Vicia faba root tips. (J. exp. Bot., 12, 75 - 84).

Audus, L.J., and M.E. Shipton, 1952. 2:4-dichloranisole-auxin interactions in root growth. (Physiol. Plant., 5, 430 - 455).

Avery, G.S., J. Berger and B. Shalucha, 1942. Auxin content of maize kernels during ontogeny, from plants of varying heterotic vigour. (Amer. J. Bot., 29, 765 - 772).

Babcock, E.B., and G.L. Stebbins jr., 1938. The American species of Crepis. Their interrelationships and distribution as affected by polyploidy and apomixis. (Publ. Carneg. Instn. Wash., 504, 1 - 199).

Bailey, W.M., 1933. Structural and metabolic after - effects of soaking seeds of Phaseolus. (Bot. Gaz. 94, 688 - 713).

Bailey, C.H., 1940. Respiration of cereal grains and flax-seed. (Plant Physiol., 15, 257 - 274).

Bailey, C.H., and A.M. Gurjar, 1918. Respiration of stored wheat. (J. agric. Res., 12, 685 - 713).

Barton, A.W., 1929. The effect of long continued soaking on seed germination. (Trans. Kans. Acad. Sci., 32, 37).

Barton, L.V., 1950. Relation of different gases to the soaking injury of seeds. (Contr. Boyce Thompson Inst., 16, 55 - 71).

Bennet-Clark, T.A., and N.P. Kefford, 1953. Chromatography of the growth substances in plant extracts. (Nature, London, 171, 645 - 647).

Bentley, J.A., 1958. The naturally-occurring auxins and inhibitors. (Ann. Rev. Plant Physiol., 9, 47 - 80).

Bernstein, L., 1943. Hybrid vigour in corn, and the mobilization of endosperm reserves. (Amer. J. Bot., 30, 801 - 809).

Berrie, A.M.M., 1959. Personal communication.

Berrie, A.M.M., 1960. Abnormal growth and development in pea, resulting from exposure to adverse conditions

during germination. (Nature, London, 185, 626 - 627).

Bitancourt, A.A., 1954. La nature des auxines des tumeurs végétales. (l'Ann. Biol., 30, 361 - 370).

Bouillenne-Walrand, M., and C. Leyh, 1963. Concerning the auxinic (IAA) metabolism of plants of Zea mays L., (Phinney dwarf varieties) treated with gibberellic acid. (Arch. Inst. Bot. Univ., Liège, 29, 1353 - 1370).

Braun, H., 1919. Presoaking as a means of preventing seed injury due to disinfectants and of increasing germicidal efficiency. (Science, 49, 544 - 545).

Braun, H., 1924. A gradient of permeability to iodin in wheat seed coats (a preliminary note). (J. agric. Res., 28, 225 - 226).

Brian, P.W.; 1959, a. Effect of gibberellins on plant growth and development. (Biol. Rev. 34, 37 - 84).

Brian, P.W., 1959, b. Morphogenetic effects of the gibberellins. (J. Linn. Soc. (Bot.), 56, 237 - 248.)

Brian, P.W., and G.W. Elson, 1963. Personal communication.

Brian, P.W., G.W. Elson, H.G. Hemming and M. Radley, 1954. The plant growth promoting qualities of gibberellic acid, a metabolic product of the fungus, Gibberella fujikuroi. (J. Sci. Fd. Agric., 5 (12), 602 - 612).

Brian, P.W., J.F. Grove and J. MacMillan, 1960. The gibberellins. (Fortschr. Chem. organ. Naturstoffe, 18, 350 - 433).

Brian, P.W., and G. Hemming, 1957. A relationship between the effects of gibberellic acid and indolylacetic acid on plant cell extension. (Nature, London, 179, 417).

Brian, P.W., and G. Hemming, 1958. Complementary action of gibberellic acid and auxins in pea internode extension. (Ann. Bot., Lond., n.s. 22, 1 - 17).

Briggs, W.R., T.A. Steeves, I.N. Sussex and R.H. Wetmore, 1955. A comparison of auxin destruction by tissue extracts and intact tissues of the fern, Osmunda cinnamomea, L. (Plant Physiol., 30, 148 - 155).

Brown, A.J., 1907. On the existence of a semi-permeable membrane enclosing the seeds of the Gramineae. (Ann. Bot., Lond., 21, 79 - 87).

Brown, A.J., 1909. The selective permeability of the coverings of seeds of Hordeum vulgare. (Proc. roy. Soc., B, 81, 82 - 93).

Brown, A.J., and F. Tinker, 1916. Selective permeability: the absorption of phenol and other solutions by the seeds of Hordeum vulgare. (Proc. roy. Soc., B, 89, 373 - 379).

Burstrom, H., 1957. On the adaptation of roots to beta indolyl acetic acid. (Physiol. Plant., 10, 187 - 197).

Butcher, D.N., 1963. The presence of gibberellins in excised tomato roots. (J. exp. Bot., 14, 272 - 280).

Butcher, D.N., and H.E. Street, 1960. The effects of gibberellins on the growth of excised tomato roots. (J. exp. Bot., 11, 206 - 216).

Carr, D.J., and K.G.M. Skene, 1961. Diauxic growth curves of seeds, with special reference to french beans (Phaseolus vulgaris, L.). (Aust. J. Biol. Sci., 14, 1 - 12).

Chippindale, H.G., 1934. The effect of soaking in water on the "seeds" of some Gramineae. (Ann. appl. Biol., 21, 225 - 232).

Cholodny, N., 1924. Über die hormonale Wirkung der Organspitze bei der geotropischen Krümmung. (Ber. d. bot. Ges., 42, 356 - 362).

Cholodny, N., 1926. Beiträge zur Analyse der geotropischen Reaktion. (Jahrb. wiss. Bot., 65, 447 - 459).

Cochran, W.G., 1938. Some difficulties in the statistical analysis of replicated experiments. (Emp. J. exp. Agric., 6, 157 - 175).

Cossins, E.A., and E.R. Turner, 1963. The metabolism of ethanol in germinating pea seedlings. (J. exp. Bot.,

14, 290 - 298).

Crocker, W., and L.V. Barton, 1957. Physiology of seeds. Chronica Botanica Coy., Waltham, Mass., U.S.A.

Denny, F.E., 1917. Permeability of certain plant membranes to water. (Bot. Gaz., 63, 373 - 397).

Documenta Geigy, 1956. Documenta Geigy Scientific Tables. Geigy Pharmaceutical Co. Ltd., Manchester.

Drennan, D.S.H., 1960. Studies in the physiology of germination in the genus Avena. (Ph. D. thesis, Glasgow, 1960).

Drennan, D.S.H., A.M.M. Berrie and G.A. Armstrong, 1961. Prevention of manganese deficiency in oat by soaking the grain in solutions of manganous chloride. (Nature, London, 190, 824).

Evenari, M., 1957. The physiological action and biological importance of germination inhibitors. (Symp. Soc. Expt. Biol., 12, 21 - 43).

Eyster, H.C., 1936. Sensitivity of seeds to soaking. (Amer. J. Bot., 23, 691).

Eyster, H.C., 1939. Cause of decreased germination of bean seeds soaked in water. (Amer. J. Bot., 26, 18s).

Eyster, H.C., 1940. The cause of decreased germination of bean seeds soaked in water. (Amer. J. Bot., 27, 652 - 659).

Fang, S.C., P. Theisen and J.S. Butts, 1959. Metabolic

studies of applied indoleacetic acid-1-C^{14} in plant tissues as affected by light and 2:4D treatment. (Plant Physiol., 34, 26 - 32).

Finney, D.J., 1947. Probit analysis. Cambridge University Press.

Fisher, R.A., and F. Yates, 1948. Statistical tables for biological, agricultural and medical research. 3rd Edition. Oliver and Boyd, Edinburgh.

Folkes, B.F., and E.W. Yemm, 1958. Respiration of barley plants. X. Respiration and the metabolism of amino acids and proteins in germinating grain. (New Phytol., 57, 106 - 131).

Fruton, J.S., and S. Simmonds, 1958. General Biochemistry. 2nd Edition. Chapman and Hall Ltd., London.

Furuya, M., and A.W. Galston, 1961. Effect of in vitro preincubation with cofactors on the activity of the indoleacetic acid oxidase of peas. (Physiol. Plant., 14, 750 - 766).

Furuya, M., A.W. Galston and B.B. Stowe, 1962. Isolation from peas of co-factors and inhibitors of indolyl-3-acetic acid oxidase. (Nature, London, 193, 456 - 457).

Galston, A.W., 1956. Some metabolic consequences of the

administration of indoleacetic acid to plant cells. In : R.L. Wain and F. Wightman, The chemistry and mode of action of plant growth substances, P. 219.
Butterworth, London.

Galston, A.W., 1957. Studies on indoleacetic acid oxidase and its inhibitor in light grown peas. (Plant Physiol., 32, xxi).

Galston, A.W., 1959. Studies on indoleacetic acid oxidase inhibitor and its relation to photomorphogenesis. In : Photoperiodism and related phenomena in plants and animals. (A.A.A.S., Washington, 137 - 157).

Galston, A.W., and R.S. Baker, 1949. Studies on the physiology of light action. II. The photodynamic action of riboflavin. (Amer. J. Bot., 36, 773 - 780).

Galston, A.W., and R.S. Baker, 1951. Studies on the physiology of light action. III. Light activation of a flavoprotein enzyme by reversal of a naturally occurring inhibition. (Amer. J. Bot., 38, 190 - 195).

Galston, A.W., J. Bonner and R.S. Baker, 1953. Flavoprotein and peroxidase as components of the indoleacetic acid oxidase system of peas. (Arch. Biochem.

Biophys., 42, 456 - 470).

Galston, A.W., and L.Y. Dalberg, 1954. The adaptive formation and physiological significance of indoleacetic acid oxidase. (Amer. J. Bot., 41, 373 - 380).

Galston, A.W., and M.E. Hand, 1949. Studies on the physiology of light action. I. Auxin and the light inhibition of growth. (Amer. J. Bot., 36, 85 - 94).

Galston, A.W., and H. Warburg, 1959. An analysis of auxin-gibberellin interaction in pea stem tissue. (Plant Physiol., 34, 16 - 22).

Goldacre, P.L., 1949. On the mechanism of action of 2:4 dichlorophenoxyacetic acid. (Aust. J. sci. Res., B, 2, 154 - 157).

Goldacre, P.L., and W. Bottomley, 1959. A kinin in apple fruitlets. (Nature, London, 184, 555 - 556).

Goldacre, P.L., A.W. Galston and R.L. Weintraub, 1953. The effect of substituted phenols on the activity of the indoleacetic acid oxidase of peas. (Arch. Biochem. Biophys., 43, 358 - 373).

Good, N.E., W.A. Andreae, and M.W.H. van Ysselstein, 1956. Studies on 3-indoleacetic acid metabolism. II. Some products of the metabolism of exogenous 3-indoleacetic acid in plant tissues. (Plant Physiol., 31, 231 - 235).

Gordon, S.A., 1954. Occurrence, formation and inactivation of

auxins. (Ann. Rev. Plant Physiol., 5, 341 - 378).

Gortner, W.A., and M.J. Kent, 1953. Indoleacetic acid oxidase and an inhibitor in pineapple tissue. (J. biol. Chem., 204, 593 - 603).

Gortner, W.A., and M.J. Kent, 1958. The coenzyme requirement and enzyme inhibitors of pineapple indole acetic acid oxidase. (J. biol. Chem., 233, 731 - 735).

Goulden, C.H., 1952. Methods of Statistical Analysis. 2nd Edition.
Chapman and Hall Ltd., London.

Griffith, M.M., 1957. Some anatomical effects of gibberellic acid on dwarf peas. (Quart. J. Florida Acad. Sci., 20, 238 - 242).

Griffiths, A.E., 1937. Observations on the germination of lettuce seed. (Contr. Boyce Thompson Inst., 9, 329 - 337).

Gruen, H.E., 1959. Auxins and fungi. (Ann. Rev. Plant Physiol., 10, 405 - 440).

Hamilton, R.H., R.S. Bandurski and B.H. Grigsby, 1961. Isolation of indole-3-acetic acid from corn kernels and etiolated corn seedlings. (Plant Physiol., 36, 354 - 359).

Harrington, G.T., and W. Crocker, 1923. Structure, physical characteristics and composition of the pericarp

and integument of Johnson grass seed in relation to its physiology. (J. agric. Res., 23, 193 - 222).

Hatcher, E.S.J., 1945. Studies in the vernalization of cereals. IX. Auxin production during development and ripening of the anther and carpel of spring and winter rye. (Ann. Bot., Lond., n.s., 9, 235 - 266).

Hillman, W.S., and A.W. Galston, 1956. Interaction of manganese and 2:4 dichlorophenol in the enzymatic destruction of indoleacetic acid. (Physiol. Plant., 9, 230 - 235).

Hillman, W.S., and A.W. Galston, 1957. Inductive control of indole-3-acetic acid oxidase activity by red and near infra-red light. (Plant Physiol., 32, 129 - 135).

Hora, F.B., 1936. The development of saccharase and amylase during the course of germination of barley grains. (D. Phil. thesis, Oxford, 1936).

Housley, S., and N.M. Griffiths, 1962. The occurrence of 3-indolylacetic acid in seeds and shoots of pea. (ϕYton, 19 (2), 85 - 93).

Howell, P.J., 1961. Personal communication.

Hughes, E.W.D., and H.E. Street, 1960. Effects of inhibitory concentrations of 3-indolylacetic acid and

3-indolylacetonitrile on cell division and tissue differentiation in excised tomato roots. (J. exp. Bot., 11, 198 - 205).

Jacques, R., 1957. Quelques données sur le photopériodisme de Chenopodium polyspermum, L.; influence sur la germination des grains.
In : International symposium on photo-thermoperiodism. The action of different radiations, of gibberellins and other active substances. (International union of biological sciences, Série B, colloque 34, 125 - 130).

James, A.L., 1940. The carbohydrate metabolism of germinating barley. (New Phytol., 39, 133 - 144).

James, W.O., and A.L. James, 1940. The respiration of barley germinating in the dark. (New Phytol., 39, 145 - 176).

Johansen, D.A., 1940. Plant microtechnique.
McGraw Hill Book Coy. inc., New York and London.

Kato, J., and M. Katsumi, 1958. Effect of gibberellins on IAA oxidase. (Naturwissenschaften, 45, 344).

Katsumi, M., 1962. Physiological effects of kinetin effect on the thickening of etiolated pea stem sections. (Physiol. Plant., 15, 115 - 121).

Kefford, N.P., 1962. Auxin-gibberellin interaction in rice coleoptile elongation. (Plant Physiol., 37,

380 - 386).

Kefford, N.P., and P.L. Goldacre, 1961. The changing concept of auxin. (Amer. J. Bot., 48, 643 - 650).

Kenten, R.H., 1955. The oxidation of indolyl-3-acetic acid by waxpod bean root sap and peroxidase systems. (Biochem. J., 59, 110 - 121).

Kenten, R.H., and P.J.G. Mann, 1949. The oxidation of manganese by plant extracts in the presence of H_2O_2. (Biochem. J., 45, 255 - 263).

Kenten, R.H., and P.J.G. Mann, 1950. The oxidation of manganese by peroxidase systems. (Biochem. J., 46, 67 - 73).

Kidd, F., 1915. The controlling influence of carbon dioxide. Part III. The retarding effect of carbon dioxide on respiration. (Proc. roy. Soc., B, 89, 136 - 156).

Kidd, F., and C. West, 1918, a. Physiological predetermination : The influence of the physiological condition of the seed upon the course of subsequent growth and upon the yield. I. The effects of soaking seeds in water. (Ann. appl. Biol., V, 1 - 10).

Kidd, F., and C. West, 1918, b. Physiological predetermination : The influence of the physiological condition of the seed upon the course of

subsequent growth and upon the yield. II. Review of the literature. Chapter I. (Ann. appl. Biol., V, 112 - 142).

Kidd, F., and C. West, 1919, a. Physiological predetermination : The influence of the physiological condition of the seed upon the course of subsequent growth and upon the yield. III. Review of the literature. Chapter II. (Ann. appl. Biol., V, 157 - 170).

Kidd, F., and C. West, 1919, b. Physiological predetermination : The influence of the physiological condition of the seed upon the course of subsequent growth and upon the yield. IV. Review of the literature. Chapter III. (Ann. appl. Biol., V, 220 - 251).

Kidd, F., and C. West, 1919, c. Physiological predetermination : The influence of the physiological condition of the seed upon the course of subsequent growth and upon the yield. V. Review of the literature. Chapter IV. (Ann. appl. Biol., VI, 1 - 26).

Kidd, F., and C. West, 1919, d. The influence of temperature on the soaking of seeds. (New Phytol., 18, 35 - 39).

Kraemer, L.M., E.E. Conn and B. Vennesland, 1951.

The β-carboxylases of plants. III. Oxalacetic carboxylase of wheat germ. (J. biol. Chem., 188, 583 - 591).

Kuraishi, S., and R.M. Muir, 1962. Increase in diffusible auxin after treatment with gibberellin. (Science, 137, 760 - 761).

Kuraishi, S., and R.M. Muir, 1963. Diffusible auxin increase in a rosette plant treated with gibberellin. (Naturwissenschaften, 50, 337 - 338).

Lahiri, A.N., and L.J. Audus, 1960. Growth substances in the roots of Vicia faba. (J. exp. Bot., 11, 343 - 350).

Lahiri, A.N., and L.J. Audus, 1961. Growth substances in the roots of Vicia faba. II. The effects of ageing and excision of the main tap root meristem. (J. exp. Bot., 12, 364 - 377).

Larsen, P., 1936. Über einen wuchstoffinaktivierenden Stoff aus Phaseolus - Keimpflanzen. (Planta, 25, 311 - 314).

Larsen, P., 1940. Untersuchungen über den thermolabilen, wuchsstoffoxydierenden Stoff in Phaseolus - Keimpflanzen. (Planta, 30, 673 - 682).

Larsen, P., 1955. Growth substances in higher plants. In : Modern methods of plant analysis. III, 565 - 625. Ed. K. Paech and M.V. Tracey.

Springer-Verlag, Berlin.

Leach, W., 1943. Studies on the metabolism of cereal grains. II. The effect of age and kernel size on the course of respiration of wheat during early germination stages. (Canad. J. Res., C, 21, 289 - 296).

Lek, H.A.A. van der, 1934. Over den invloed der knoppen op de wortelvorming der stekken. (With summary : On the influence of the buds on root-development in cuttings). (Meded. Landbouwhooge-school, Wageningen, 38 (2), 1 - 95).

Levitt, J., 1956. Osmosis and permeability. In : Encyclopedia of Plant Physiology. III, 22 - 29. Ed. W. Ruhland. Springer-Verlag, Berlin.

Levitt, J., 1957. The significance of "apparent free space" (A.F.S.) in ion absorption. (Physiol. Plant., 10, 882 - 888).

Levitt, J., 1959. An interpretation of free space in ion absorption. (Recent Advances in Botany. From lectures and symposia presented to the IX international congress, Montreal. II, 1131 - 1132).

Luckwill, L.C., 1959. Factors controlling the growth and form of fruits. (J. Linn. Soc. (Bot.), 56, 294 - 302).

McLachlan, G.A., and E.R. Waygood, 1956. Catalysis of indole-

acetic acid oxidase by manganic ions. (Physiol. Plant., 9, 321 - 330).

MacLeod, A.M., 1957. Raffinose metabolism in germinating barley. (New Phytol., 56, 210 - 220).

McManus, M.A., 1960. Certain mitotic effects of kinetin, gibberellic acid, indole acetic acid and maleic hydrazide on the root of Allium cepa. (Nature, London, 185, 44 - 45).

MacMillan, J., J.C. Seaton and P.J. Suter, 1959. A new plant growth promoting acid - Gibberellin A_5 - from the seed of Phaseolus multiflorus. (Proc. Chem. Soc., Lond., 1959, 325 - 326).

MacMillan, J., and P.J. Suter, 1958. The occurrence of Gibberellin A_1 in higher plants : isolation from the seed of runner bean (Phaseolus multiflorus). (Naturwissenschaften, 45, 46).

Malhotra, R.C., 1931. Periodic permeability of iodine solution and water in the protoplasm of Zea mays seeds. (Protoplasma, 13, 374 - 388).

Malhotra, R.C., 1932. Biochemical studies of seeds during germination. (Bot. Contr., 50, 1 - 19).

Manos, G.E., 1961. The effects of growth substances on attached and detached root tips of Pisum sativum L. (Physiol. Plant., 14, 697 - 711).

Mather, K., 1949. Statistical analysis in biology. 3rd. Ed'n.

Methuen and Co. Ltd., London.

Miller, C.O., 1958. The relationship of kinetin and red light promotion of lettuce seed germination. (Plant Physiol., 33, 115 - 117).

Miller, C.O., 1960. An assay for kinetin-like materials. (Plant Physiol., 35, xxvi).

Miller, C.O., 1961. Kinetin and related compounds in plant growth. (Ann. Rev. Plant Physiol., 12, 395 - 408).

Miller, C.O., F. Skoog, M.K. von Saltza and F.M. Strong, 1955. Kinetin, a cell division factor from deoxyribonucleic acid. (J. Amer. chem. Soc., 77, 1392).

Miller, C.O., F. Skoog, F.S. Okumura, M.H. von Saltza and F.M. Strong, 1955. Structure and synthesis of kinetin. (J. Amer. chem. Soc., 77, 2662 - 2663).

Miller, C.O., F. Skoog, F.S. Okumura, M.H. von Saltza and F.M. Strong, 1956. Isolation, structure and synthesis of kinetin, a substance promoting cell division. (J. Amer. chem. Soc., 78, 1375 - 1380).

Milner, M., and P. Linko, 1958. Gas exchange induced in dry wheat embryos by wetting. (Plant Physiol., 33, xxxv).

Moewus, F., 1948. Ein neuer quantitativer Test für pflanzliche Wuchstoffe. (Naturwissenschaften, 4, 124).

Moulton, J.E., 1942. Extraction of auxin from maize, from smut tumours of maize and from Ustilago zeae.

(Bot. Gaz., 103, 725 - 739).

Mudd, J.B., B.G. Johnson, R.H. Burris and K.P. Buchholtz, 1959. Oxidation of indoleacetic acid by quackgrass rhizomes. (Plant Physiol., 34, 144 - 148).

Muir, R.M., 1942. Growth hormones as related to the setting and development of fruit in Nicotiana tabacum. (Amer. J. Bot., 29, 716 - 720).

Naylor, A.W., and B.N. Rappaport, 1950. Studies on the growth factor requirements of pea roots. (Physiol. Plant., 3, 315 - 333).

Nitsch, J.P., and C. Nitsch, 1955. Séparation chromatographique des auxins de l'ovule féconde de Haricot à differents stades de son développement. (Bull. Soc. bot., Fr., 102, 528 - 532).

Noirfalise, A., 1940. Recherches sur le développment des racines de Vicia faba traitées a l'hétéroauxine. (La Cellule, 48, 307 - 334).

Novikov, V.A., and R.O. Sadovskaja, 1939. Soaking of cotton seeds in boric acid as a means going to satisfy the boron requirement and increase salt resistance. (C.R. Acad. Sci., U.R.S.S., 23, 276 - 279).

Ochoa, S., A.H. Mehler and A. Kornberg, 1948. Biosynthesis of dicarboxylic acids by carbon dioxide fixation. I. Isolation and properties of an enzyme from

pigeon liver catalysing the reversible oxidative decarboxylation of l-malic acid. (J. biol. Chem., 174, 979 - 1000).

Ochoa, S., and E. Weisz-tabori, 1948. Biosynthesis of tricarboxylic acids by carbo dioxide fixation. II. Oxalo-succinic carboxylase. (J. biol. Chem., 174, 123 - 132).

Overbeek, J. van, 1956. Absorption and translocation of plant regulators. (Ann. Rev. Plant Physiol., 7, 355 - 372).

Overbeek, J. van, 1959. Auxins. (Bot. Rev., 25, 269 - 350).

Phinney, B.O., and C.A. West, 1960. Gibberellins as native plant growth regulators. (Ann. Rev. Plant Physiol., 11, 411 - 436).

Pilet, P.E., 1951. Etude de la circulation des auxines dans la racine de Lens culinaris. (Ber. schweiz. bot. Ges., 61, 410 - 424).

Pilet, P.E., 1952. Problème hormonal concernant l'Endophyllum sempervivi Lév., parasite du Sempervivum tectorum L. (Ber. schweiz. bot. Ges., 62, 269 - 274).

Pilet, P.E., 1957. Action des gibbérellines sur l'activité auxin-oxydasique de tissus cultivé in vitro. (C.R. Acad. Sci., Paris, 245, 1327 - 1328).

Pilet, P.E., and W. Wurgler, 1958. Action des gibbérellines

sur la croissance et l'activité auxines-oxydasique du Trifolium ochroleucum Hudson. (Ber. schweiz. bot. Ges., 68, 54 - 63).

Pilet, P.E., and G. Collet, 1959. Acide gibbérellique et destruction auxinique in vitro. (C.R. Acad. Sci., Paris, 249, 298 - 299).

Piper, C.S., 1941. Marsh spot of peas : a manganese deficiency disease. (J. agric. Sci., 31, 448 - 453).

Platt, R.S.jr., and K.V. Thimann, 1956. Interference in Salkowski assay of indoleacetic acid. (Science, 123, 105 - 106).

Poljakoff-Mayber, A., and A.M. Mayer, 1955. Some quantitative changes in the fat metabolism of germinating lettuce seed. (Physiol. Plant., 10, 14 - 19).

Pólya, L., 1961. Injury by soaking of Populus alba seeds. (Nature, London, 189, 159 - 160).

Ragai, H., and W.E. Loomis, 1954. Respiration of maize grain. (Plant Physiol., 29, 49 - 55).

Ray, P.M., 1958. Destruction of auxin. (Ann. Rev. Plant Physiol., 9, 81 - 118).

Ray, P.M., 1960. The destruction of indoleacetic acid. III. Relationships between peroxidase action and indoleacetic acid oxidation. (Arch. Biochem. Biophys., 87, 19 - 30).

Reinhold, L., 1954. The uptake of indoleacetic acid by pea

epicotyl segments and carrot discs. (New Phytol., 53, 217 - 239).

Resühr, B., 1941. Ueber die Bedeutung konstitutioneller Mängel für das Auftreten von Keimlinschäden bei Soja hispida Moench. II. Beitrag. (Zeitschr. f. Pflanzen-krankheiten (Pflanzen-pathologie) u. Pflanzenschutz, 51, 161 - 192).

Rhine, L.E., 1924. Divergence of catalase and respiration in germination. (Bot. Gaz., 78, 46 - 67).

Rudolfs, W., 1925, a. Effect of seeds upon hydrogen-ion concentration equilibrium in solution. (J. agric. Res., 30, 1021 - 1026).

Rudolfs, W., 1925, b. Selective absorption of ions by seeds. (Soil Sci., 20, 249 - 252).

Sacher, J.A., 1961. An IAA oxidase inhibitor system in bean pods. I. Physiological significance and source of the inhibitor. (Amer. J. Bot., 48, 820 - 828).

Sacher, J.A., 1962. An IAA oxidase inhibitor system in bean pods. II. Kinetic studies of oxidase and natural inhibitor. (Plant Physiol., 37, 74 - 82).

Sachs, J. von, 1887. Lectures on the Physiology of Plants. English translation by H.M. Ward, Oxford, 1887.

Sági, F., and A.S. Garay, 1961. Interaction of indoleacetic

acid oxidase, phenol content and gibberellic acid in the photoperiodically controlled growth of Lupinus albus L. (Physiol. Plant., 14, 488 - 496).

Salkowski, E., 1885. Über das Verhalten der Skatolcarbonsaure in Organismus. (Zeitschr. Physiol. Biochem, 9, 23 - 33).

Scarth, G.W., 1924. The toxic action of distilled water and its antagonism by cations. (McGill Univ. Publ., Series 2 (Botany) No. 28).

Sharman, B.C., 1943. Tannic acid and iron alum with safranin and Orange G in studies of the shoot apex. (Stain Tech., 18, 105 - 111).

Shull, C.A., 1913. Semi-permeability of seed coats. (Bot. Gaz., 56, 169 - 199).

Shull, C.A., 1916. Measurements of the surface forces in soils. (Bot. Gaz., 62, 1 - 31).

Shull, C.A., 1920. Temperature and rate of moisture intake of seeds. (Bot. Gaz., 69, 361 - 390).

Siegel, S.M., and A.W. Galston, 1953. Experimental coupling of indoleacetic acid to pea root protein in vivo and in vitro. (Proc. Nat. Acad. Sci., Wash., 39, 1111 - 1118).

Siegel, S.M., and A.W. Galston, 1955. Peroxide genesis in plant tissues and its relation to indoleacetic

acid destruction. (Arch. Biochem. Biophys., 54, 102 - 113).

Sircar, S.M., 1957. Auxin relations of rice plant. In: Modern developments in Plant Physiology. Ed. P. Maheshwari, Botany Dept., University of Delhi, P. 76 - 80.

Sircar, S.M., T.M. Das and A.N. Lahiri, 1955. Germination of rice embryo under water and its relation of growth to endosperm fractions. (Nature, London, 175, 1046 - 1047).

Skene, K.G.M., and D.J. Carr, 1961. A quantitative study of the gibberellin content of seeds of Phaseolus vulgaris at different stages in their development. (Aust. J. Biol. Sci., 14, 13 - 25).

Skipper, H.E., and L.L. Bennett jr., 1958. Biochemistry of cancer. (Annu. Rev. Biochem., 27, 137 - 166).

Skoog, F., and C.O. Miller, 1957. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. (Symp. Soc. Exp. Biol., 11, 118 - 131).

Snedecor, G.W., 1946. Statistical Methods, 4th Edition. Iowa State College Press, Ames, U.S.A.

Sondheimer, E., and D. Griffin, 1960. Activation and inhibition of indoleacetic acid oxidase activity from peas. (Science, 131, 162).

Stanley, R.G., 1958. Gross respiratory and water uptake patterns in germinating sugar pine seed. (Physiol. Plant., 11, 503 - 515).

Stiles, W., and W. Leach, 1932. The course of respiration during the germination of the seed and the early development of the seedling of Lathyrus odoratus. (Proc. roy. Soc., B, 111, 338 - 355).

Stiles, W., and W. Leach, 1933. Researches on plant respiration II. Variations in the respiratory quotient during germination of seed with different food reserves. (Proc. roy. Soc., B, 113, 405 - 429).

Stowe, B.B., 1958. Growth promotion in pea epicotyl sections by fatty acid esters. (Science, 128, 421 - 423).

Stowe, B.B., and T. Yamaki, 1957. The history and physiological action of the gibberellins. (Ann. Rev. Plant Physiol., 8, 181 - 216).

Street, H.E., 1958. Regulation of growth and differentiation in roots. (Proc. Internat. Congr. Biochem., Symposium VI. Biochemistry of morphogenesis. Pergamon Press, London, P. 98 - 104).

Street, H.E., D.J. Griffiths, C.L. Thresher, and M. Owens, 1958. Ethanol as a carbon source for the growth of Chlorella vulgaris. (Nature, 182, 1360 - 1361).

Strong, F.M., 1958. Topics in Microbial Chemistry. pp. 98 - 157, Chapman and Hall, Ltd., London.

Stutz, R.E., 1957. The indole-3-acetic acid oxidase of Lupinus albus L. (Plant Physiol., 22, 31 - 39).

Tang, Y.W., and J. Bonner, 1947. The enzymatic inactivation of indole acetic acid. I. Some characteristics of the enzyme contained in pea seedlings. (Arch. Biochem., 13, 11 - 25).

Tang, Y.W., and J. Bonner, 1948. The enzymatic inactivation of indole acetic acid. II. The physiology of the enzyme. (Amer. J. Bot., 35, 570 - 578).

Thimann, K.V., 1957. Growth and growth hormones in plants. (Amer. J. Bot., 44, 49 - 55).

Thimann, K.V., and Koepfli, 1935. Identity of the growth-promoting and root-forming substances of plants. (Nature, 135, 101).

Thomas, M., 1957. Plant Physiology. 4th Edition. J. and A. Churchill Ltd., London.

Tilford, P., C.F. Able and R.P. Hibbard, 1924. An injurious factor affecting the seeds of Phaseolus vulgaris soaked in water. (Papers Mich. Acad. Sci., Arts and Letters, 4, 345 - 356).

Toole, E.H., S.B. Hendricks, H.A. Borthwick and V.K. Toole, 1956. Physiology of seed germination. (Ann. Rev. Plant Physiol., 7, 299 - 324).

True, R.H., 1914. Harmful action of distilled water. (Amer. J. Bot., 1, 255 - 273).

Wagenknecht, A.C., and R.H. Burris, 1950. Indole acetic acid inactivating enzymes from bean roots and pea seedlings. (Arch. Biochem., 25, 30 - 53).

Wager, H.G., 1954. The effect of artificial wilting on the sugar content and respiration rate of maturing green peas. (New Phytol., 53, 354 - 363).

Wager, H.G., 1957. The effect of artificial wilting on the carbon dioxide production of developing peas. (New Phytol., 56, 230 - 245).

Wager, H.G., 1959. The effect of artificial wilting on the production of ethanol by ripening pea seeds. (New Phytol., 58, 68 - 74).

Warburg, O., 1930. The Metabolism of Tumours. English Edition, Constable and Co. Ltd., London.

Watanabe, R., and R.E. Stutz, 1960. Effect of gibberellic acid and photoperiod on indoleacetic acid oxidase in Lupinus albus L. (Plant Physiol., 35, 359 - 361).

Went, F.W., 1928. Wuchsstoff und Wachstum. (Rec. Trav. bot., Néer., 25, 1 - 116).

Went, F.W., and K.V. Thimann, 1937. Phytohormones. The Macmillan Co., New York.

West, C.A., and B.O. Phinney, 1959. Gibberellins from flowering plants. I. Isolation and properties of a gibberellin from Phaseolus vulgaris L. (J.

Amer. chem. Soc., 81, 2424 - 2427).

Whaley, W.G., and J. Kephart, 1957. Effect of gibberellic acid on growth of maize roots. (Science, 125, 234).

Willaman, J.J., and J.H. Beaumont, 1928. The effect of accumulated carbon dioxide on plant respiration. (Plant Physiol., 3, 45 - 49).

Wolfe, F.T., 1952. The production of indoleacetic acid by Ustilago zeae and its possible significance in tumour formation. (Proc. nat. Acad. Sci., Wash., 38, 106 - 111).

Yabuta, T., and T. Hayashi, 1939. Biochemistry of the Bakanae fungus. II. Isolation of gibberellin, a metabolic product of Gibberella fujikuroi Wr. which promotes the slender growth of rice seedlings. (J. Agric. Chem. Soc. Japan, 15, 257 - 266).

Yabuta, T., Y. Sumiki, K. Fukunaga, and M. Horiuchi, 1951. Biochemistry of the Bakanae fungus. XXII. The effect of gibberellin on the chemical composition of rice seedlings. (J. Agric. Chem. Soc. Japan, 24, 396 - 397).

Yamada, N., 1954. Auxin relationships of the rice coleoptile. (Plant Physiol., 29, 92).

Yates, F., 1933. The analysis of replicated experiments when the field results are incomplete. (Emp. J. exp.

Agric., 1, 129 - 142).

Younis, A.F., 1954. Experiments on the growth and geotropism of roots. (J. exp. Bot., 5, 357 - 372).

Yule, G.U., and M.G. Kendall, 1950. An introduction to the theory of statistics, 14th Edition. C. Griffin & Co. Ltd., London.