Ph.D

STUDIES IN THE GROWTH AND RESPIRATION OF ROOT TISSUE, with particular reference to auxin action.

by

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INTRODUCTION

INTRODUCTION

The "growth process" which takes place in plant cells is a complex series of events and has been studied as a whole from the earliest times. While these studies have yielded most valuable data for the total functioning of plant processes, more recent studies have been directed towards particular aspects of the whole process of growth. When attention is directed towards the "growth" of a stem or root apex, it is evident that three phases take place in sequence from the tip. The cells are at first meristematic, actively dividing and characterised by high protoplasmic and protein content and the presence of very small vacuoles.(Bailey, 1930; Zirkle, 1932) When these cells have ceased to divide they undergo rapid elongation involving both rapid water uptake and rapid extension of the cell wall. Cell-wall extension, according to Burstrom (1941, 1942), takes place in two stages. Firstly the cell-wall stretches (due to increased swelling of intermicellar colloids) and secondly new micelles are interspersed among already existing ones by intussusception. The second stage of the elongating phase accounts for two thirds of the "stretching growth". The main characteristic of this period is the increased vacuolation of these cells which is accompanied by increase in protein nitrogen and in dry weight (Brown and Broadbent, 1951) Maturation of these

cells takes place subsequent to elongation or may even begin before it is completed thus terminating the latter process.

It is very probable that the three regions of meristematic/activity, cell extension and maturation are differently affected by the internal and external conditions prevailing in the neighbourhood of these cells (Thompson, 1945), for these phases involve very different types of metabolic activity. They also may have some controlling or directive influence on one another. The inhibitory action of the root tip was emphasised by Cholodny (1924, 1934), Hawker (1932), Boysen Jensen (1933), Thimann (1934) and others. The effect of cotyledons and aerial tissue as a source of food material and hormone precursors must also be considered (Went 1928). To simplify the problem of studying growth in a complex environment, special growth regions have been isolated and studied apart from stimulating, retarding or directive influences of other tissue.

The phase of growth to which the majority of work has been directed is the second phase of cell elongation (Streckungswachstum). It is in a sense the simplest one for systematic study, since it involves only a small amount of protein and protoplasmic synthesis (Brown and Broadbent) and is co-incident with increased water uptake, cell wall extension and intussusception of new micelles. The more complex maturation processes involving apposition of a large number of layers on the primary cell wall succeed this phase.

Since early workers found that the tip of a stem or of an Avena coleoptile had a stimulating action on the growth of elongating cells, it was cut off in studies of the effect of auxin on cell wall properties during growth (Heyn, 1940; Soding, 1937; Ruge, 1937; Diehl et al, 1939). Soon the idea of cutting sections of a given length from the elongating zone was adopted (Bonner, 1935). If sections of etiolated Avena coleoptiles were taken from the elongating zone and supplied with suitable growth materials in the form of sugars, a very good and uniform elongation resulted. This method has become standard for the study of straight growth and from results obtained extensive theories on growth processes in plant material have been elaborated. The elongation of Avena coleoptile sections is dependent on the presence of both an auxin (e.g. β indolyl acetic acid) and a sugar (e.g. sucrose). If either becomes depleted during experimental work, growth will cease (Schneider, 1938). Sections of pea-stem tissue, however, have the advantage of not being dependent on a sugar supply (Christiansen and Thimann, 1950). These sections have been taken from the third internode of etiolated pea-stem tissue, and results have in part substantiated previous studies with Avena coleoptile tissue.

Until 1949 root section material had not been used for systematic studies of the type to which the Avena coleoptile had been subjected. Brown and Sutcliffe (1949)

found they could obtain a very much greater extension growth than in the Avena coleoptile by using short, strictly-located sections and a particular technique. They used Zea and Cucurbita root sections but were unable to obtain any consistent results for the effect of auxin on their tissue. By chance, in the experiments described below we have used pea roots which are quite consistent in their auxin response. They can therefore be used in experiments similar to those carried out for the Avena coleoptile, and inhibitor action can be more extensively studied.

It has been firmly established for some time that root growth is inhibited by those concentrations of auxin which stimulate shoot or coleoptile growth. Root growth of cereals was inhibited by an extract of Rhizopus culture media, (Nielsen, 1930) the active part of which was indolyl acetic acid (Thimann, 1935). Later workers used this substance in a pure state showing that the occurence of root inhibition was general for all the particular concentrations tried (Kogl et al, 1934; Meesters, 1936; Faber, 1936; Marmer, 1937). In fact root inhibition is so quantitatively related to the concentration of added auxin (unless it is very low), that it has been made the basis of a number of auxin-assay methods (Swanson, 1945; Ready and Grant, 1947; Audus, 1948).

However the root growth of the pea can be accelerated by the supply of a low concentration of auxin

from above (Thimann, 1936). Under similar conditions the root growth of Avena is unchanged or is inhibited. By assuming that the sensitivity to auxin of pea and oat roots was the same, Thimann explained his results on the basis that the concentration of natural auxin is sub-optimal in the pea but supra-optimal in Avena root tissue. In fact root behaviour parallels that of the shoot in that an optimum concentration of auxin does exist for growth, but differs in that it is often surpassed in roots and is of a much lower order than that for stem tissue.

In spite of contrary evidence it seems that an optimum concentration for root growth must exist although it may be difficult to demonstrate since it is very low and critical, varying with species and with age and past environment of the plant. Also it may generally be exceeded in the normal root. It seems that roots which are naturally poor in auxin are the best test materials for the demonstration of the existance of an auxin-optimum. However, claims stimulation of growth by low concentrations of auxin have been made for a large range of test materials. Fiedler (1936) found a concentration of 10^{-10} indolyl acetic acid was stimulatory to root growth. A concentration of 10^{-9} was inhibitory. Here the test material was Zea roots. Using Vicia Faba roots, Amlong (1940) obtained a stimulation of growth with 1.75×10^{-10} indolyl acetic acid. Slight stimulation was observed with this auxin by Bonner and

Koepfli (1939), and with other auxins (Macht and Grumbein, 1937). Seed treatment with auxin may cause subsequent stimulation of root growth (Thimann and Lane, 1938). Low concentrations of auxin which were stimulatory in the initial stages of growth became inhibitory in later stages (Geiger-Huber and Burlet, 1942).

Roots and coleoptiles may give different qualitative responses to the same quantitative amount of auxin. For instance, a very low concentration may be stimulatory to root growth but will have no effect whatsoever on shoot or coleoptile elongation. A moderately low concentration (10^{-6} indolyl acetic acid) will be stimulatory to coleoptile growth, but it will be extremely inhibitory if applied to roots. It may seem that auxin differs in function in root and stem tissue. The ~~root~~ tip is a potential source of auxin. If it is removed from the coleoptile growth is retarded. However if it is removed from the root growth may be accelerated (Cholodny 1926) or it may have no effect (as in the pea, Bünning, 1928). Different relative concentrations are needed to produce the same effects in the two types of tissue. However Czaja (1935) assumed that the function of auxin in roots was fundamentally the same as in shoots and this function was to cause elongation. The retardation frequently observed was due to the presence of both an "upward" and a "downward" auxin stream. This theory as it stands may seem quite

absurd in view of recent work, but the concept of an analagous function of auxin in root and shoot tissue is valuable.

The original statement of Went (1928) that without "growth-substance" no growth is possible, can be taken as referring to root as well as other tissue. All growth occurring in the absence of added auxin must then be attributable to naturally occurring auxin. But in root tissue the presence of naturally occurring auxin in a concentration above the somewhat critical optimum will be manifested in the form of an inhibition of root growth. It will be useless in such tissue to expect an auxin-stimulation of growth. The concentration is already supra-optimal. The roots will grow better in the absence of added auxin, in which there is a chance that dilution of this relatively high concentration will occur with progressive water uptake. Since however there are some roots in which acceleration by auxin has been observed, it seems that these at least possess a concentration of natural auxin which is sub-optimal.

The above is illustrated by the work of Thimann (1936). Avena roots which have been inhibited by the application of auxin, were then transferred to water. Progressive dilution of the high auxin concentration (by water uptake) coupled with the rapid disappearance of auxin, resulted in a considerable reduction of the internal auxin concentration.

The root growth was accelerated far above that of the uninhibited control. At the same time Thimann found that low concentrations of auxin would accelerate root growth in pea (but not in Avena).

Now it is generally agreed that when growth takes place work must be done in the plant cell. Considering elongatory growth alone, work may be done in the increase of cell-wall materials (Burstrom, 1941 and 1942), the maintenance of osmotic pressures inside the cell during water uptake, (Burstrom, 1942), "Active water uptake" (cf. Reinder's work on potato tissue, 1938), increased protein content or dry weight (Brown and Broadbent, 1950) etc. These processes involve an expenditure of energy above that of the so-called "energy of maintenance" continuously utilised in every living cell.

An obvious source of the excess energy needed in growth is by increased respiration. Hence when auxin was found to augment the respiration at the same time as augmenting growth, it was considered that the energy for the increased growth was made available by a simultaneous increase in respiration (Bonner, 1933). Unfortunately this was not repeatable by van Hulssen (1936), Kogl et al (1936) and by Bonner himself using purified indolyl acetic acid instead of a cruder preparation from Rhizopus. Pre-treatment of wheat grains with auxin prior to germination caused an increase in respiration of the embryo as a whole, but had no correlation

with immediate cell elongation (Pratt, 1938). However Bonner (1949) has now been able to find an increased respiration of 15-25% in Avena coleoptile sections treated with a concentration of auxin which is stimulatory to growth. Similarly Berger et al. (1946) obtained 28-45% increase in respiration.

There seems to be some evidence that auxin does cause an increase in the respiration of Avena coleoptile sections, concentrations which are at the same time stimulatory to growth. Christiansen and Thimann (1950) have found this also to be true for pea stem tissue. It must be emphasised that this stimulation is not found in root tissue (Burstrom, 1942). Apart from this, results using 2 4 dichlorophen-oxyacetic acid instead of indolyl acetic acid as an auxin are very confusing.

Brown (1946) using a concentration of 10^{-3} 2 4 D obtained a respiratory stimulation of between 18 and 80% in bean seedlings four days after spraying. It is not imaginable that such a high concentration would have any growth promoting activity. This is an instance of auxin stimulation of respiration! However this cannot possibly be connected with the supply of increased energy for growth. Growth must be simultaneously inhibited. Taylor (1947) using somewhat more probable concentrations of 0.25 to 10 p.p.m. found decreased oxygen uptake of wheat and mustard seedlings during the first hour after application. He does

not state the effect of this concentration on growth. Similarly Hsueh and Lou (1947) found an inhibition of 33% of the respiration of rice and barley seeds during the first day of application. Smith, Hamner and Carlson again obtained stimulation with concentrations of 2.4.D which were in all probability toxic to growth, by spraying whole plants of bindweed and measuring the oxygen absorption of their roots. Smith (1948) obtained comparable stimulation in bean stems. He pointed out the importance of specifying the basis on which respiratory readings are taken. Using his own results, he showed a progressive stimulation of respiration by 2.4.D estimating results on a unit dry weight basis but the same results showed a progressive inhibition estimating on a unit nitrogen basis. Valuable as the above results may be in giving an indication of the phytocidal action of 2.4.D on plant tissue, it is felt that they have no relevance here. It is only if an auxin can be shown to cause a stimulation of respiration, <u>simultaneous</u> with increased growth that a direct connection between auxin action in growth and respiration can be claimed. The authors quoted above give no indication of the effect of 2.4.D on growth, and in general the concentrations employed are much too high to suppose that it was anything but inhibitory! Kelly and Avery (1948) studied the action of relatively low concentrations of 2.4.D on the growth and respiration of pea stem and Avena coleoptile tissue. However all the

concentrations used (10^{-6} to 10^{-3}) were inhibitory to growth. In spite of this stimulation of respiration was obtained using the lower concentrations (10^{-6} to 10^{-4}). Christiansen and Thimann (1949) gave simultaneous figures for growth, respiration and content of reducing sugars in pea stem sections during treatment with low concentrations of 2.4.D. They found that whereas the growth increased by $2\frac{1}{2}$ x the original the respiration increased by only 1/7. This is roughly of the same order as the results obtained by Bonner (1949).

It seems therefore that in <u>aerial tissue</u> auxins may cause a stimulation of respiration at the same time as growth is stimulated. It is however firmly established that auxin-stimulation of respiration does occur at concentrations which are much too high for growth-stimulation. These facts alone are not sufficient evidence to suppose that auxin action is effected by means of a simultaneous increase in respiration and growth rates, the former providing the energy for the latter. It is not essential that the energy for growth be immediately derivable from concurrent respiration. Respiratory energy is not always used directly but may be transformed into high-energy phosphate bonds, which remain as a store until the need for work to be done arises. Previous respiratory activity may result in the presence of an adequate store of high-energy phosphate which is utilisable as an energy source for growth <u>without</u> any

immediate increase of energy being essential. Conversely if the cell has become depleted of high-energy phosphate, it may have to increase its store until a certain level is reached (by means of increased respiration) before the growth rate can be increased. In either case there would be a time-lag between the two processes and to recognise this calls for careful checking of growth rates and respiration rates at intervals after auxin has been added to the tissue. This has been attempted in the following work.

"Growth is a property of living protoplasm". This statement would receive support from all but a few workers (e.g. Ruge, 1937, who showed that the extensibility of cell walls still increased under auxin action if they were dead or narcotised.) In higher plants it seems that aerobic respiration is normally essential for growth as well as living protoplasm. If this process is inhibited by potassium cyanide, phenylurethane or by replacement of oxygen by nitrogen, growth is inhibited to the same extent as respiration. (Bonner, 1933). Growth cannot therefore continue without aerobic respiration but it is obvious that respiration frequently occurs in the absence of growth (for instance in cells which have already completed elongation and maturation processes). It was therefore suggested that growth was controlled by a specific fraction of respiration: that fraction which is observable as a stimulation when auxin is added to the tissue. Moreover the dehydrogenase

inhibitor, mono-iodoacetic acid, has the property of markedly inhibiting growth and at the same time only inhibiting the respiration by 10%. Now 10% was the percentage stimulation of respiration obtained on the addition of β indolyl acetic acid to Avena tissue soaked in 1% sucrose (Commoner and Thimann, 1941). This concentration of auxin caused stimulation of the growth of this tissue. It was therefore concluded that iodoacetate ions specifically inhibit the increased 10% of respiration due to the presence of auxin and this is the specific fraction controlling growth. The inhibition of growth and respiration was antagonised by certain dicarboxylic acids of the Szent-Gyorgyi respiratory acid cycle (e.g. fumaric, malic or succinic acids). This provided a method by which the supposed part of respiration controlling growth could be identified. This auxin-stimulated C_4 acid cycle is the connecting link between the two processes.

If there be any objection to this type of reasoning it will surely be in pointing out the large number of inhibitors which show differential effects on growth and respiration. Much of the work does give some support to the acid-cycle theory but in general it shows the immensity of the problem which cannot be solved by reference to one inhibitor at a selected concentration range!

For instance, the increased respiration due to auxin is abolished not only by iodoacetate but by malonate,

arsenite, arsenate, sodium fluoride, canavanine, sodium fluoracetate and dichloroanisole (Bonner, 1948, 1949). Dinitrophenol may increase respiration at the same time as growth is inhibited. Emetine and berberine inhibit growth but have no effect whatsoever on the respiration (Goddard, 1948).

The iodoacetate results are supported by the fact that other -SH combining inhibitors seem to have a similar effect i.e. they inhibit growth with very little or no effect on respiration. Such substances are iodoacetamide, arsenite, mapharsen, p-chloromercuribenzoate, and phenylmercuric salts (Bonner, 1948, 1949) (Thimann and Bonner, 1949) (Thimann, 1948). However other inhibitors which are probably -SH combining such as protoanemonin and coumarin inhibit growth and respiration to the same extent. Their action is reversed by B.A.L. (1 2 dimercaptopropane).

The picture has been even further complicated by the discovery of Bonner (1948) that adenylic acid and arginine both stimulate the processes of growth and respiration. This led to the elaboration of a theory by which the respiration controlling growth was pictured as a number of steps each of which may be stimulated by certain substances and inhibited by their antagonists. These steps were not in general identifiable with any familiar part of the known respiratory cycle (Bonner, 1949). Until some attempt has been made to identify these hypothetical steps this theory

cannot be treated with any seriousness.

All the hypotheses above have been based on results for Avena coleoptile tissue or for pea stems. No work has been done on the effect of inhibitors on sections taken from the elongating zone of roots. It seemed that a full investigation of the behaviour of root sections under comparable conditions to those to which Avena coleoptile sections have been subjected might throw some light on possible connecting links between growth and respiration in the metabolism of root tissue.

The elongating zone in uniform pea roots has been strictly located and sections have been taken from the zone of maximum elongation. A technique for their culture ensuring uniform growth has been devised. Changes in growth rates and respiration rates over a period of 48 hours have been noted by taking samples at intervals. The effect of sucrose and sodium hydrogen malate as metabolites in these processes have been determined. Auxin action has been observed at stimulatory and inhibitory concentrations. Three inhibitors were used - sodium arsenate, dinitrophenol and iodoacetate. The results were strictly compared with those previously obtained for Avena tissue and the following were taken strictly into account:-

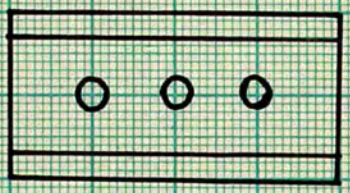
(a) the time-drift of respiratory and growth measurements

(b) concentration effects of inhibitors

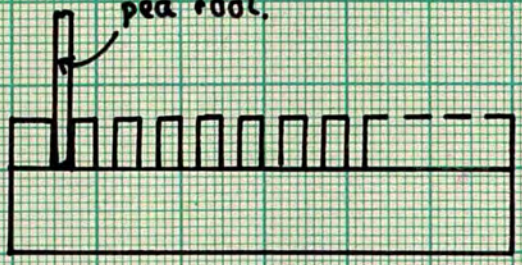
(c) the effects of inhibitors in varying auxin concentrations.

PRELIMINARY EXPERIMENTS ON WHOLE ROOTS

(a) "van der Weij type" cutter.

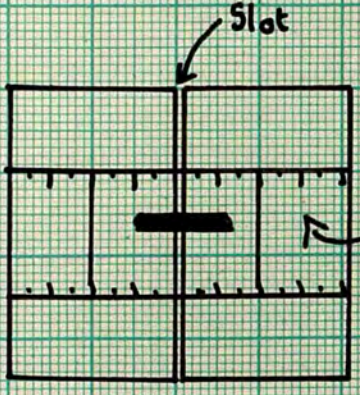


Blade

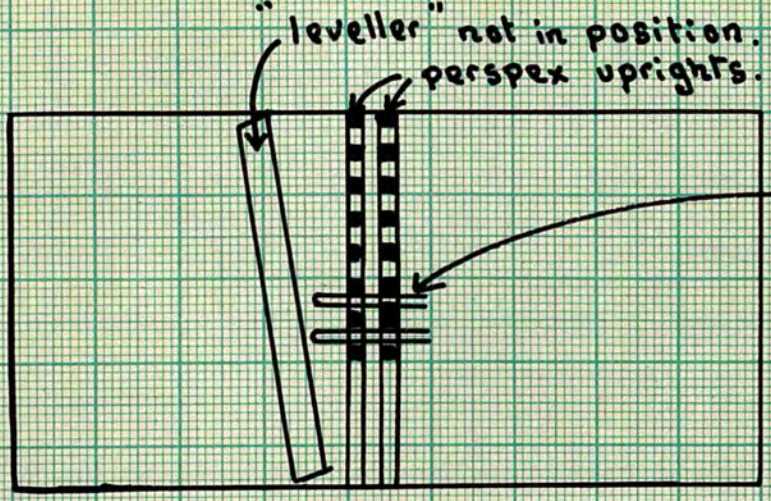


Stand

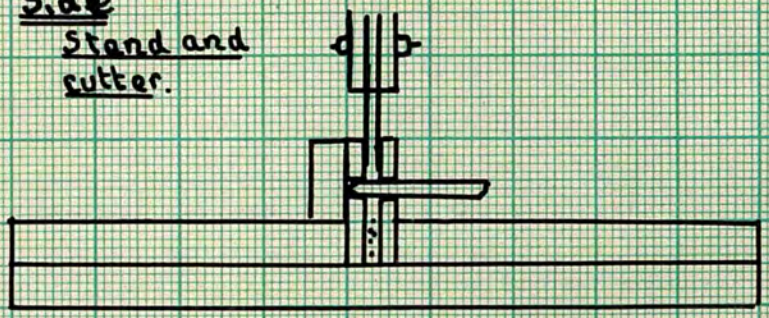
Rough single-bladed guillotine cutter



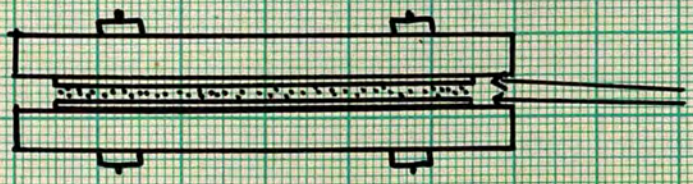
Stand for "double-bladed guillotine" cutter.



Side
Stand and cutter.



Cutter.



From below.

Materials and methods

Seeds of Pisum sativum (variety "Meteor"), supplied at regular intervals by Messrs Sutton and Sons, were surface-sterilised by placing for 2 minutes in 80% alcohol, followed by 2 minutes in 0.2% mercuric chloride solution and by thorough washing in tap water containing one drop of saturated sodium sulphide solution. They were soaked overnight in running tap water and planted in a dark cupboard in frequently sterilised sand, damped with glass-distilled water.

This method of germination was found to be far superior to ones soaking seed in still, not running water, or using vermiculite or filter paper instead of sand. An adequate number of uniform straight roots could be produced at any one time.

The seedlings were always used on the third day of planting. At this time they have a single primary root and the plumule has not yet emerged. Straight roots of similar size were carefully selected, 3.5 - 4.5 cm. long.

For the early experiments roots or root sections were placed in a culture solution similar to the familiar A-Z solution of Hoagland and Snyder, with modifications to adjust the pH to 6.5 as advised by Audus (1948).

In later experiments it was considered advisable to use glass-distilled water or glass-distilled water plus sucrose as a control, since possible effects of various nutrients remained an unknown factor. For instance,

van Santen (1940) showed sodium ions inhibit the growth of root sections; and Brown and Sutcliffe (1950) that potassium stimulates respiration and growth. The stimulating effect of phosphate is well-known. All solutions were neutralised to pH 7.0 by use of N/10 potassium hydroxide and hydrochloric acid solutions where necessary. It was found that the time drift over the 48 hour experimental period was very small indeed, never exceeding 0.5 pH units.

Preliminary experiments on whole isolated roots

Respiratory measurements were made using constant-volume Warburg manometers following the method elaborated by Dixon (1943) and by Umbreit et al. (1949). The water bath was maintained at 26°C and the manometers were shaken at 65 r.p.m. Isolated three-day old roots gave very constant respiration readings over a 10 hour period. Roots taken from the same batch of seedlings were less variable than those taken from different batches of seedlings, planted at different times. All roots were washed with glass-distilled water and transferred directly to Warburg flasks, containing 5 ml. medium.

The following are means of 6 experiments on different batches of seedlings (with the calculated standard error).

Table 1.

	Oxygen absorption/0.1 g. tissue/hour
Glass-distilled water	15.7 ± 2.3 c.mm.
Glass-distilled water 2% sucrose.	20.3 ± 1.6 c.mm.

	Oxygen absorption/0.1 g. tissue/hour
Culture solution	46.6 ± 6.7
Culture solution 2% sucrose.	37.9 ± 1.7

The oxygen absorption / 0.1 g tissue fresh weight was three times as great in culture solution as in glass distilled water. This effect is an initial effect, apparent from the first reading and hence cannot be attributed to different final length of the two samples. The effect of sucrose is not significant, for whole roots, in either instance.

The culture medium was buffered and contained potassium, ferric and calcium phosphates. These substances were doubtless partly responsible for the very high respiration rate in culture solution. It is noted that the addition of 2% sucrose had no effect. In further experiments on sections taken from the elongating zone of the root, it will be shown that sucrose had a highly significant initial effect. It seems that the initial carbohydrate supply of a whole root is adequate, and does not become depleted even over a 10 hour period. However the carbohydrate supply of the sections used is very soon depleted to a very low level, and the addition of sucrose has a highly significant effect on respiration (and growth).

Experiments on 14 day roots

Isolated 14 day roots in glass-distilled water have

a significantly higher respiration rate /0.1g fresh weight. This is doubtless due to the greater proportion of elongating and meristematic tissue in the lateral roots. This is borne out by respiration rates of component parts.

Table 2.

	Oxygen absorption/0.1 g tissue
Whole 14 day root (g.d. water)	30.7 ± 8.6c.mm.
Root stripped of laterals	17.5 ± 1.6
Laterals only	37.8 ± 8.6
Root tips (3.39 mm. long)	50.3 ± 2.4

For one experiment the actual respiration rates of parts from a single root were:

(a) Whole root	34.4 c.mm. /hour
(b) Main root	4.0 c.mm. /hour
(c) Laterals	27.4 c.mm. /hour
Total (b) and (c)	31.4 c.mm. /hour

The apparent inhibition of respiration by cutting off the laterals is well within the experimental error.

The interesting point about these experiments is that they do confirm the many reports of concentration of respiratory activity towards the apex of the root. At a 14 day old stage the laterals are the zone of most active elongation and meristematic activity. It has been shown here that the respiration /unit fresh weight is higher in the laterals than in the main root. The root tips were relatively long (3.39 mm.) and as will be shown later

included a large part of the elongating (as well as all the meristematic) zone.

Experimental possibilities are infinite here. By various combinations of cutting off tips, laterals and leaving both or either intact it should be possible to obtain a picture of the distribution of respiratory activity throughout this fairly mature root system. This has been well attempted in primary roots by Berry and Brock (1946) and by Brown and Broadbent (1951) but as yet we have found no reference to work on maturer root tissue. This problem is however outside our thesis but, if such studies were paralleled by observations on growth, might throw some light on the inter-relations of growth and respiration in root tissue.

Effect of sectioning on subsequent respiration

It was proposed to study the elongatory growth and, to simplify the problem as much as possible, cells undergoing elongation were isolated by taking sections from the appropriate zone of the primary root. Such sections are isolated from the possible retarding influences of the tip (Bünning has been unable to prove their existance in the particular instance of Pisum sativum) and also from the modifying influences and nutritive sources of maturer parts. It is however possible that sectioning itself may have a not negligible effect on future growth and development of the cells. Increases in respiration following shock treatments

such as handling, burning or cutting are well known, and may persist for several hours. It is desirable to ascertain whether this particular type of tissue tends to give such a response to the handling and cutting processes which are, of necessity, used to isolate the parts to be studied.

Several experiments have been carried out to that end. It has been noted above that cutting off lateral roots in a well developed root system had no significant effect on the sum of subsequent respiration rates of the parts. Further, cutting a primary 3-day old root along its length into "long" or "short" sections produced no significant change or only slight inhibition of subsequent respiration rate. The following is a typical experiment ---- two roots were selected as near as possible similar and their fresh weights obtained. They were placed in manometer flasks and their oxygen absorption recorded over a one hour period. After one hour one root was removed from its flask and cut along its length into 3.39 mm. sections. The sections were replaced in the flask and the respiration of the two samples determined side by side.

Table 3. Readings c.mm. oxygen absorbed/15 minutes/0.1g tissue

Time	1st hour	2nd hour	3rd hour	4th hour
Whole root A	10.0±0.4	9.9±0.1	11.5±0.4	12.0±0.0
Whole root B	10.3±0.3	8.5*±0.6	9.3±1.0	9.2±1.0

* from this point the root was removed and sectioned.

The standard errors were calculated from the four readings

over the period indicated.

The control root showed a slight drift upwards in oxygen absorption over a 4 hour period and readings were very constant over each experimental period. The second root B had nearly the same initial respiration rate but sectioning was followed by inhibition (barely significant). No rise was observed with time.

The above experiment was performed in glass-distilled water. Other experiments in glass-distilled water plus sucrose gave similar results. No stimulation was ever observed by this method of sectioning.

It may be mentioned that Audus (1935, 1938) obtained stimulation of the respiration of isolated cherry, laurel and other leaves starved in the dark simply by rubbing or bending. This stimulation lasted over a relatively long period (2 to 3 days) and varied in intensity from 18.8 to 182.8% in different species. Since plant physiological work very often involves handling of plant tissue, it is important to distinguish between any effects due to handling, and effects due to subsequent treatment.

Brown and Broadbent (1950) have also determined the effect of sectioning on root tissue respiration, using pea roots. They find that over the first 4.0mm. from the tip the combined respiration rates of successive segments does not differ significantly from that of the intact fragment. Their segments were only 0.4 mm. long. They did however

find a significant lowering on sectioning 4.0 to 9. 0 mm. from the tip, using 0.4 mm. sections. However using longer sections - i.e. cutting across and damaging fewer vacuolated cells - they found no significant difference.

In the experiments described above we have used a relatively long root compared with that used by Brown and Broadbent, and we have made no distinction between sections taken prior to or after the critical 4.0 mm. point (from the tip). A larger amount of maturer tissue has been included, and the overall effect has been slightly inhibitory. Brown and Broadbent (1951) measured the respiration of samples of sections taken at equal distances from the tip, and the total respiration on sectioning was taken as the sum of the respiration rates of these samples.

It can be seen that this technique may give slightly varying results (due to sampling error) but it seems unlikely that there is an excessive inhibition or any stimulation of respiration rate on cutting pea roots. Any effect is probably accounted for by damage to the cells, local exudation of toxic materials etc. not to any fundamental metabolic change as one may suppose takes place during shock responses in the cherry laurel (Audus, 1935, 1938).

USE OF SECTIONS AND ELABORATION OF THE FINAL TECHNIQUE

Technique of section cutting

In our experiments we used sections of two different lengths (a) long sections 3.39 ± 0.05 mm. long sliced off in a simplified cutter of the type employed by van der Weij (1932). It consisted of two sheets of perspex; the lower being unperforated and serving as a leveller of the root tips and an upper one having 1 mm. perforations. The roots were cut off about 2 cm. long and stood upright in the holes under their own weight - the tips resting on the lower plate. The apices were sliced off by a blade on the surface of the upper plate. This was repeated for subterminal sections and other sections as required.

The length of the apex was varied by using a blade guillotine-wise on a graduated scale. The roots were placed over a slot between two glass slides affixed to this scale (see diagrams).

An accurate 2.0 mm. apex was obtained by (b).

(b) short sections 1.983 ± 0.005 mm. long using a double-blade guillotine cutter. The cutter consists of a perspex base in which there are two celluloid uprights of constant thickness. These are bored with holes placed opposite to one another 1 mm. in diameter. The roots are supported in a horizontal position passing through these holes. The tips were levelled off flush with the edge of a perspex block to the outer edge of one celluloid upright. The apices

and sub-terminal segments were severed by one single cutting motion of the guillotine. The guillotine consisted of two blades clamped at a constant distance from one another. The severed apices were equal in length to the thickness of one celluloid upright i.e. 2.0 mm. long and the sub-terminal segments were about 1.983 mm. i.e. just under 2.0 mm. long.

Location of the zone of maximum elongation

Three day old roots 3.5 to 4.5 cm. long were selected for uniformity and sectioned into "long" (3.39) sections from the tip. Four sections were taken from each root and designated as sections 1. (terminal), 2. (sub-terminal), 3., 4. Each sample consisted of twelve sections taken from the same region. They were placed in petri-dishes with only sufficient liquid to half cover them and kept at 26°C in an incubator. Samples were removed at intervals and the sections measured using a travelling microscope. Means were taken of these readings for length and elongation calculated as a % of the original mean length (3.39mm.)

Table 4. Actual and % elongation

mm from tip	0.0-3.39		3.39-6.78		6.78-10.17		10.17-13.56		
Sections	1		2		3		4		
I Culture only	mm	%	mm	%	mm	%	mm	%	Total %
120 mins.	0.38	12.9	0.12	4.2	0.05	1.7	0.00	0.0	4.7
240 mins.	0.47	16.3	0.28	9.8	0.08	2.7	0.03	1.0	7.5
330 mins.	0.49	16.7	0.36	12.6	0.13	4.4	0.06	2.1	8.9
1440 mins.	0.54	18.4	0.35	12.2	0.20	4.8	0.09	3.1	9.6
2880 mins.	-	-	0.41	14.3	-	-	0.08	2.8	-

Actual and % elongation (cont'd)

mm from tip	0.0- 3.39		3.39-6.78		6.78-10.17		10.17-13.56		
Sections	1		2		3		4		
II Culture ½% sucrose	mm	%	mm	%	mm	%	mm	%	Total %
120 mins.	0.12	4.1	0.11	3.8	0.01	0.3	0.06	2.2	2.6
240 mins.	0.40	13.9	0.30	10.4	0.09	3.1	0.06	2.2	7.4
360 mins.	0.37	12.9	0.29	10.1	0.13	4.4	0.06	2.2	7.4
1470 mins.	0.38	13.2	0.28	9.7	0.20	6.0	0.08	2.7	7.9
III Culture 2% sucrose									
90 mins.	0.09	2.6	0.11	3.2	0.07	2.1	0.01	0.3	2.1
210 mins.	0.35	11.3	0.05	(1.7)	0.10	2.8	0.02	0.6	3.7
300 mins.	0.40	11.4	0.17	4.9	0.07	2.1	0.07	2.8	5.3
390 mins.	0.39	11.4	0.16	4.6	0.12	2.9	0.10	2.9	5.5
1440 mins.	0.39	11.4	0.16	4.6	0.12	2.9	0.10	2.9	5.5

The table (No. 4) shows that in the three media employed section 1. always shows maximum elongation. The zone of maximum elongation is hence located quite near the tip. It is evident that in these experiments total growth takes place in the first 5 to 6 hours. These results are for "long" sections in still culture. Later experiments with short sections, shaking dishes and glass-distilled water show a much longer growth period.

Since we proposed to use sub-terminal sections in our final experiments it became evident that apices of less than 3.39 mm. must be removed to locate the zone of maximum elongation.

Another series of experiments were performed specifically on the elongation of sub-terminal segments severing apices of 0.0, 1.0, 2.0, 3.0, 3.39 mm. long with

the guillotine cutter. The actual sections were cut in the van der Weij cutter as before.

The table No 5. shows that in the pea root the elongation zone is entirely located in the first 6 mm. It is much more strictly located than the corresponding zone in the pea stem internode. (Christiansen and Thimann (1950) were able to cut 20.0 mm. sections) or in the Avena coleoptile (Bonner (1933) cut two almost identical 3 mm. sections.)

Table No.5 Percentage elongation of sections (3.39 ± 0.05 mm)

Root tip removed (approx)	0mm	1mm	2mm	3mm	3.39mm
2 hours	4.1	8.9	5.2	9.8	3.8
4 hours	12.9	26.8	22.9	19.8	10.4
6 hours	13.9	23.8	39.0	31.0	10.1
24 hours	14.0	-	42.3	-	9.7
48 hours	-	67.1	45.6	33.2	-

The maximum elongation in 6 hours is obtained if a tip of 2mm. is cut off. At 24 hours it is obtained using a 1.0mm. tip but it has been shown anatomically that mitosis may occur as far as 1.5 mm. from the apex. If 1 mm. tips were used the number of cells would not necessarily remain constant throughout the experiment, and moreover the 6 hour readings show that the cells are not immediately ready for elongation.

It was concluded that it is best to sever a 2.0 mm. tip and this was done for "long" and "short" sections used in the main part of the investigation.

Effect of distance from the tip on oxygen absorption

In the previous experiment the four hour values are those found after determining the oxygen absorption in shaken Warburg flasks (63 r.p.m.). Readings for other time periods were for samples in still media. Over the short period of four hours the growth measurements are not significantly different from those in still culture. This enables a valid estimate of respiration to be obtained.

	0mm.	1mm.	2mm.	3.mm.	3.4mm	6.8mm	10.2mm apex removed
% elongation in 4 hrs. (still media)	12.9	26.8	22.9	19.8	10.4	-	-
% elongation in 4 hrs. (in Warburg)	13.9	26.8	30.0	19.6	11.0	3.1	2.2
Oxygen absorption in c.mm /hour/12 sections	33.3	31.4	31.0	35.7	28.2	34.9	33.9

It is interesting to note there is no significant effect of distance from the tip on respiration rate placed on a unit section basis. However using pea roots Brown and Broadbent (1950) found that the respiration rate/ section falls rapidly after a preliminary rise up to 2.0 mm. from the tip. They used sections only 0.4 or 0.8 mm. long and their results are quite different from those of Berry and Brock (1946), who found a ~~polar distribution~~ gradient of respiration rate from tip to base of Allium cepa roots. Berry and Brock used longer sections 5.0, 10.0 and 15.0 mm. long and always included the tip so as to have only one cut surface

per section. Their results confirmed those of Lund and Kenyon (1927) who, finding the root tip reduced methylene blue at a greater rate than the base, stated it was more electropositive. Further phenol red tests showed a greater apparent carbon dioxide production in the tip. Leonard Machlis (1944) also obtained a respiratory gradient using 10.0mm. sections in Barley roots, but Gregory and Woodford (1939) dispensed with the use of sections by devising an apparatus for studying 1 cm. sections on the intact root. Unfortunately the results are not consistent enough to be of use here.

Expressing our respiration rates on a unit section basis we find increasing distance from the tip has no effect on respiration. On a dry weight basis the rate decreases so that it is less in the meristematic zone. This confirms the results of Broadbent - the preliminary rise is probably obscured here due to the length of the section.

All authors, who have found a higher respiration rate at the tip on a unit section or a fresh weight basis, have used relatively long sections (greater than 5.0 mm.). The divergence of results is most probably due to the length of the sections taken. If the length of section is small, the size of wounded surfaces is large compared with the total surface area and the damaging effect is relatively great. Conversely the difference in growth rate and in respiration rate of cells at basal and apical ends of a long

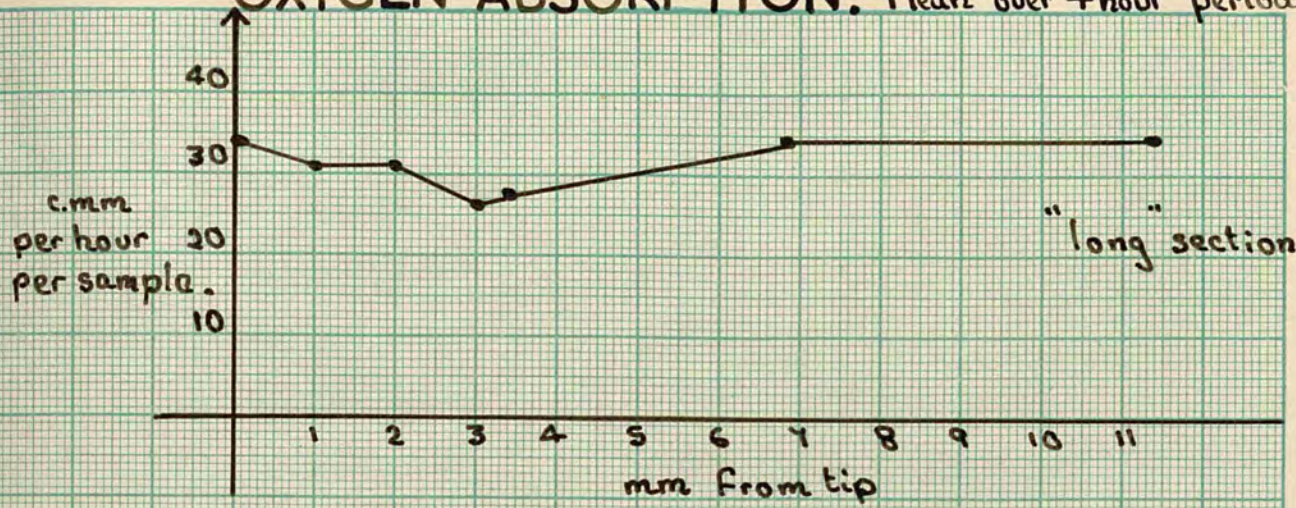
Table No. 6.

% ELONGATION OF "LONG" SECTIONS.

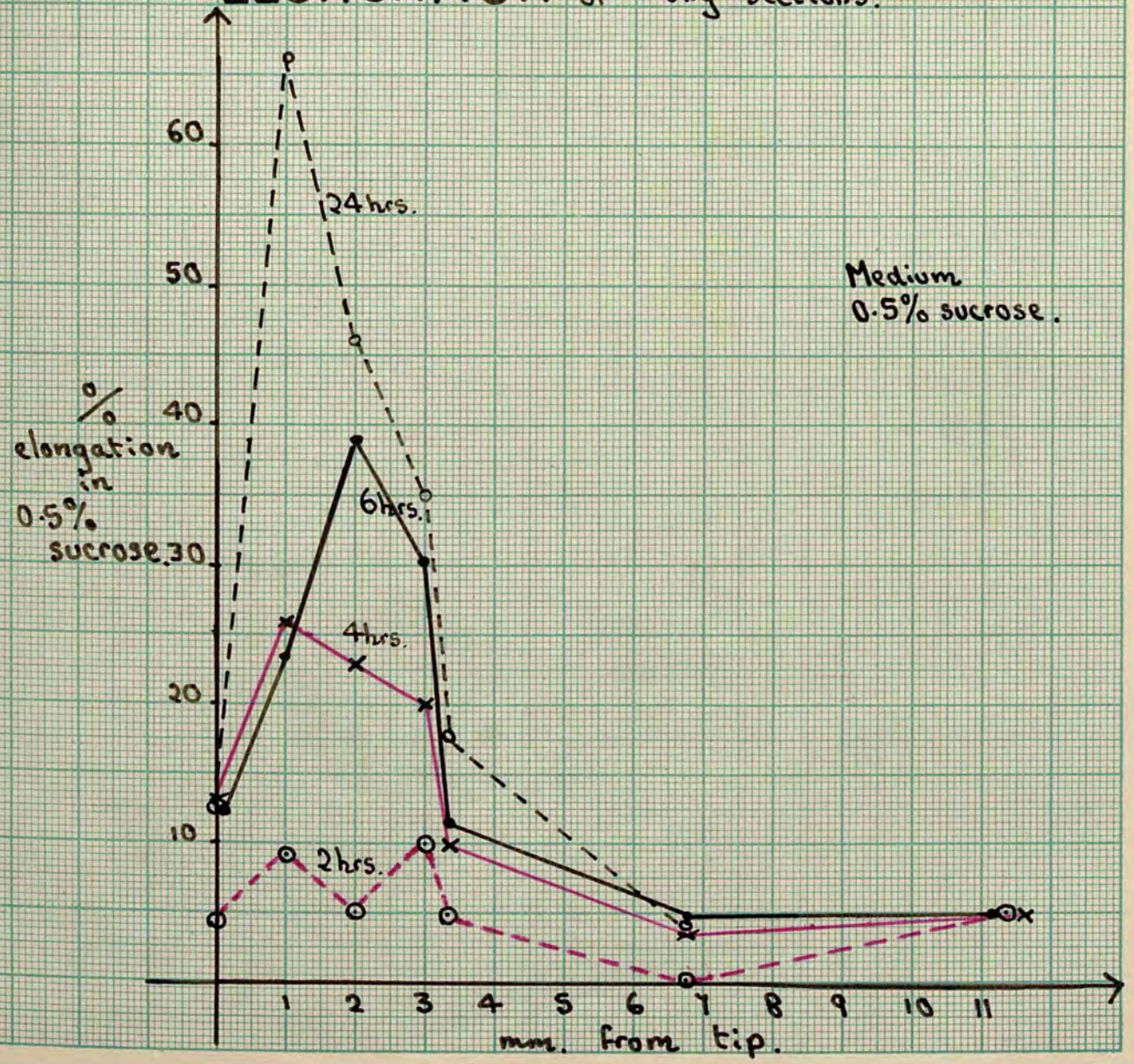
mm. apex removed	0	1	2	3	3.39	6.78	10.17
NO SUCROSE							
2 hours.	12.9	-	8.7 17.7	-	4.2	1.7	0.0
4 hours.	16.3	-		-	12.6	2.7	1.0
6 hours.	16.7	-	17.7	-	12.6	4.4	2.1
24 hours.	18.0	-	33.7	-	16.5	5.0	-
0.5 % SUCROSE							
2 hours.	4.1	8.9	5.2	9.8	3.8	0.3	2.2
4 hours.	13.9	26.8	22.9	19.6	10.4	3.1	2.2
6 hours.	12.9	23.8	39.1	31.3	10.1	4.4	2.2
24 hours.	13.2	67.0	45.6	35.0	18.4	3.4	-
1.0 % SUCROSE							
2 hours.	-	-	3.5	-	-	-	-
4 hours.	-	-	17.7	-	-	-	-
6 hours.	8.8	-	20.3	13.2	8.8	-	-
24 hours.	5.2	-	32.4	17.0	-	-	-
2.0 % SUCROSE							
2 hours.	3.4	-	2.9	-	4.1	2.1	-
4 hours.	6.0	-	11.2	-	-	2.3	-
6 hours.	11.4	-	19.5	-	4.7	2.5	-
24 hours.	14.4	-	21.7	-	10.2	6.0	-

Graph 1.

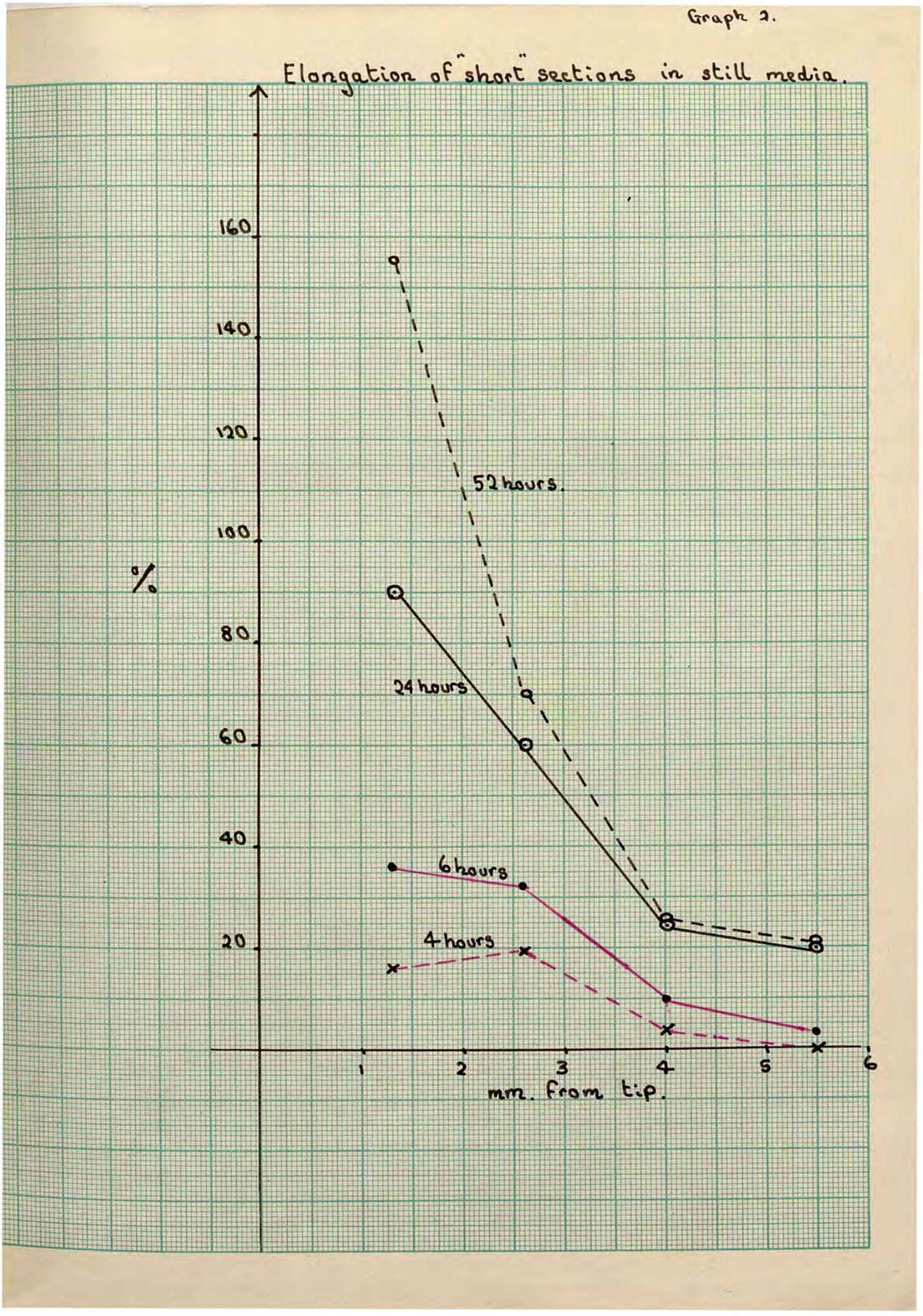
OXYGEN ABSORPTION. Mean over 4 hour period.



ELONGATION of "long" sections.



Graph 2.


Elongation of "short" sections in still media.
160
140
120
100
80
60
40
20
%
52 hours.
24 hours
6 hours
4 hours
1
2
3
4
5
6
mm. from tip.

section is likely to be greater than between those of a short section. In experiments we found shorter sections gave more uniform results, and, since they were taken from a more strictly located elongating region, gave a higher actual and % elongation.

Relationship between growth and respiration with varying distance from the tip. (Table No. 6)

The results can be collected into a table showing corresponding respiration and growth measurements. The following trends are evident.

(a) Maximum growth is obtained with a 2 mm. apex.

(b) Maximum growth is obtained using a solution of ½% sucrose.

(c) Respiration rates tend to be lower in the absence of sucrose.

The table given is incomplete and gives little indication of errors. It is in fact only the outcome of preliminary experiments. The results are best considered in the light of further, more extensive results (See graph 1. and graph 2. for short sections).

Effects of light and darkness

Root sections were always cut in normal laboratory light and manometric measurements were carried out in flasks exposed to diffuse light. According to Galston (1949) /3 indolyl acetic acid is destroyed by light in tissue in which riboflavin is present. Stress has been laid in Avena

assay work on the use of monochromatic red light. Since this precaution is expensive and tedious, preliminary experiments were carried out to determine if this is really necessary. Some of the flasks were covered with black cloth bags and others, placed alternately in position were illuminated, by means of two 150 Watt bulbs partially emersed in the Warburg tank. (This illumination greatly exceeded any normally used in the laboratory)

It was found that neither the growth rate nor the respiration rate was significantly altered by exposure to light; and light did not significantly change the sensitivity of the root sections to auxin. (see later).

Table no. 4.

	Control	10^{-11} IAA	10^{-10} IAA	10^{-9} IAA	
Light	54.2±1.4	77.8±3.0	27.8±2.1	2.1±1.1	% elongation
Darkness	53.3±2.0	71.6±2.8	21.6±3.1	6.4±4.0	% elongation
Light	26.6±1.1	27.8±1.0	25.2±1.7	26.6±0.8	cmm oxygen) respiration over 4 hr. period
Darkness	28.0±0.8	29.3±1.3	26.2±0.5	25.4±1.2	cmm oxygen)

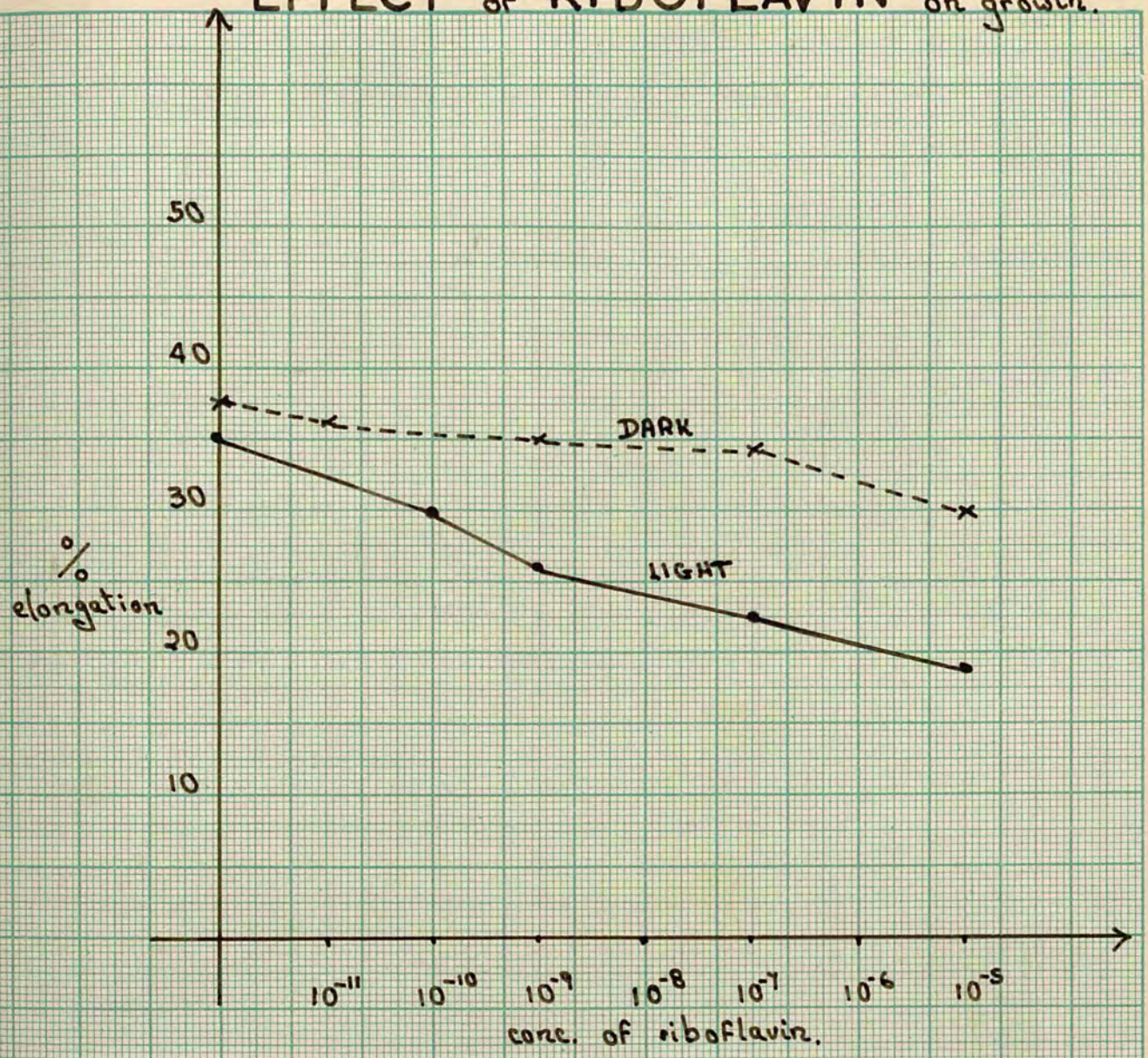
Effect of riboflavin* (Graph 3.)

Riboflavin sensitises the "in vitro" photooxidation of β indolyl acetic acid and a similar effect occurs "in vivo" in pea stem sections (Galston and Baker 1949).

Although riboflavin was inhibitory in all concentrations used (10^{-11} to 10^{-5}) it was considerably more so in the light. By Galston and Bakers' theory this was attributed to the destruction of naturally occuring auxin in pea roots in the presence of riboflavin. Presumably

Graph 3.

EFFECT of RIBOFLAVIN on growth.



naturally occuring riboflavin is in too low a concentration to appreciably inhibit growth by auxin destruction.

% elongation in 6 hours	Control (½% sucrose)	10^{-11}	10^{-10}	10^{-9}	10^{-7}	10^{-5} riboflavin
Light	35.1±2.2	--	29.8±1.7	26.6±1.1	22.6±2.7	18.6±3.2
Darkness	38.8±2.0	36.8±2.1	--	35.1±2.4	34.3±1.6	29.7±2.9

It is concluded the diffuse laboratory light has little or no effect on auxin destruction and hence on subsequent growth. This may be due to the low level of naturally occuring riboflavin - we have no direct evidence of this. From the above figures it was considered permissible to use diffuse light for the measurement of sections and for respiratory measurements. Except for these operations the sections were kept in the dark at 26°C.

* The riboflavin was in the form of BEFLAVIT produced by Roche Products Ltd.

Elaboration of the final technique

1. Sampling technique

Brown and Sutcliffe (1950) studied the metabolism of Zea and Cucurbita root sections by means of a sampling technique over a period of 48 hours.

Following their method we used samples of 25 short or 12 long sections in various media in petri dishes at 26°C. The dishes were removed at intervals of 6 - 12 hours and the sections placed in Warburg flasks for the determination of their oxygen absorption by the direct method. After 2 hours the sections were removed and their lengths obtained using

a travelling microscope. Thus oxygen absorption and elongation values could be obtained from the same sample. A new sample was used for each time interval determination.

2. Use of short sections

Short sections undergo a greater elongation than long sections in all media employed. The maximum elongation of long sections in unshaken petri dishes with 5 ml. media was only 46% (½% sucrose) whereas short sections under the same conditions elongate up to 80%. Short 1.983 mm. long were used for all standard experiments, and the results compared favourably with the elongation obtained in root sections by van Santen (1942) and by Brown and Sutcliffe (1950). These workers used 1.5 mm. sections.

3. Tilting of petri dishes

An apparatus was constructed by which the petri dishes were slowly tilted on trays attached to an electric motor. This had a highly significant effect on subsequent growth and respiratory measurements. Under these conditions "long" sections elongate upto 100% (in ½% sucrose) and "short" sections upto 200%. Two speeds of tilting were investigated of 60 vibrations/minute and 2 vibrations/minute. Both these had a highly significant effect. The speed of vibration did not significantly affect the result; the slower speed having the same effect as the faster speed. Increase in elongation due to tilting is therefore unlikely to be due to mechanical stimulation - in such an instance

one would expect effects to be proportional to the speed of shaking. Both media do however ensure adequate stirring of the medium and constant aeration.

Coult (1939) showed that shaking caused a highly significant increase in the growth of plants of Sinapsis Alba. The greater single factor was aeration. Schneider (1938) found how important it was not to completely submerge sections of Avena coleoptile to obtain maximum elongation. However we have found that with root tissue this precaution is in itself insufficient. Root sections undergo a much higher % elongation than pea stem or Avena coleoptile sections. Brown and Sutcliffe (1950) obtained elongation up to 4 times the original length in Zea root segments. In Pisum root tissue we obtained elongation upto 3 times the original length; whereas pea stem sections elongate only 22% (Christiansen and Thimann, 1950) - this is increased to 55% in the presence of auxin. Avena coleoptile sections elongate only 40-50% (Bonner 1949). It should be noted that with stem and coleoptile tissue:-

(a) longer sections have been invariably used

(b) the tissue has not been tilted or shaken in the medium In unshaken media the % elongation for root tissue is noticeably less (see 2. and van Santen, 1942).

It seems that aeration plays a large part in the promotion of elongation of root segments. Brown and Sutcliffe (1950) were able to obtain as great a % elongation

not by shaking or tilting but by growing on still sintered glass discs. This ensured maximum aeration. They also demonstrated the retardation of growth under reduced oxygen pressure.

4. Selection of the auxin concentration to be used

Root cell elongation is normally inhibited by those concentrations of auxin which cause a stimulation in stem or coleoptile growth. However we find a stimulation of root section growth using 10^{-11} and 10^{-10} indolyl acetic acid and 2.4 dichlorophenoxy-acetic acid (2.4.D.). There have been varied reports of stimulation of the growth of root tissue by the addition of low concentrations of auxins. Here with root sections we find consistent increases. Root sections are probably more suited for auxin studies than whole roots, since they are isolated from possible tip influences. Also, in dealing only with elongating cells we are presented with only one aspect of growth. Meristematic activity and maturation may possibly have different auxin optima which would tend to obscure the auxin effect in experiments with the complex tissue of a whole root.

For comparative study it was thought desirable to deal with respiration and elongation at three auxin levels:-

(a) in the absence of any added auxin; here growth may be dependent on the naturally occuring auxin.

(b) in the presence of a concentration of auxin stimulatory

to growth. (10^{-11} or 10^{-10} indolyl acetic acid of 2.4.D.)

(c) in the presence of a concentration of auxin inhibitory to growth (10^{-8} IAA or 2.4.D.)

Factorial experiments on the effect of sucrose and auxin

The main part of the investigation consisted of a large number of factorial experiments which were carried out in the following manner.

(a) each experiment was limited by the number of nearly identical 3.5 to 4.5 cm. long pea roots which could be grown at one time and by the number of Warburg manometers available.

(b) the roots were washed in glass-distilled water and segments excised 2.0-4.0 mm. from the tip. These were placed in samples of 25 segments in petri dishes containing 10 ml. of the particular medium.

(c) petri dishes were placed in an incubator at 26°C in the dark, on a shaker tilting the dishes at 2 vibrations/minute.

(d) serial samples were taken after various time intervals and the sections transferred to Warburg flasks together with 1 ml. of the original medium.

(e) the oxygen absorption of sections was determined by the direct method over a period of 2-4 hours.

(f) the sections were removed from the Warburg flasks, placed in petri dishes and their lengths determined.

(g) the sample was then discarded and new ones taken from the incubator for other time intervals.

Notes

1. 10 ml. medium in the petri dishes only half covers the sections. Tilting ensures a time sequence of complete emersion followed by complete aeration of the sections. This can be compared with the "comb" method of Schneider (1938). Avena coleoptile sections were "threaded" onto a comb which could be alternately lowered into and raised above the medium. Unfortunately this method cannot be used for roots since they are not hollow!

2. Oxygen absorption and elongation were always determined on the same sample. The process of transferring from petri dishes to Warburg flasks had little effect on subsequent elongation, whereas the time taken to measure the lengths of the sections - of necessity performed at a temperature of less than 26°C - did seem to have some effect on subsequent oxygen absorption. Hence respiratory measurements were always taken prior to length measurements.

3. The experimental period never exceeded 48 hours. Danger of bacterial contamination prevented further extension of this period. All 48 hour values were checked for contamination in the manner of Brown and Sutcliffe (1950). After a respiratory measurement had been taken the sections were removed from the medium and further results were obtained for the medium alone. The oxygen absorption of

the sections was taken to be that of the original total minus that of the medium alone. In a case of bad contamination the sample was discarded. However contamination was never found until after a period of 36 hours - reasonable precautions were taken to ensure that this was so. Even at 48 hours contamination was only encountered in a small % of the samples. This was fortunate since Brown and Sutcliffe's method is only an approximation. Most of the contamination is probably on the root surface and is removed with the sections.

4. Preliminary experiments showed there was no appreciable change in diameter over the experimental period. Length changes were taken to be a valid estimate of growth.

Expression of results

It is proposed to give the results of two factorial experiments in full to show the general experimental design. Nearly all the results were obtained from similarly designed experiments. These have been lumped together to show the effect of various added metabolites or inhibitors. Since every factorial experiment included at least one ½% sucrose control and/or other relevant controls it is possible to single out effects by means of the ratios. This entirely eliminates the variation due to the particular batch of peas employed. These ratios are particularly valuable for the singling out of effects but should be used cautiously in conjunction with the actual data. This is particularly

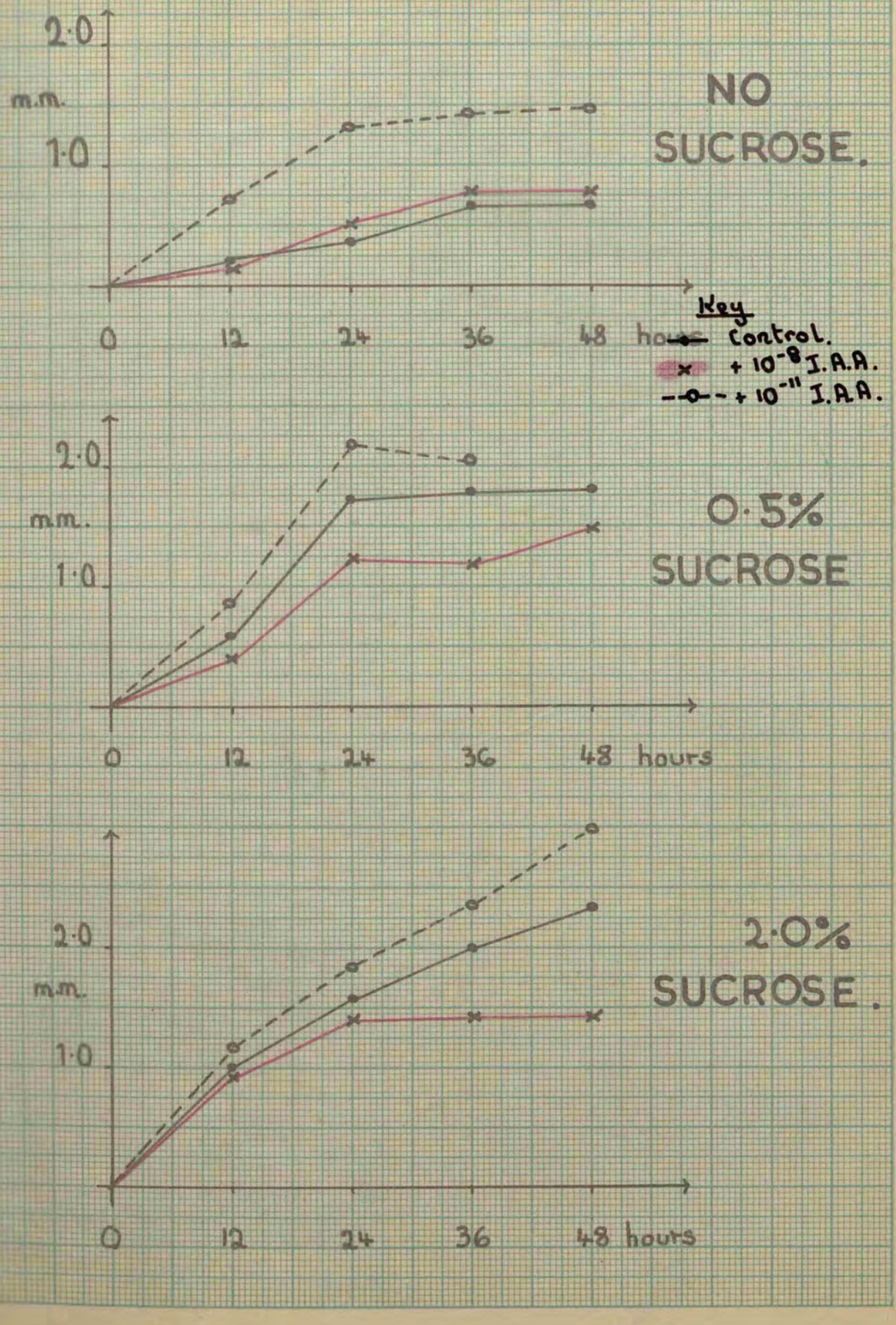
Still media

Table No. 8. OXYGEN ABSORPTION IN C.MM. / ½ HOUR/ 25 SECTIONS

	0 SUCROSE			½% SUCROSE			2% SUCROSE		
	0 IAA	10^{-11} IAA	10^{-8} IAA	0 IAA	10^{-11} IAA	10^{-8} IAA	0 IAA	10^{-11} IAA	10^{-8} IAA
0 hours	17.6	17.4	16.0	21.8	18.0	17.8	25.0	19.2	15.4
12 hours	17.6	16.8	16.2	31.4	25.0	16.2	27.2	23.2	24.0
24 hours	17.6	16.0	16.6	22.6	17.8	12.8	23.2	23.0	23.8
36 hours	17.8	15.2	16.0	19.2	10.6	8.4	15.2	15.0	15.0
48 hours	17.2	14.0	15.0	17.4	16.8	9.2	7.2	7.0	6.0
				MEAN LENGTH (in mm.)					
0 hours	1.950	1.970	1.966	1.981	1.881	1.901	1.929	1.967	1.943
12 hours	2.150	2.670	2.189	2.588	2.853	2.397	2.550	2.600	2.487
24 hours	2.364	3.263	2.488	3.635	4.170	3.181	3.151	3.327	2.884
36 hours	2.660	3.302	2.755	3.700	4.007	3.200	3.475	4.542	2.894
48 hours	2.720	3.392	2.795	3.785	3.927	3.451	3.831	4.550	2.900
				GROWTH RATE (mm./12 hours)					
0 hours	0.200	0.700	0.223	0.607	0.972	0.496	0.621	0.633	0.546
12 hours	0.214	0.593	0.299	1.047	2.317	0.784	0.601	0.727	0.397
24 hours	0.296	0.039	0.267	0.065	-	0.019	0.324	1.215	0.010
36 hours	0.060	0.090	0.040	0.085	-	0.251	0.356	0.008	0.006

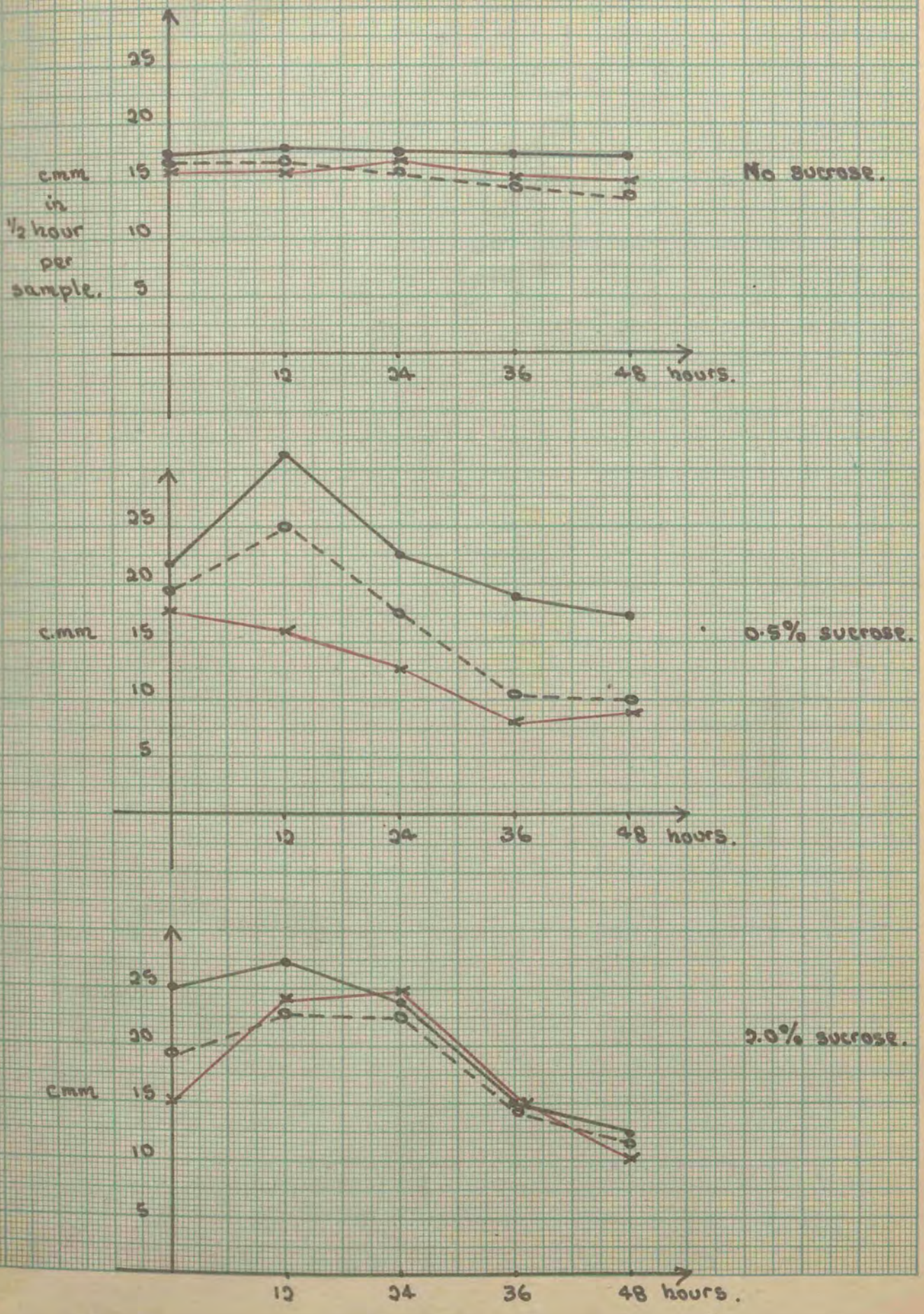
Graph 4.

ELONGATION. STILL MEDIA.



Graph 5.

OXYGEN ABSORPTION. STILL MEDIA.



true when several substances are causing independent or interacting variations.

Factorial experiment in still media

This was the only experiment with untilted dishes. The sections were, of necessity, shaken over the 2 hour period of respiratory determination. Corresponding length and respiratory measurements refer to the same sample.

This set of results illustrates some important points peculiar to still media and short sections.

(a) 2.0% sucrose is the medium which allows maximum growth. The samples doubled their length in this medium compared with a much smaller growth when no sucrose was present. The maximum elongation of "long" sections under these conditions was 70%.

(b) Growth continues up to 24 hours or (in 2% sucrose) 36 hours. Although growth continues for a longer period in the higher sucrose concentration, the maximum growth rate is obtained over the period 12-24 hours in ½% sucrose. The auxin effect is more marked in this medium.

(c) Whereas 10^{-11} indolyl acetic acid (IAA) is always stimulatory to growth, 10^{-8} is only markedly inhibitory if sucrose is present.

(d) The correspondence between growth rate, respiration rate and auxin action is obscure.

(1) growth and respiration rate corresponded in that increase in length tended to result in immediate

increase in respiration rate e.g. rise from the initial value during the 12-24 hour period with respect to both these values, and absence of this rise if sucrose is absent.

(2) regarding auxin action there was no relative correspondence between growth rate and respiration rate. Both concentrations of auxin tended to be inhibitory to respiration; whereas growth rate was stimulated in 10^{-11} IAA, and 10^{-8} had no effect or was inhibitory.

Several miscellaneous points can be noted here

(1) still media are not satisfactory for optimum growth - even if the precaution of not completely submerging the sections is taken. Sections in still media tend to become distorted or bent, and root hair growth which commences in profusion on the aerated side is absent or irregular on the unaerated part.

(2) the sections although still while in the petri dishes were shaken during the period of respiratory determination.

(3) the highest growth rate - although not the maximum length - is obtained in ½% sucrose.

Factorial experiment in shaken media (Table 42)

In this experiment procedure was similar to the previous experiment but the petri dishes were tilted at a constant rate as has been described. Samples were removed and their respiration rates obtained after the elapse of certain time intervals. For the experiment whose results

Shaking media

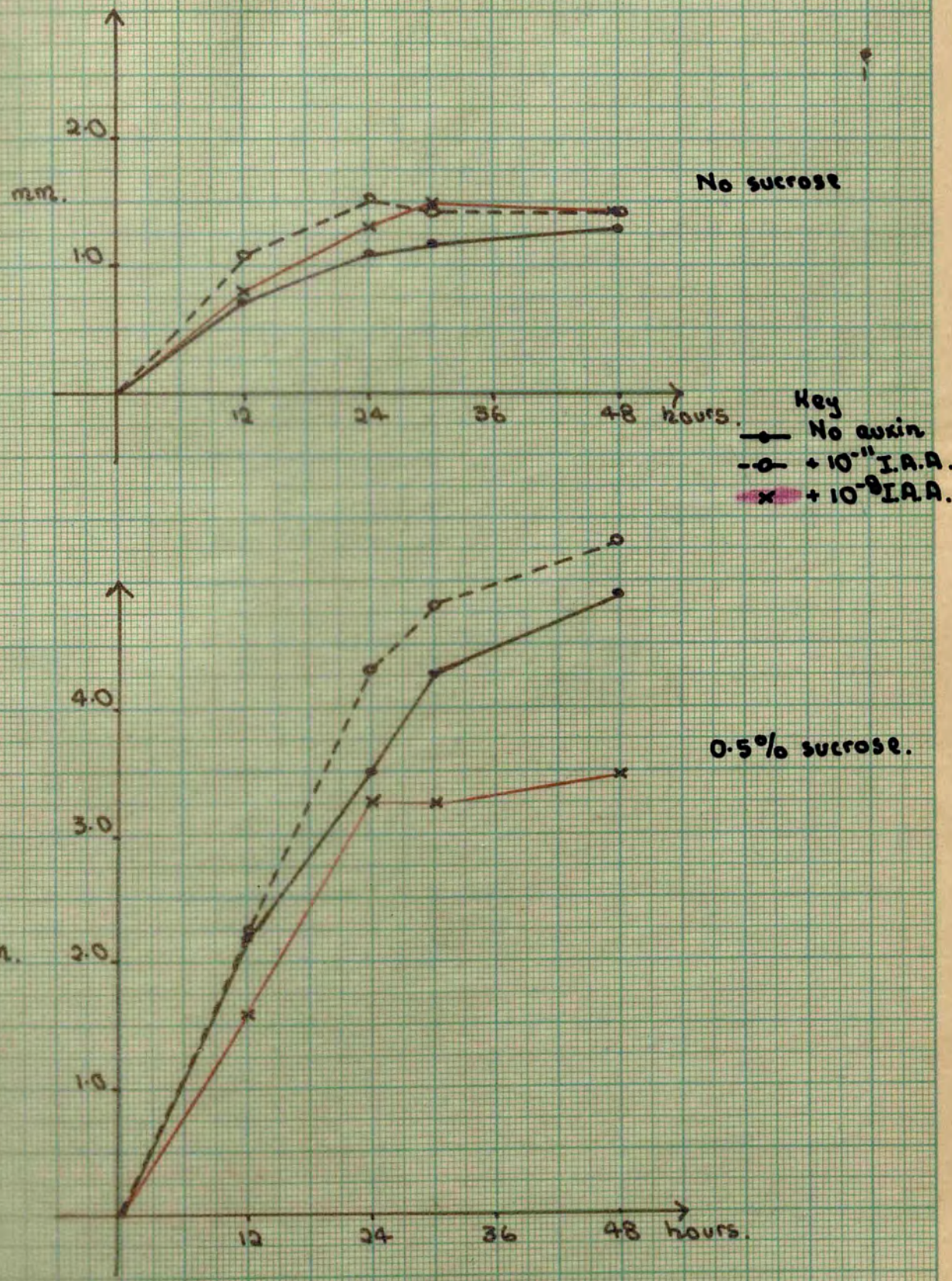
Table No. 9

OXYGEN ABSORPTION IN C.MM/ ½ HOUR/ 25 SECTIONS

	0 SUCROSE			½% SUCROSE			2% SUCROSE		
	0	10^{-11}	10^{-8} IAA	0	10^{-11}	10^{-8} IAA	0	10^{-11}	10^{-8} IAA
0 hrs.	17.6	17.6	16.0	22.0	18.0	18.0	24.0	16.0	15.4
12 hrs.	17.8	15.2	18.0	29.0	20.4	-	24.0	16.6	20.0
24 hrs.	16.2	17.0	13.8	39.4	35.6	20.6	56.8	40.4	23.6
30 hrs.	14.0	14.2	10.0	25.4	32.8	26.8	27.8	34.6	20.0
48 hrs.	16.6	6.8	8.0	25.4	14.4	6.0	8.6	11.0	11.6
				MEAN LENGTH (in mm.)					
0 hrs.	1.982	1.990	1.906	1.981	1.981	1.986	1.908	1.987	1.958
12 hrs.	2.724	3.060	2.779	4.291	4.244	3.636	4.495	4.954	3.849
24 hrs.	3.154	3.520	3.320	5.569	5.927	5.309	5.404	6.412	4.820
30 hrs.	3.214	3.457	3.466	6.823	6.327	5.230	7.126	6.942	5.381
48 hrs.	3.389	3.400	3.436	6.934	7.409	5.380	7.400	7.121	5.751
				GROWTH RATE (in mm./ 12 hour period)					
0-12 hrs.	0.742	1.070	0.673	2.310	2.263	1.650	2.487	1.967	1.891
12-24 hrs.	0.430	0.460	0.541	1.278	2.683	1.673	1.009	1.458	0.971
24-30 hrs.	0.120	-	0.290	2.508	0.880	-	3.044	1.010	1.122
30-48 hrs.	0.116	-	-	0.066	0.708	0.100	0.182	0.119	0.246

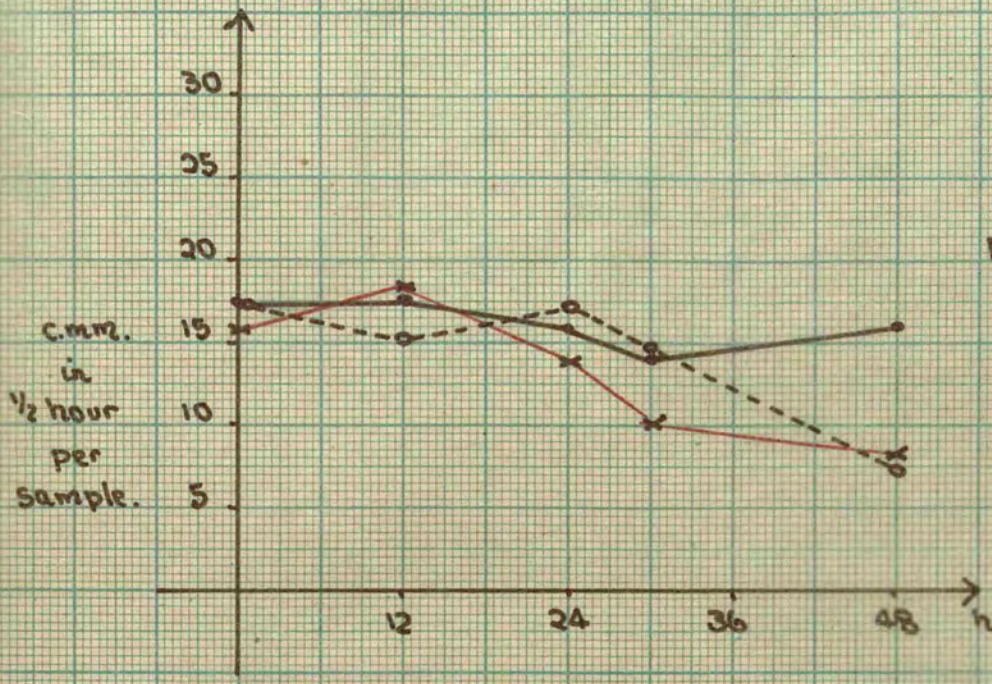
Graph 6.

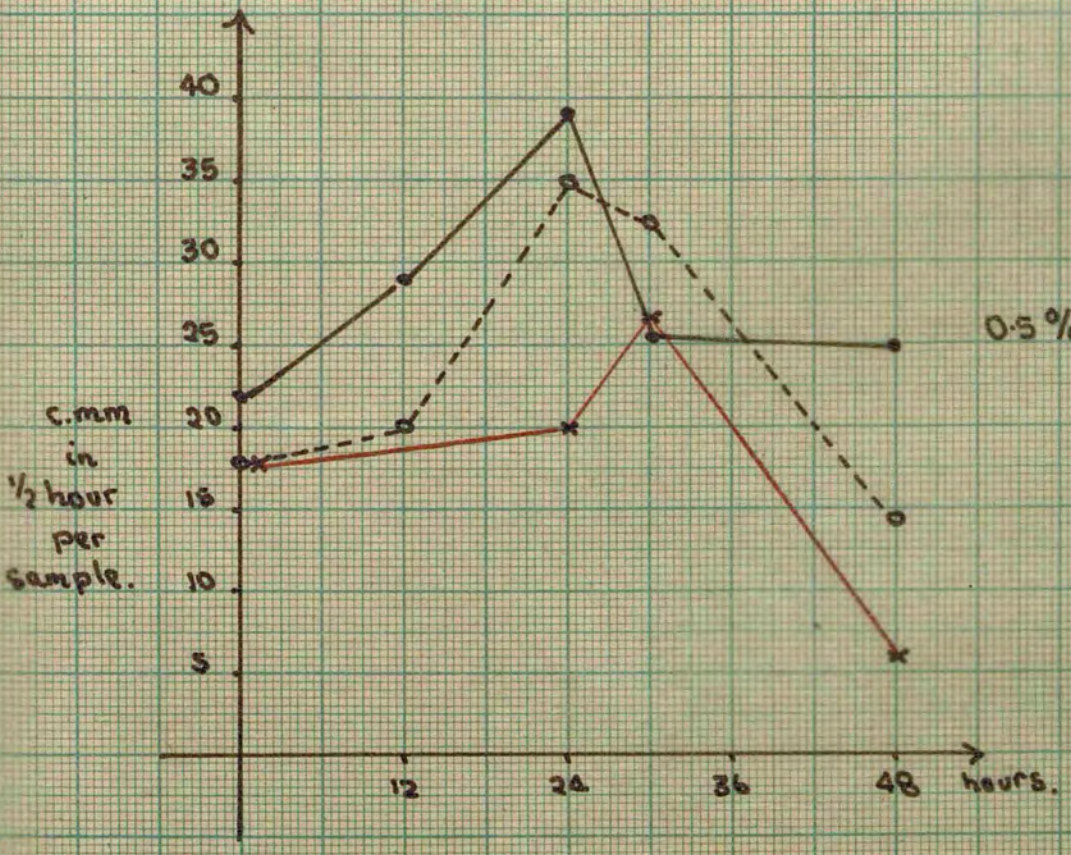
ELONGATION. SHAKEN MEDIA.



Graph Y.

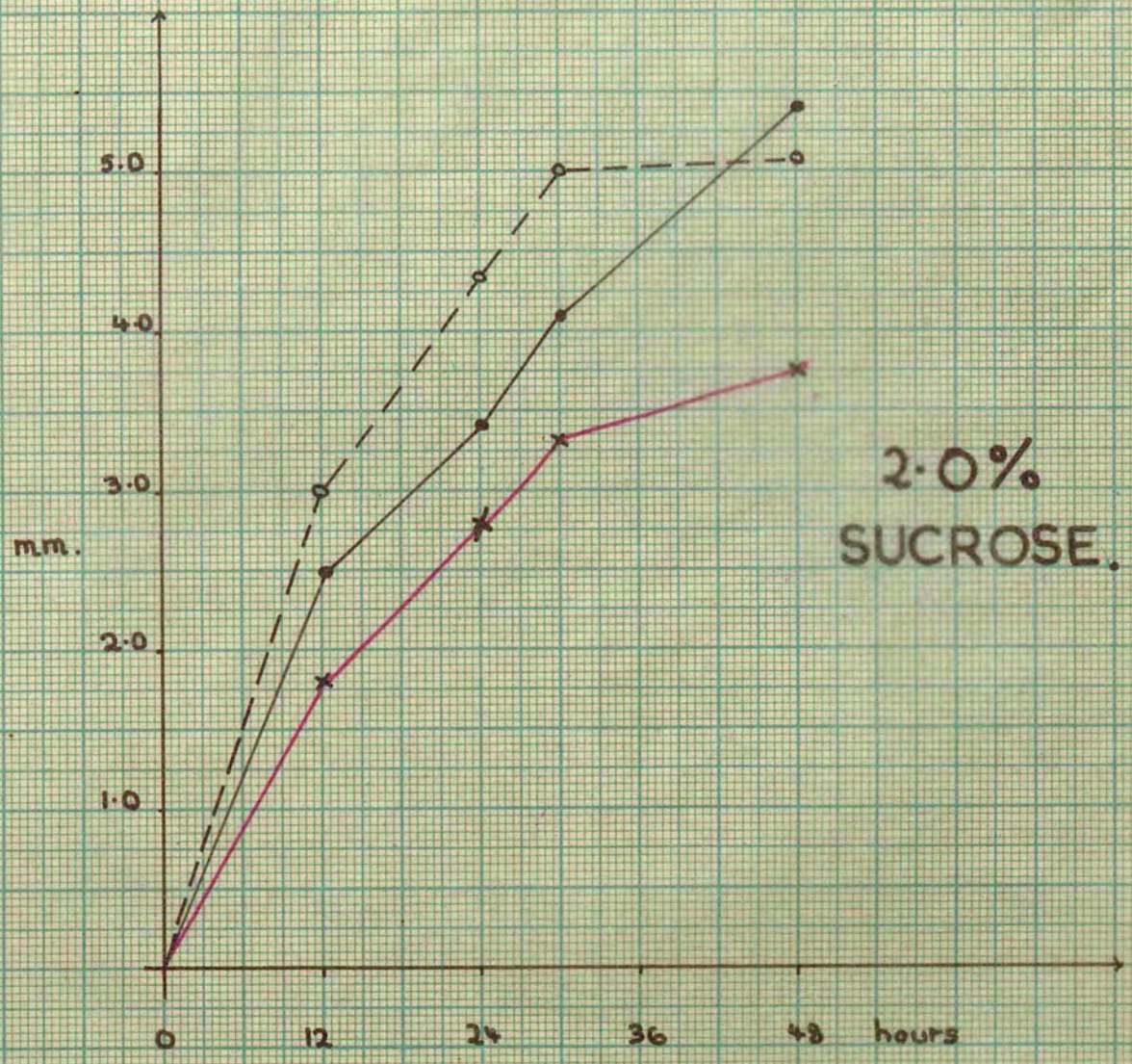
OXYGEN ABSORPTION. SHAKEN MEDIA.





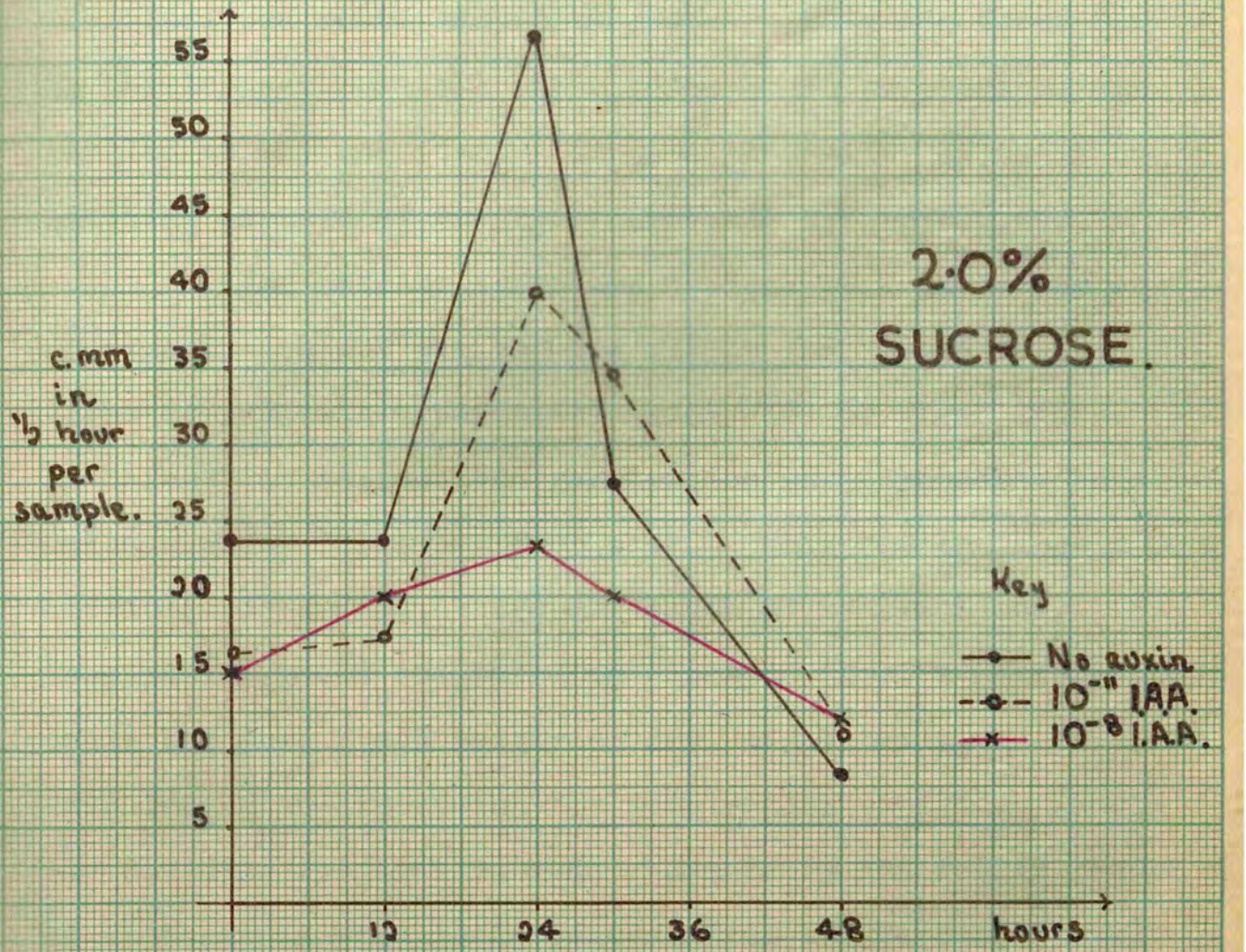
Graph 8.

ELONGATION. SHAKEN MEDIA.



Graph 9.

OXYGEN ABSORPTION. SHAKEN MEDIA.



are given below it was necessary to cut and set up 45 original samples of 25 sections each i.e. 1125 near identical pea roots had to be grown. This was found to be the largest possible batch which could be produced and handled at any one time. In general smaller experiments, omitting certain of the time intervals were attempted.

The results shown are typical of a large number of experiments and can be more fairly considered in the light of numerous experiments which will be given later. As before, in sucrose the respiration rate increases to a maximum which falls off with time. This may be largely attributable to the change in length of the sections. Respiration rates are based on a sample (i.e. a unit section) basis NOT on a fresh weight or dry weight basis. Calculating an expression for respiration rate/unit length (by dividing respiration rate / sample by the actual length of that sample when it is removed from the Warburg flask) yields the following results:-

Table No 10.

	0 sucrose			½% sucrose			2% sucrose			
	0	10^{-11}	10^{-8}	0	10^{-11}	10^{-8}	0	10^{-11}	10^{-8}	IAA
0 hrs.	8.8	8.8	8.0	11.0	9.0	9.0	12.0	8.0	7.7	
12 hrs.	8.6	4.9	6.5	6.8	4.8	-	5.3	3.3	5.2	
24 hrs.	5.1	4.9	4.2	7.1	6.1	3.9	10.5	6.3	5.0	
30 hrs.	4.2	4.1	2.9	3.8	5.3	5.1	3.9	5.0	3.7	
48 hrs.	4.9	2.0	2.4	3.7	2.0	1.1	1.0	1.5	2.0	

These results show a general trend downwards of

respiration rate / unit length whether sucrose is present or absent. Confirmation of this with a large number of results will be given subsequently.

The results are quite compatible with the identification of the elongating phase with cell-wall extension and water uptake. Each cell does undertake some protein and protoplasmic synthesis (Brown and Broadbent, 1951). This synthesis must involve the establishment of some new centres of enzymatic activity but the increase in cell-length proceeds at a much more rapid rate. In consequence respiration rate / unit length falls with time. The respiration rate/cell is a fraction of the respiration rate/sample, since in the sections used the number of cells remains constant. No meristematic tissue is included.

EFFECTS OF SUCROSE

Effects of Sucrose

Effects have been considered under four heads:-

(a) effect on actual elongation

(b) effect on actual respiration rate / sample of 25 "short" or 12 "long" sections.

(c) effect on growth rate, calculated as elongation / time

(d) effect on respiration rate / unit length

Possible correlation between any two of these effects must be considered.

It is to be emphasised that all these values show a definite trend with time, and each time level must be considered individually for any valid comparison of effects to be made. The practice (particularly of certain American workers) of comparing initial respiration rates with the final length after 24 hours is to be deplored. The time drifts encountered are as follows:-

(a) elongation takes place until a maximum length for the particular added metabolite or combination of metabolites is reached. Then it slackens off until it finally ceases, giving the typical S-shaped curve of growth against time. In the case of an inhibitor of growth being present, the elongation will depend on the rate of penetration of the inhibitor. In general elongation will take place at a decreasing rate until the concentration inside and outside the section cells has reached an

equilibrium. This rate of elongation may be maintained, but, more likely, elongation will soon cease, following again an S-shaped curve. Penetration effects are very important if a true picture of the action of any substance on plant tissue is to be obtained. Unless penetration is immediate, the curve (elongation / time) follows the time course of the sections in absence of that substance; and as the concentration inside the section cells increases gradually takes up its final form typical of the concentration studied.

A total length / time curve is of the same form as the normal S-shaped curve of growth, taking the initial value not as the origin but as the original length (for short sections 1.983 mm.). This curve is important since, if the curve (b) (respiration rate / sample) is divided by the length / time curve, curve (d) (respiration rate / unit length) is obtained.

(b) respiration rate / sample may show various drifts with time. It may remain fairly constant, falling off slightly with time (as in glass-distilled water).

It may fall off relatively rapidly to the new level set by an inhibitor (as in arsenate, iodiacetate, or dinitro-phenol).

It may rise to a maximum value, from which it falls generally to below the original level (as in ½%. 2% or 5% sucrose).

The 4th possibility of rising to a maximum which is maintained over a 48 hour period was never encountered in our experiments. Brown and Sutcliffe obtained this type of curve using Zea and Cucurbita root segments in 2% sucrose and 5% sucrose. This is a deviation from our results, since in glass-distilled water and in 1/4% sucrose their curves corresponded to our first and third types respectively. It is possible that their high 48 hour values were due to bacterial contamination. In dealing with high sugar concentrations the greatest care is necessary to prevent this occurring; and even their method of checking the absorption of the medium, as has been previously pointed out, is somewhat unsatisfactory.

The typical rise and fall of oxygen absorption (our third type curve) is similar to that observed by Ståfelt, who found that respiration of isolated root tissue placed on an organ basis increased during the first day. This rise was followed by a fall on the second. He called this the "Grand Period of Oxygen Absorption" but, as we will show, this is only indirectly correlated with the "grand period of growth".

(c) growth rate / time curves represent curves of the slope of (a) curves against time.

They may remain constant for a time and then fall off (as in glass-distilled water).

They may fall rapidly to a zero value (as in inhibitors).

They may remain constant for a time, then increase rapidly reaching a maximum value from which a falling away occurs (as in sucrose or other metabolites).

(d) respiration rate / unit length. Since the respiration rate / sample generally only increases over a period when length is increasing and, since respiration rate changes are more than accounted for by the change in length, respiration rate / unit length falls off with time.

Results of the sucrose effect will now be given in full.

A) SHORT SECTIONS (Table No 1K)

Respiration rate / sample / 15 minutes

INITIAL

0 sucrose		½% sucrose			2% sucrose
8.8	8.0	11.0	9.9	10.8	12.0
10.4	10.5	12.5	11.1	11.0	11.0
10.0	10.2	11.0	11.7	9.0	11.7
8.8	8.9	9.3	10.0	12.0	
9.8		10.9	10.1	10.1	
				15.0	
Mean and standard error					
9.48 ± 0.29		10.96 ± 0.36			11.57 ± 0.58
Number of individuals (N)					
9		16			3

Sucrose has a significant effect on the initial respiration / sample. Comparing the means of no sucrose and ½% sucrose a value for "t" = 3.58 is obtained. "p" is nearly equal to 0.001. Only three results were obtained for 2% sucrose and they do not appear to be significantly higher than ½% sucrose.

Table No 11 b

	0 sucrose	½% sucrose	2% sucrose
12 HOURS	8.9	10.2	12.0
	8.0	15.5	
	8.7	18.2	
	9.0	18.0	
		15.0	
		10.0	
Mean and standard error	8.65 ± 0.24	14.45 ± 1.65	
N.	4	6	
24 HOURS	8.1	17.8 15.4	13.9
	10.8	16.7 17.5	14.4
	10.3	15.4 16.8	
	11.2	21.0 23.5	
	9.7	17.6 19.0	
	9.9	22.0 13.0	
	8.0	12.5	
Mean and standard error	9.71 ± 0.50	17.55 ± 0.90	
N.	7	13	
30 HOURS	7.0	12.7	13.9
		15.0	
Mean and standard error		13.85 ± 1.15	
N.		2	
48 HOURS	8.3	12.7 6.5	4.3
	9.2	9.2 5.0	5.0
	7.0	7.7 5.2	
	9.0	13.6 4.5	
	10.2	9.3 4.0	
	7.6		
	7.5		
Mean and standard error	8.40 ± 0.43	7.77 ± 1.07	

Table No 11 c.

FINAL TABLE

	0 sucrose				½% sucrose	
	Mean & st.error	N	"t"	"p"	Mean & st.error	N
0 hrs.	9.48 ± 0.29	9	3.58	0.001	10.96 ± 0.36	16
12 hrs.	8.65 ± 0.24	4			14.45 ± 1.65	6
24 hrs.	9.71 ± 0.50	7			17.55 ± 0.90	13
30 hrs.	7.00	1			13.85 ± 1.15	2
48 hrs.	8.40 ± 0.43	7			7.77 ± 1.07	10

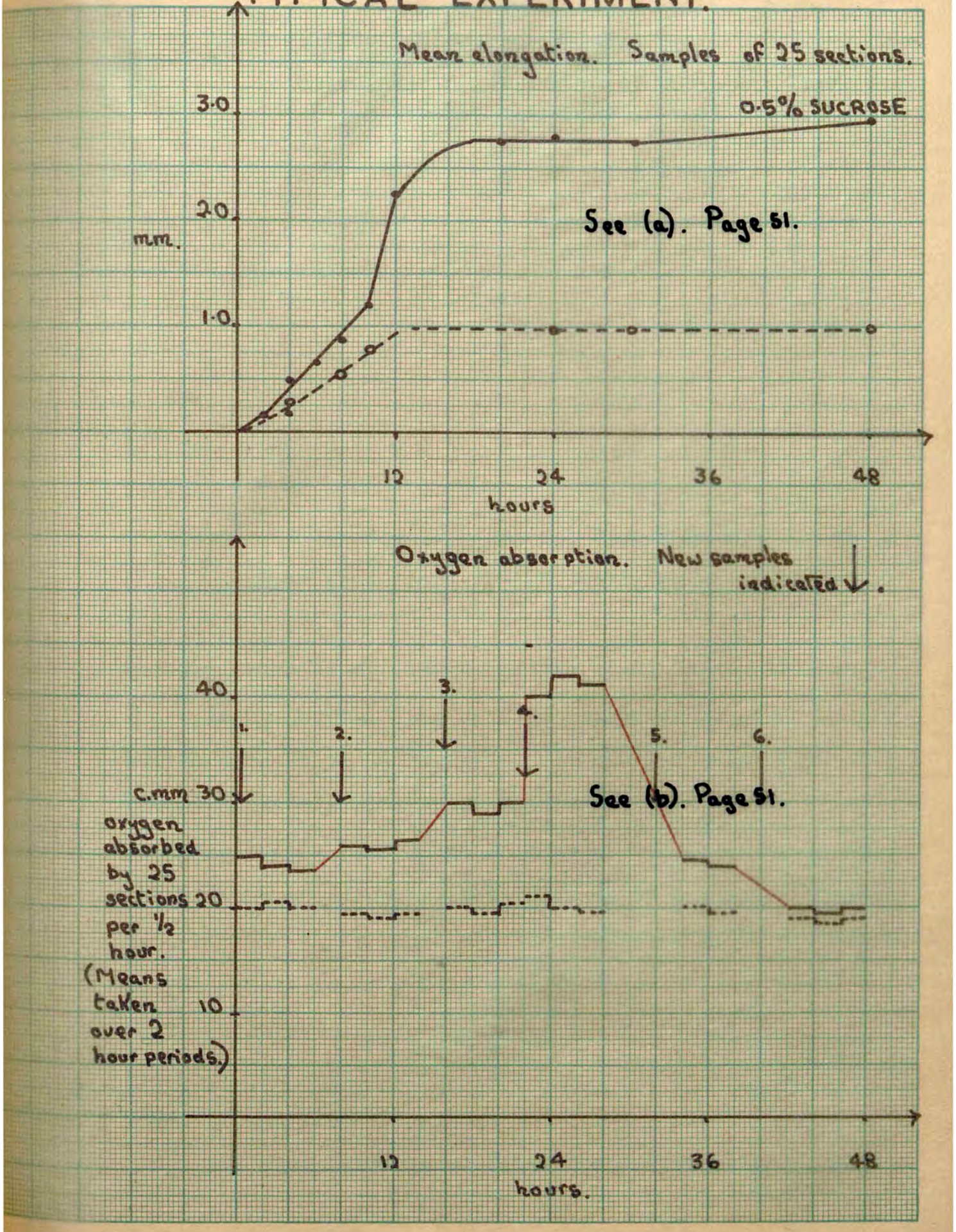
Table No 11 d

Mean elongation of "short" sections (total length minus 1.983 ± 0.005 mm.)

	0 sucrose	½% sucrose	
1 hr.	0.295	0.286	
2 hrs.	0.300	0.158	
		0.196	
4 hrs.	0.365	0.547	
		0.440	
		0.353	
		0.290	
		0.058	
		0.176	
		0.408	
		0.256	
5 hrs.		0.580	
		1.117	
		0.566	
6 hrs.	0.653	0.603	
		0.708	
		0.640	
		0.598	
8 hrs.	0.645	0.858	
9 hrs.		1.116	
12 hrs.	0.724	1.651	
	1.127		
		2.291	
		2.127	
24 hrs.	0.886	1.578	2.500
	0.727	2.273	2.260
	1.137	2.199	2.520
	1.150	2.450	3.004
	0.824	2.521	3.268
	1.024	1.531	2.478
	1.193	2.609	1.955
48 hrs.	1.119	2.373	2.592
	0.786	2.527	2.970
	1.189	2.958	3.227
	1.208	2.530	3.134
	0.923	2.958	3.210
	1.119	1.904	1.992
	0.862	3.456	2.888
		2.846	2.466
		3.106	

Graph 10.

TYPICAL EXPERIMENT.



ELONGATION

FINAL TABLE - Effect of sucrose on sections

	0 sucrose Mean and standard error	N	½% sucrose Mean and standard error	N
0 hrs.	0.30	1	0.18 ± 0.02	2
4 hrs.	0.37	1	0.31 ± 0.17	8
5 hrs.	-		0.75 ± 0.32	3
6 hrs.	0.65	1	0.63 ± 0.03	4
8 hrs.	0.65	1	0.86	1
9 hrs.	-		1.12	1
12 hrs.	0.92 ± 0.15	2	2.02 ± 0.38	3
24 hrs.	0.99 ± 0.05	7	2.36 ± 0.13	14
48 hrs.	1.16 ± 0.09	8	2.89 ± 0.10	16

From these results the following conclusions were drawn:-

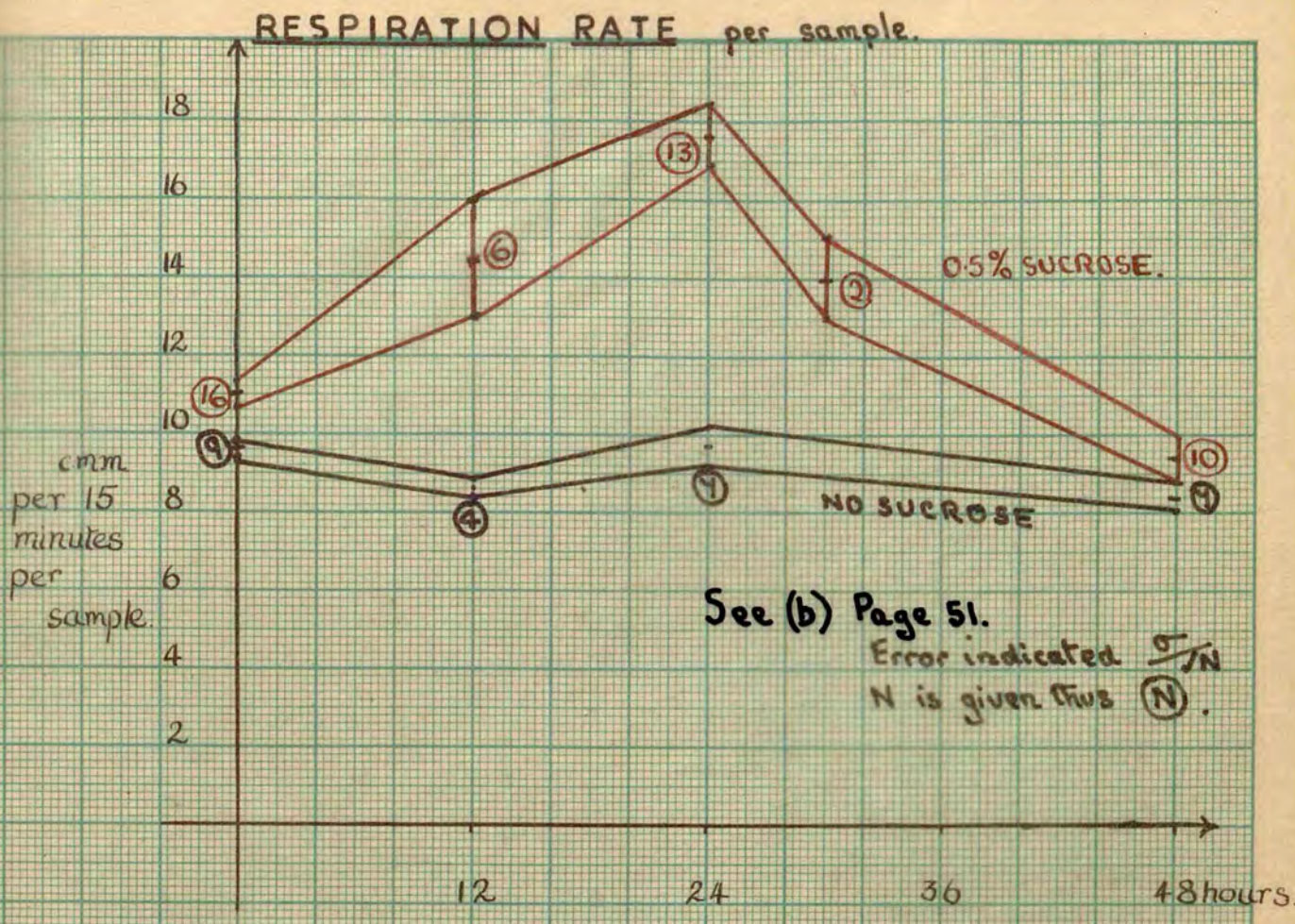
(a) the presence of sucrose causes a highly significant increase in growth. Elongation continues up to 12 hours in the absence of sucrose but even continues (slowly) over the 24-48 hour period if it is present.

(b) respiration rate / sample remains fairly constant in the absence of sucrose; but, when it is present, increases to a maximum at 24 hours and then falls to BELOW the original value. (It is 10.96 ± 0.36 originally but at 48 hours 7.77 ± 1.07 c.mm. / 15 minutes).

(c) a growth rate curve / time was taken as the differential curve of the smoothed elongation curve / time.

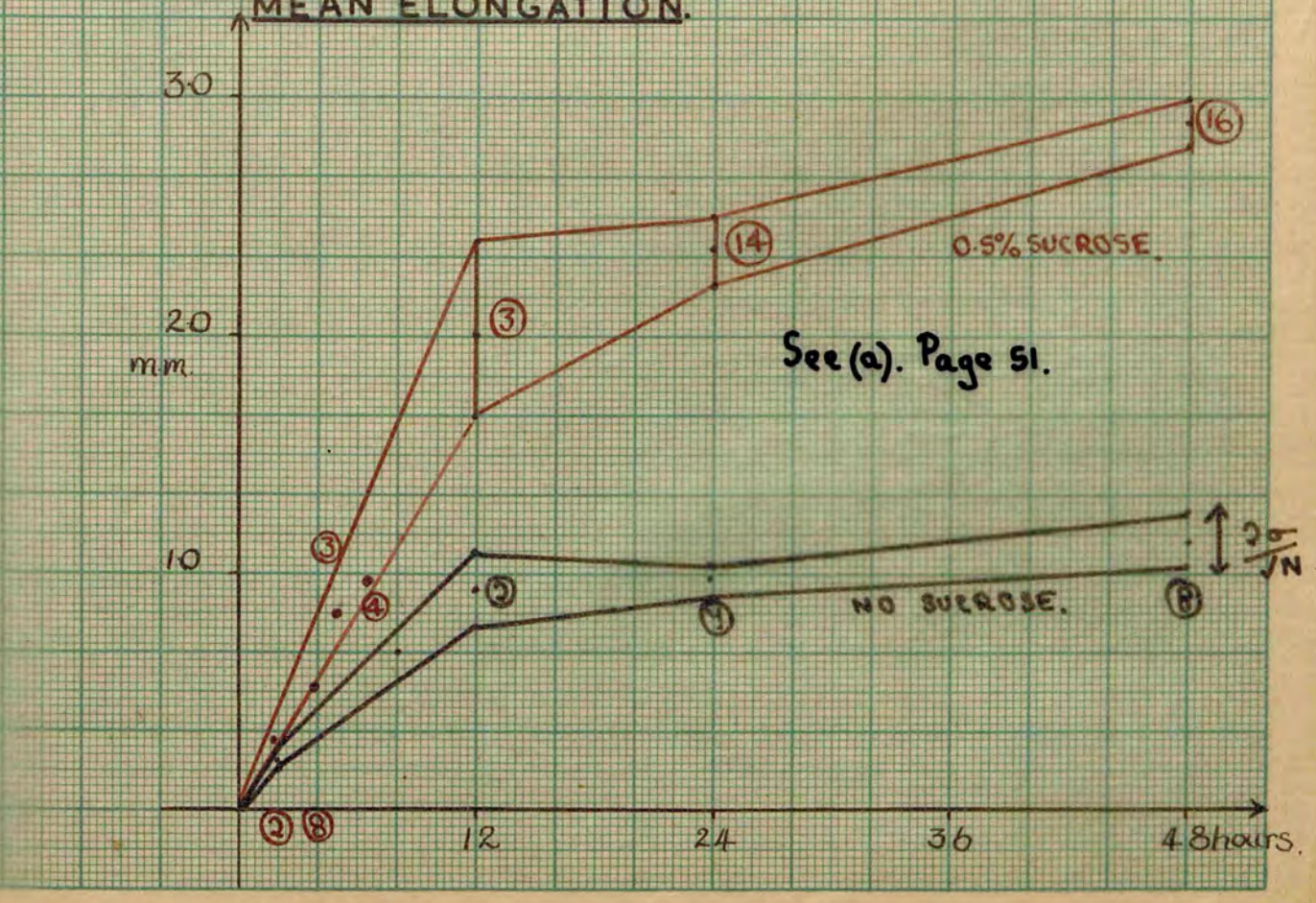
From comparison of the respiration rate / sample /

Graph II.



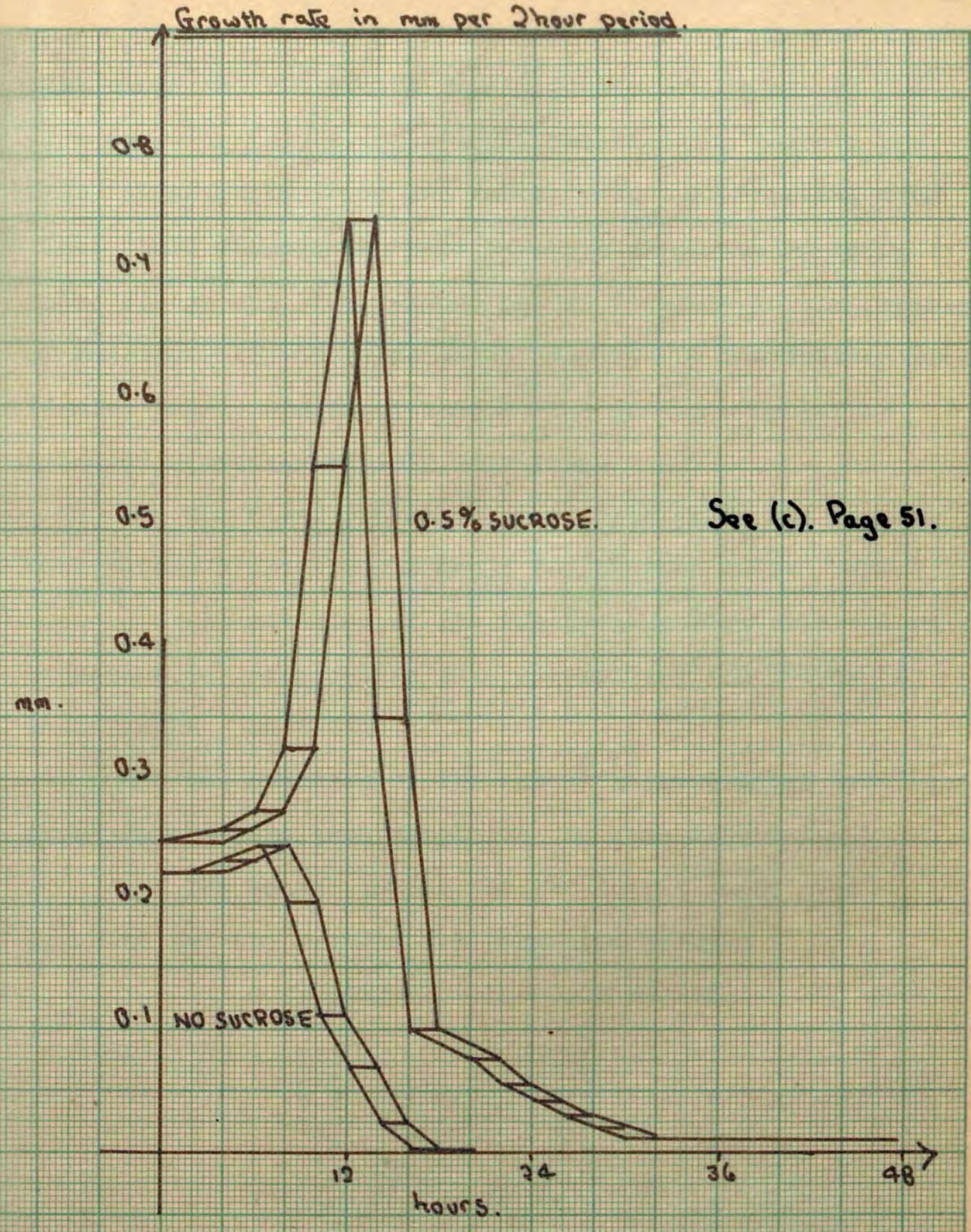
See (b) Page 51.

Error indicated $\sigma/\sqrt{N}$ N is given thus (N).



See (a). Page 51.

Graph 12.



See (c). Page 51.

time curve and the growth rate / time curve it can be seen there is only a superficial relationship between the two. Respiration rate and growth rate both increase with time; but, whereas the respiratory maximum occurs at 24 hours the growth rate maximum occurs 12 hours in the presence of sucrose. In the absence of sucrose the respiration rate remains fairly constant, whereas the growth rate (after 6 hours) decreases rapidly with time.

The final presentation of results in the form of respiration / unit length could be obtained in two ways:-

(1) division of the two gross curves obtained to represent (a) respiration rate / sample by the curve, (b) mean length / unit time.

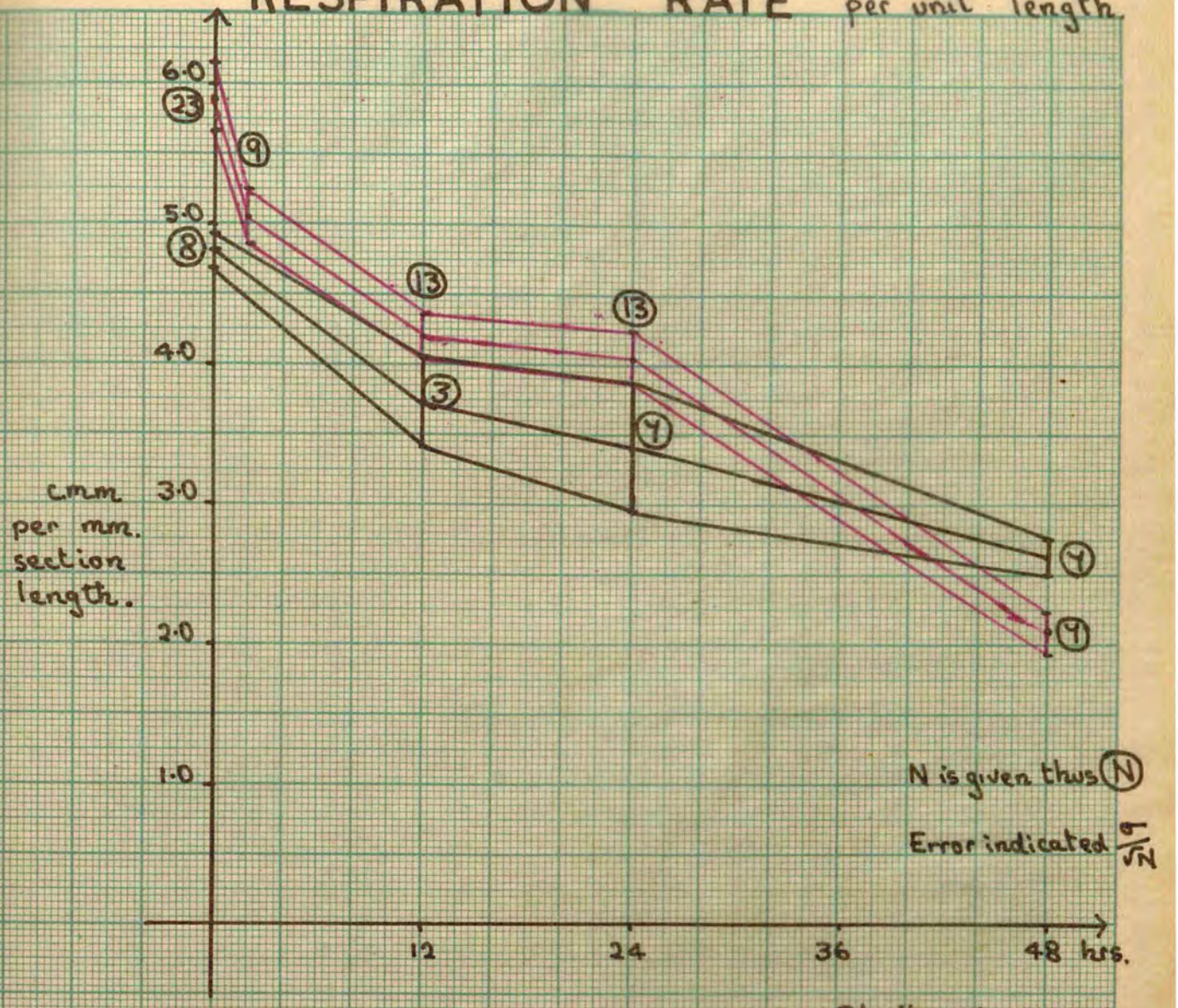
(2) division of the actual corresponding results obtained from the same sample in the same experiment.

The second course was considered to be more accurate and the results are presented below.

	0 SUCROSE	0.5% SUCROSE	
0 hours	4.4	5.5	5.1
	5.2	5.5	5.4
	4.4	5.5	7.8
	5.0	4.7	5.5
	4.9	5.5	5.9
	4.0	4.9	5.3
	5.3	5.5	4.5
		5.0	6.0
	5.1	7.5	4.3
		5.0	8.9
		6.5	7.0
		5.7	

Graph 13

RESPIRATION RATE per unit length.



N is given thus (N)

Error indicated $\sigma/\sqrt{N}$

Black = No sucrose
Red 0·5% sucrose.

	0 SUCROSE	0.5% SUCROSE	
2 hours	4.0	4.8	5.5
		5.5	5.5
		5.7	4.4
		4.8	
		4.1	
		5.4	
12 hours	3.9	5.5	3.6
	4.1	3.4	4.1
	3.2	4.3	4.2
		5.2	4.1
		4.2	4.4
		5.2	3.1
		2.9	
24 hours	3.5	3.5	5.8
	4.0	4.4	4.3
	3.5	3.7	4.2
	3.5	3.6	3.3
	3.4	4.5	3.5
	3.3	3.9	3.4
	2.7	4.6	
48 hours	2.5	2.8	
	3.0	2.1	
	2.1	2.0	
	2.8	2.2	
	2.9	2.0	
	2.6	1.4	
	2.6	2.1	

FINAL TABLE OF RESPIRATION / UNIT LENGTH

	0 SUCROSE	0.5% SUCROSE
0 hours	4.79 ± 0.16	5.80 ± 0.23
2 hours		5.05 ± 0.19
12 hours	3.73 ± 0.29	4.17 ± 0.22
24 hours	3.41 ± 0.46	4.06 ± 0.19
48 hours	2.64 ± 0.11	2.09 ± 0.15

It seems that the following relationships are evident for short sections:-

(a) in sucrose:-

	respiration/ sample	mean length	respiration/ unit length	growth rate during the period
0- 6 hours	increases	increases	falls	remains constant
6-12 hours	increases	increases	falls	increases
12-24 hours	increases	increases	constant	falls
24-48 hours	falls to below original	nearly constant	falls	falls to zero

(b) no sucrose

0- 6 hours	decreases	increases	falls	remains constant
6-12 hours	decreases	increases	falls	falls
12-24 hours	decreases	constant	falls	falls
24-48 hours	decreases	constant	falls	falls

In sucrose the respiration rate / sample is increasing while the growth rate is falling, and this increase can be more than accounted for by a change in length.

In the absence of sucrose the two rates fall off but the slope is not the same. It seems here that we have little evidence that growth rate and respiration rate are directly related. An increase in length tends to cause increase in respiration rate / sample but not / unit length, if sucrose is present; and this increase is delayed so that growth rate maxima precede respiration rate maxima. In the absence of sucrose, change in length does not of itself prevent the falling off of both respiration rate / sample and respiration rate / unit length. Therefore a length increase must be accompanied by the presence of sufficient respiratory substrate to have a marked increasing power over the

respiration rate. It is tentatively suggested that when elongation takes place it is not accompanied or initiated by any detectable increase in respiration rate: however, such elongation is accompanied by increase in respiratory centres. In the presence of a substrate these may be utilised giving a detectable increase. This is supported by the fact that the initial respiration rate is higher in sucrose than in glass-distilled water - i.e. before any growth has taken place. Also it is to be noted that the respiration rate / unit length is higher in sucrose than in its absence.

LONG SECTIONS

Respiration rates and change in length was followed over the initial 6 hours only. The following results were obtained (these are based on 5 samples of 12).

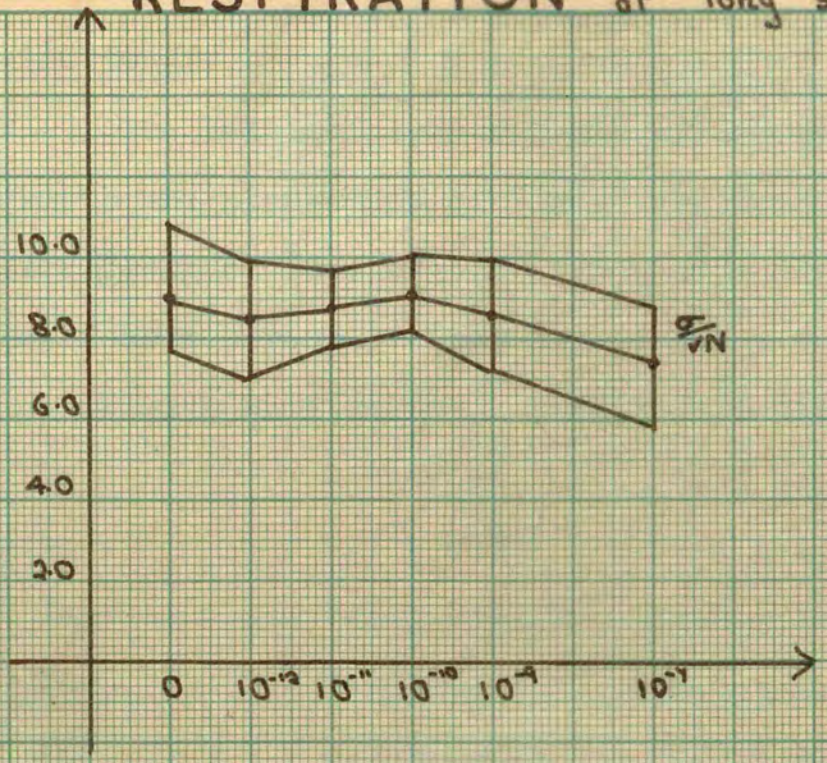
	0 sucrose	0.5% sucrose
Respiration rate / sample of 12 as mean over 6 hours.	9.7 ± 0.72	9.1 ± 1.41 c.mm.
original length	3.39 ± 0.05	mm.
Final length after 6 hours.	4.07 ± 0.36	4.29 ± 0.22 mm.

As can be seen above, 0.5% sucrose has no effect on the initial respiration rate or the initial growth rate of these sections. Since these "long" sections undoubtedly include cells which have passed their maximum rate of elongation, it seems that sucrose is less important in the late elongating or maturing phases of the cell.

EFFECTS OF AUXIN

Graph 14

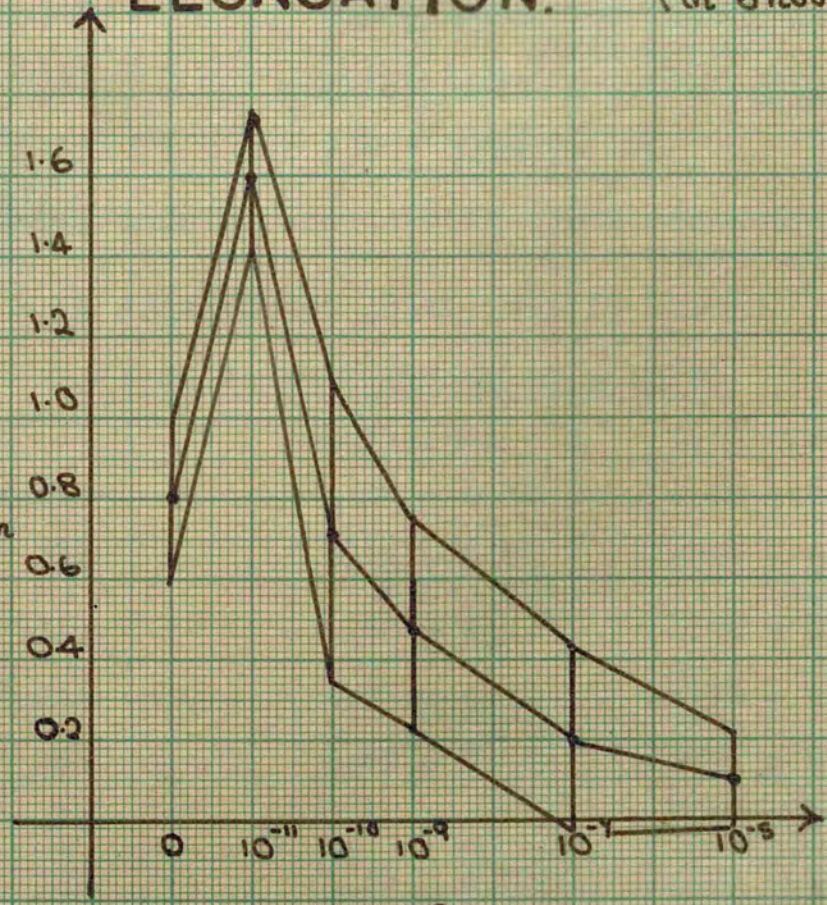
RESPIRATION of "long" sections. 0.5% SUCROSE



c mm oxygen /15 mins. per sample.

Means over 6 hour period, of 4 experiments.

ELONGATION. (in 6 hours)



mm elongation in 6 hours.

conc. of β indolyl acetic acid.

LONG SECTIONS

The results given for the effect of sucrose on the growth and elongation of "long" sections were part of a series designed to show the effect of auxin over an initial 6 hour period. They are given in full below.

INITIAL RESPIRATION / SAMPLE / 15 minutes

	0 sucrose	0.5% sucrose
0 auxin	9.7 ± 0.72	9.1 ± 1.41 c.mm.
10^{-12} IAA	7.1 ± 0.41 *	8.6 ± 1.56
10^{-11} IAA	7.4 ± 0.74 *	8.8 ± 1.02
10^{-10} IAA	8.5 ± 0.81	9.2 ± 1.55
10^{-9} IAA	8.9 ± 1.06	8.6 ± 1.26
10^{-7} IAA	-	7.5 ± 0.50
10^{-5} IAA	7.0 ± 1.84	-

IAA = indolyl acetic acid (note concentrations actual not molal.)

* = significant inhibition by auxin.

FINAL MEAN LENGTH AT 6 HOURS

	0 sucrose	0.5% sucrose
0 auxin	4.07 ± 0.36	4.29 ± 0.22
10^{-12} IAA	4.10 ± 0.21	4.34 ± 0.33
10^{-11} IAA	4.30 ± 0.11	5.00 ± 0.18 **
10^{-10} IAA	4.06 ± 0.10	4.11 ± 0.49
10^{-9} IAA	4.20 ± 0.10	3.87 ± 0.27
10^{-7} IAA	-	3.60 ± 0.23 *
10^{-5} IAA	3.52 ± 0.03*	3.51 ± 0.12 *

** = significant stimulation by auxin

* = significant inhibition by auxin

Even in as short a period as 6 hours the effect of auxin on growth can be fully represented. It is seen that in the presence of sucrose 10^{-11} IAA causes a significant stimulation of growth, whereas higher concentrations are definitely inhibitory. In sucrose too, it can be seen that auxin has no effect on the initial respiration rate. This is certainly a different result from that obtained with Avena coleoptile segments (see introduction).

With "long" sections and the short time period of 6 hours the auxin effect is not fully represented in the absence of sucrose. Since substrate is limiting the growth reactions, it is not surprising that it is not obtained. The effect has however been obtained, in the absence of sucrose, using longer time periods and shorter sections. Again auxin has no significant effect on the respiration rate of these sections, but lowest concentrations are inhibitory to the order of 20%. It is noted that errors are high - especially in the presence of sucrose and may be due to:-

(a) the variation in uniformity between the five different batches of seedlings used.

(b) variability of "long" sections which are not completely uniform over their length.

(c) the limit of accuracy of the Warburg manometers. It is not possible to read these to more than one place of decimals - hence changes of the order of 5% would remain

undetected.

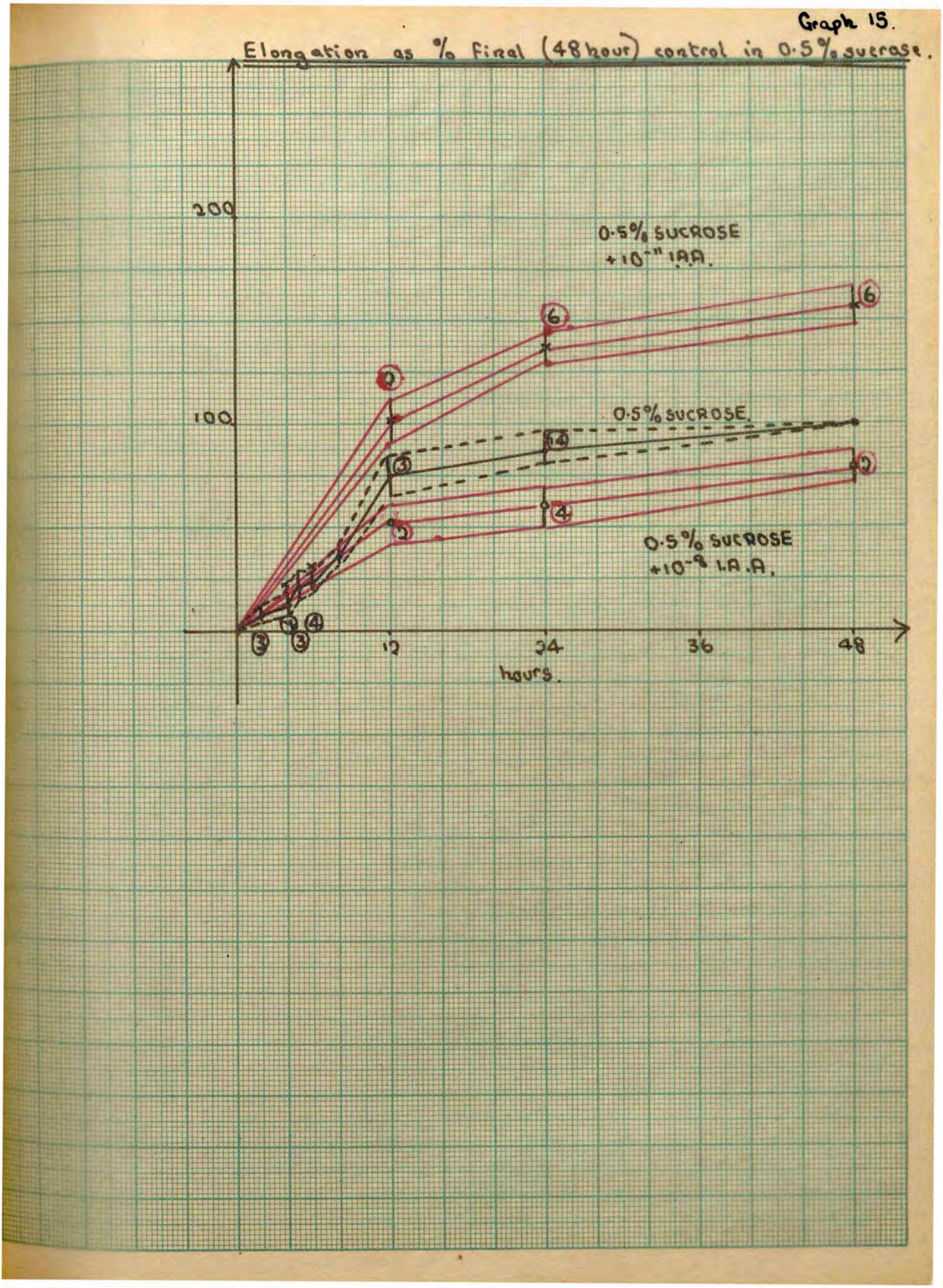
(d) high errors in sucrose may be due to differential responses of the different batches of seedlings, the initial sugar content of which may be slightly different.

These effects are not exclusive to indolyl acetic acid. Using the more stable substance 2.4 dichlorophenoxy-acetic acid, the following results are obtained:-

Respiration rate/sample	0 sucrose	0.5% sucrose
0 auxin	8.1 ± 0.92	8.6 ± 1.25
10^{-11} 2.4.D	9.5 ± 0.85	8.3 ± 1.54
10^{-10} 2.4.D	8.8 ± 1.50	9.4 ± 0.86
10^{-9} 2.4.D	8.4 ± 0.99	7.0 ± 0.89
10^{-5} 2.4.D	6.9 ± 0.26	4.7 ± 1.43*
Mean length		
0 auxin	4.07 ± 0.36	4.29 ± 0.22
10^{-11} 2.4.D	4.09 ± 0.47	5.66 ± 0.11**
10^{-10} 2.4.D	4.01 ± 0.31	3.72 ± 0.04*
10^{-9} 2.4.D	3.76 ± 0.33	-
10^{-5} 2.4.D	3.59 ± 0.05*	3.35 ± 0.01

(~~see graph~~ ~~and~~)

Graph 15.


Elongation as % final (48 hour) control in 0·5% sucrose.
200
100
0·5% SUCROSE
+10⁻¹¹ I.A.A.
0·5% SUCROSE.
0·5% SUCROSE
+10⁻⁹ I.A.A.
12
24
36
48
hours.

SHORT SECTIONS

Typical experiments on the growth and respiration rates of short sections have been previously represented. Pooling all results on this topic the following is obtained:-

Effect of auxin on total elongation (means of samples of 25)

NO SUCROSE

	Control	10^{-11} IAA	Ratio/control	10^{-8} IAA	Ratio/control
12 hrs.	2.31	2.46	1.07	-	
	2.72	3.06	1.13	2.78	1.02
	3.12	-		2.75	0.88
24 hrs.	2.89	-		-	
	2.73	2.99	1.09	-	
	3.14	3.52	1.12	3.32	1.06
	3.15	-		2.68	0.86
	2.82	3.28	1.16	-	
	3.02	-		2.94	0.98
	3.19	-		3.15	0.99
	2.41	2.84	1.18	-	
30 hrs.	3.21			3.10	0.96
48 hrs.	3.32	-		-	
	2.79	2.97	1.07	-	
	3.39	3.40	1.01	3.44	1.01
	3.21	-		2.65	0.82
	2.92	3.19	1.10	-	
	3.12	-		-	
	3.55	-		2.87	0.81
	2.86	-		-	

0.5% SUCROSE

	Control	10^{-11} IAA	Ratio/ control	10^{-8} IAA	Ratio/ control
12 hours	3.65	4.48	1.29	-	
	4.29	4.24	0.99	3.64	0.84
	4.13	-		2.77	0.67
24 hours	4.50	-		-	
	4.26	5.57	1.31	-	
	6.57	5.93	0.91	5.31	
	4.52	-		-	
	5.00	-		-	
	5.27	-		-	
	4.48	5.80	1.30	-	
	3.96	-		-	
	4.61	5.08	1.10	-	
	3.53	4.46	1.26	-	
	4.45	-		-	
	4.52	-		-	
	4.20	-		3.36	0.80
	4.27	-		4.20	0.98
	3.58	4.38	1.23	2.85	0.80
48 hours	4.37	5.59	1.28	-	
	4.53	7.41	1.63	5.38	1.19
	5.00	5.32	1.06	-	
	5.53			4.50	0.81
	4.95	6.03	1.22	-	
	3.90	4.23	1.08	-	
	5.46	5.41	1.00	-	
	4.84	-		2.76	0.58
	4.59	-		4.13	0.90
	4.97	-			
	5.23	-			
	5.13	-			
	5.21	-			
	3.99	-			
	4.89	-			

INITIAL RESPIRATION RATE

0 SUCROSE	Control	10^{-11} IAA	Ratio/ control	10^{-8} IAA	Ratio/ control
	8.8	8.8	1.0	8.0	0.91
	10.4	-		11.5	1.11
	8.8	8.8	1.0	-	
	10.0	11.0	1.1	11.3	1.13
	9.8	8.7	0.89	-	
	8.0	-		8.0	1.00
	10.5	10.0	0.95	10.5	1.00
	10.2	10.3	1.01	10.0	0.98
	8.9	-		-	
0.5% SUCROSE					
	11.0	9.0	0.82	9.0	0.82
	12.5	-		11.9	0.95
	11.0	9.0	0.82	-	
	9.3	10.5	1.13	11.1	1.19
	10.9	9.0	0.82	8.9	0.82
	9.9	9.6	0.97	9.3	0.94
	11.1	10.9	0.98	10.6	0.95
	10.0	10.0	1.00	10.0	1.00
	10.1	-		7.5	0.74
	10.8	10.0	0.93	-	
	11.7	-		8.5	0.55
	11.0	-		11.0	0.85
	9.0	9.7	1.08	10.5	0.95
	12.0	12.0	1.00	-	
	10.1	-		10.2	1.01
	15.0	14.5	0.97	12.0	0.80

RESPIRATION RATES / SAMPLE to show auxin effect

NO SUCROSE	Control	10^{-11} IAA	Ratios/ control	10^{-8} IAA	Ratios/ control
12 hours	8.9	9.0	1.02	7.6	0.87
	8.0	-		7.6	0.95
	8.7	11.0	1.26	8.0	0.92
	9.0	9.8	1.09	6.7	0.74
24 hours	8.1	8.5	1.05	6.9	0.85
	10.8	-		10.9	1.01
	10.3	11.2	1.09	10.0	
	11.2	11.3	1.01	10.0	0.89
	9.7	10.8	1.11	-	
	9.9	-		9.5	0.96
	8.0			-	
30 hours	7.0	7.1	1.02	5.0	0.72
48 hours	8.3	3.4	0.48	4.0	0.48
	9.2	-		3.2	0.35
	7.0	4.7	0.67	-	
	9.0	-		8.0	0.89
	10.2	7.8	0.77	10.0	0.98
	7.6	-		-	
	7.5	-		1.5	0.20

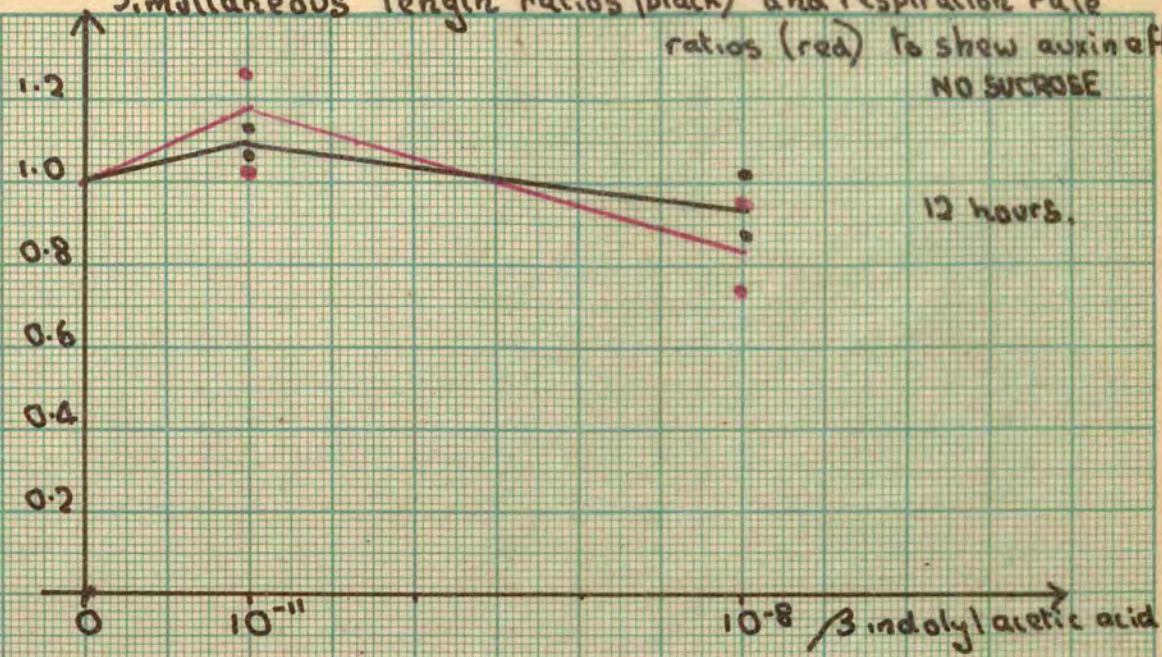
RESPIRATION RATES / SAMPLE

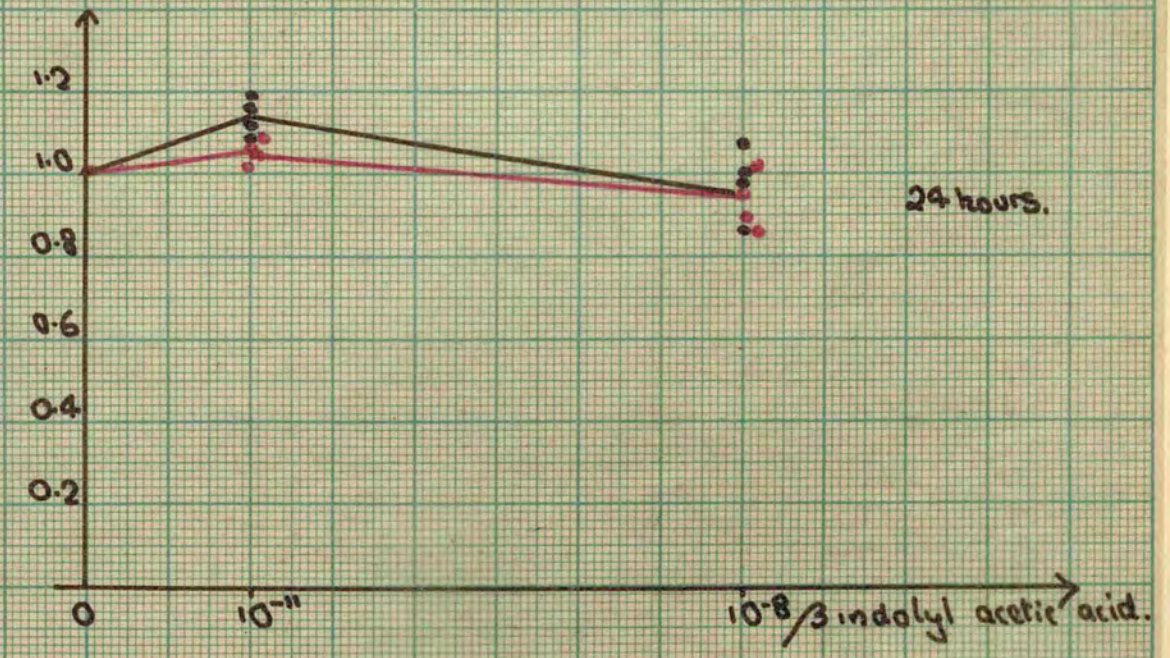
0.5% SUCROSE	Control.	10^{-11} IAA	Ratio/ control	10^{-8} IAA	Ratio/ Control
12 Hours	10.2	14.5	1.42	13.0	
	15.5	-	1.06	13.0	0.84
	18.2	19.2	1.06	17.7	0.97
	18.0	18.1	1.02	17.0	0.94
	15.0	17.8	1.19	-	
	10.0	11.0	1.10	-	
24 hours	17.8	19.7	1.11	10.3	0.58
	16.7	-		13.0	0.88
	15.4	20.4	1.30	-	
	1.0	23.7	1.13	18.4	0.87
	17.6	-		17.5	0.99
	22.0	27.5	1.25	19.3	0.87
	15.4	-		-	
	17.5	-			
	16.8	18.0	1.07	-	
	23.5	25.5	1.09	20.0	0.85
	19.0	-		-	
	13.0	-		-	
	12.5	14.5	1.17	-	
30 hours	12.7	16.0	1.26	13.4	1.06
	15.0	16.4	1.10	12.0	0.80
48 hours	12.7	7.2	0.57	3.0	0.23
	9.2	-		6.0	0.65
	7.7	12.5	1.66	-	
	13.6	8.5	0.62	10.7	0.88
	9.3	11.6	1.24	6.5	0.70
	6.5	-		-	
	5.0	-		-	
	5.2	6.0	1.16	8.0	1.40
	4.5	5.5	1.22	-	
	4.0	6.5	1.63	-	

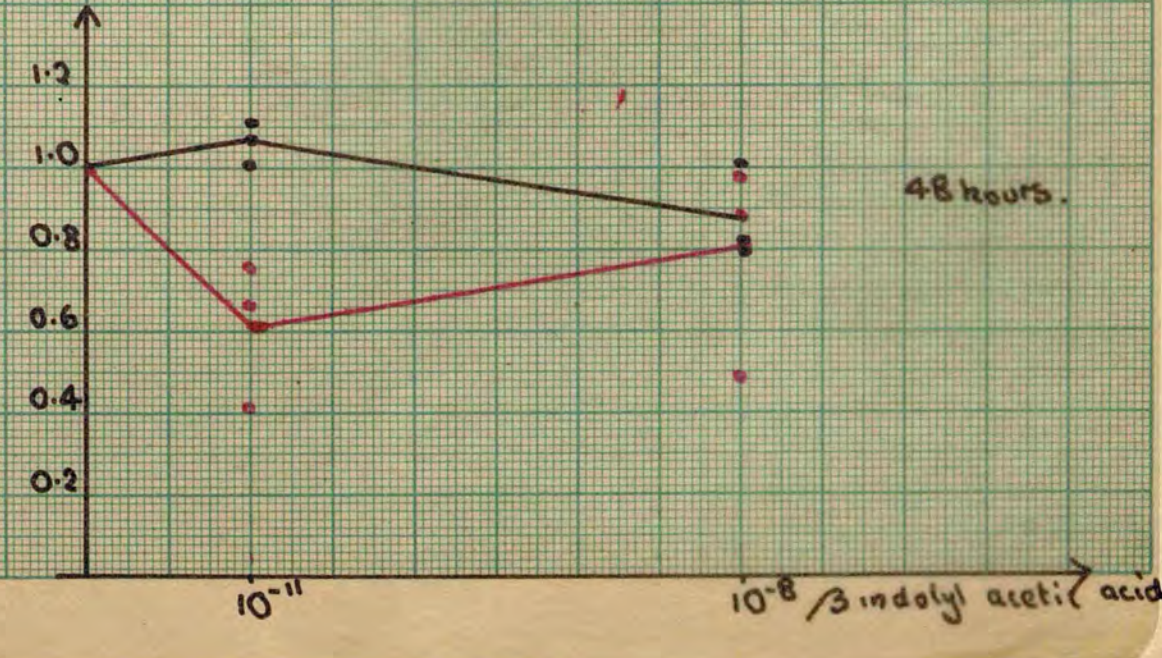
Graph 16.

Simultaneous length ratios (black) and respiration rate ratios (red) to show auxin effect.

NO SUCROSE



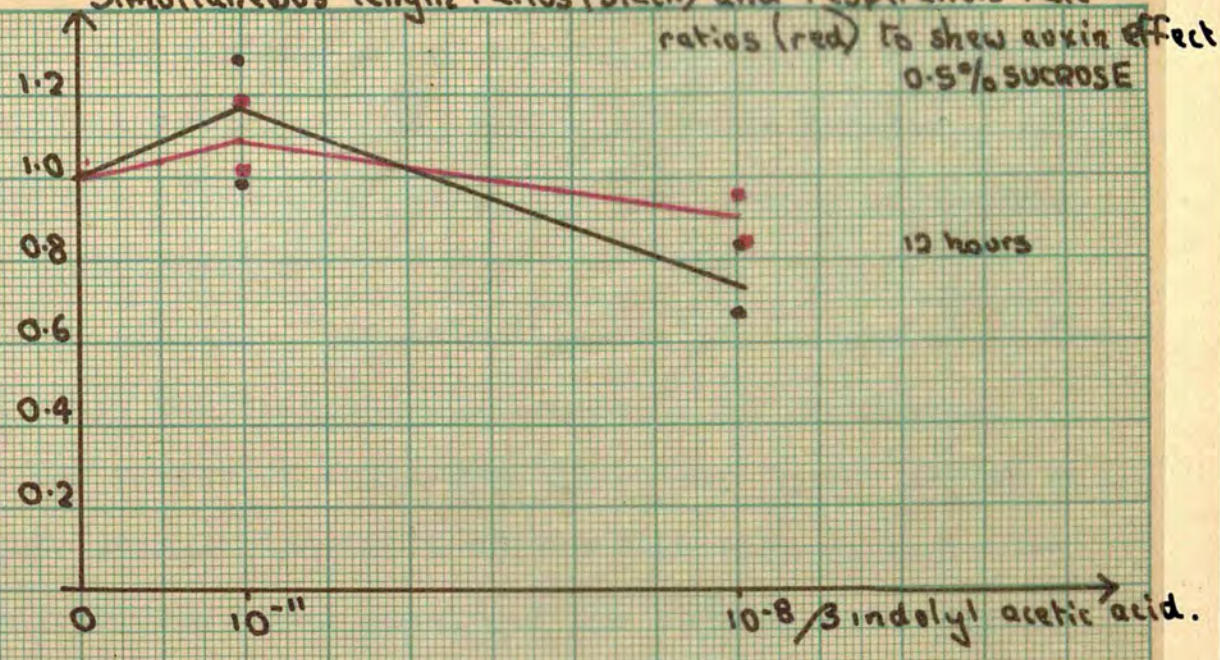




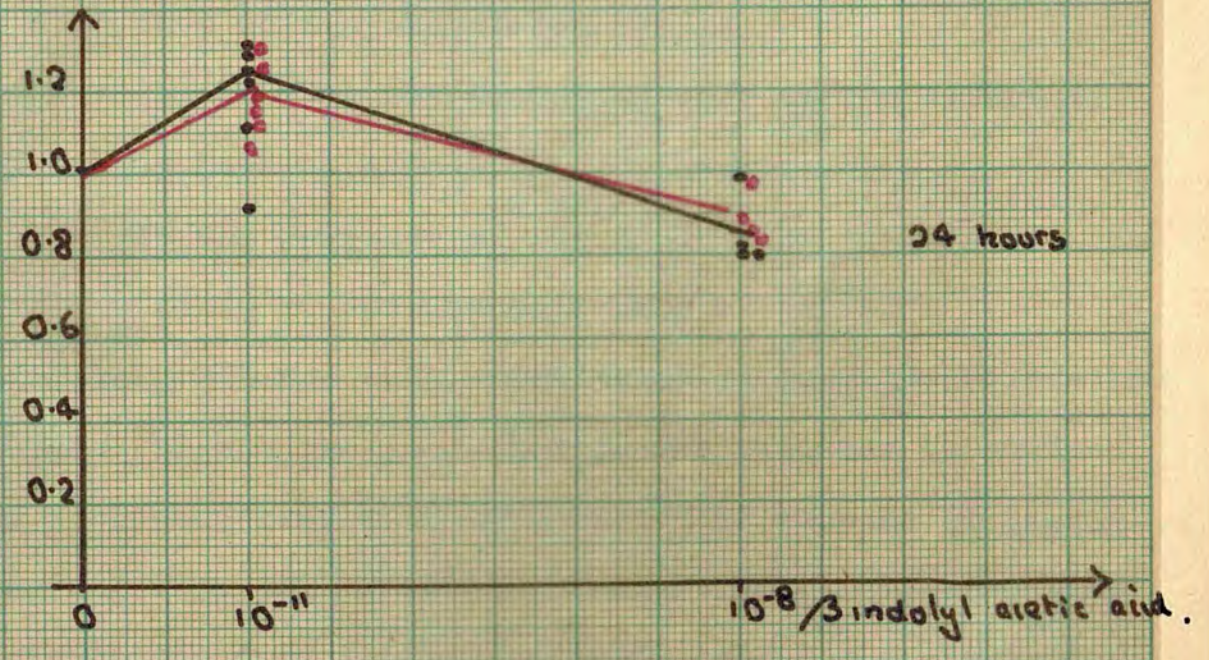
Graph 14

Simultaneous length ratios (black) and respiration rate ratios (red) to shew auxin effect.

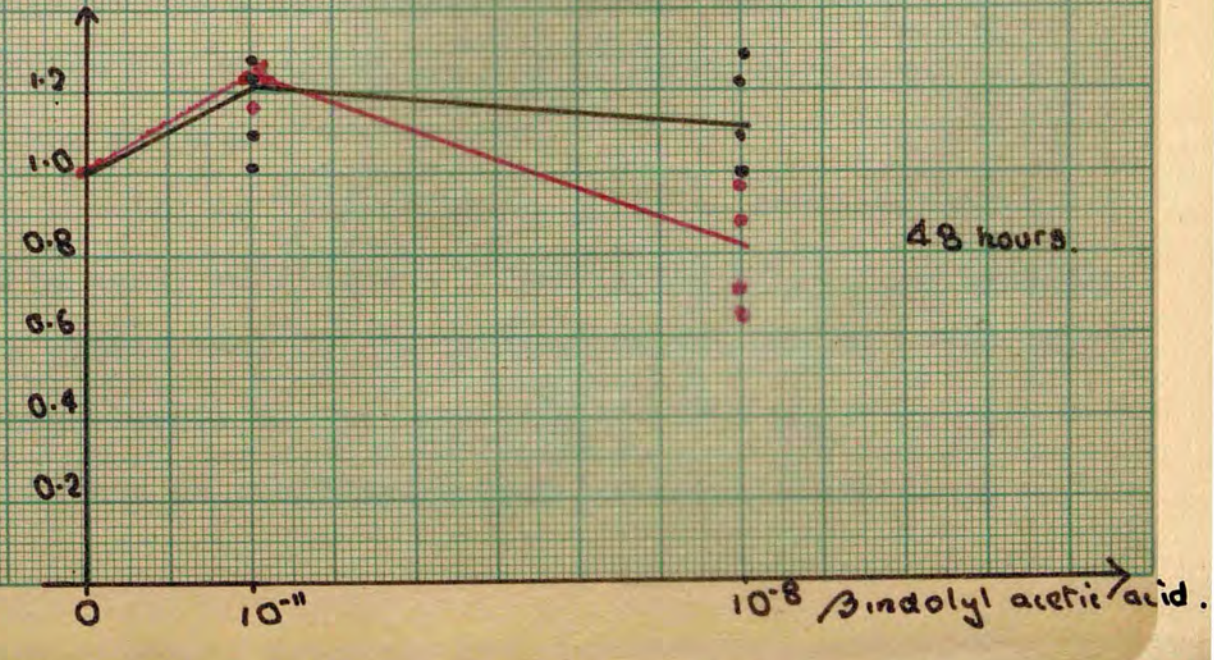
0·5% SUCROSE



12 hours



24 hours



48 hours.

Owing to the variability of batches the effect of auxin was best illustrated by taking ratios of auxin treated / control. Considering elongation ratios, in the absence of sucrose, it is seen that:

(a) 10^{-11}IAA stimulates to the extent of 10-20% in 24 hours but this stimulation falls off with time. Hence the effect of auxin here is really on the growth rate, and this stimulation falls off so that with an increased time-period the total length of the control may become equal with that of the stimulated sample.

(b) 10^{-8}IAA may be stimulatory to the extent of 10-14% or may have no effect.

In the presence of sucrose:-

(a) 10^{-11} stimulates to the extent of approximately 25% and this stimulation is normally maintained with time. Hence the increased growth rate due to auxin can be continued over a 48 hour period if sucrose is present. This agrees very well with the results for Avena coleoptile segments obtained by Schneider (1938), who found that elongation was dependent on a sufficient supply of <u>both</u> auxin and sucrose.

(b) 10^{-8}IAA causes a definite 20% inhibition which is again maintained. The action of sucrose here is on the growth rate of the control, which can be maintained at a high level.

With respect to respiration rate ratios - there is no

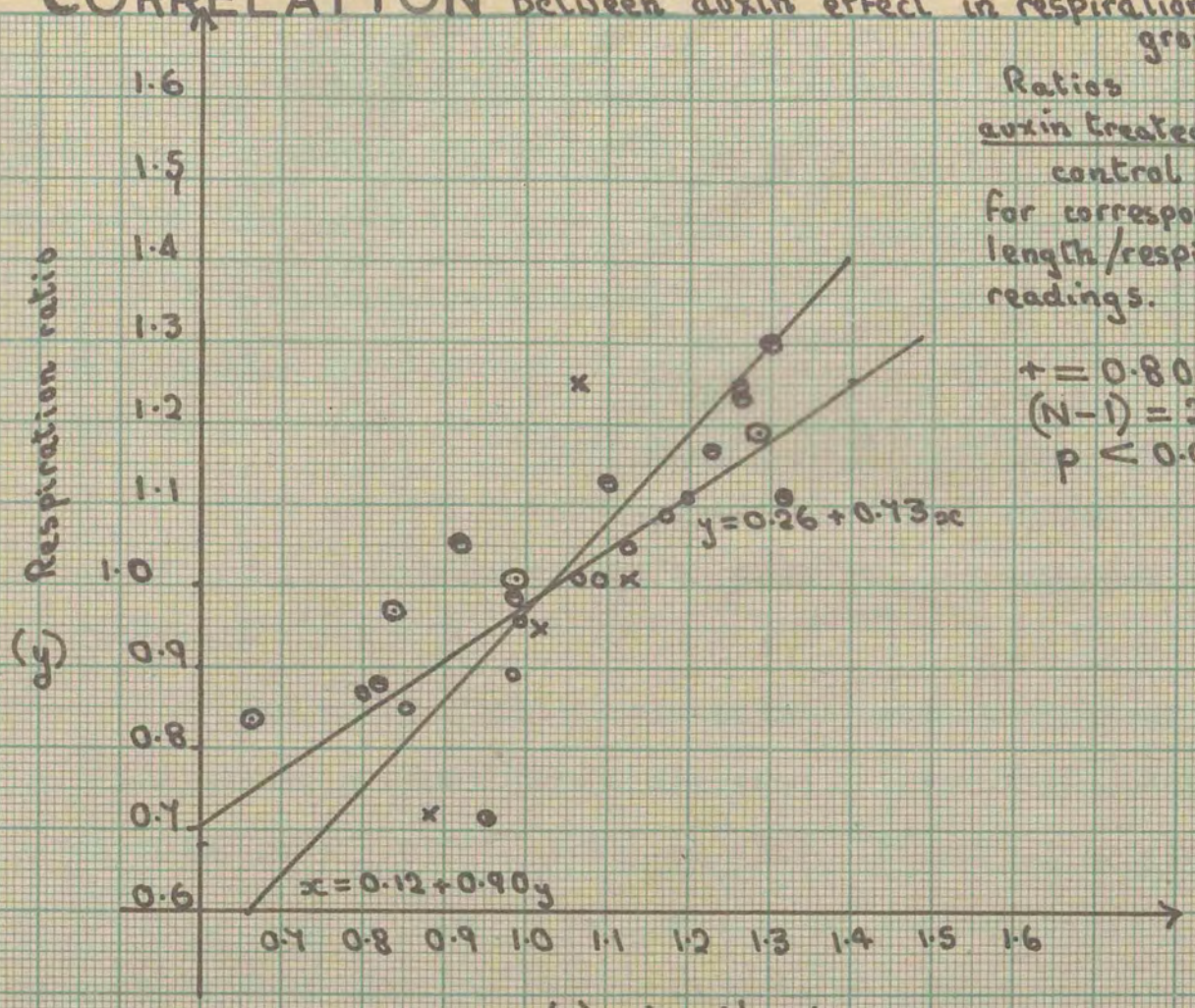
significant initial effect of auxin on respiration, unless both sucrose and 10^{-8} IAA is present when it is inhibitory. The following table giving the "t" values obtained in comparing the ratio means with the control (mean = 1.00), makes this clearer

	0 SUCROSE		
	0 auxin	10^{-11} IAA	10^{-8} IAA
Mean standard error	1.00	0.99 ± 0.03	1.03 ± 0.04
"t"		0.30	0.74
"p"		0.7-0.8	0.5-0.6
n	9	6	5
	0.5% SUCROSE		
	0 auxin	10^{-11} IAA	10^{-8} IAA
Mean and standard error		0.96 ± 0.03	0.92 ± 0.04
"t"		1.42	2.30
"p"		0.2-0.1	0.05
n	16	11	12

To establish the relationship between respiration rate and length ratios, the graphs (14-17) are invaluable in showing the main trends. There seems to be some correlation between the auxin effect on change in length and its effect on respiration rates, except at the final interval, 48 hours in the absence of sucrose. Pairing corresponding readings for length and respiration, a correlation coefficient can be obtained. Using the ratios mentioned above (note for growth expression length not elongation

Graph 18

CORRELATION between auxin effect in respiration and growth.



Ratios
auxin treated
control
for corresponding length/respiration readings.

$r = 0.8098$
$(N-1) = 26$
$p < 0.001$

× No sucrose 12 hrs.
○ No sucrose 24 hrs.
● No sucrose 36 hrs.
⊙ ½% " 12 hrs.
◉ ½% " 24 hrs.

ratios were used) a correlation coefficient of r = 0.8098 was found, omitting the puzzling 48 hour results. Since N-1 = 26, and hence P = 0.001, there is highly significant correlation between the auxin effect on the change in length of the section and that on change in respiration. By using ratios we have largely eliminated the time effect.

The scatter diagrams plotting growth ratios as abscissa and respiration ratios as ordinate are interpretable as follows:-

(a) added auxin in an optimal concentration of 10^{-11}IAA causes a simultaneous increase in growth rate and respiration rate / sample over and above the normal drifts of these values. This is represented on the scatter diagram in that both the ratios tend to be simultaneously greater than 1.00.

(b) when auxin has no effect (as in 10^{-8}IAA in the absence of sucrose), the scatter points are concentrated on the point (1.1).

(c) when auxin is inhibitory to both growth and respiration both ratios are less than 1.00.

The scatter shows linear regression with both regression lines of positive slope. Since auxin does not alter the form of growth rate and respiration rate / sample, but simply increases or decreases the maximum rates the same thesis as has been put forward for these processes in the

absence of added auxin can be held. It must be emphasised that the maximum for growth rate precedes that for respiration rate / sample. If we are to accept Went's tenet that without auxin no growth is possible, the growth rate curve of the control is an auxin induced curve. The maximum for growth rate (at 12 hours in 0.5% sucrose) may be increased by 10^{-11}IAA or decreased by 10^{-8}IAA. The respiration rate / sample may also be increased or decreased by auxin; but occurs not at 12 hours but at 24 hours. Hence it seems that the auxin effect on growth precedes that in respiration.

Growth is not caused by an increase in respiration (as the American workers believe to be the case for Avena coleoptile tissue) but WHEN GROWTH HAS TAKEN PLACE respiration may be increased by virtue of the increase in length.

This is further substantiated by the fact that auxin has no initial effect on respiration (except in 10^{-8} plus sucrose) when the lengths of the sections are the same.

It is to be concluded that auxin has no basic effect on the respiration rate.

(1) It certainly has no stimulatory effect initially.

(2) Its effect at 12 or 24 hours is paralleled by its stimulation or inhibition of the growth ~~rate~~ of the

sections. Since respiration rate / unit length falls with time, irrespective of the presence or absence of added auxin it seems that the primary effect is on the change in length which may, in turn, be reflected on the respiration rate / sample. The effect may be sufficiently great to prevent, to some extent, the normal fall away of respiration rate / unit length (see 12 - 24 hours in 0.5% sucrose).

AUXIN HAS NO EFFECT ON THE RESPIRATION OF THIS TISSUE WHICH CANNOT BE FULLY ACCOUNTED FOR BY LENGTH CHANGES.

EFFECT OF ARSENATE

Effect of arsenate

Many research workers (see introduction) have found auxin causes an initial increase of the respiration of Avena tissue of the order of 25-30%. This stimulation was entirely removed in the presence of arsenate ions. Hence arsenate is regarded by Bonner (1950) as a selective inhibitor of auxin action - since it inhibits only this stimulated part of the total respiration together with the whole of growth. (At approximately 100 mg. / litre).

We have not found auxin action in growth to be accompanied by any initial respiratory rise in root section material BUT auxin still has an action on growth similar to that observed in Avena sections. It was thought that some light may be thrown on the differences in response in stem and root tissue by the use of arsenate as an inhibitor.

It should be noted that Bonner obtained respiration rates for the period 2-3 hours after cutting, whereas he obtained a measure of growth by measuring the sections after 24 hours. This is a very unwise procedure -

(a) as will be shown later, arsenate penetration is not immediate and hence the respiration rates measured may correspond to a state of incomplete penetration - the concentrations inside and outside the cells not having reached an equilibrium state. The lengths on the other hand will correspond to cells which have undergone this initial period of a few hours plus a much longer period when

there has been complete penetration.

(b) it is unlikely that the growth rate will remain constant over a 24 hour period. Also if it can be assumed that the respiration rate of Avena sections follows a similar time course as do root sections, the respiration rate is not constant over this period.

From his results Bonner deduced that respiration and growth are correlated in the following way.

(1) no growth takes place in the absence of added (or natural) auxin. 10^{-6} indolyl acetic acid causes a stimulation of the normal respiration of 25-30% and induces the growth of the sections.

(2) arsenate ions remove the 25-30% stimulation by auxin and inhibit the auxin induced growth.

(3) Bonner believes that growth is controlled by the 25-30% respiration which is removed in the presence of arsenate.

Now according to Needham and Pillai (1937) arsenate prevents the accumulation of phosphoric esters of glucose during fermentation of this carbohydrate by yeast. Without arsenate the breakdown of hexose-diphosphate is slow. Two possible mechanisms of arsenate action have been suggested.

(a) that of Harden and Young that arsenate activates a hexose-diphosphate-phosphatase with the continuous formation of free phosphoric acid. As has been

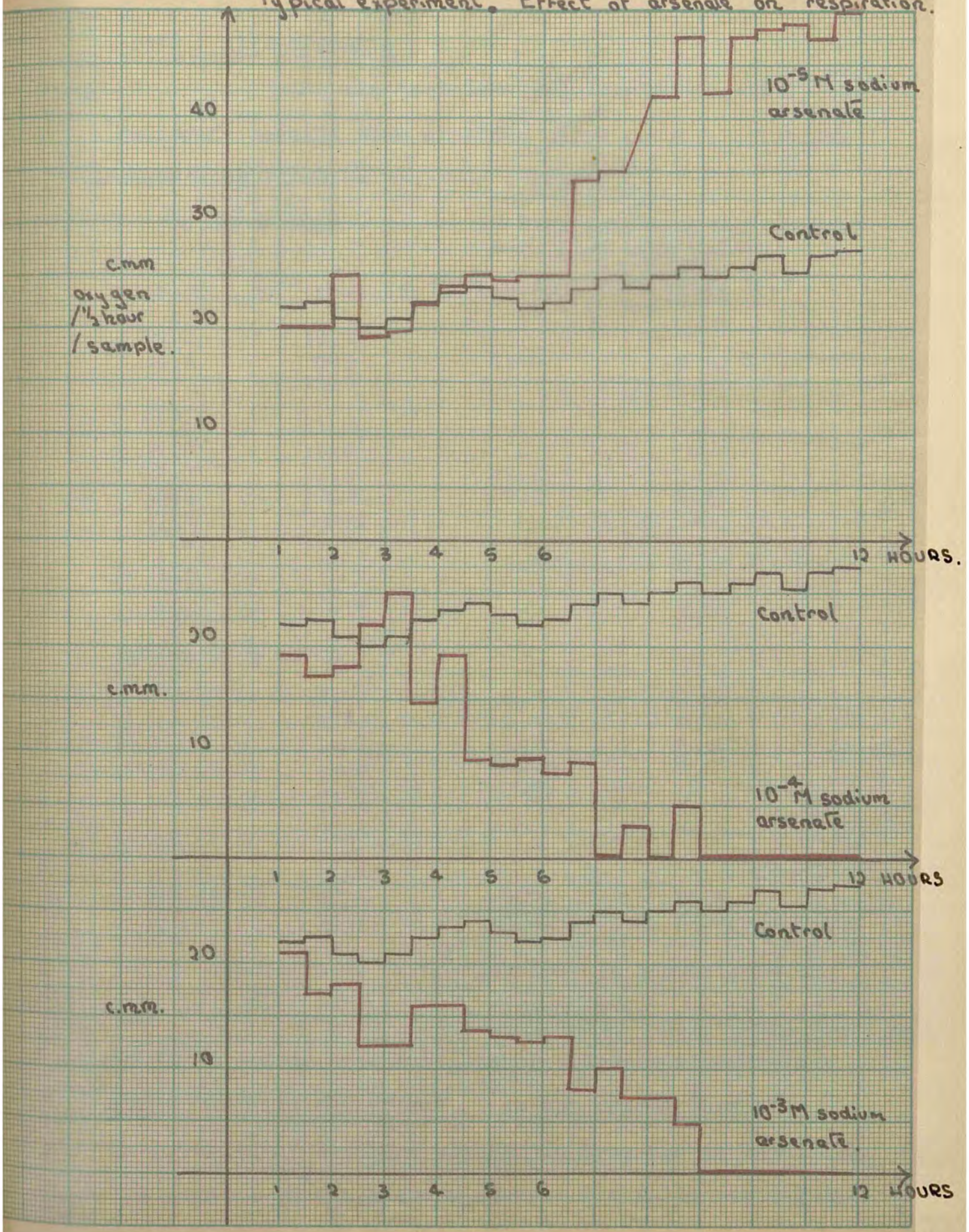
pointed by Pett and Wynne phosphatases are normally inhibited by arsenate, but there is an exception to this in the form of adenyl-phosphatase (Meyerhof and Kiessling, 1936).

(b) the last fact brings us to the theory of Warburg and Christian (1939) that arsenate forms an intermediate compound with 3-phosphoglyceric aldehyde. This compound 1-arseno 3-phosphoglyceraldehyde immediately forms the glyceric acid derivative, which is very unstable and breaks down in aqueous solution giving 3-phosphoglyceric acid. Normally 3-phosphoglyceric acid is only formed by a coupling reaction between 1 3-diphosphoglyceric acid and the Adenosine - diphosphate (ADP) / Adenosine triphosphate (ATP.) mechanism.

1 3-diphosphoglyceric acid does not undergo spontaneous decomposition in solution unlike the compound, 1-arseno 3-phosphoglyceric acid. Therefore in the absence of arsenate the ADP$\rightleftharpoons$ATP mechanism is essential for the continuation of the respiratory processes. When uncoupling occurs the cells become depleted of ATP since it is soon all dephosphorylated in the reverse ATP$\rightarrow$ADP reaction. If a supply of ATP is an essential energy source for growth, this may account for the differential action of arsenate. For a time at least the supply of 3-phosphoglyceric acid remains normal and respiration can continue unhindered.

Graph 19.

Typical experiment. Effect of arsenate on respiration.



Results with arsenate

Using the technique previously described, the following results were obtained using neutral solutions containing disodium hydrogen arsenate.

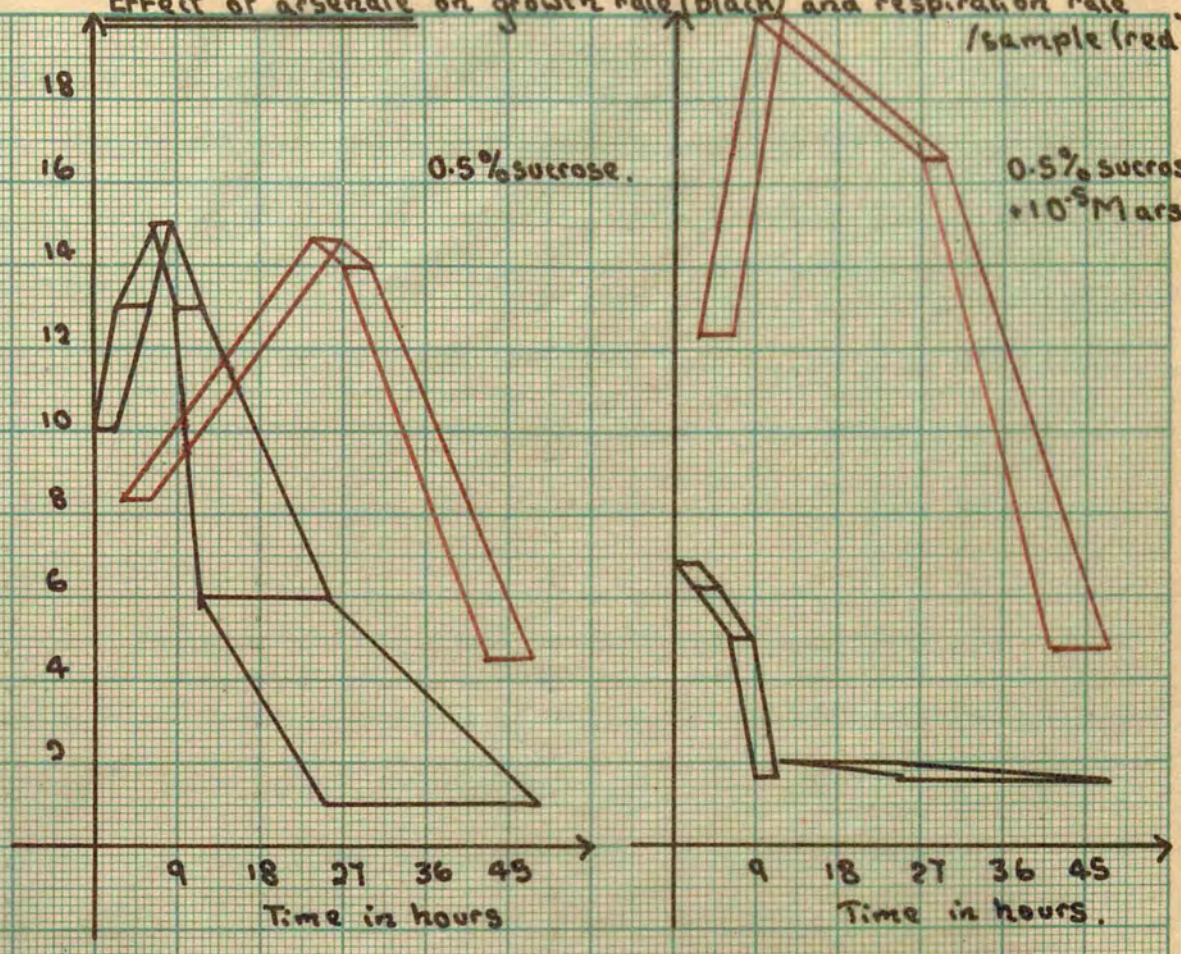
0.5% SUCROSE - no auxin. "INITIAL" RESPIRATION RATE (3-5 hour period)

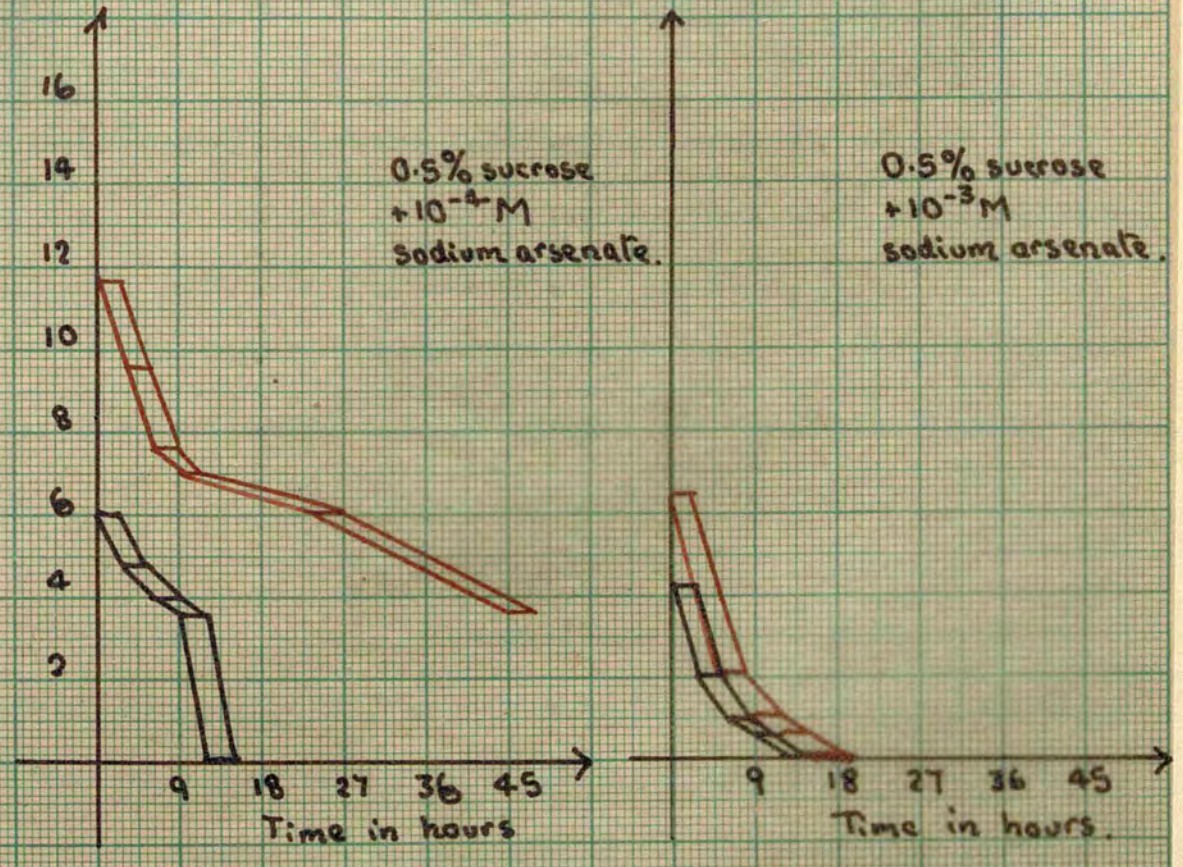
Control	10^{-5} arsenate	10^{-4}M	10^{-3}M
10.5	10.2	-	-
15.5	8.5	11.0	9.5
	11.0	-	-
10.8	-	-	-
11.0	11.0	7.5	-
17.9	13.9	-	-
10.0	12.0	11.6	-
13.3	14.4	9.7	8.5
8.5	10.0	-	-
7.5	10.0	-	-
13.0	-	9.0	6.4
11.7	-	8.6	-
11.3	-	-	7.5
Ratios to control			
	0.97	0.71	0.60
	0.55	0.68	0.64
	1.02	1.16	0.49
	1.00	0.73	0.67
	0.78	0.69	
	1.20	0.74	
	1.09		
	1.18		
	1.33		
TOTAL	9.12	4.11	2.40
Mean	1.01	0.68	0.60

While it is not possible to demonstrate in this tissue that the respiration rate is initially changed by 10^{-5}M sodium arsenate, higher concentrations have a highly significant effect. As the graphs show the respiration of sections emersed in arsenate is initially the same as in the sucrose control but falls rapidly in

Graph 20.

Effect of arsenate on growth rate (black) and respiration rate /sample (red.)





inhibitory concentrations reaching a new level. This new level, which was established after a period of approximately 3 hours, was taken to be "initial respiration rate" of these sections. In view of the variable time of penetration of the lowest concentration used it was extremely difficult to obtain any consistant results, hence the same period (3-5 hours after cutting and treatment) was taken as representative.

Bonner (1950) failed to find any effect of arsenate on the respiration of Avena sections in the absence of auxin. He used a concentration range of 10^{-5}-10^{-4}M. His procedure differed from ours in several important respects:-

(A) the Avena sections were grown in 2% sucrose in a 0.0025 maleate buffer at p H 4.5. Our sections were in an unbuffered solution of 0.5% sucrose at pH 7.0.

(B) Bonner gives no indication that penetration is complete over the period he has studied.

Rietsema (1949) has found using critical dye methods that external pH has very little effect on the internal pH. Hence whether arsenate acts differently with different internal pH is not likely to concern us here. The most likely effect is on the rate and extent of penetration of the ions or molecules. Our solutions at pH 7.0 will certainly contain more arsenate ions and less sodium arsenate molecules than a solution at pH 4.5.

Due to the charged nature of cell membranes one would expect a greater penetration if the substance is present in the uncharged state, as molecules. It is therefore to be expected that the effect of arsenate would be greater at a lower pH. However Bonner finds arsenate does not inhibit the respiration of Avena even at a concentration of 10^{-3}M at pH 4.5. We obtain inhibition of the respiration of our root sections at pH 7.0 with a concentration of 10^{-4}M. It must be concluded that the effect of arsenate is different in root tissue.

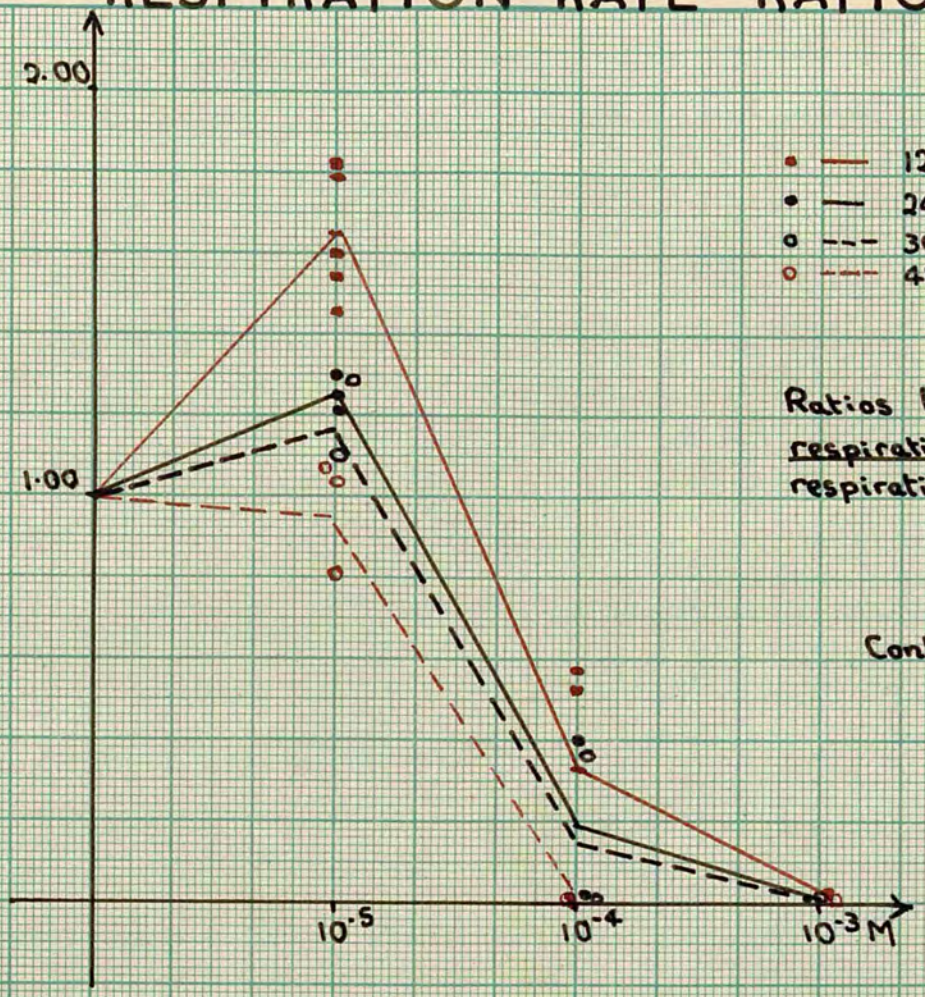
EFFECT OF ARSENATE ON THE RESPIRATION RATE ctd.

0.5% SUCROSE.	Control	10^{-5}M arsenate	10^{-4}M arsenate	10^{-3}M arsenate
12 hours	15.0	24.0	0.0	0.0
	11.0	20.0	6.3	0.0
	9.5	17.3	5.0	0.0
	10.0	14.5		
	9.1	14.0		
24 hours	11.0	14.0	0.0	0.0
	15.4	20.0	6.0	0.0
	13.0	16.0		0.0
30 hours	12.7	14.0	0.0	0.0
	14.0	18.0	5.0	0.0
48 hours	10.0	10.5	0.0	0.0
	11.6	2.5		0.0
	6.0	4.8	3.6	0.0
	5.5	6.0		
	6.5	13.4		

It is evident that, whereas 10^{-4} and 10^{-3} molal solutions of sodium arsenate in time completely inhibit the respiration of these root sections, 10^{-5}M has a highly stimulatory effect.

Graph 21.

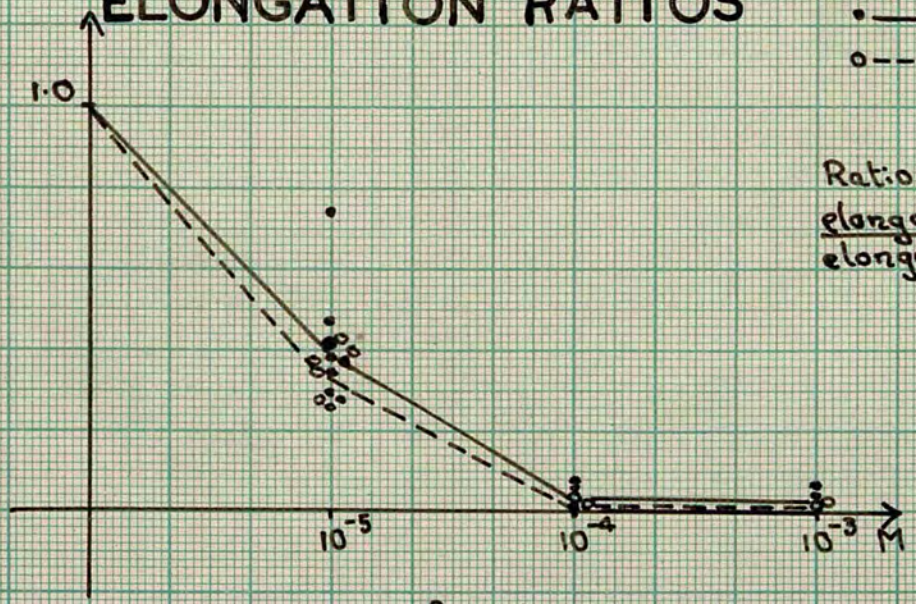
RESPIRATION RATE RATIOS.



Ratios taken
respiration rate in arsenate,
respiration rate in control.

Control 0.5% sucrose.

ELONGATION RATIOS



Ratios taken
elongation in arsenate
elongation in control.

conc. of sodium arsenate.

Since Warburg and Christian have shown the breakdown of the arseno compound of phosphoglyceric acid is more rapid than the normal 1 3 diphosphoglyceric acid⇌ATP⇌ADP mechanism an initial stimulation would be explainable. However it seems unlikely that this would persist for 24-30 hours. Low concentrations of inhibitors frequently stimulate respiration rate and the mechanism is often obscure.

The stimulation by 10^{-5}M sodium arsenate does not occur unless sucrose is present. The results above were in 0.5% sucrose and the following results are for other concentrations:-

	0 SUCROSE	+10^{-5}M arsenate	+10^{-4}M arsenate	+10^{-3}M arsenate
0 hours	11.5	11.5	0.0	0.0
12 hours	9.5	8.0	0.0	0.0
24 hours	8.0	8.0	0.0	0.0
30 hours	7.5	7.5	0.0	0.0
48 hours	7.6	7.7	0.0	0.0
	2% SUCROSE			
0 hours	11.0	20.0	0.0	0.0
12 hours	12.0	25.0	0.0	0.0
24 hours	18.9	20.0	0.0	0.0
30 hours	13.9	11.0	0.0	0.0
48 hours	4.3	1.0	0.0	0.0
	10% SUCROSE			
0 hours	10.5	10.0	0.0	0.0
12 hours	12.5	9.0	0.0	0.0
24 hours	13.0	12.5	0.0	0.0
30 hours	13.5	11.0	0.0	0.0
48 hours	15.0	10.0	0.0	0.0

2% sucrose increases the stimulation to greater than 100%, whereas in 10% sucrose the arsenate is inhibitory. 10% sucrose is hypertonic to the plant cell sap, a condition

Each point is mean of 25 section lengths.

which will involve a large number of new physiologic factors. A different type of result is not unexpected.

Apart from sucrose the stimulation is considerably reduced by the presence of auxin of a concentration which is normally inhibitory or normally stimulatory to growth, whereas Bonner found auxin had a stimulatory effect on respiration rate which can be eliminated in the presence of arsenate; we find that auxin has no significant effect on the respiration rate not attributable to change in length and in the presence of auxin the arsenate effect is ~~increased~~ cut out.

INITIAL RESPIRATION RATE/SAMPLE

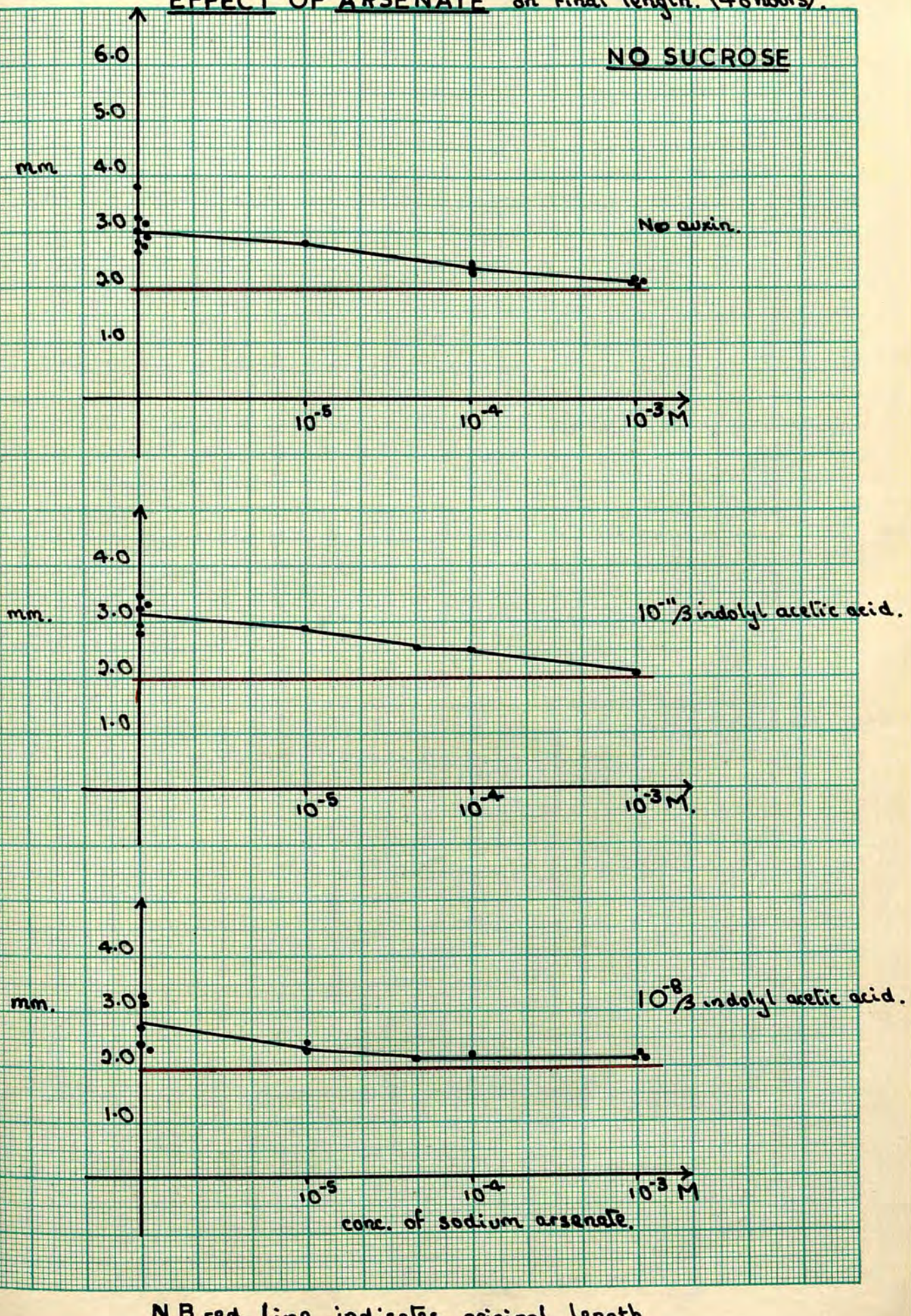
Medium 0.5% sucrose

	$+10^{-11}$ IAA	$+10^{-11}$ IAA $+10^{-5}$ M ARSENATE	$+10^{-8}$ LAA	$+10^{-8}$ IAA $+10^{-5}$ M ARSENATE
	12.0	11.0	8.0	8.0
	13.0	12.0	9.8	8.4
	13.4	9.1		
	10.0	7.5		
	12.0	10.5		
	9.7	7.7		
12 hours	9.8	12.7	7.6	8.0
	11.0	13.0	8.0	8.4
	9.8	14.0	6.7	7.0
24 hours	25.5	22.7	20.0	19.0
	20.4	19.0	18.4	15.4
30 hours	14.5	15.0		
	16.0	17.0	13.4	14.0
48 hours	11.6	12.7	6.5	5.0
	6.0	6.0	8.0	10.3

Each point is mean of 25 section lengths.

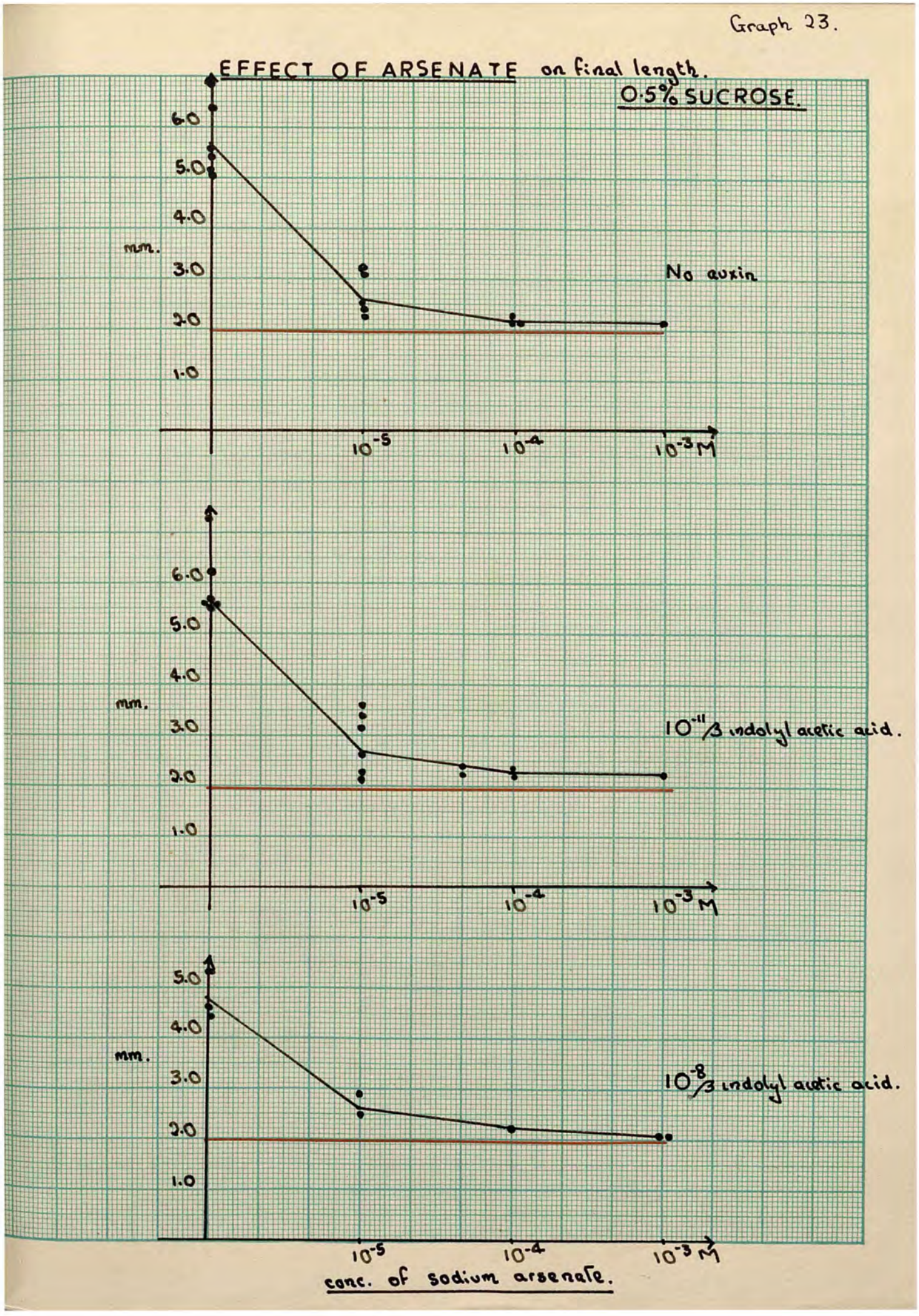
Graph 22

EFFECT OF ARSENATE on final length. (48 hours).



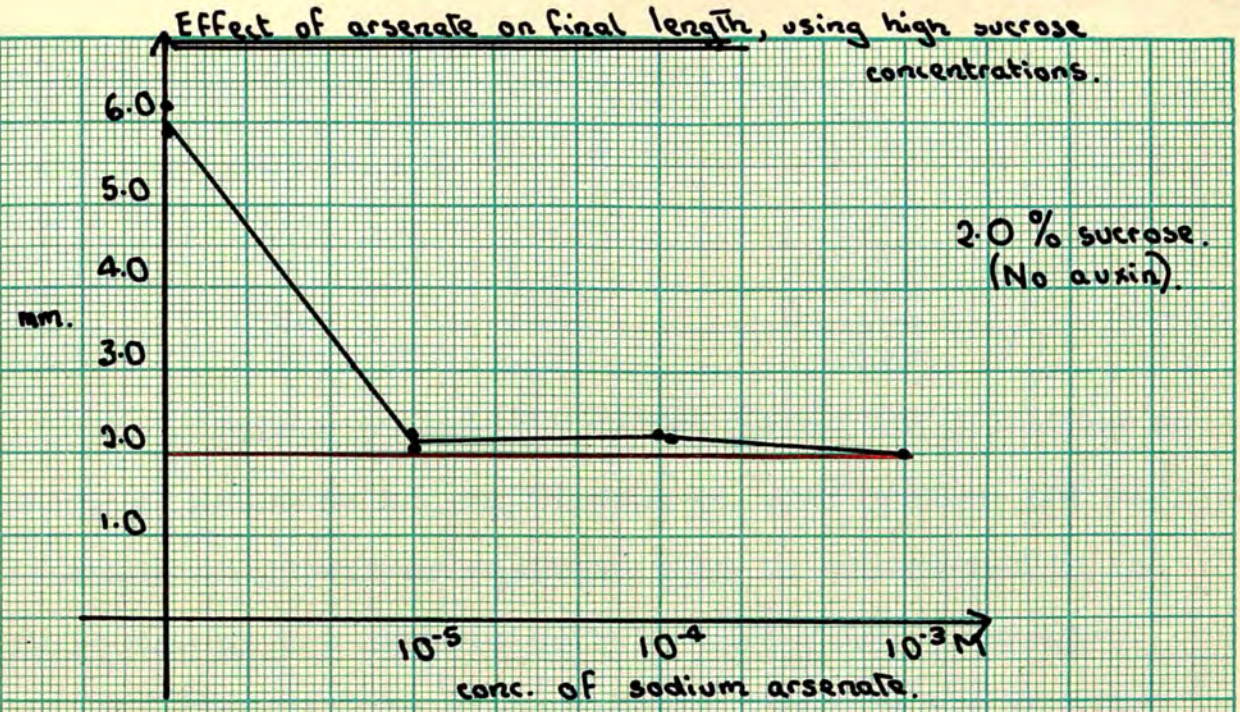
N.B. red line indicates original length.
Each point is mean of 25 section lengths.

Graph 23.

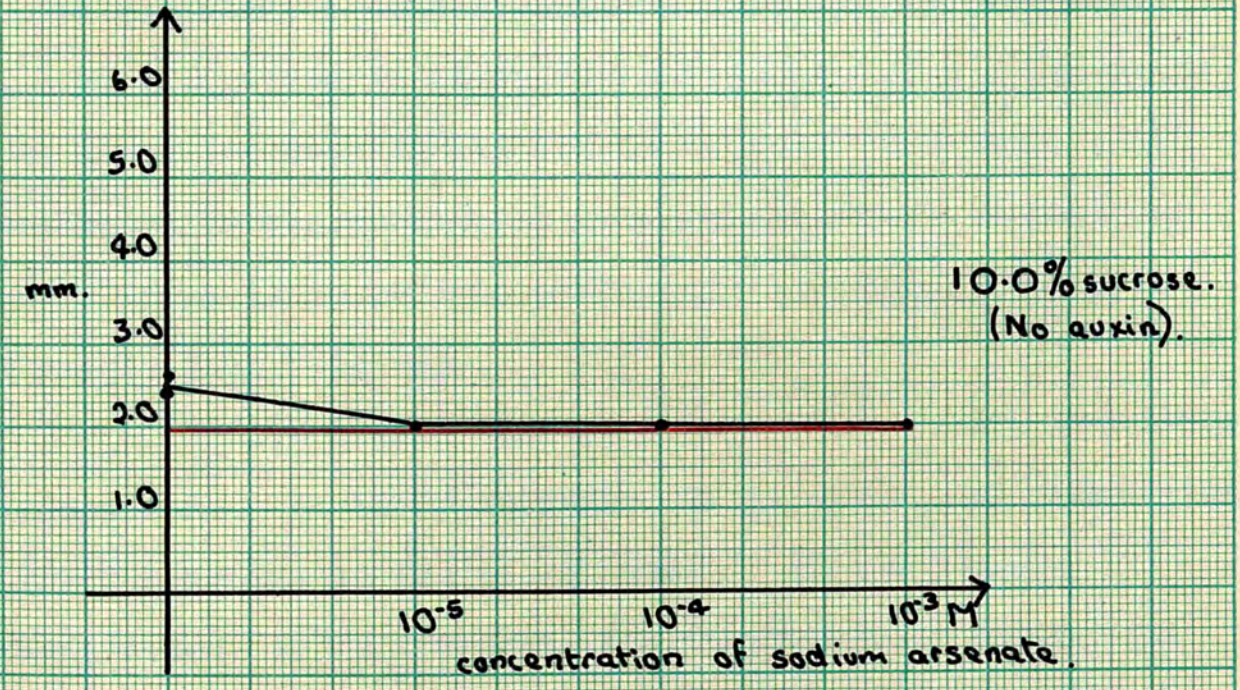

EFFECT OF ARSENATE on final length.
0·5% SUCROSE.
6·0
5·0
4·0
mm.
3·0
2·0
1·0
No auxin
10^{-5}
10^{-4}
10^{-3} M
10^{-11}/3 indolyl acetic acid.
10^{-8}/3 indolyl acetic acid.
conc. of sodium arsenate.

Graph 23 (a).

Effect of arsenate on final length, using high sucrose concentrations.



2·0 % sucrose. (No auxin).



10·0 % sucrose. (No auxin).

MEAN RATIOS IN ARSENATE / CONTROL

	0 auxin	10^{-11} IAA	10^{-8} LAA
0 hours	1.01	0.81	0.93
12 hours	1.59	1.27	1.04
24 hours	1.29	0.91	0.89
30 hours	1.19	1.05	1.05
48 hours	1.07	1.05	1.05

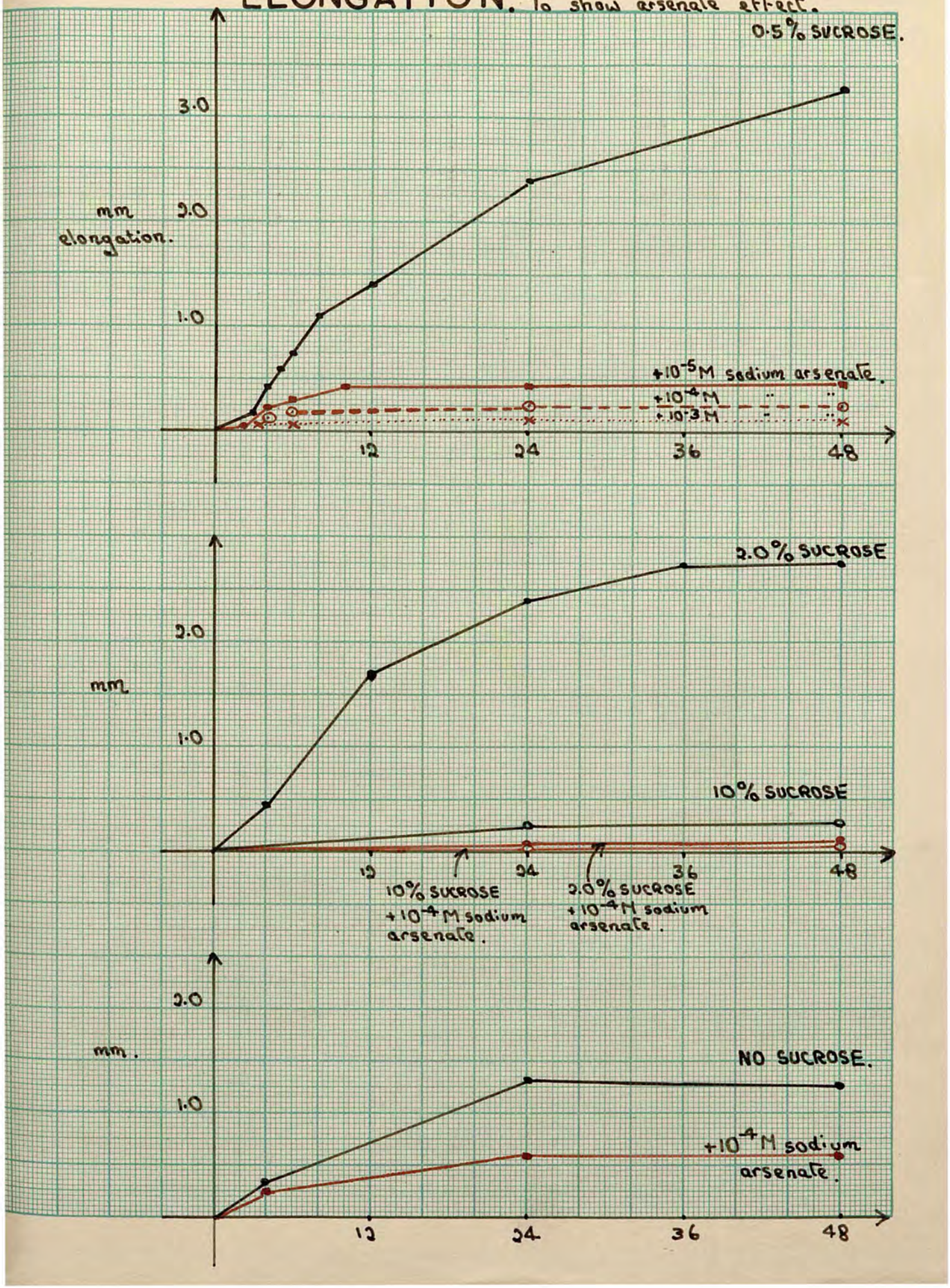
Before proceeding to the effect of phosphate on arsenate stimulation and inhibition of respiration, the effects of arsenate on growth of sections must be considered to see if it is in any way correlated with respiratory response.

As will be seen from the graphs (21,23) in 0.5% sucrose solution, a concentration of 10^{-5}M sodium arsenate inhibits elongation in 24 hours to 35% (mean of 9 experiments). In 48 hours the mean inhibition is to 33%. Now this ~~stimulation~~ inhibition is accompanied by STIMULATION of the respiration rate. There is no obvious correlation between growth rate and respiration rate here.

With higher concentrations of arsenate, inhibition is increased. In 10^{-4}M arsenate, at 24 hours, the inhibition is 92% increasing to 96% at 48 hours. In the highest concentration, 10^{-3}M arsenate, the inhibition of elongation is 94% and this increases to 98% with time. Although we observe progressive inhibition of elongation with increasing arsenate concentration it is never complete and growth is not stopped while respiration continues normally (Bonner). In fact here the inhibition of elongation is paralleled by that of ~~growth~~ respiration. It has been shown that in arsenate most of

Graph 24

ELONGATION. To show arsenate effect.



the elongation, which goes to make up the 24 hour values, occurs in the initial 4 hours before penetration is complete. The growth which occurs has nothing to do with the presence of arsenate which finally inhibits growth completely. Similarly 10^{-4}M and 10^{-3}M arsenate completely inhibit respiration after 9 - 12 hours emersion of the sections.

It may well be that arsenate has a differential effect on growth; and respiration, but as far as the results above can be taken as indicative of the normal response of plant tissue, the state of affairs is not as simple as Bonner would have us believe. Again it is emphasised that arsenate takes time to penetrate the plant cell. The effect is to reduce the growth rate to nearly zero after 4 hours, but its effect on respiration rate is delayed. Certainly after 4 hours the respiration rate is reduced to a new lower level, and this slowly drifts down to zero in the 12 - 24 hours period. The reason for this may be that as the sections get older they become more sensitive to arsenate poisoning. This was confirmed by adding arsenate 24 hours after cutting and placing in sucrose. The respiration rate was rapidly reduced to zero.

With respect to the stimulation of respiration rate by 10^{-5}M arsenate, this is over and above the normal increase in respiration rate per sample which takes place with increasing length, and is in fact independent of it. If this concentration of arsenate is added after 24 or even

48 hours, stimulation as great as is obtained over and above the 24 hour maxima is observed.

As was pointed out no respiratory maximum was observed in the absence of sucrose. [see p. 51 (b)] No stimulation by 10^{-5}M arsenate was observed ~~when measuring the respiration rate~~ in the absence of sucrose. ~~Just as~~ The inhibition of respiratory rate is less if no sucrose is present and the inhibition of elongation compared to the control is reduced.

Although auxin reduces the stimulation of respiration in 0.5% sucrose by 10^{-5}M arsenate solution, it does not effect the growth inhibition (on a percentage basis / control). It may be that when an inhibitor causes there to be a stimulation of one process accompanied by an inhibition of the other it is acting on one part of the general metabolic unit. For instance if this low concentration of arsenate can stimulate the formation of 3-phosphoglyceric acid at the expense of the formation of some substance essential to growth this type of result is entirely explicable.

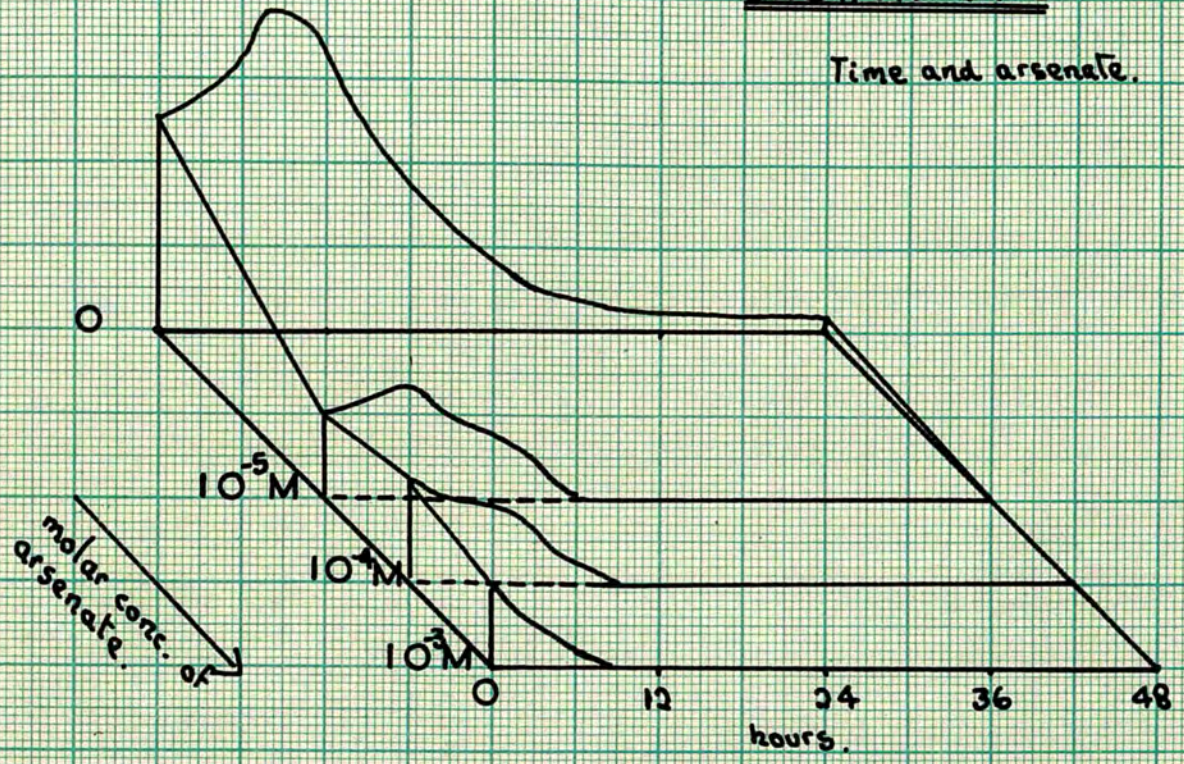
With respect of auxin action we have the paradox that, whereas Bonner finds that arsenate removes the stimulation of respiration due to auxin, we find auxin partly removes the stimulation due to arsenate.

We thought it might be possible to imitate Bonner's results for root sections using much lower concentrations of sodium arsenate. As many workers have shown, roots tend to be considerably more sensitive to added toxic substances

Graph 25.

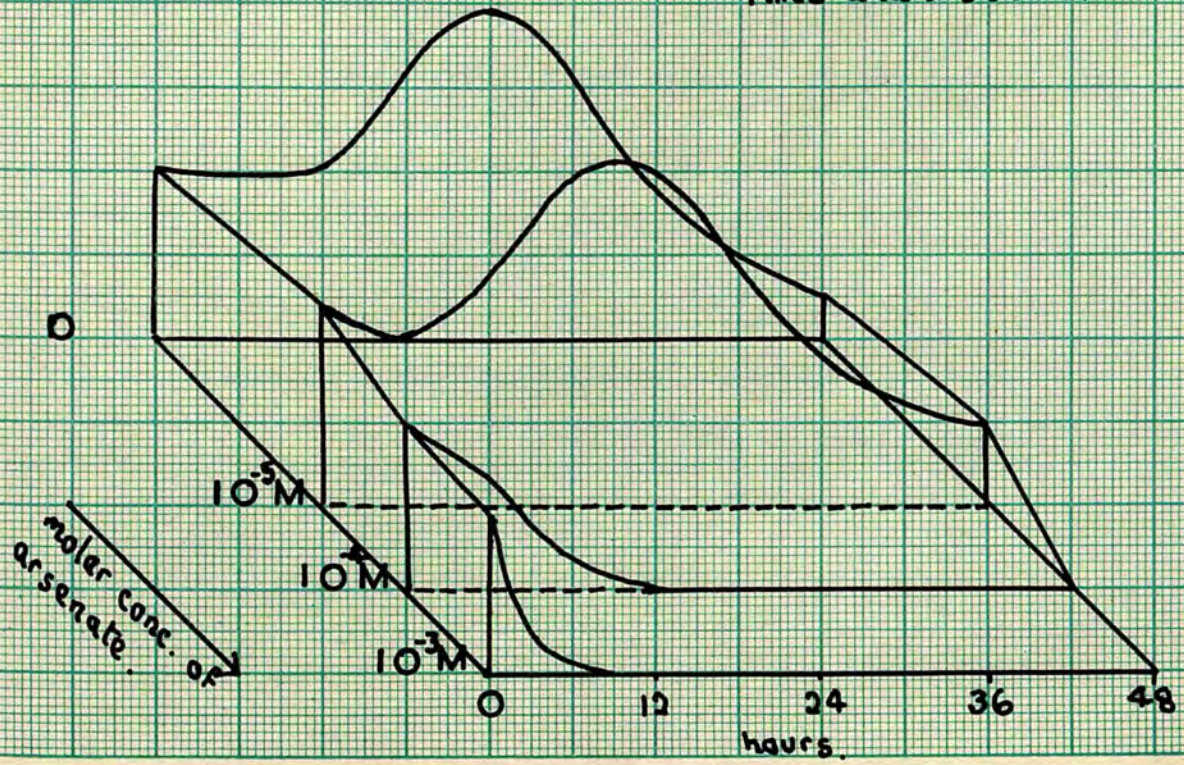
GROWTH RATE

Time and arsenate.



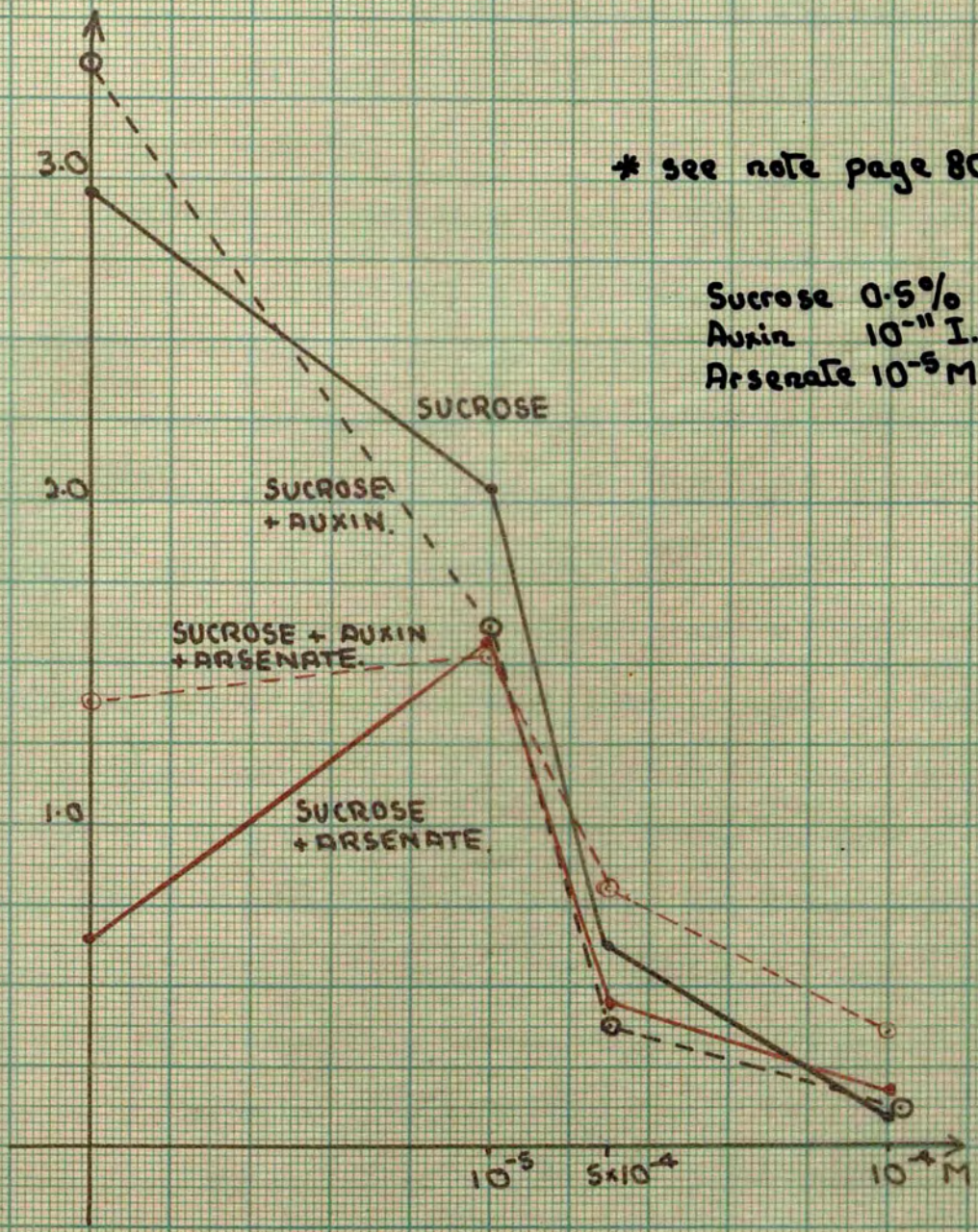
RESPIRATION RATE

Time and arsenate.



Graph 26.

Effect of ammonium dihydrogen phosphate on elongation and on arsenate inhibition of elongation.



* see note page 80.

Sucrose 0·5%
Auxin 10^{-11} I.A.A.
Arsenate 10^{-5} M.

than do shoot tissue. Using a range of 10^{-9}M to 10^{-6}M arsenate we found arsenate had no effect on the respiration of root sections but it had no effect on the elongation either. We could not repeat Bonner's results and we feel they should be repeated using his tissue, taking into account

(1) the time of penetration of the arsenate.

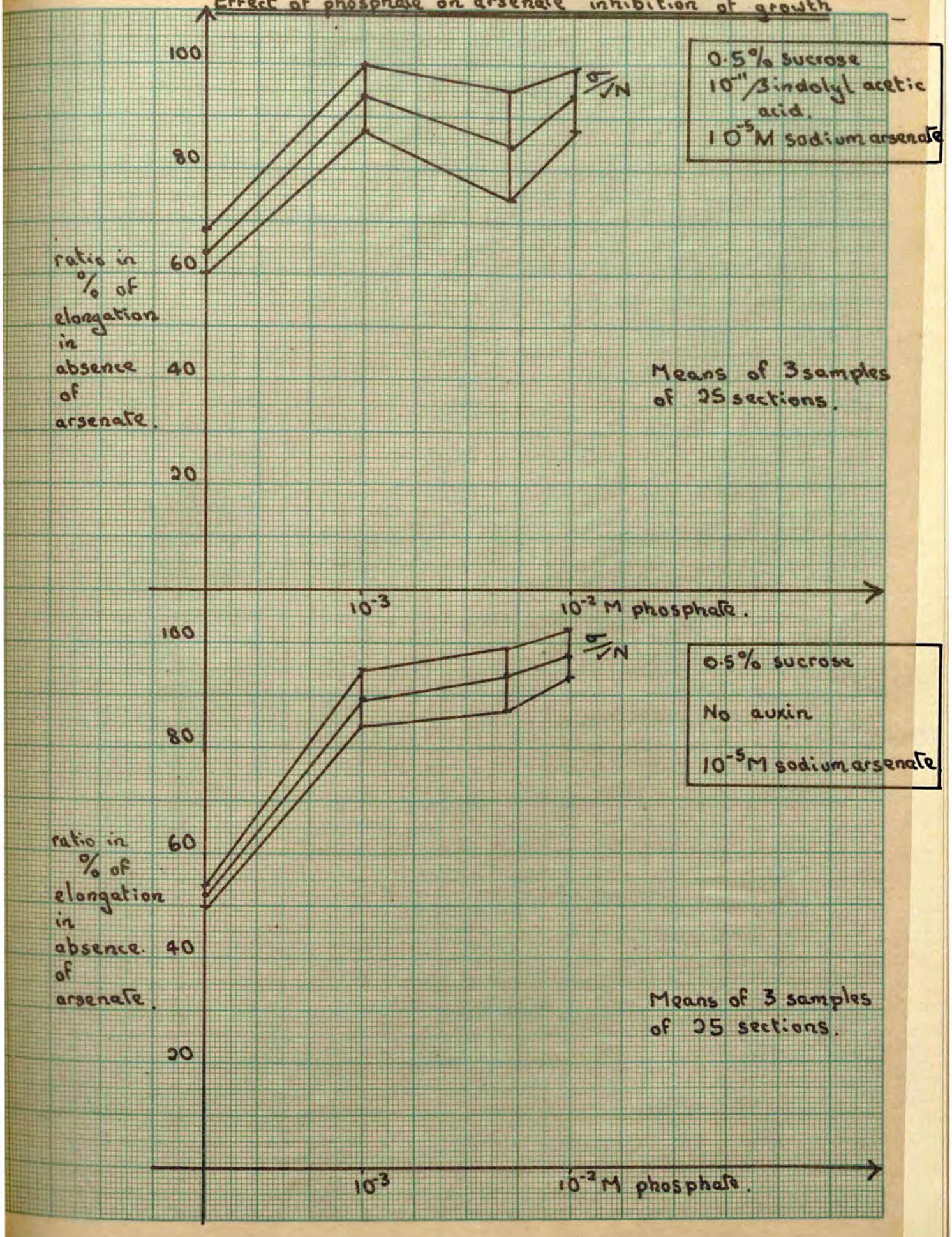
(2) the time factor involved in growth and respiration measurements.

EFFECT OF PHOSPHATE ON ARSENATE INHIBITION

Since arsenate probably acts in the metabolism of the plant by substituting for phosphate in the respiratory cycle it is possible that its effect may be overcome by adding sufficient excess of inorganic phosphate ions. In the course of preliminary experiments it was found that ammonium ions from ammonium dihydrogen phosphate had a highly inhibitory effect on the growth of root sections.* This entirely swamped any effect of the phosphate ions when added together with arsenate. It was thus found necessary to introduce potassium ions in the form of potassium dihydrogen phosphate (sodium is also an inhibitory ion.) All solutions of this salt were carefully neutralised to pH 7.0 and the pH was checked at 12 hour intervals using a pH meter. It was found that the inhibition of growth by 10^{-5}M arsenate in 0.5% sucrose was partially reversed by 10^{-4}M phosphate and completely reversed by 10^{-3}M and higher concentrations. The same reversal takes place in solutions containing auxin.

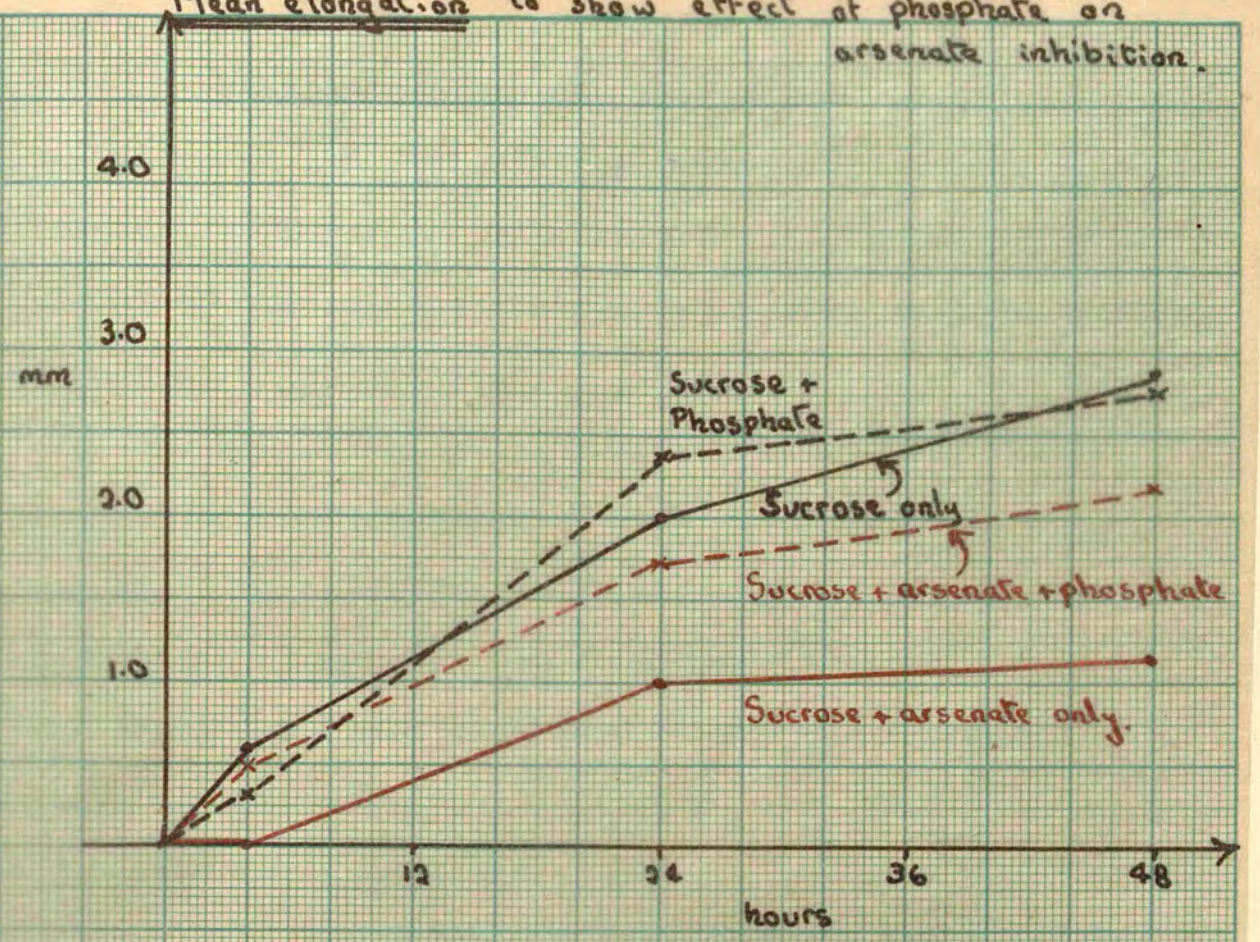
Graph 24

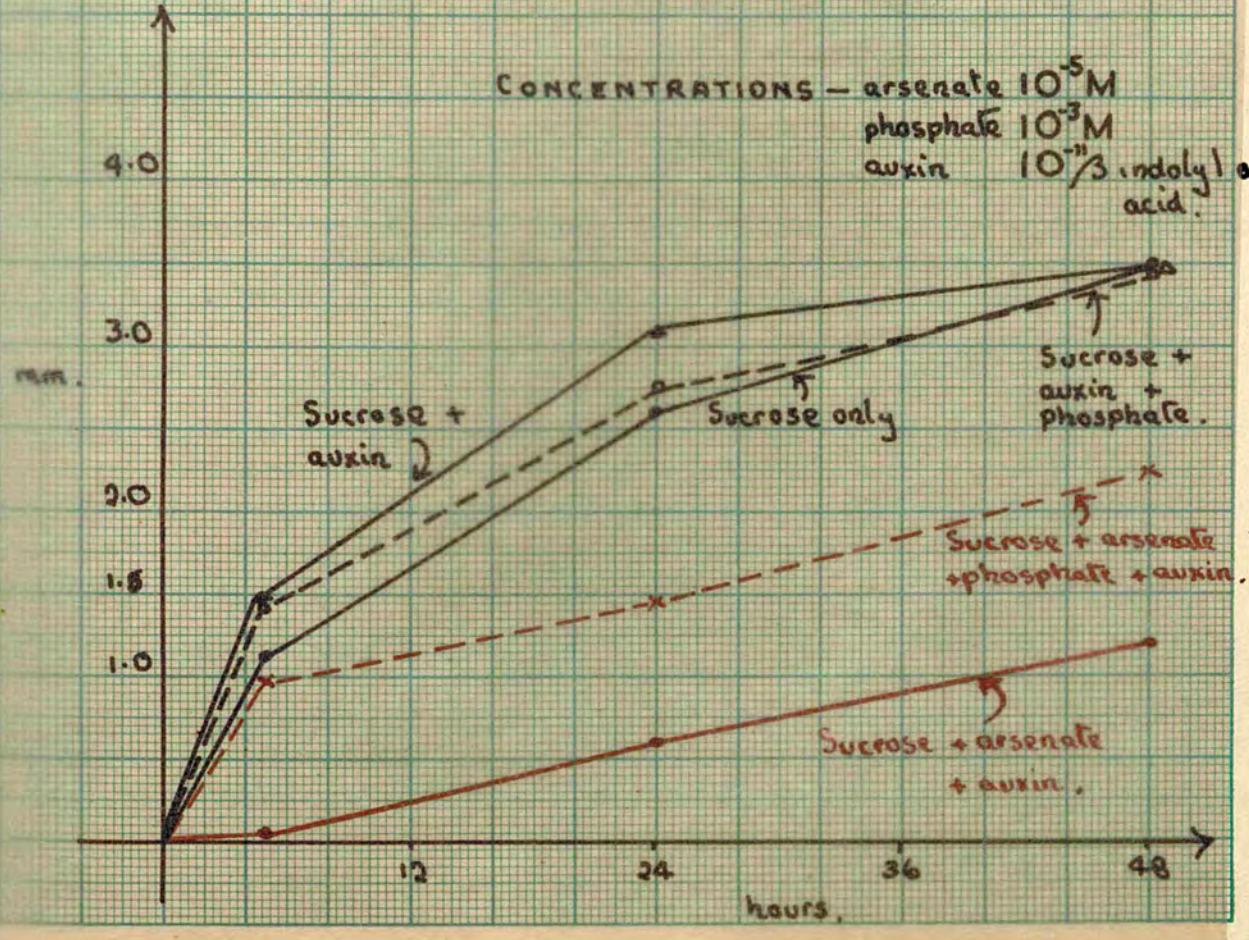
Effect of phosphate on arsenate inhibition of growth



Graph 28.

Mean elongation to show effect of phosphate on arsenate inhibition.





Potassium phosphate does itself stimulate growth considerably and this stimulation occurs even in the presence of arsenate. Therefore the elongation is not merely brought to a level of the sucrose control but surpasses this becoming equal to that in phosphate.

Now 10^{-5}M sodium arsenate stimulates the respiration of root sections. This stimulation is also removed by 10^{-3}M phosphate. It seems that arsenate action in suppressing elongation and in stimulating respiration is in both cases concerned with substitution for inorganic phosphate i.e. the theories of Warburg and Christian are substantiated for this tissue.

The importance of these results is to explain auxin action. Indolyl acetic acid has no effect on the inhibition of growth by arsenate. It has no effect on the restoration of this inhibition by phosphate. However indolyl acetic acid considerably reduces the stimulation of respiration by a low concentration of arsenate. When phosphate reverses this inhibition, its effect is additive. It seems that auxin may be acting as a regulator in the respiratory cycle prior to the arsenate regulated stage. If in the presence of auxin the formation of 3-phosphoglyceric aldehyde cannot exceed a certain near normal rate, a concentration of arsenate which in the absence of auxin can be stimulatory to a later process, can have no effect. A previous reaction is limiting.

Therefore the following possibilities occur:-

(a) auxin if present prevents any stimulation above normal by any reactant whose action is after the auxin controlled one.

(b) if auxin is not there, a low concentration of arsenate may stimulate respiration by substituting for inorganic phosphate - a reaction which disturbs the ATP⇌ADP mechanism and eliminates the growth reaction.

(c) phosphate of a concentration one hundred times that of the arsenate completely outweighs any arsenate effect causing reversal of both respiratory stimulation and growth inhibition.

EFFECT OF 2 4 DINITROPHENOL

Effect of dinitrophenol

In 1922 Plantefol found that 2.4. dinitrophenol in an acid medium inhibited the growth of Sterigmatocystis nigra. The effect of this substance on cell division seems to be widespread; for instance, Woodward, Kingery and Williams (1934) found a solution in 1% sodium carbonate was toxic to the growth of Monilia tropicalis: Clowes and Krahl (1936) obtained blockage of the normal division of sea urchin eggs, etc.

Woodward et al. had observed that in their alkaline solution the toxicity was considerably less than that of o-nitrophenol. In an acid solution the reverse is true (Plantefol 1922). pH must be considered very carefully in experiments with these inhibitors. Since the pK value of 2 4 dinitrophenol is 4.0 the toxicity decreases rapidly as the pH is raised. Finding this to be so, Clowes and Krahl (1931) attributed the toxicity due to undissociated molecules only. It seems now that this view is partly erroneous (Simon and Blackman, 1949). Even if the weak acid is completely dissociated, it may still be toxic. This may be because the pH of the cells is less than that of the surrounding medium. The dissociated molecules are in equilibrium with undissociated ones at the lower internal pH.

The effect of pH on the dissociation of 2 4 dinitrophenol is as follows (Newcombe 1950).

at pH 4.0 50% molecules are undissociated

at pH 5.0 only 10% molecules are undissociated

at pH 6.0 1% molecules are undissociated

As Simon and Blackman point out it is theoretically desirable that when comparative studies of the effectiveness of different compounds are to be made, a pH should be used at which there is very little dissociation. They point out that for a weak acid at a pH two units below the pK value toxicity is uninfluenced by ionisation. Using plant material it would be physiologically impossible to use such a pH. In spite of the high degree of dissociation we have used a pH of 7.0 (as in previous experiments) - a pH previously used by Stenlid (1949) for wheat roots.

Dinitrophenol as well as being an effective blocking agent of mitosis also inhibits subsequent elongation and differentiation. Bonner (1949) found dinitrophenol inhibitory to the growth of Avena coleoptile segments which did not, of course, include any meristematic tissue. Other research workers were more concerned with the effect of this compound on the respiration of plant tissue and their accounts did not (unlike Bonner's) include simultaneous measurements of the growth or elongation of the tissue.

The effect on respiration also concerns us here, because (as was the case with low concentrations of sodium arsenate) inhibition of growth processes is again accompanied by a stimulation of respiration. Clowes and Krahl (1936) found that as low a concentration as 10^{-6}M stimulated

the respiration and blocked the division of sea urchin eggs. A concentration of 3×10^{-5}M gave 292% stimulation. Apparently the active group was the phenolic -OH since the nitro groups could be substituted by halo-groupings without altering the characteristic activity. (Krahl and Clowes, 1939).

Turning to the effect on plant material, Stenlid obtained stimulation of the respiration of carrot leaves and of young wheat roots at a pH of 6.5 - 7.0 with the high concentration of 10^{-3}M dinitrophenol. However using the same concentration at a pH of 4.5 - 5.0 inhibition was observed. This is in keeping with the original remarks made on the effect of pH on dissociation. Bonner's paper on the effect of dinitrophenol on Avena sections unfortunately does not mention the pH at which the experiments were carried out. However he had previously used "a maleate buffer of pH 4.5"; so we can possibly assume that this was the pH of the experiments to which we refer. He obtained stimulation of respiration together with inhibition of growth at a much lower concentration than did Stenlid (above). In a 2% sucrose medium 1.5×10^{-6} dinitrophenol caused initial stimulation of the respiration, whereas higher concentrations inhibited it. However in a pyruvate medium a concentration as high as 10^{-5} stimulated respiration. (Note concentrations not molar.) A further paper on the effect of dinitrophenol on plant tissue results from Newcombe's work on tobacco callus tissue. He

obtained stimulation of the respiration of this tissue during the first hour with concentrations of 10^{-6}M to 10^{-5}M but this fell off with time rapidly returning to normal. The stimulation was accompanied by an increase in the respiratory quotient.

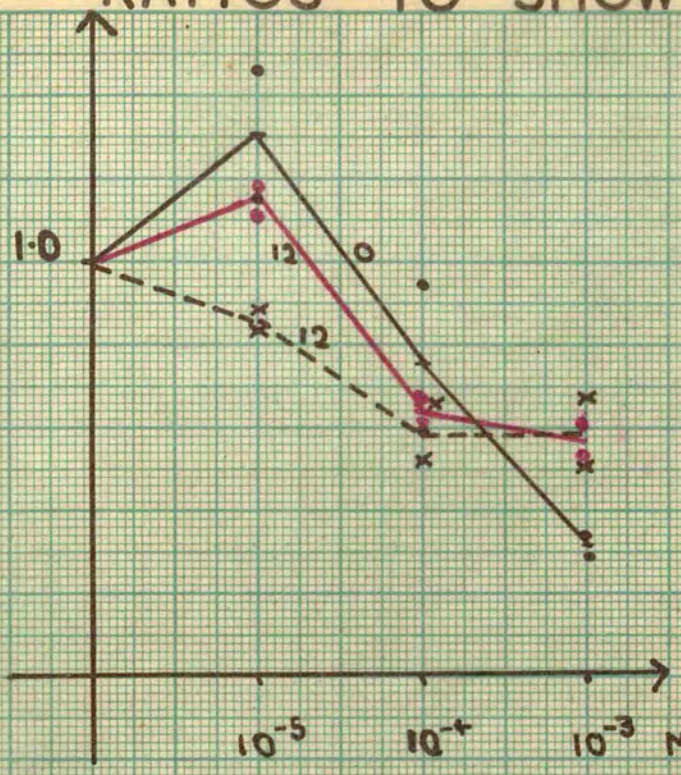
Theories of the possible mechanism of dinitrophenol action have been elaborated for plant and animal tissue ever since the original work of Clowes and Krahl (1936). Winzler and Burk (1949) observed that the drug inhibited the nitrogen assimilation of bacteria; and Spiegelmann obtained evidence that there was prevention of the formation of adaptive enzymes. Evidence has been accumulating that the mechanism of action is very much connected with the phosphate metabolism of the cell. For instance, when respiration is stimulated in yeast cells, the uptake of phosphate ions from the surrounding medium is inhibited (Hotchkiss 1944). Clifton (1948) found that dinitrophenol can "replace" inorganic phosphate in the respiration of micro-organisms. Phosphate deficient systems responded to the drug, whereas systems complete in phosphate requirements were less responsive. Loomis and Lipmann (1948) found that washed kidney particles could oxidise glutamate in the absence of phosphate (this component was previously considered obligatory). In 1949 Teply pointed out that dinitrophenol has the property of releasing inorganic phosphate from prepared phosphate gels. Hence he suggests that of

dinitrophenol does not act in substituting for inorganic phosphate as previous writers stated, but acts by releasing inorganic phosphate from phosphate esters present in the protoplasm. This causes stimulation of the respiration. He does not explain why in this case growth is inhibited - presumably a "double action" must be invoked. Anyway this question must seem to plant physiologists to be largely academic.

From results on the Avena coleoptile, Bonner (1949) postulated a "dual mechanism" of dinitrophenol action. The stimulation of respiration at low concentrations coupled with an inhibition of growth, was the effect of replacement by the drug of the adenylic acid ortho-phosphate system. At higher concentrations there was interference with the production of pyruvate from a hexose substrate. If however pyruvate was used as the respiratory source, this inhibition was reversed. The stimulation of respiration by dinitrophenol was completely inhibited by sodium fluoride and 80% inhibited by malonic acid. Bonner concludes from this that dinitrophenol is affecting the parts of respiration affected by these inhibitors. Sodium fluoride is believed to affect the action of enolase in the formation of (enol)-phosphopyruvic acid from 2-phosphoglyceric acid. Malonate is an acknowledged inhibitor of succinic dehydrogenase - which acts on the Krebs acid cycle, but also affects other systems. It is very difficult to draw any concrete conclusions from this data.

Graph 29

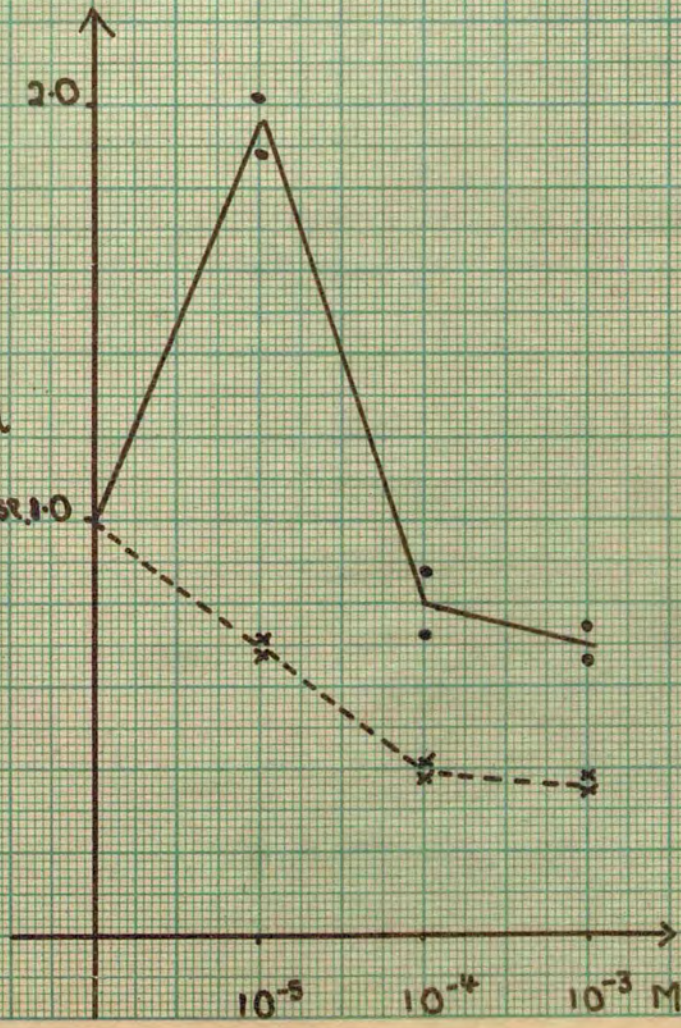
RATIOS TO SHOW D.N.P. EFFECT.



•— Ratios of mean respiration rate (0–2 hours)

•— Ratios 10–12 hours.

x -- Length ratios 12 hours.

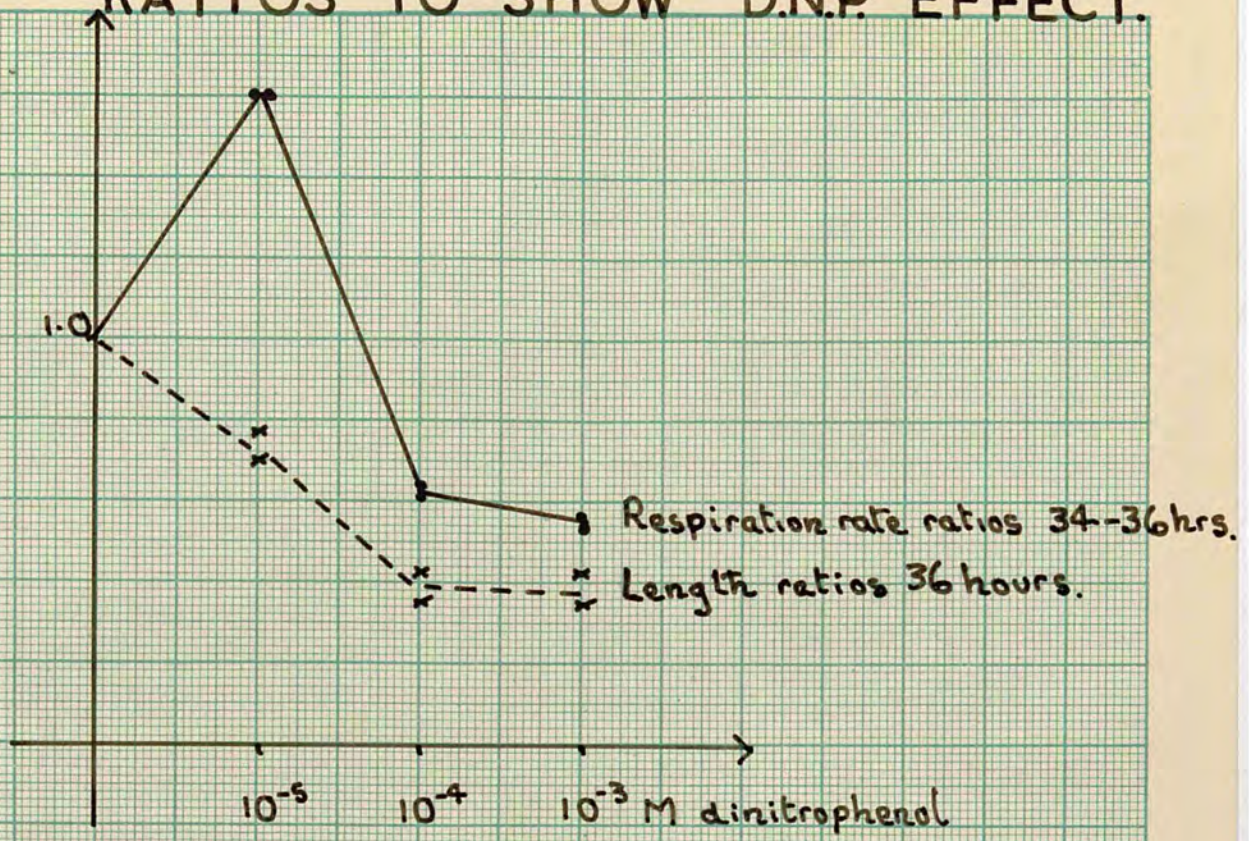


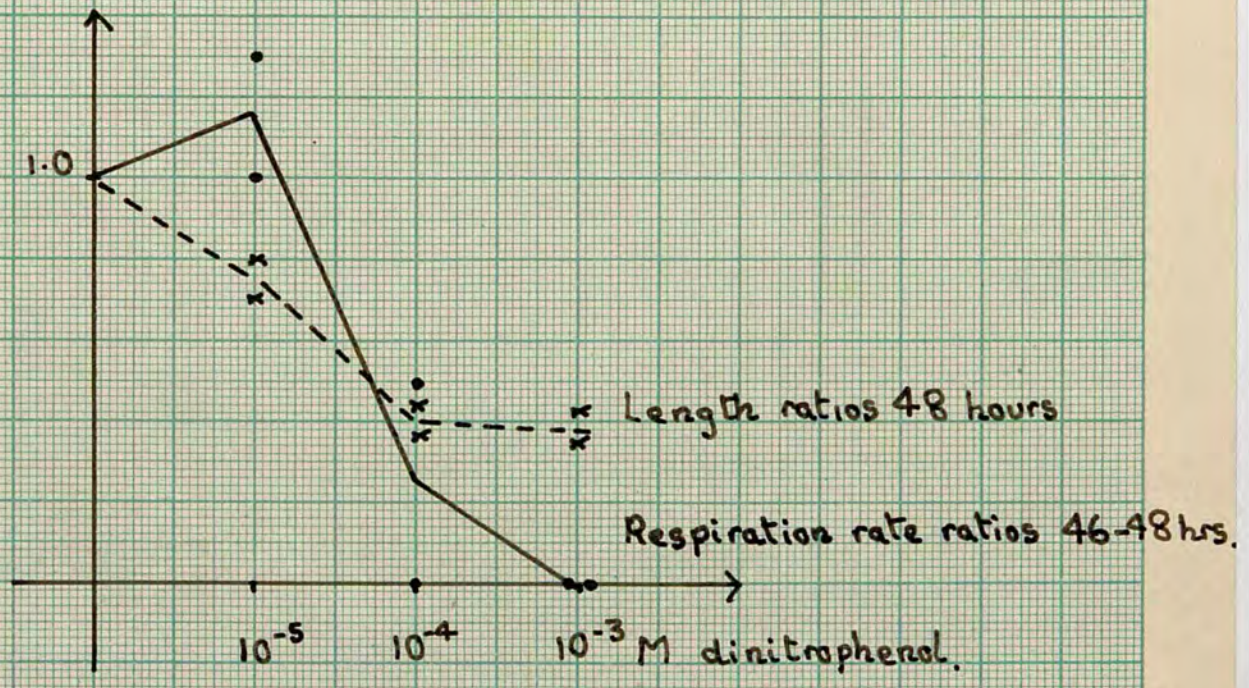
•— Respiration rate ratios (22–24 hours.)

x— Length ratios (24 hours.)

Graph 30

RATIOS TO SHOW D.N.P. EFFECT.





Kelly and Avery (1949) have obtained some interesting results on the effect on pea and oat stem segments. They found a concentration of 0.2 - 1.0 mg./litre stimulated the respiration 20-30%, whereas 10 mg./litre was inhibitory to the extent of 50%. An interesting fact was that the stimulation was additive to any stimulating action of 2 4 D.

In our experiments we have attempted to show the effect of dinitrophenol on pea root segments, measuring simultaneous elongation and respiration. We hoped to throw some light on the connection with phosphate metabolism and determine how these results were affected by the presence of auxin.

Experiments with 2 4 dinitrophenol

The graphs show respiration and length ratios / control in 0.5% sucrose. It can be seen that with a low concentration (10^{-5}M) there is an initial stimulation (in the first two hours) of approximately 30%. A concentration of 10^{-4}M is inhibitory to the extent of about 20%, and a concentration of 10^{-3}M is very inhibitory. It is to be noted that with a stimulatory concentration the degree of stimulation increases up to 24 hours after which it falls off to a negligible value at 36 hours. This concentration is always inhibitory to elongation of the sections. Higher concentrations are increasingly inhibitory to respiration and no elongation at all takes place. This last fact is probably due to the immediate penetration of dinitrophenol. Whereas

in experiments with arsenate the respiration rate gradually decreased to the final value, in these experiments response was immediate, occurring after 1/2 to 1 hour. No growth is possible at those concentrations which are inhibitory to respiration. The lower concentration 10^{-6}M is stimulatory to respiration and causes only partial growth inhibition.

It seems therefore that with these root sections a similar response is obtained to this drug action as has been mentioned above (Bonner 1949). It has been noted that Hotchkiss (1944) and Loomis and Lipmann (1948) stated that response was only a property of phosphate deficient systems. The following experiments have been designed to test this thesis.

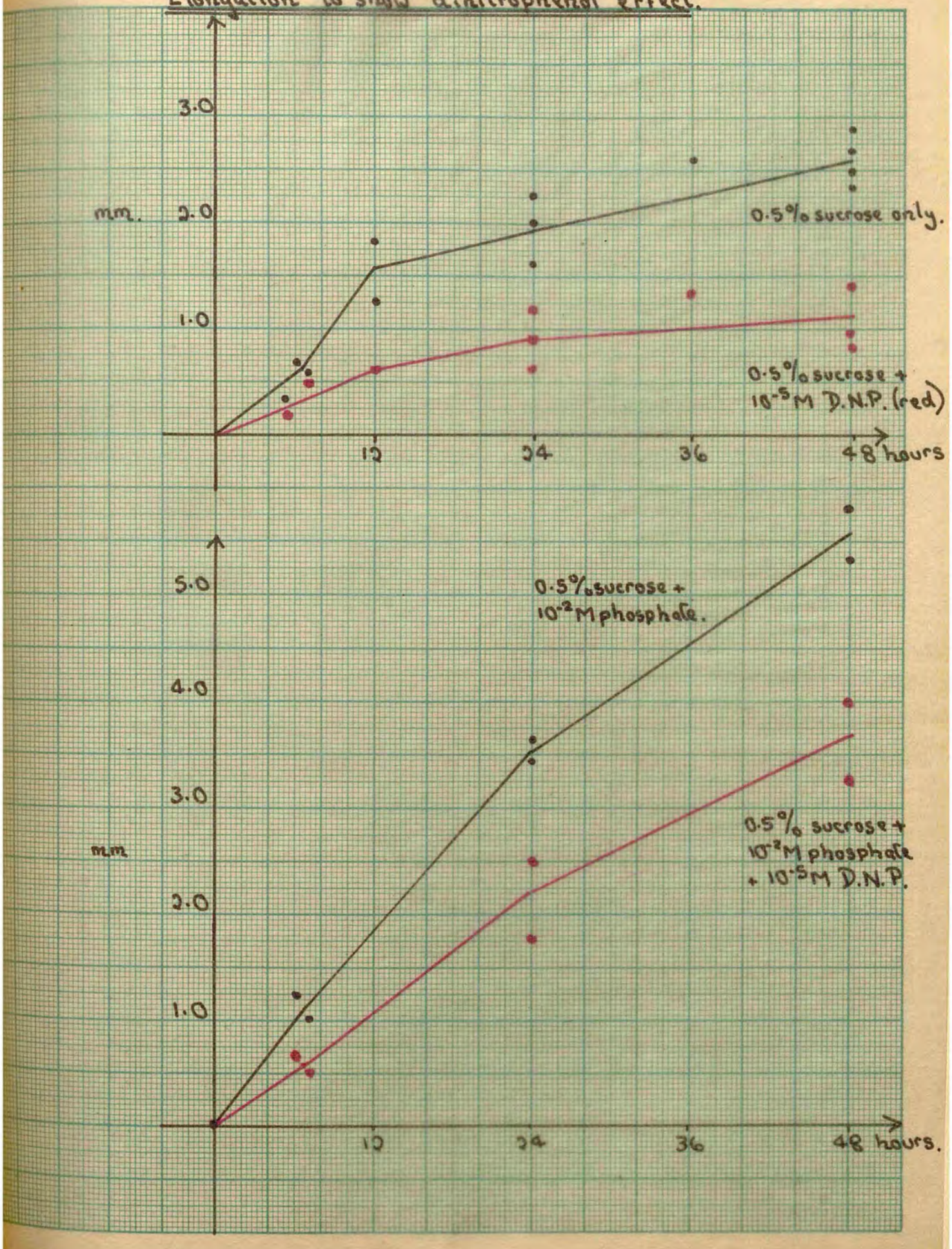
Response of dinitrophenol in the presence of phosphate

We progressively tested higher and higher concentrations of potassium phosphate in an attempt to reverse the 10^{-6}M dinitrophenol stimulation but, as the graphs will show, a concentration as high as 10^{-2}M had no effect and even increased the stimulation due to dinitrophenol. Nor was the inhibitory effect reversed. A concentration of 10^{-4}M remained about 20% inhibitory.

We have noted that the penetration of dinitrophenol is very rapid and we suggest it is even more rapid than the normal penetration of phosphate ions. Once the drug has established an attachment within the cell even a high concentration of phosphate may be unable to reverse the effect.

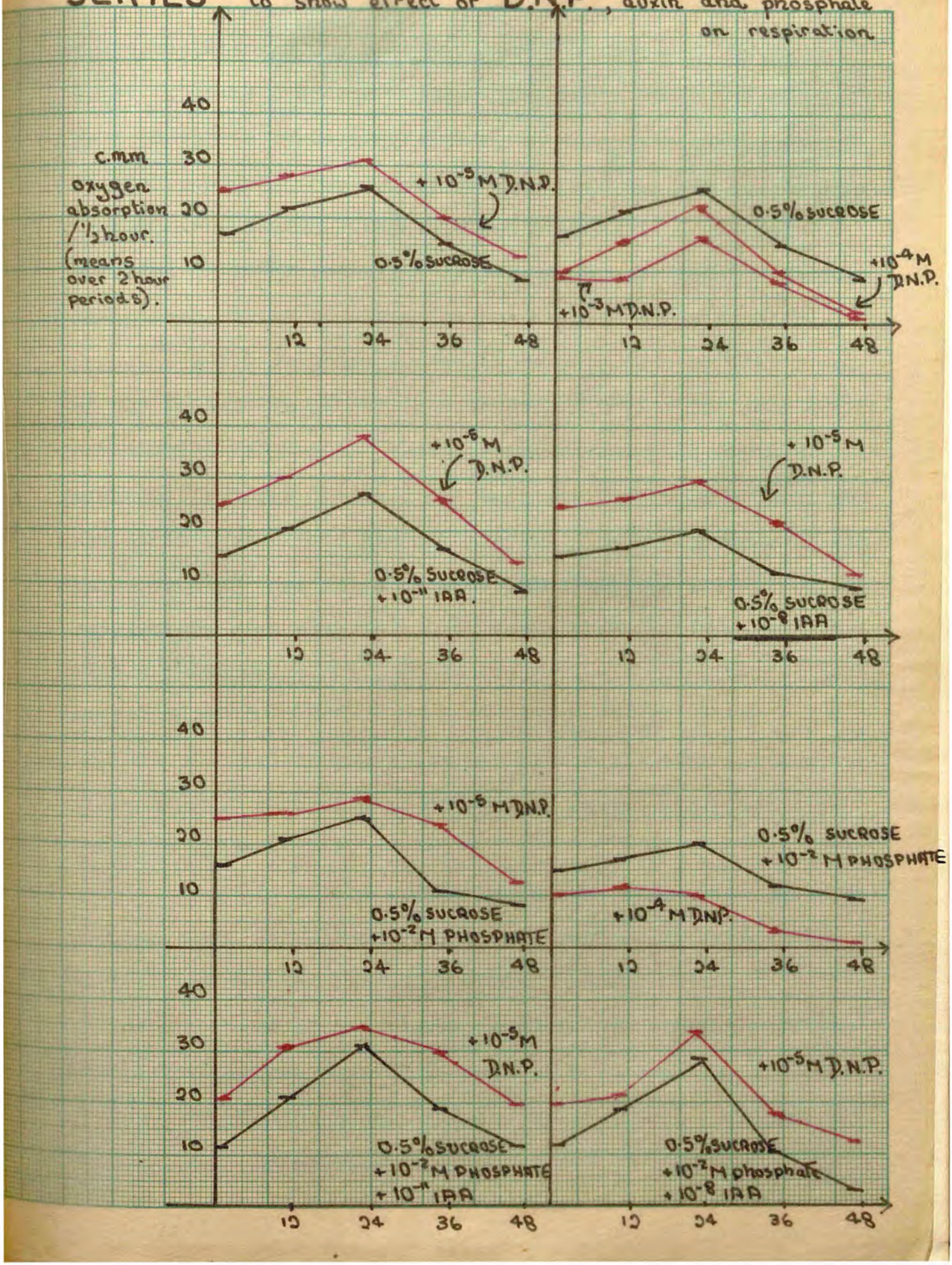
Graph 31

Elongation to show dinitrophenol effect.



Graph 32.

SERIES to show effect of D.N.P., auxin and phosphate on respiration



The phosphate and the drug were added at one and the same time; whereas previous workers have grown their micro-organisms in a phosphate containing medium, to which the dinitrophenol was later added. We therefore attempted one set of experiments in which the sections were cut and emersed in a neutral solution of 10^{-3}M potassium dihydrogen phosphate. This sample was placed in the incubator on the shaker for 24 hours as usual. After 24 hours the sections were removed with 1 cc. of the medium and placed in a Warburg flask. The side arm contained 1 cc. 2 x 10^{-6}M dinitrophenol solution (in 0.5% sucrose). After the original respiration rate was obtained the dinitrophenol was tipped into the flask. In none of the three samples used for this experiment was the characteristic respiratory rise observed. It seems then that dinitrophenol action is connected with phosphate metabolism in the following way:-

(a) if phosphate has previously been present, dinitrophenol is unable to effect the system and exert its characteristic effect.

(b) if the system has become "phosphate deficient" dinitrophenol can occupy respiratory centres and stimulate or inhibit respiration according to its concentration.

(c) if the tissue is placed in a mixed solution of the two and has previously been in a "phosphate deficient" system, the dinitrophenol will exert its effect by virtue of its superior penetration rate.

Hotchkiss used only tissue which by past history was "phosphate rich" or "phosphate deficient" i.e. he had only the data of (a) and (b) on which to base his theory. It seems that dinitrophenol can act in the presence of phosphate, providing it penetrates first.

It is useful to compare the effects above with the previous investigation of arsenate action. Contrary to the above, if arsenate and phosphate are added simultaneously the action of arsenate is suppressed. This is because the penetration of arsenate ions is very slow, presumably slower than that of the phosphate ions. Hence penetration rates are in the following order:-

dinitrophenol is faster than

phosphate which is faster than

arsenate.

Effect of auxin on dinitrophenol action, in the presence and absence of phosphate

The graphs show that auxin (IAA), while having no initial effect on respiration itself, does not reverse and even slightly increases the stimulatory effect due to dinitrophenol. When phosphate is present the effect of increasing the stimulation is even more marked.

Taking into account Kelly and Avery's on the additive effect in conjunction with 2 4 D. it seems that auxin works in the same system as does dinitrophenol. The difference is here that whereas 2 4 D (and IAA) has an initial

stimulatory effect on the respiration of Avena and Pisum stem tissue, in root sections there is no such initial effect. Auxin does however have an effect which results in increase in stimulation in the presence of dinitrophenol.

The results above for auxin effect are the reverse of those obtained when arsenate is used as an inhibitor. Auxin tends to remove the low concentration stimulation due to arsenate. The following is very tentatively suggested. Assuming that Bonner is correct in concluding that the action of auxin is mainly within the Krebs acid cycle, it is unlikely that it should augment the stimulatory action of arsenate whose action is well removed from this cycle. In fact the two may act in opposition so that the stimulatory effect is reduced. Dinitrophenol may owe its action to stimulation of succinic dehydrogenase (malonate removes its stimulatory action). Hence it is possible that the effects of auxin and dinitrophenol are additive. There are a large number of difficulties to be resolved:-

1. Bonner's observation that arsenate removes the stimulation of respiration due to auxin. Criticisms of his technique should be remembered here.

2. The respiratory stimulation by dinitrophenol is also removed by sodium fluoride, and apart from the rather dubious action of malonic acid there is no evidence that it has any action in the Krebs cycle.

3. The action of IAA itself in the Krebs cycle is not proven.

EFFECT OF IODOACETATE

Effect of iodoacetate

In 1941 Commoner and Thimann found that iodoacetate could be used as an inhibitor of growth in the Avena coleoptile, at concentrations which were too low to appreciably inhibit respiration. Moreover a concentration of this inhibitor which gave complete inhibition of growth (5×10^{-5}M) reduced the respiration by only 10%. It was therefore thought possible that only part of the respiratory processes was intimately coupled with growth. Since 90% of respiration can continue when growth is completely inhibited, growth may only be dependent on the remaining 10%. It was assumed that the iodoacetate-sensitive fraction of respiration was the growth-controlling system. Since the iodoacetate inhibition of growth can be eliminated if malate, pyruvate, fumarate or succinate happen to be present, the growth-controlling system was thought to be the C_4 carboxylic respiratory cycle of Szent-Gyorgyi.

The identification of the iodoacetate sensitive system was confirmed by the work of Albaum and Commoner on intact seedlings of oat. The iodoacetate inhibition did not occur in the presence of malate and fumarate. Albaum, Kaiser, Guttentag and Eichel (1941) studied the effects of iodoacetate on intact roots of two varieties of oat. Using the variety "Fulgham" low concentrations of iodoacetate were found to stimulate growth, whereas high concentrations inhibited it. However when the variety "Black Norway" was

used there was no stimulation by low concentrations, although inhibition was still obtained with high concentrations. Albaum et al. correlated the response with the higher auxin content of "Fulgham". There is therefore an antagonism between iodoacetate and auxin in root response. The authors state that the iodoacetate acts by "quantitavely inactivating the auxins originally present". They believe that the normal inhibition by auxin of root growth in high auxin-containing roots (e.g. "Fulgham") is released in the presence of low concentrations of iodoacetate. The antagonism of auxin action by iodoacetate is mentioned by Thimann (1941) but no data is given. Indeed nearly all the work on the inhibition of growth by iodoacetate has been done in the presence of auxin. Thimann and Bonner (1948) working on Avena coleoptile segments always used a 1% sucrose solution containing 10^{-6} indolyl acetic acid. In view of the possible interaction of auxin and iodoacetate it seems unwise to perform such experiments without proper auxin-lacking controls.

It may be difficult to ensure complete absence of auxin using any plant tissue, but some light may be thrown on the problem by adding solutions containing a range of auxin concentrations. This is more profitable when root tissue is used since this range may include concentrations both inhibitory and stimulatory to root growth, whereas with stem tissue the higher "inhibitory" concentrations would be outside the normal "physiological" range of the plant.

It also seemed inconsistent to study the protection by C_4 acids without a full investigation of the effects of these acids on the tissue in the absence of inhibitor. Unfortunately we have only had time to investigate fully one of these viz. malic. After this we proceeded to investigate the effect on iodoacetate inhibition in the presence and absence of auxin.

As before we paid due attention to time drifts. Commoner and Thimann (1941) while following the elongation of sections over a 48 hour period, had taken respiratory measurements over only the initial four hours. Subsequent workers (Thimann and Bonner, 1948) did not include any respiratory measurements in their results, but assumed that the concentrations they used were too low to appreciably inhibit respiration. In view of the stimulation of respiration by low concentration which have been found with arsenate and dinitrophenol and the stimulation by low concentrations of auxin found by a number of workers, it seemed unwise to assume that the effect will be negligible. It seemed that a full investigation, including possible time-drifts and auxin interactions, was needed to clear up these points.

Christiansen and Thimann have published some interesting papers (1950) giving values for respiration for two time periods (2-4 and 12-14 hours) after cutting, for pea stem material. However here again all investigation has been done in the presence of only one concentration of ausin. No

indication of iodoacetate-auxin interaction can be obtained from these data.

Results with iodoacetate

I. EFFECT OF IODOACETATE IN THE ABSENCE OF ADDED AUXIN

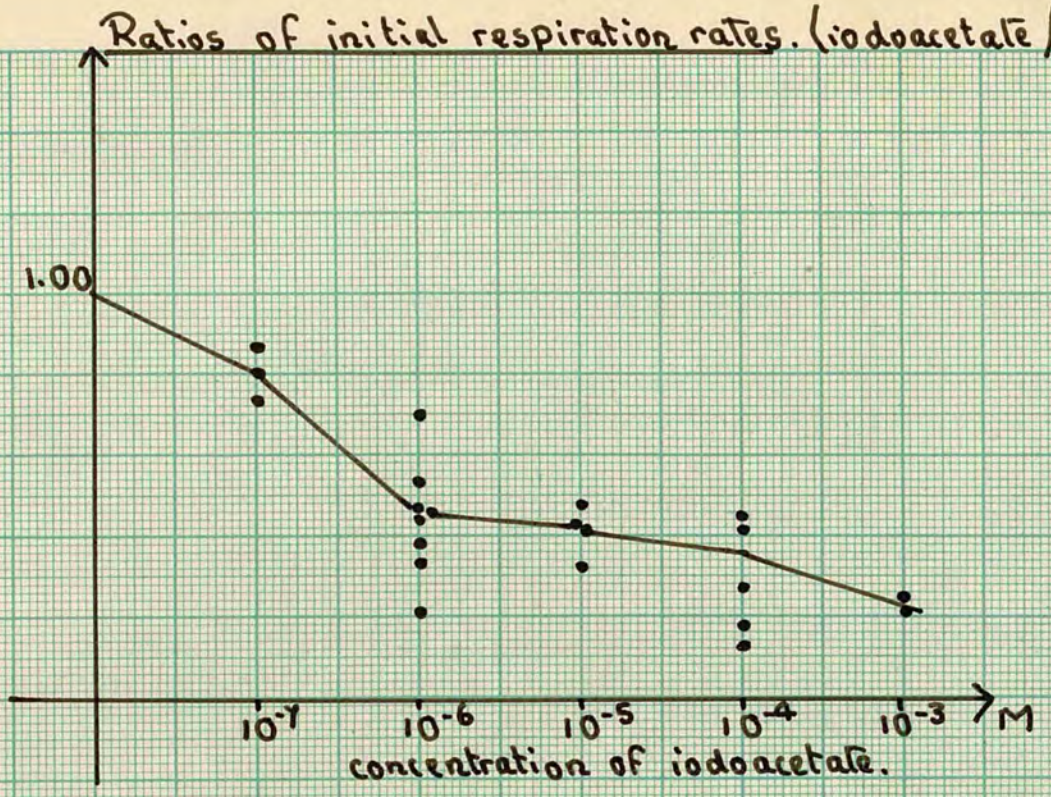
(a) on initial respiration.

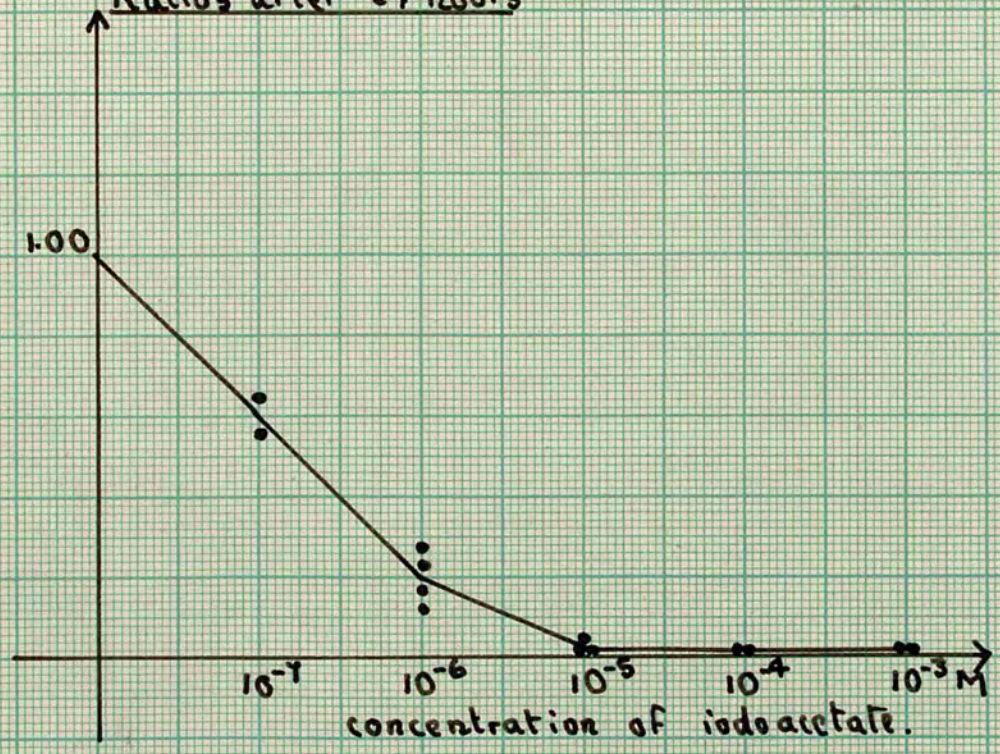
The graph plotting initial respiration (1-3 hours after cutting) has the following features. All the concentrations used between 10^{-6}M and 10^{-3}M were inhibitory to respiration. With 10^{-7}M 20% inhibition was obtained, with 10^{-6}M it was 55% (mean of 8 readings). With 10^{-5}M it was 57%, with 10^{-4}M 64% and with 10^{-3}M 74%. Now this is in contrast to the results obtained by Commoner and Thimann (1941). Using roughly the same range they obtained no effect or only 10% inhibition at the concentration which inhibited elongation 50% (5×10^{-5}M). Taking 24 hour values this concentration is about 10^{-6}M for roots, as will be shown below. Hence with this tissue the concentration causing a 50% inhibition of growth also inhibits respiration 55% i.e. respiration is just as sensitive to inhibition by iodoacetate as is growth.

Disregarding this inconsistency it was proposed to reduce the concentration of iodoacetate until the effect on initial respiration was only of the order of 10%. This effect was obtained using a concentration of 5×10^{-8}M but in this case the inhibition of growth was only 12%.

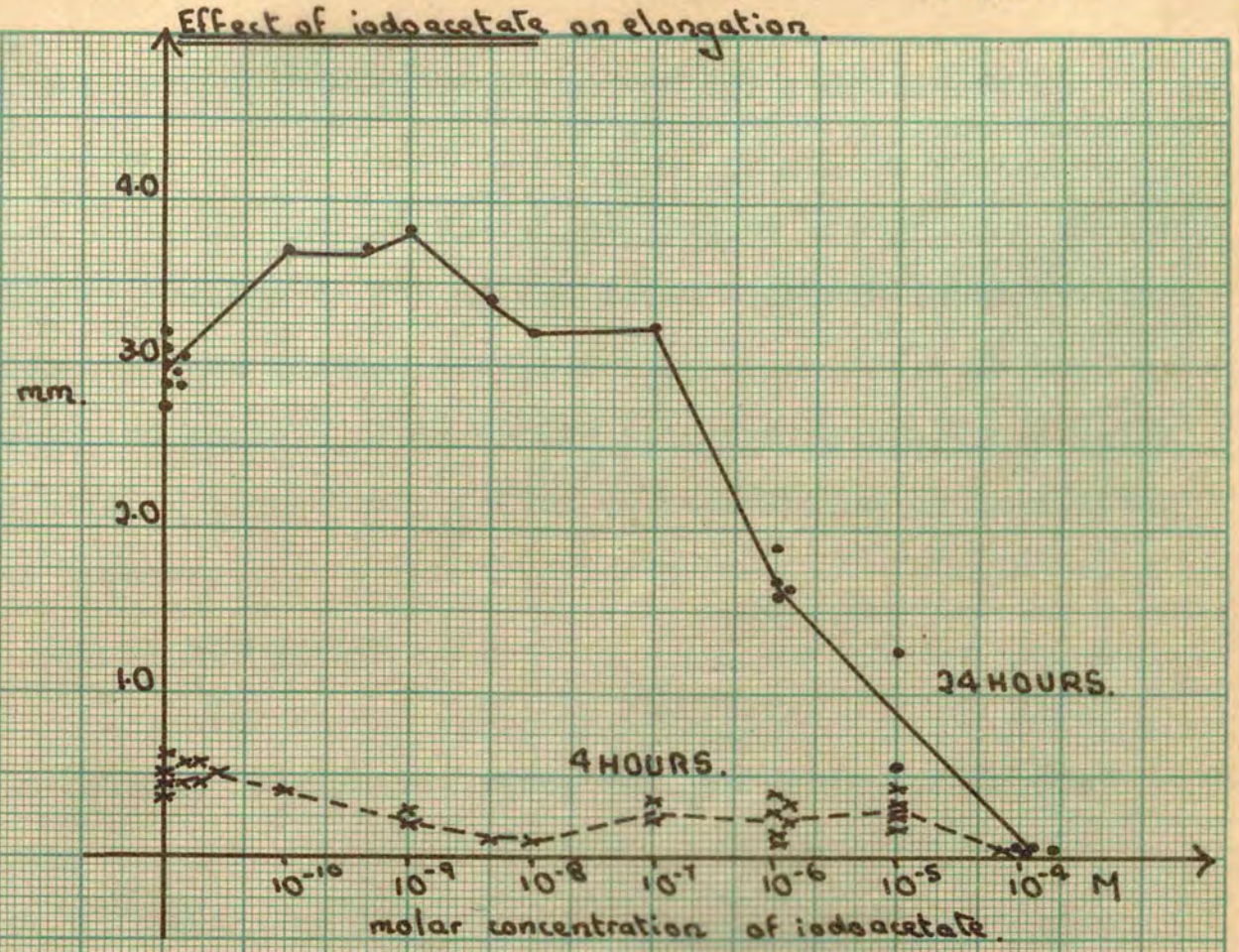
Further even lower concentrations were stimulatory

Graph 33.


Ratios of initial respiration rates. (iodoacetate / control).
1.00
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
M
concentration of iodoacetate.


Ratios after 24 hours
1.00
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
M
concentration of iodoacetate.

Graph 34.


Effect of iodoacetate on elongation.
4·0
3·0
2·0
1·0
mm.
24 HOURS.
4 HOURS.
10⁻¹⁰
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
10⁻⁴ M
molar concentration of iodoacetate.

to growth and 10^{-10}M iodoacetate was 10% stimulatory to respiration.

It must be concluded that IN THE ABSENCE OF ADDED AUXIN the results do not in the least resemble those obtained by Commoner and Thimann (1941) for Avena segments in the presence of auxin. Both growth and respiration are equally sensitive to iodoacetate action in stimulating or inhibiting these processes.

(b) on elongation

The values obtained for sections taken after their initial respiration rates had been obtained in the Warburg apparatus were too variable to give anything but a vague indication of iodoacetate action. However 24 hour values gave a graph which corresponds very well with that obtained by Thimann and Bonner (1948) for "surfaced" sections. Using the low concentrations 10^{-10}M to 10^{-9}M a 23% stimulation of elongation in 24 hours is obtained. This stimulation is reduced, but still continues, up to 10^{-8}M. 5×10^{-8}M gives an inhibition of 12% and 10^{-6}M a 50% inhibition. Complete inhibition of growth is obtained with 10^{-4}M iodoacetate and it is to be noted that this corresponds to an inhibition of initial respiration of 64%.

(c) of respiration after 24 hours

When the sections have been emersed in iodoacetate for as long a period as 24 hours the respiration levels are extremely low. The critical concentration of

10^{-6}M (50% inhibition value initially for respiration and for 24 hour elongation values) has increased its effectiveness to 77%. The respiration in samples of any higher concentration is reduced to nil. Stimulation is never observed after 24 hours. The concentration 10^{-10}M being 4% inhibitory.

Experiment on penetration of iodoacetate

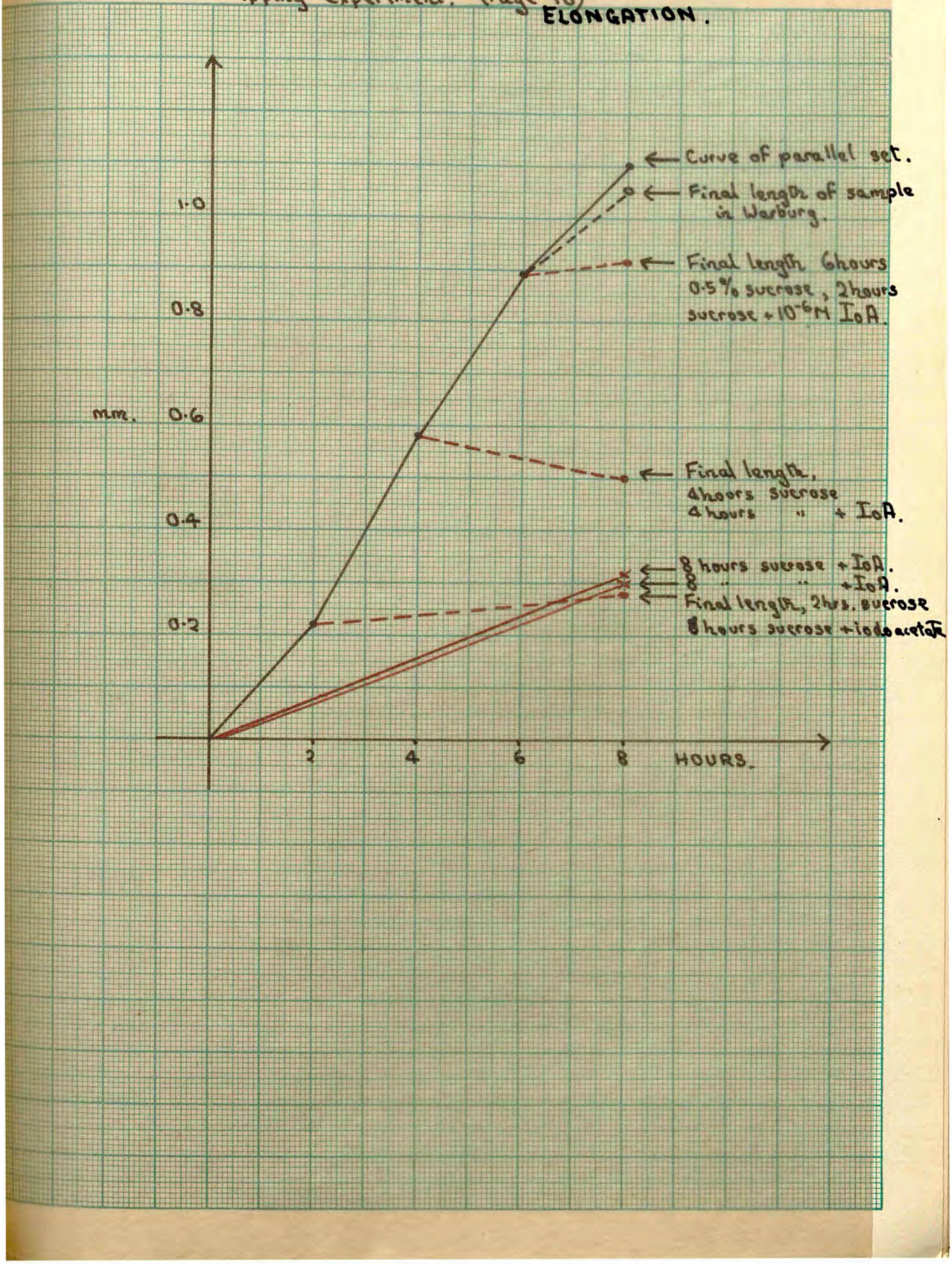
A large number of "tipping experiments" on the respiratory drift had shown that the response to iodoacetate takes place within one hour providing the inhibitor is added not less than two hours after cutting. If it is added within this time period or initially it may take anything up to two hours before the full effect is observed. Even this penetration rate is relatively fast compared with that of arsenate molecules. Since too, with arsenate, the effect of the inhibitor on growth preceded that on respiration; it was thought desirable to determine if this was also the case when iodoacetate was used.

The six manometers were utilised in the following way.

1. 0.5% sucrose control, readings were taken over an 8 hour period.
2. 0.5% sucrose, iodoacetate was tipped after two hours to give a concentration of 10^{-6}M.
3. 0.5% sucrose, iodoacetate tipped after four hours.
4. 0.5% sucrose, " " " six "
5. 0.5% sucrose + 10^{-6}M iodoacetate.

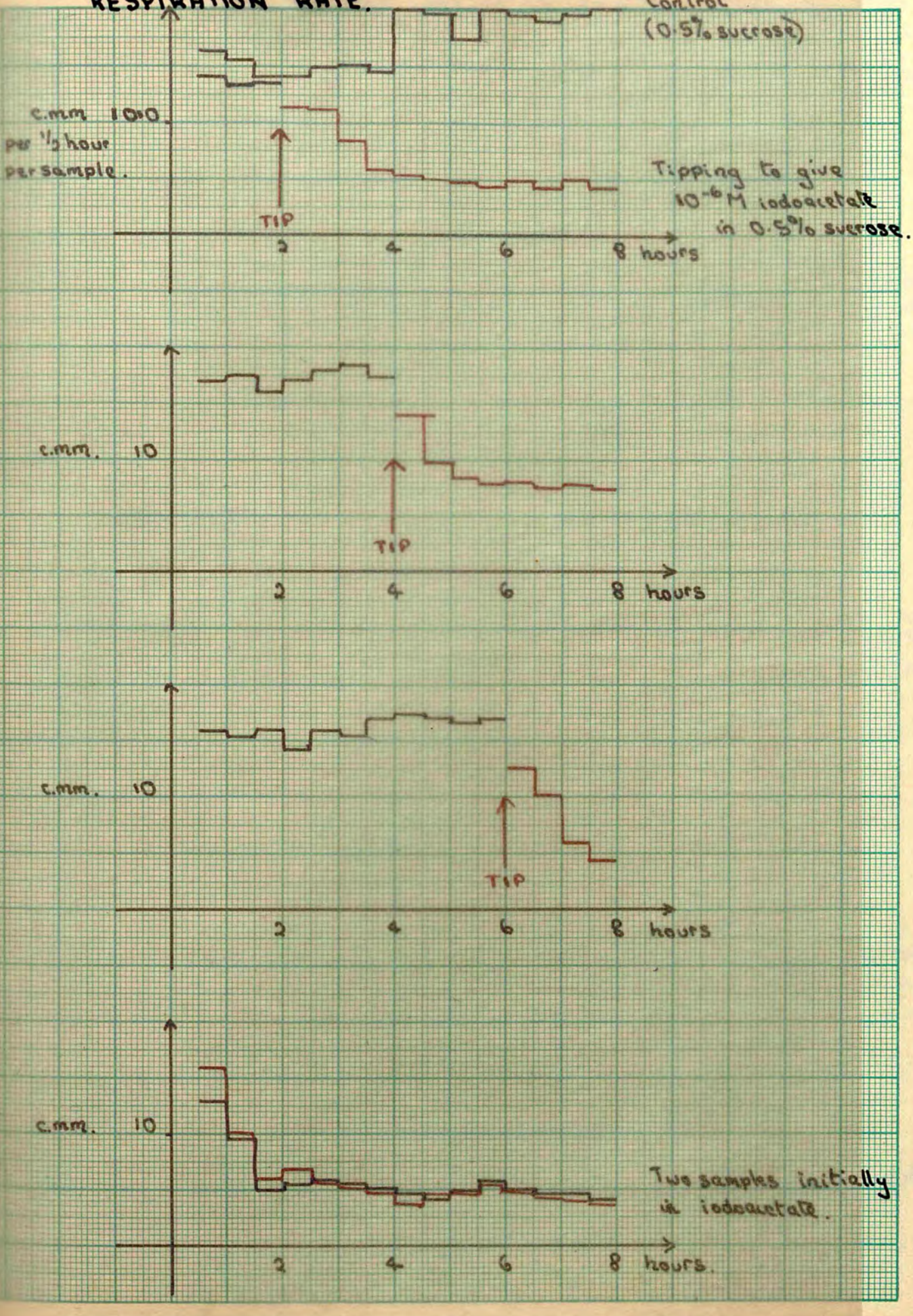
Graph 35.

"Tipping experiment". (Page 98)

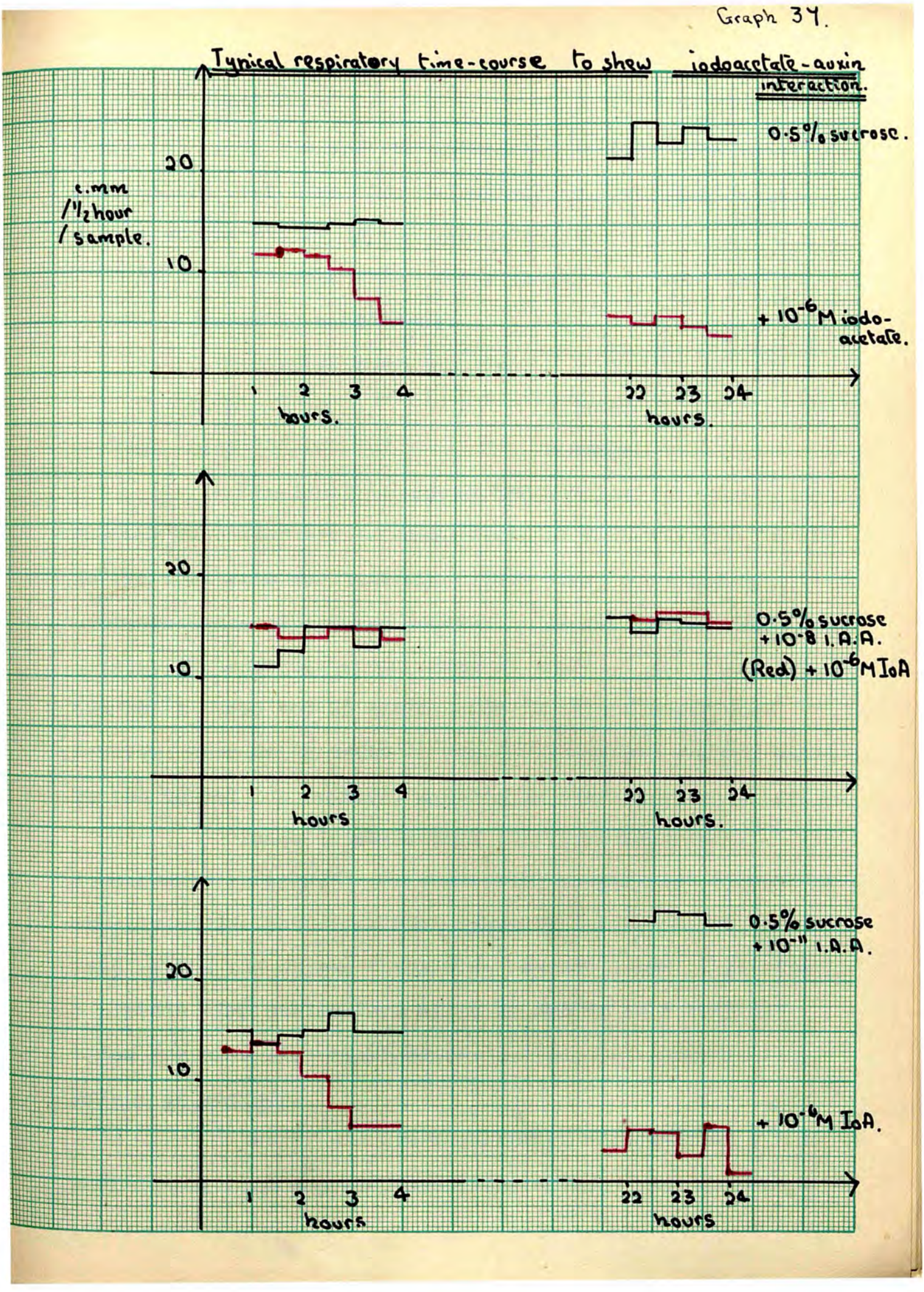


Graph 36.

RESPIRATION RATE.



Graph 34.


Typical respiratory time-course to show iodoacetate-auxin interaction.
c.mm /½ hour / sample.
20
10
1 2 3 4
hours.
22 23 24
hours.
0·5% sucrose.
+ 10⁻⁶M iodo-acetate.
0·5% sucrose + 10⁻⁸ I.A.A.
(Red) + 10⁻⁶M IoA
0·5% sucrose + 10⁻¹¹ I.A.A.
+ 10⁻⁶M IoA.

6. Repeat 5

At the end of 8 hours all samples were measured and, at the same time, the following parallel samples were set up on the shaker in the incubator.

7. 0.5% sucrose which was a check on 1. for the incubator conditions.
8. 0.5% sucrose measured after 2 hours
9. 0.5% " " " 4 hours
10. 0.5% " " " 6 hours

The final length of the sucrose control in the Warburg manometer was 3.056mm. (mean of 25), whereas the one in the incubator was 3.105mm. This shows a very good correspondence of the two samples. As can be seen from the graphs, if iodoacetate was added at any time after the initial two hours there was no appreciable further elongation. However, when it is added initially elongation roughly equal to the normal two hours elongation in sucrose takes place.

Hence it seems that the time taken for iodoacetate to affect both growth and respiratory systems is roughly equivalent. As with arsenate inhibition of respiration the oxygen absorption reaches a new level after a certain time period from which it drifts to a very low value (or even nil) at 24 hours.

Effect of sucrose

Nearly all the experiments were carried out in a solution of 0.5% sucrose, and there was not time to make a

full investigation of the sucrose effect on iodoacetate inhibition. However a few experiments were carried out with concentrations 10^{-7}M to 10^{-4}M in the absence of sucrose. These showed that the effectiveness of the inhibitor was considerably reduced if sucrose was not present. This is a similar result to that obtained in arsenate experiments, and the remarks made there, apply here.

II. EFFECT OF IODOACETATE IN THE PRESENCE OF A CONCENTRATION OF AUXIN NORMALLY INHIBITORY TO GROWTH (10^{-8}IAA)

Very early in the investigation it was noted that iodoacetate failed to exert its normal inhibitory effect on respiration if a concentration of 10^{-8}IAA was present. This was fully investigated with experiments over the whole range of iodoacetate effect.

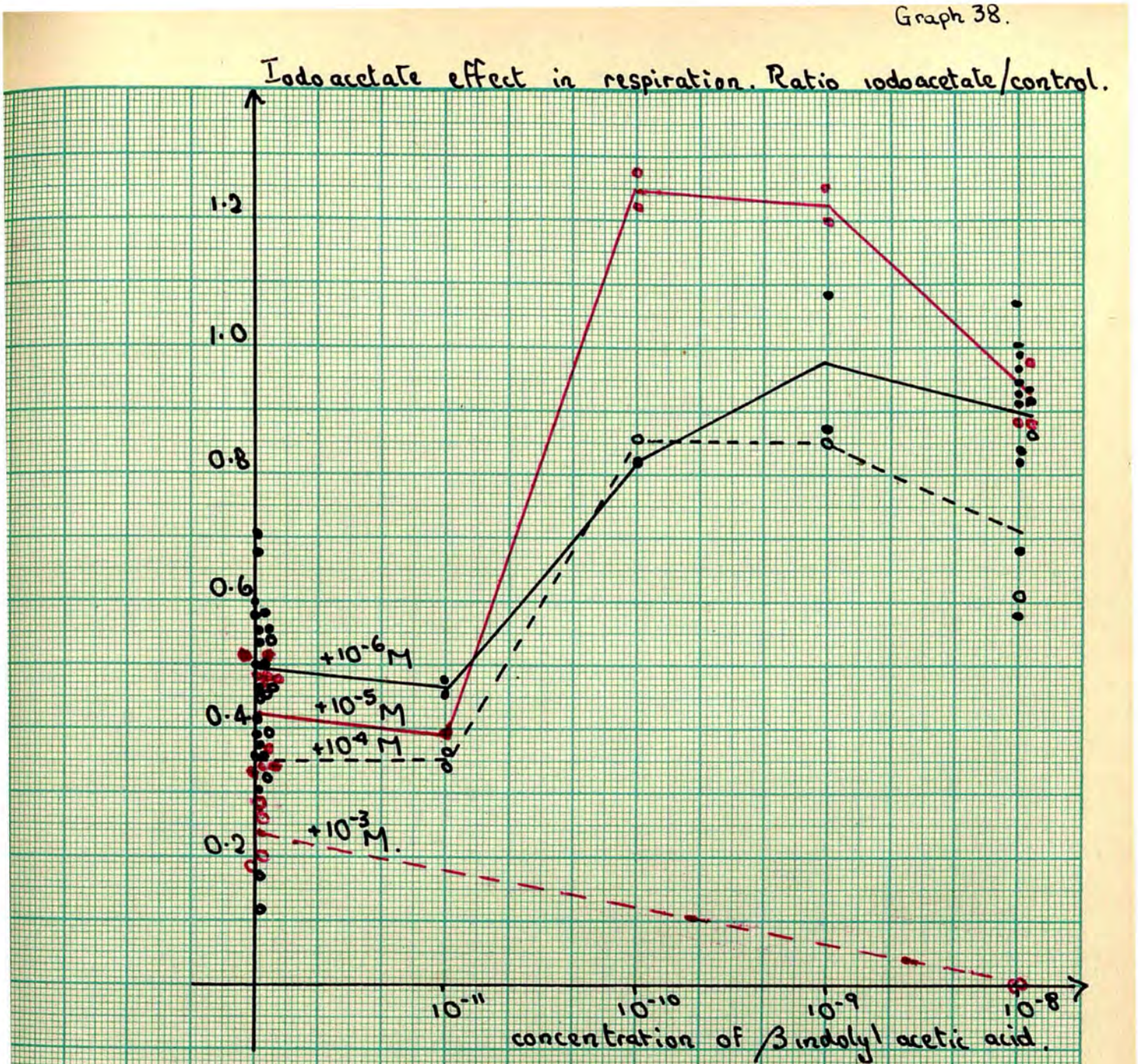
(a) on initial respiration

As will be seen from the graphs the effect of iodoacetate is considerably reduced in this concentration of auxin. Using a concentration of 10^{-6}M an inhibition of only 9.0% was observed (mean of 12 readings). Increasing the concentration to 10^{-5}M only increased the inhibition to 20%, 10^{-4}M to 21%. Lower concentrations than 10^{-6}M had no effect on respiration, stimulation was not observed.

(b) on the growth in 24 hours

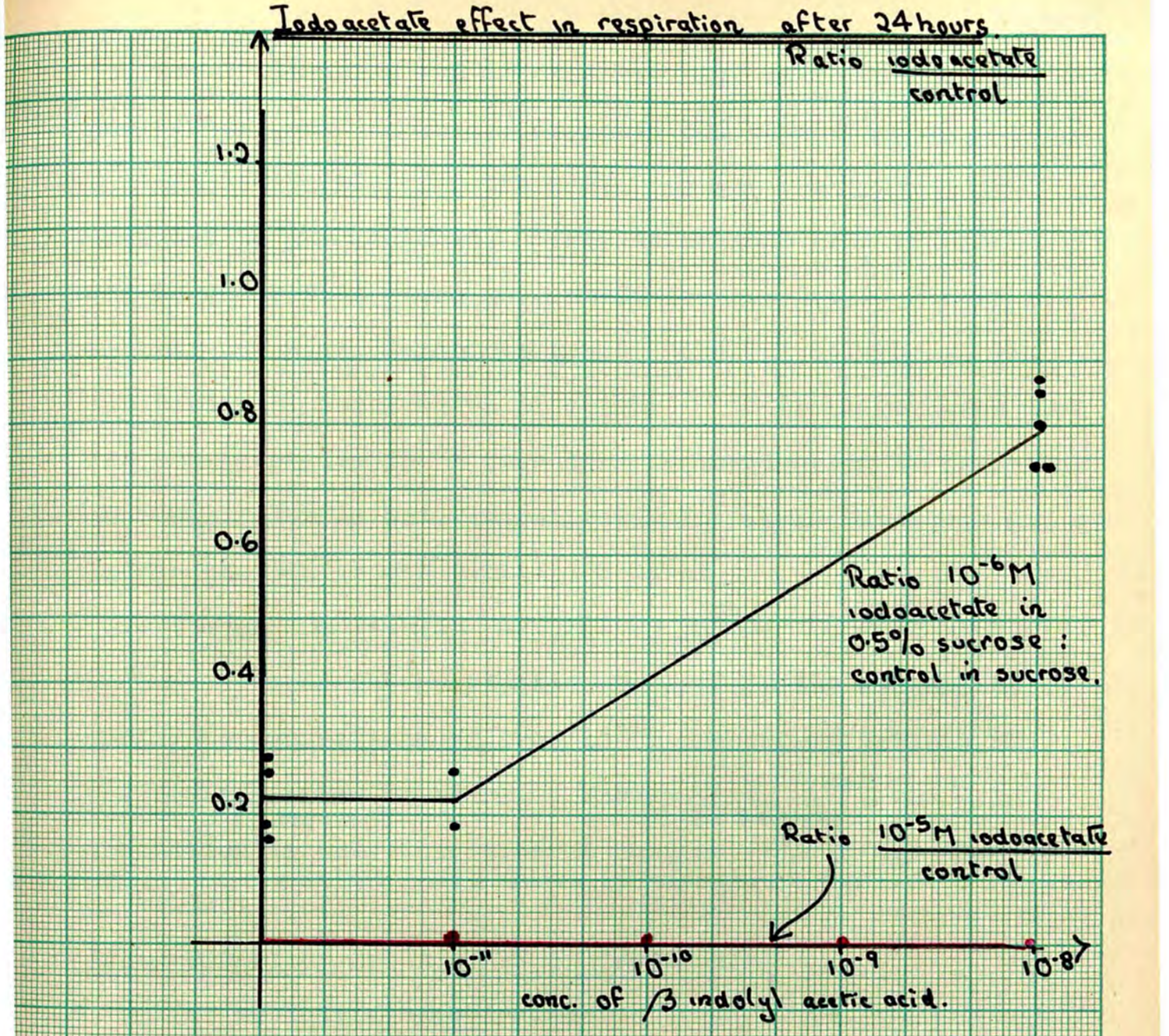
The effect on the total elongation was quite remarkable, for, compared with the auxin inhibited control, the sections in 10^{-6}M iodoacetate + auxin grew 35% more.

Graph 38.



Values given for respiration rates 2–4 hours after treatment.
Medium 0·5% sucrose.

Graph 39.


Iodoacetate effect in respiration after 24 hours.
Ratio iodoacetate / control
1.2
1.0
0.8
0.6
0.4
0.2
Ratio 10⁻⁶M iodoacetate in 0.5% sucrose : control in sucrose.
Ratio 10⁻⁵M iodoacetate / control
10⁻¹¹
10⁻¹⁰
10⁻⁹
10⁻⁸
conc. of β indolyl acetic acid.

It seems that auxin and iodoacetate were really cancelling one another out, since the expected 50% inhibition of growth by iodoacetate was not obtained; nor was the inhibition expected as due to auxin. This is a clear case of antagonism and it seems that these two substances completely cancel one another out with respect to growth inhibition, whereas the inhibition of respiration is slight. It is to be noted that the concentration 10^{-6}M does inhibit respiration to the order of roughly 10% in this concentration of auxin but it is no longer the 50% growth inhibition level.

(c) on the 24 hour respiratory values

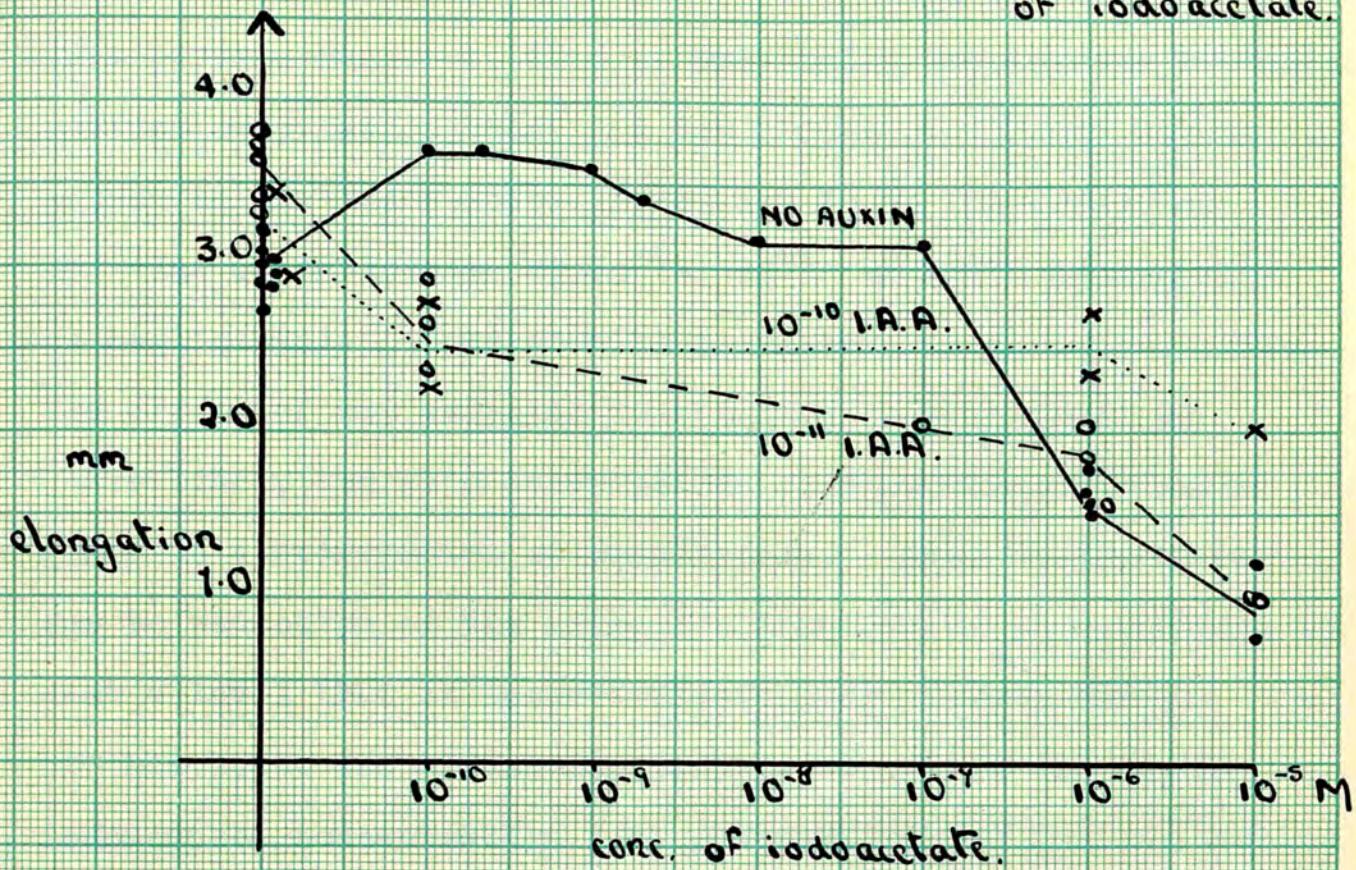
As was previously the case in the absence of auxin, concentrations of over 10^{-5}M completely inhibit the respiration after 24 hours. However with 10^{-6}M the 9.0% (at the start) inhibition is only increased to 20% (after 24 hours) (cf. 77% in the absence of auxin).

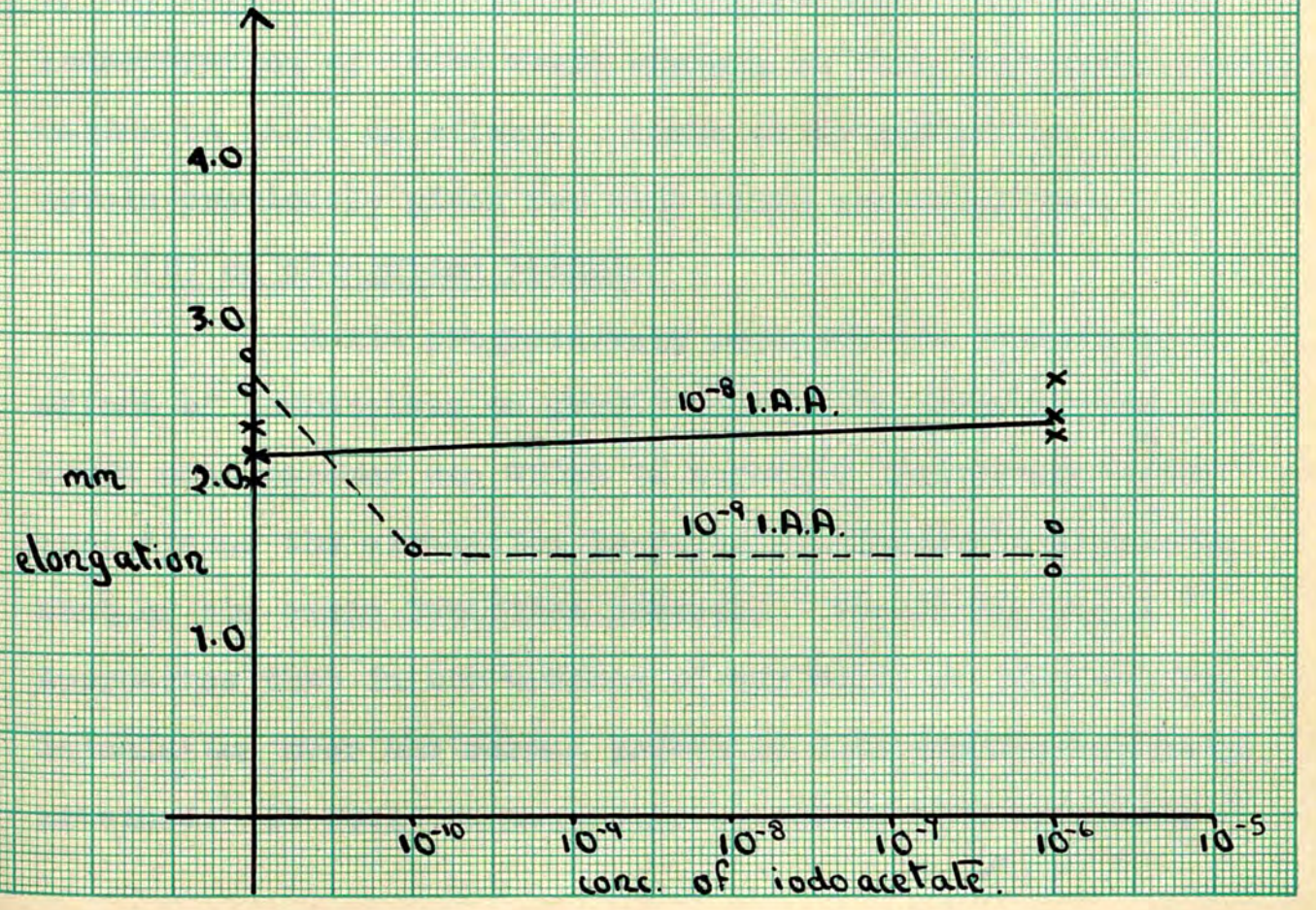
III. EFFECT OF OTHER AUXIN CONCENTRATIONS

(a) on initial respiration

It has been observed previously that auxin has no initial effect on respiration except that concentrations of 10^{-8}IAA tend to be slightly inhibitory. The normal curve in the absence of auxin has been well established. Low concentrations of iodoacetate are stimulatory to initial respiration and higher concentrations are increasingly inhibitory. If however we select any one concentration of iodoacetate and study its relative inhibition in different

AUXIN effect on the elongation in the presence of iodoacetate.





auxin concentrations we obtain a curve which corresponds in a reverse manner to that of increasing iodoacetate inhibition against respiration rate. A low concentration has little effect on the iodoacetate inhibition (e.g. the effect of 10^{-11}IAA on inhibition by 10^{-6}M, 10^{-5}M or 10^{-4}M iodoacetate is negligible). However with increasing auxin concentration the effect of any selected iodoacetate concentration is neutralised, and with intermediate concentrations of auxin there is a stimulation of respiration. This is presumably due to reduction of the effective iodoacetate concentration to such a level that it becomes stimulatory to respiration. Up to this point the effect has been due to reduction of the iodoacetate concentration i.e. respiration has been controlled by the effective iodoacetate concentration. However when the concentration of added auxin exceeds 10^{-8}IAA, the auxin itself begins to exert an effect. There is an inhibition to the levels previously given for this auxin concentration.

(b) on elongation ratios

Auxin progressively restores the inhibitory effect of iodoacetate on growth and as the concentration of auxin used becomes one which is normally inhibitory to the root tissue the elongation in auxin + iodoacetate surpasses that of the auxin inhibited control. The final length approaches that in the absence of any auxin or iodoacetate. Stimulation over the normal growth in 0.5% sucrose was never obtained: it would be necessary to reduce both effective

concentrations to a very critical level which was not found practicable in our experiments. If stimulatory iodoacetate was used in conjunction with stimulatory auxin, the concentrations were sufficiently high for the two effects to be cancelled out.

It seems therefore that β indolyl acetic acid and iodoacetic acid are acting on the same system. They both have the property of becoming attached to certain centres of metabolism and there can act in a characteristic way, peculiar to each one of them. Both have a similar action on elongatory growth. At low concentrations there is a stimulation and at higher concentrations an inhibition of this process. However their action on initial and subsequent respiration is quite different. Auxin has no initial effect on respiration beyond an inhibitory one at high concentrations. Subsequent respiration depends on the final length of the sections. However iodoacetate has a characteristic initial effect on respiration, following very much the effect on final length. Low concentrations are stimulatory and high concentrations inhibitory. Subsequent respiration ~~results in the reduction of this process~~ falls to a low level. It seems that the number of metabolic centres to which these ions can become attached is limited, so that if both iodoacetate and auxin are present they act as competitors. Since sufficient number of these centres are not available the "effective" concentration of <u>both</u> substances is reduced and characteristic

antagonistic effects are obtained.

The metabolic centres are in all probability, enzyme complexes containing available -SH groups (Quastel and Wheatley, 1942). Since malate and other C_4 acids have the property of protecting these groups against iodoacetate inhibition the effect of the threefold bombardment by auxin iodoacetate and malate will be described below.

EFFECT OF MALATE ON GROWTH AND RESPIRATION

Effect of Malate (concentration 0.001 M sodium hydrogen malate)

The effect of malate was considered alone and in the presence of 0.5% sucrose; in the absence of auxin and at two auxin levels; and at four time levels so that some idea of the time drift could be obtained. The scatter graphs illustrate the effects fully. Ratio graphs relating each treatment reading with its appropriate control have been included.

In the absence of sucrose, malate has no significant effect on the respiratory time drift, whether auxin is present or not.

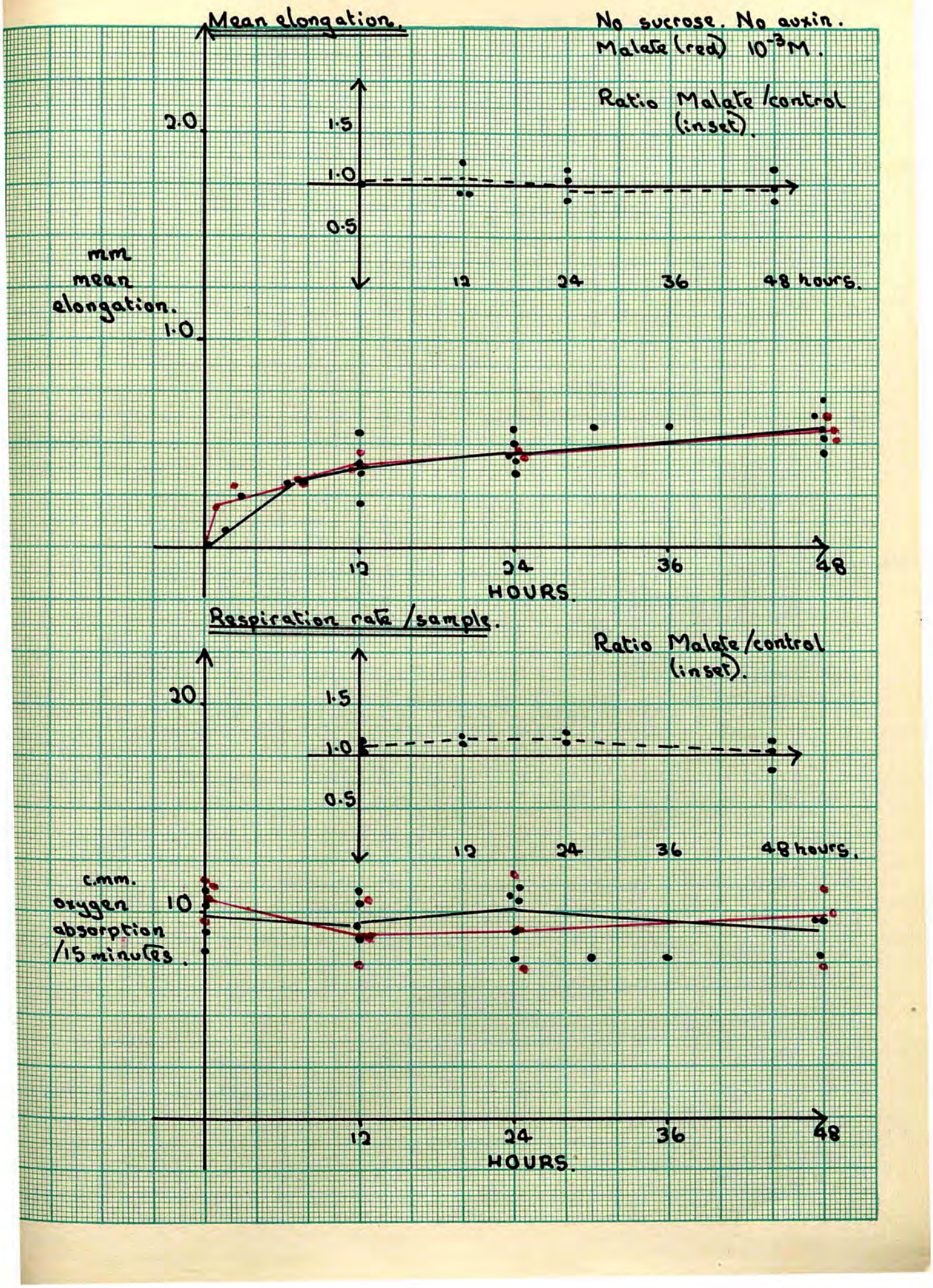
In the presence of sucrose where the characteristic respiratory maxima at 24 hours are obtained, this concentration of malate tends to increase this respiratory maxima if either stimulatory or inhibitory auxin are present, but there is no effect if auxin is absent.

Again in the absence of sucrose, malate has no effect on the elongation in the absence of auxin or in the presence of concentrations stimulatory or inhibitory to growth.

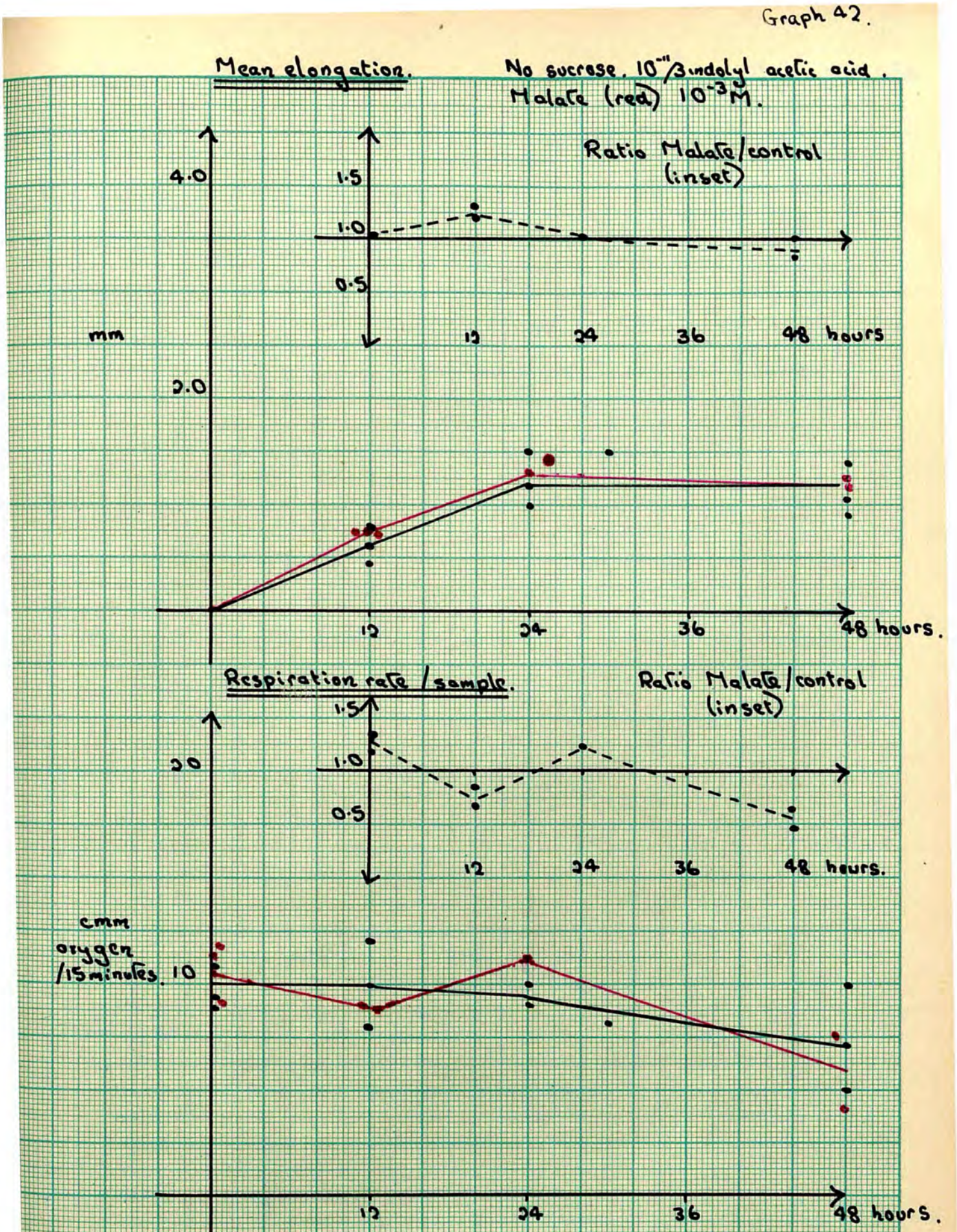
However if sucrose is present malate has the property of increasing the inhibition due to auxin.

It is obvious that malate is not acting here as a substrate in addition to sucrose. There is no significant effect in the absence of sucrose, but when there is sufficient sucrose to be used as substrate malate increases the sensitivity of the tissue to auxin action, increasing or decreasing growth

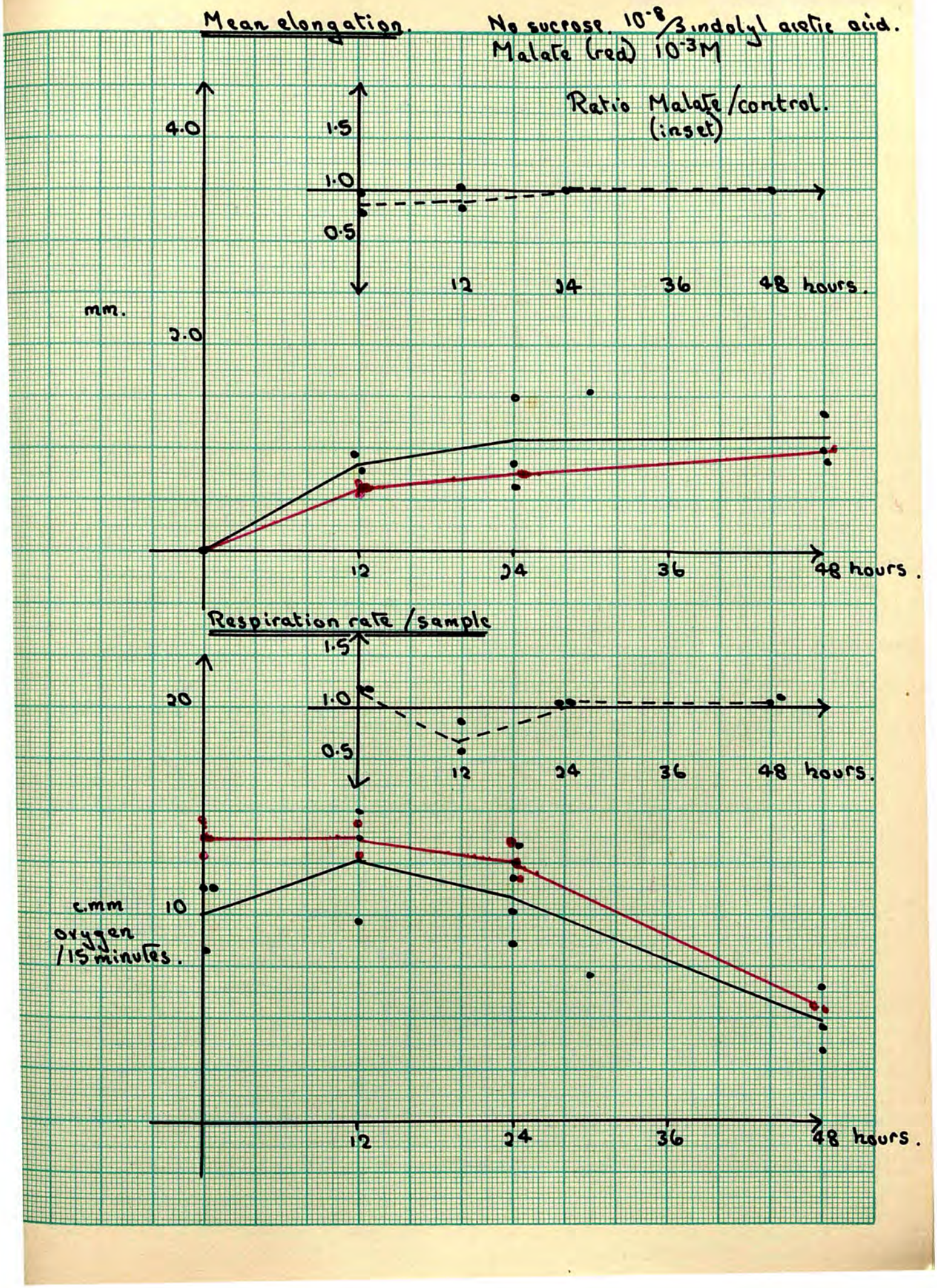
Graph 41.


Mean elongation.
No sucrose. No auxin.
Malate (red) 10^{-3}M.
Ratio Malate/control (inset).
2.0
1.0
mm mean elongation.
1.5
1.0
0.5
12
24
36
48 hours.
12
24
36
48
HOURS.
Respiration rate/sample.
Ratio Malate/control (inset).
20
10
c.mm. oxygen absorption /15 minutes.
1.5
1.0
0.5
12
24
36
48 hours.
12
24
36
48
HOURS.

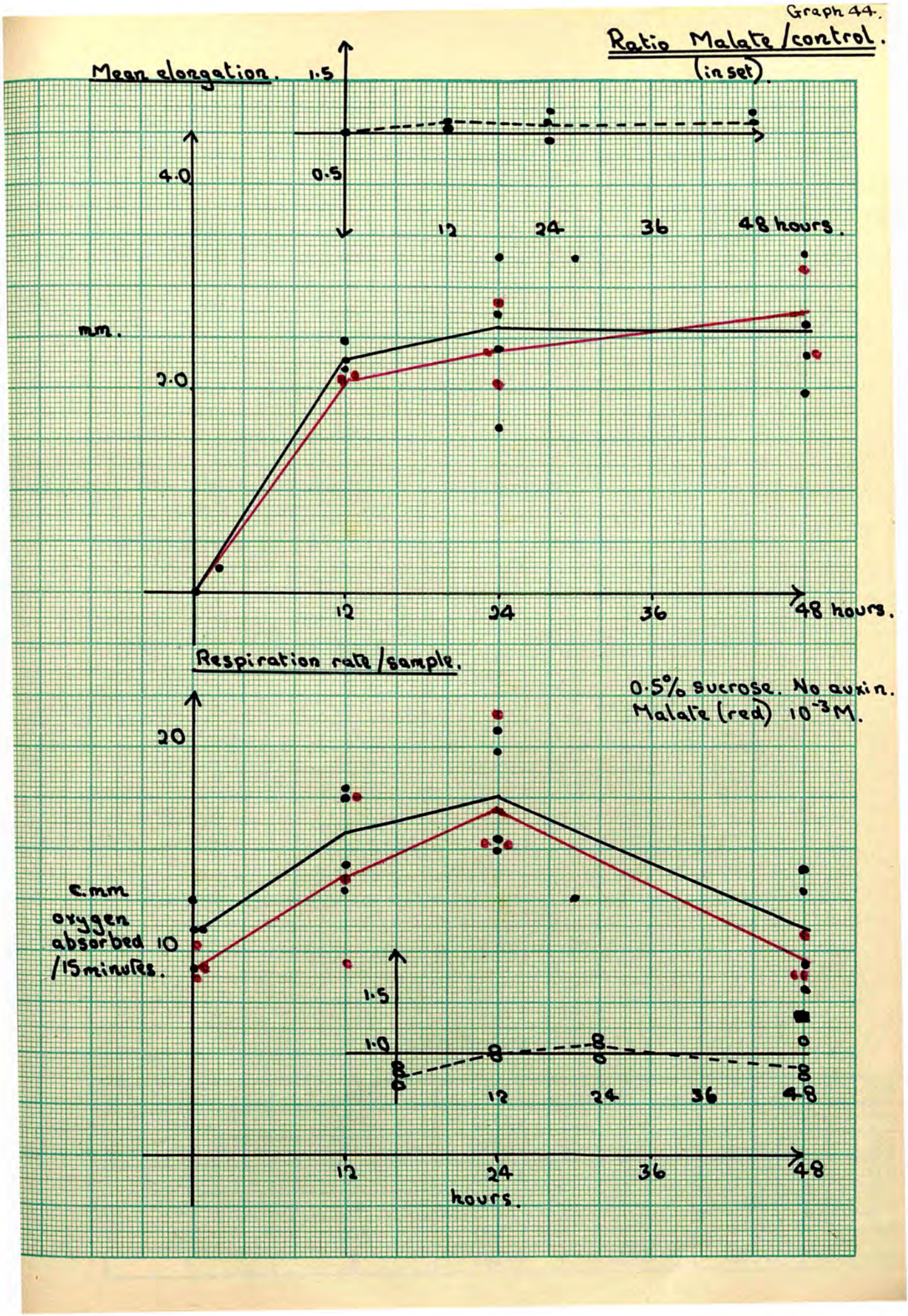
Graph 42.


Mean elongation.
No sucrose. 10^{-11}/3 indolyl acetic acid.
Malate (red) 10^{-3}M.
Ratio Malate/control (inset)
4.0
2.0
mm
1.5
1.0
0.5
12
24
36
48 hours
12
24
36
48 hours.
Respiration rate / sample.
Ratio Malate/control (inset)
20
10
cmm oxygen /15 minutes.
1.5
1.0
0.5
12
24
36
48 hours.
12
24
36
48 hours.

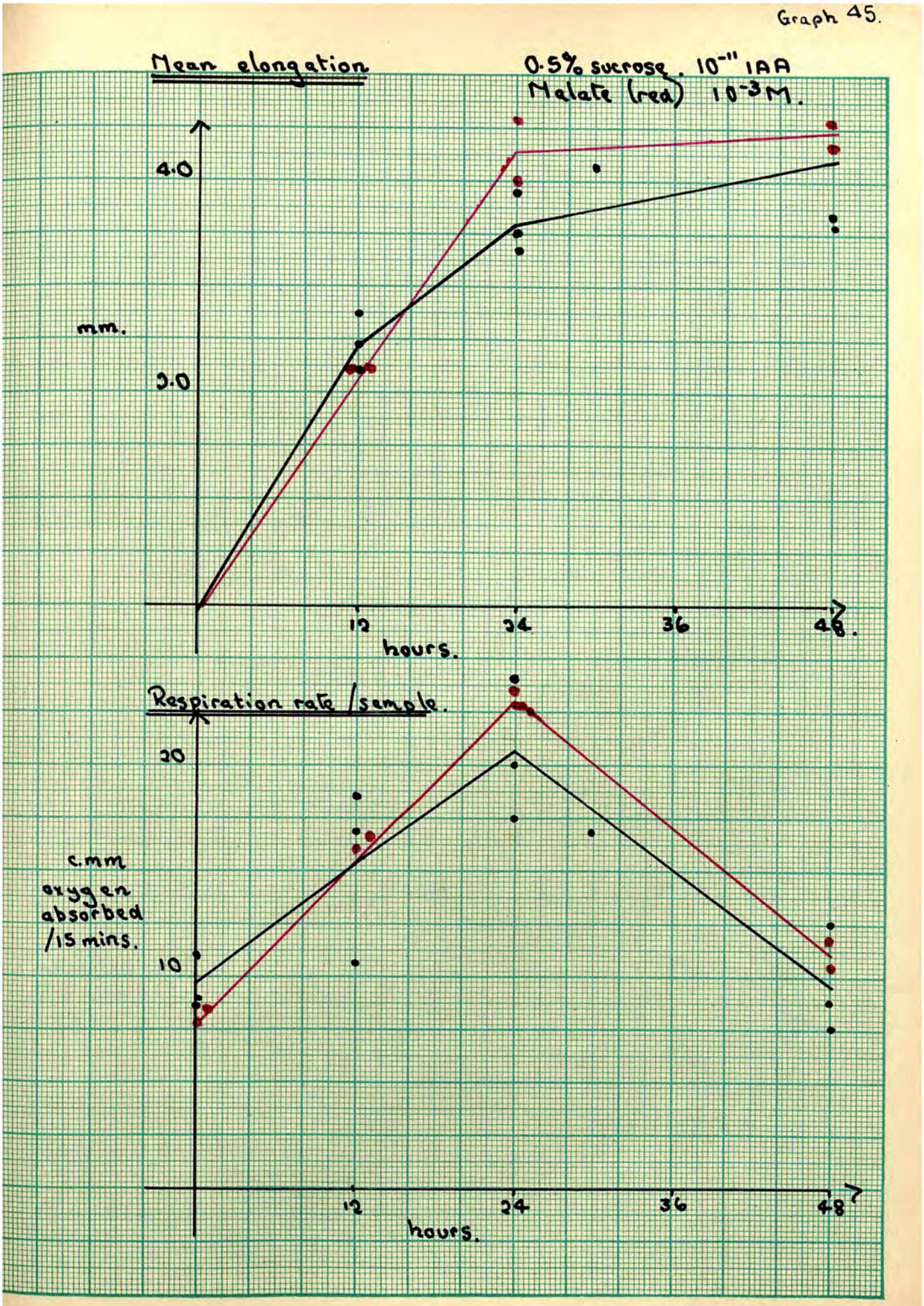
Graph 43.


Mean elongation.
No sucrose. 10^{-8} 3 indolyl acetic acid.
Malate (red) 10^{-3}M
Ratio Malate/control. (inset)
4.0
3.0
2.0
1.5
1.0
0.5
12
24
36
48 hours.
mm.
Respiration rate/sample
20
10
c.mm oxygen /15 minutes.

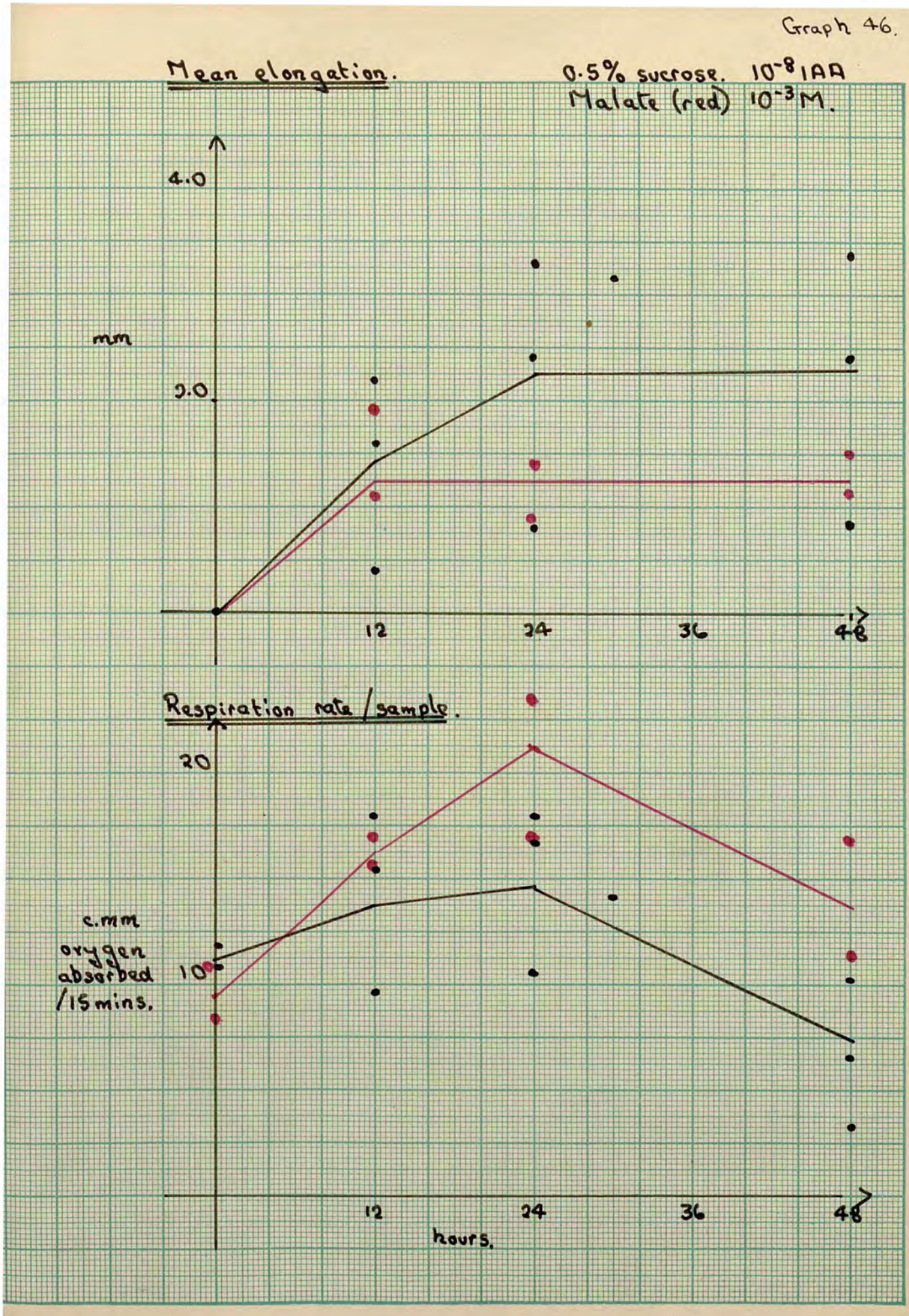
Graph 44.


Mean elongation.
Ratio Malate/control.
(in set).
1.5
0.5
4.0
2.0
mm.
12
24
36
48 hours.
Respiration rate/sample.
0.5% sucrose. No auxin.
Malate (red) 10^{-3}M.
20
10
c.mm. oxygen absorbed /15 minutes.
1.5
1.0
hours.

Graph 45.


Mean elongation
0.5% sucrose. 10^{-11} IAA
Malate (red) 10^{-3} M.
4.0
3.0
mm.
12
24
36
48
hours.
Respiration rate / sample.
20
10
c.mm oxygen absorbed /15 mins.
12
24
36
48
hours.

Graph 46.


Mean elongation.
0.5% sucrose. 10^{-8} IAA
Malate (red) 10^{-3} M.
4.0
mm
2.0
12
24
36
48
Respiration rate / sample.
20
c.mm oxygen absorbed /15 mins.
10
12
24
36
48
hours.

as the case may be. Malate and auxin are not antagonistic to each other; but malate work together increases the auxin effect.

MALATE IODOACETATE AND AUXIN

Affect of Malate on iodoacetate inhibition, in the absence of auxin

INITIAL RESPIRATION

For these experiments the concentration of 10^{-6}M iodoacetate was selected for study. It was found that when malate and iodoacetate were added together, a concentration of 10^{-5}M sodium hydrogen malate reduced the 55% inhibition to about 30%. With 10^{-4}M the inhibition was reduced to 10% and with 10^{-3}M there was a slight (insignificant) stimulation of initial respiration.

ELONGATION

As was found by Thimann and Bonner (1948) malate has the property of completely restoring the inhibition due to iodoacetate. Since malate has not the property of increasing elongation in the absence of added auxin, there is no increase of elongation over and above the normal elongation in 0.5% sucrose.

RESPIRATION AFTER 24 HOURS

Again 10^{-3}M malate has the property of returning the ~~malate~~ iodoacetate inhibition to the original level. It must however be stressed that the malate and iodoacetate were added together, at the beginning of the experiment.

Effect of malate on iodoacetate inhibition, in the presence of auxin

INITIAL RESPIRATION

The graph shows the mean respiration rates over

two initial periods 0-2 hours and 2-4 hours. There is little effect in the first two hours due to incomplete penetration of the reactants. However for the 2-4 hour period, it is shown clearly that in the presence of all four substances

(a) sucrose, here 0.5%

(b) iodoacetate, here 10^{-6}M

(c) auxin, here 10^{-8}indolyl acetic acid

(d) malate of concentration 10^{-5}M to 10^{-3}M

there is a very large reduction of the respiratory rate.

RESPIRATION AFTER 24 HOURS

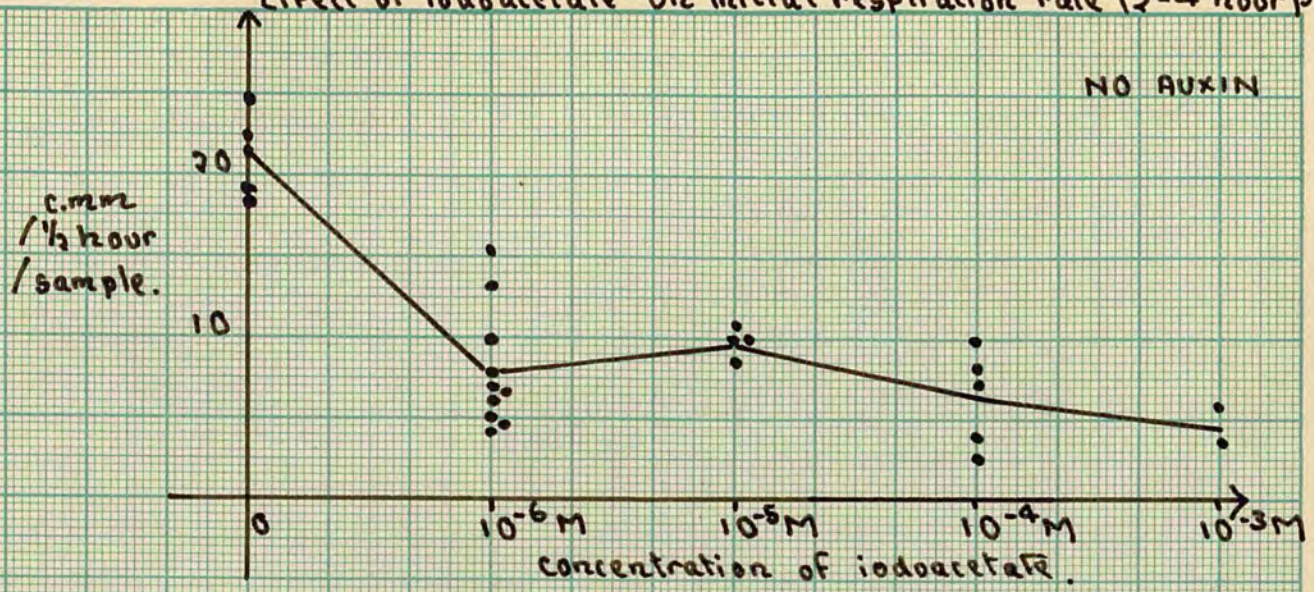
Auxin has the property in this concentration of maintaining the respiratory rate to a near normal level even if 10^{-6}M iodoacetate is present. However if malate is also present the respiration at 24 hours is reduced to nil.

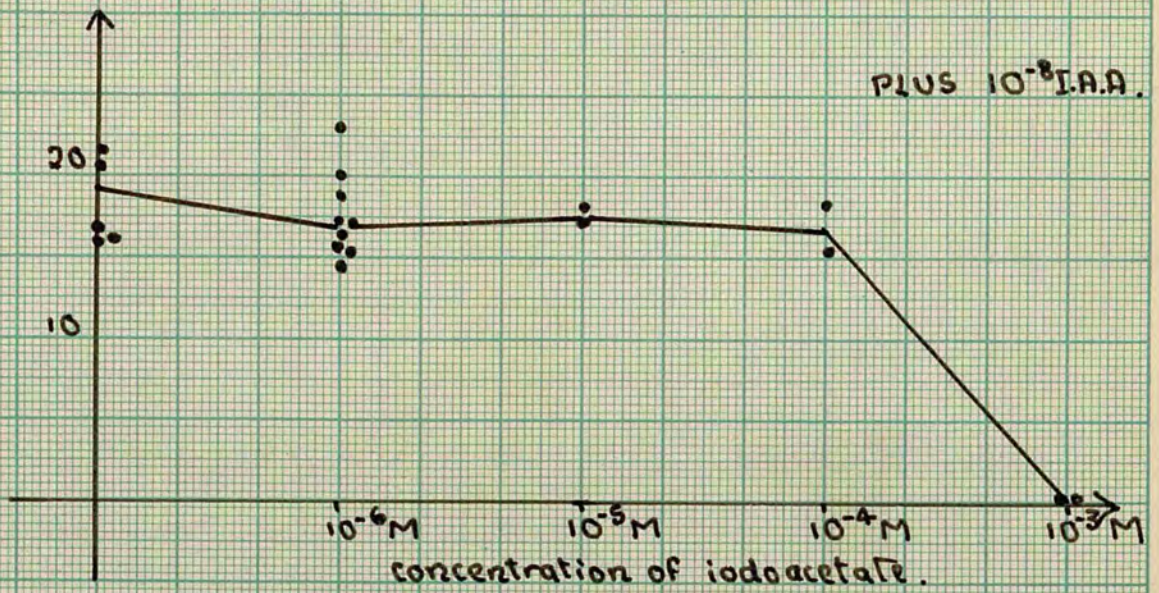
ELONGATION

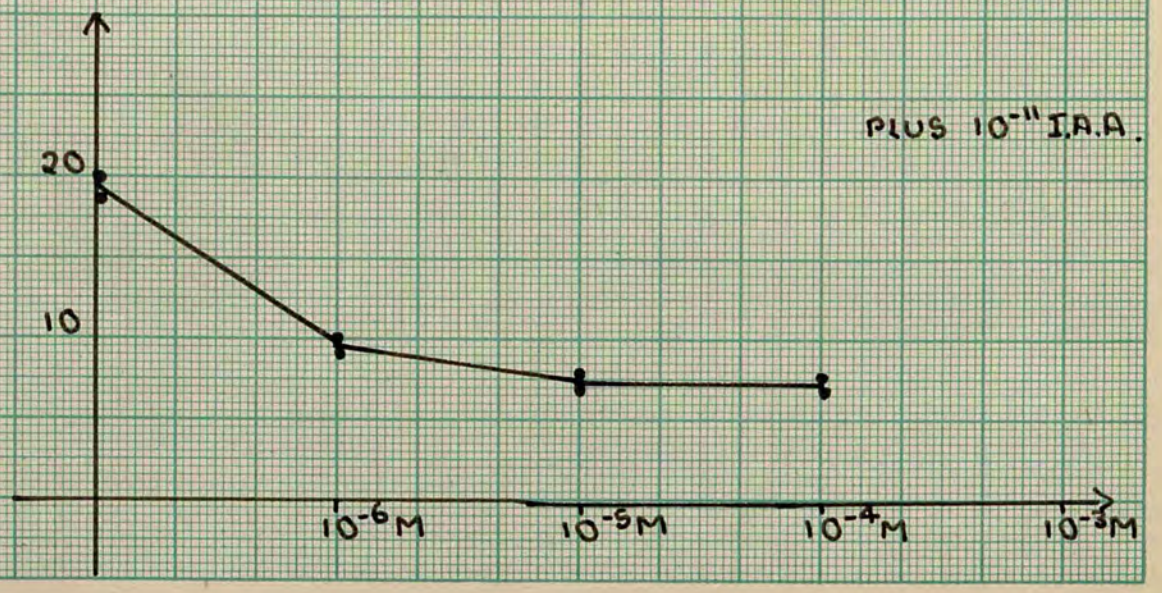
In 24 hours the actual elongation of section placed in a solution of the four components above was negligible - not more than an initial 1-2 hours normal elongation.

Graph 44

Effect of iodoacetate on initial respiration rate (2-4 hour period)

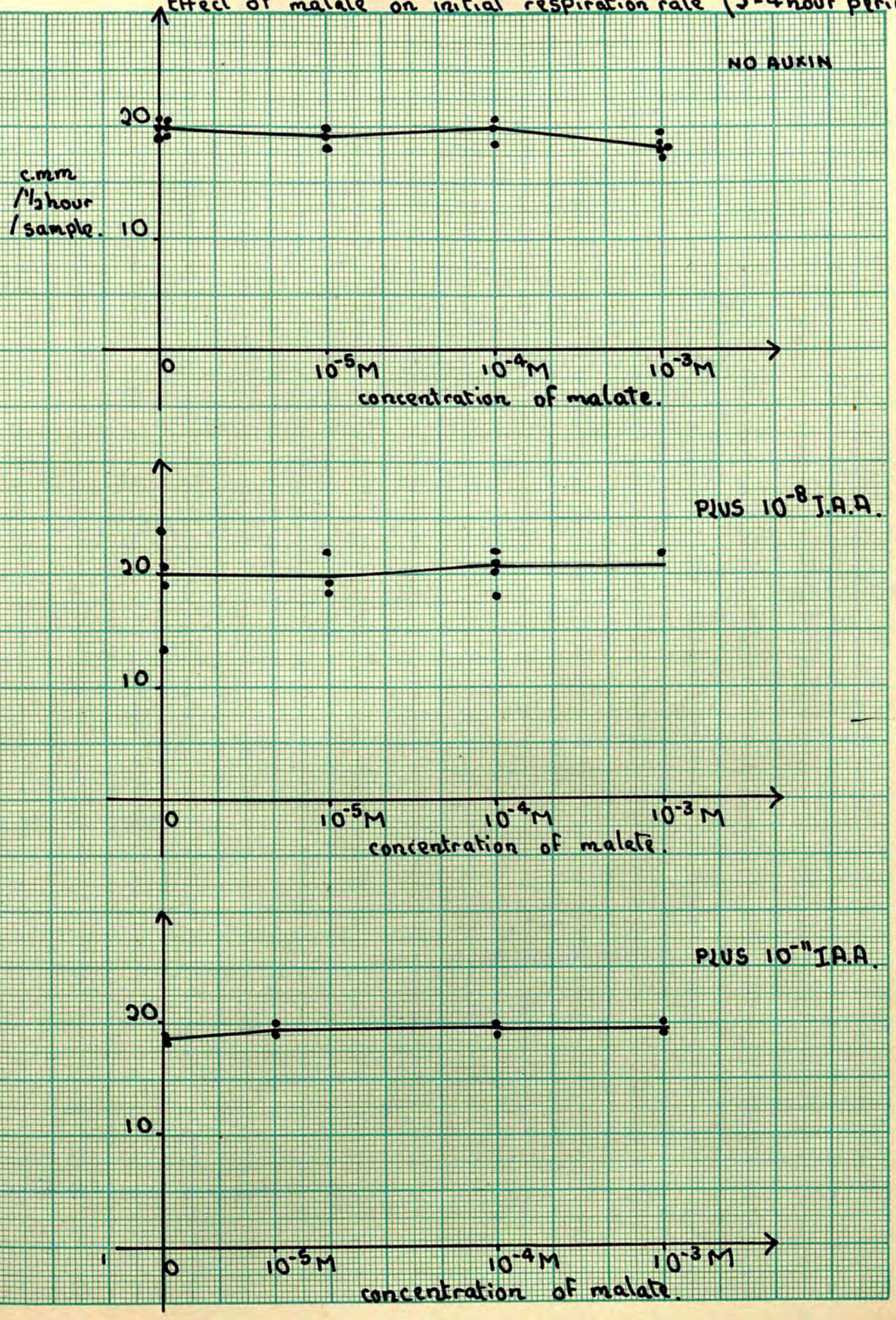






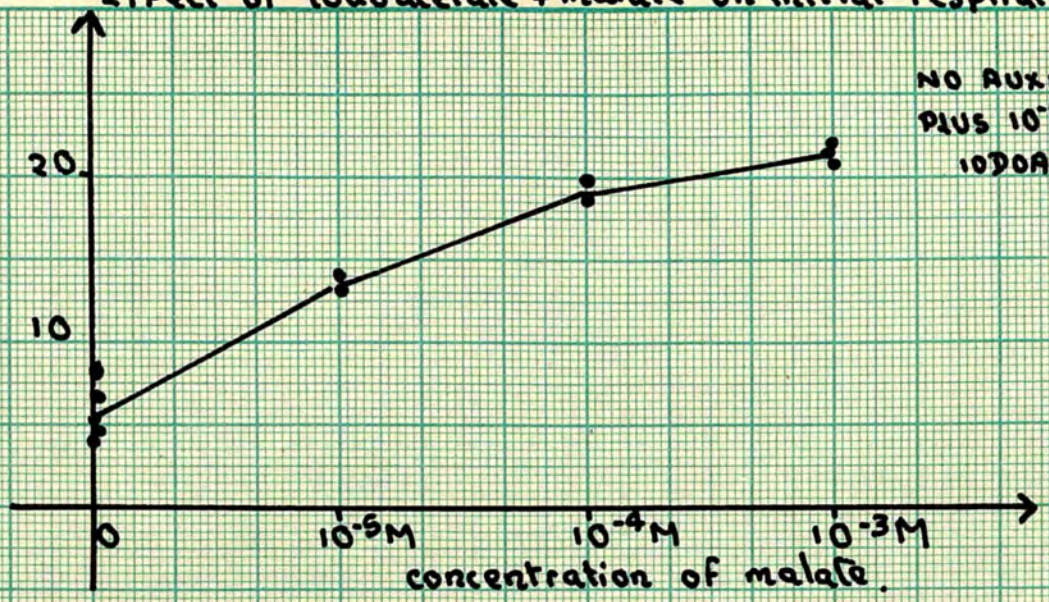
Graph 48.

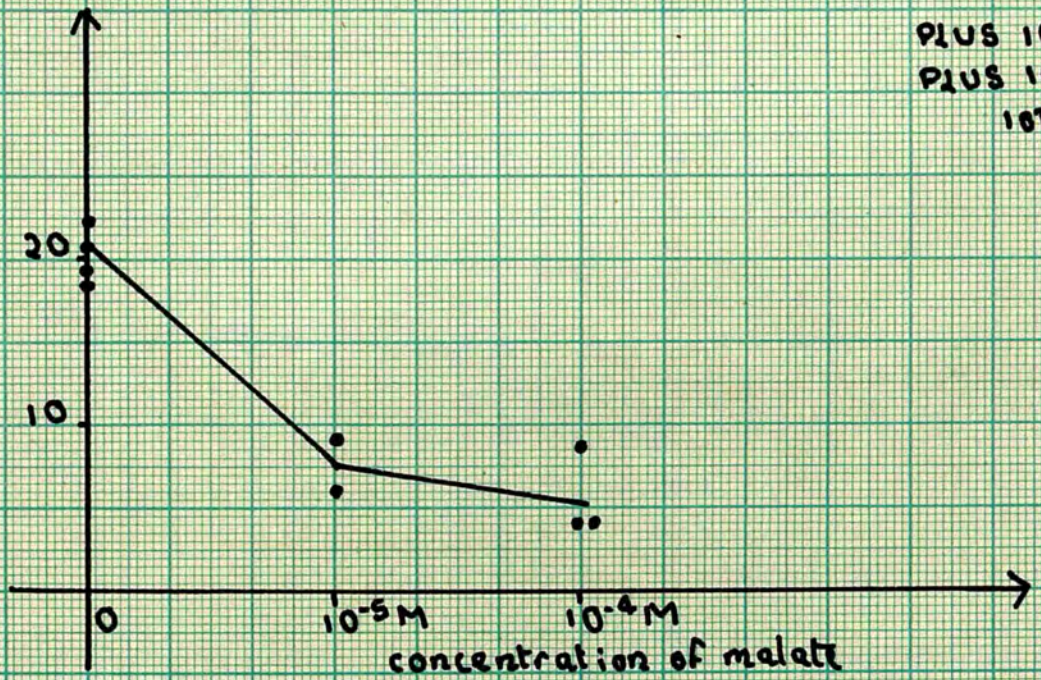
Effect of malate on initial respiration rate (2-4 hour period)



Graph 49.

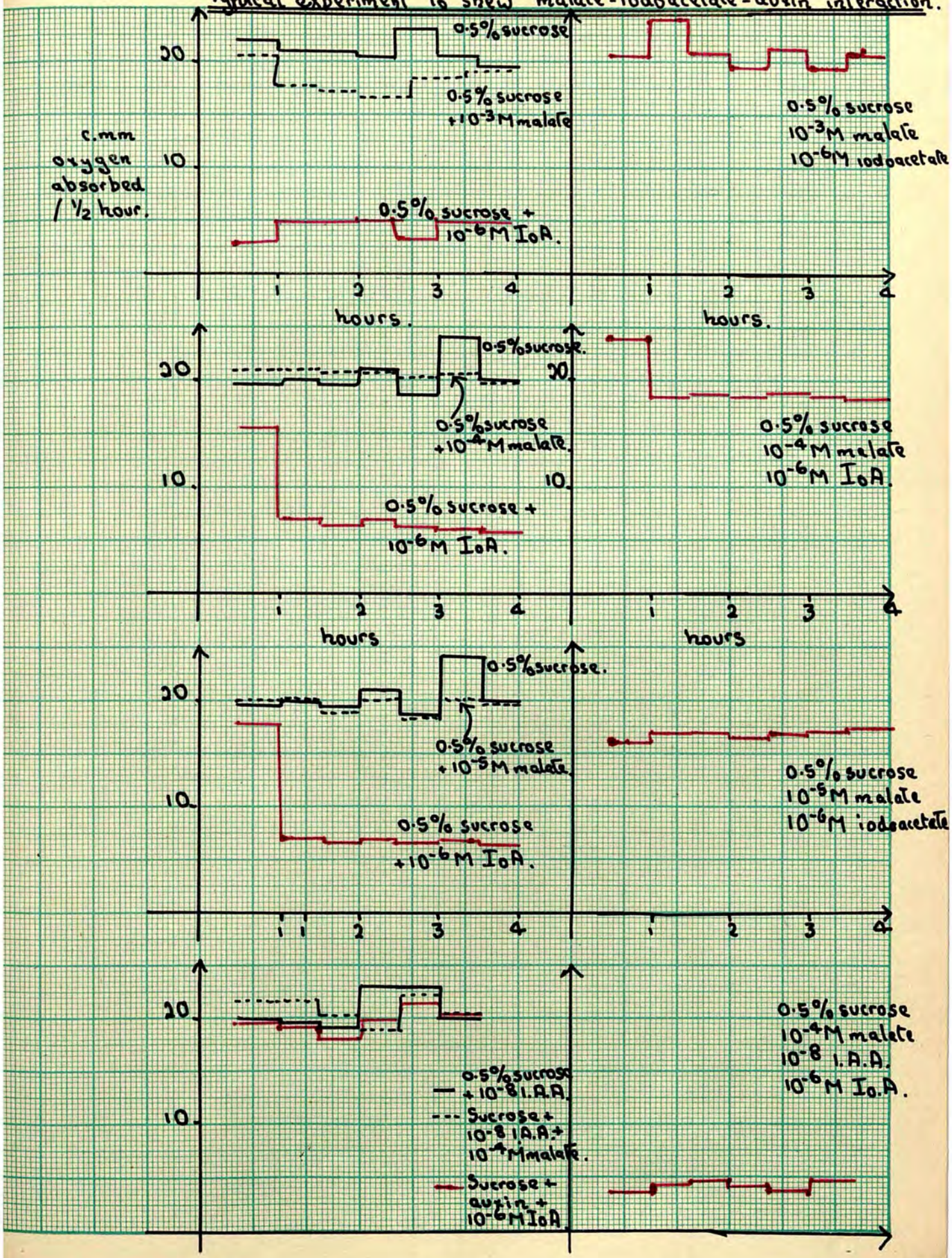
Effect of iodoacetate + malate on initial respiration rate.





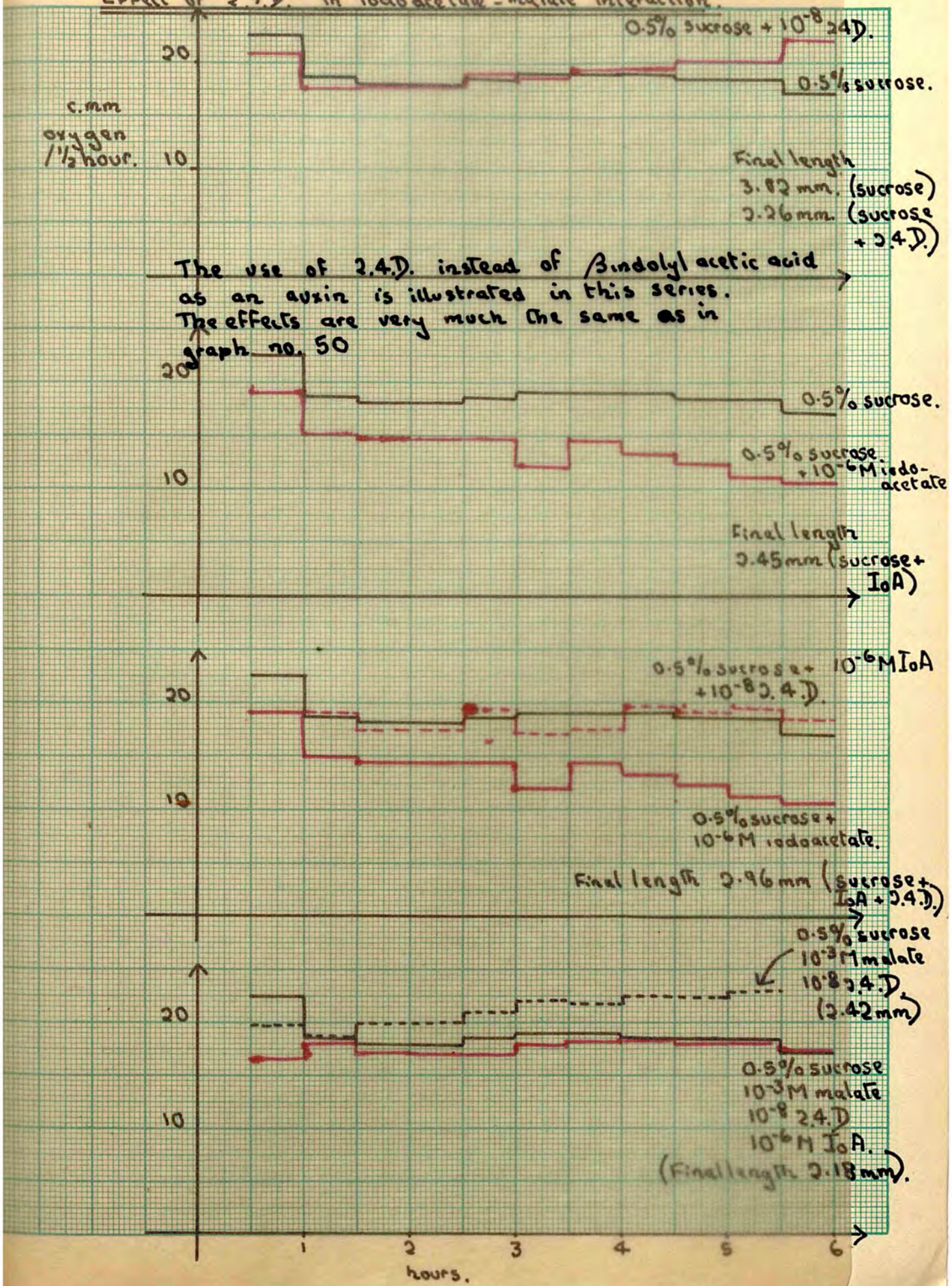
Graph 50.

Typical experiment to shew malate-iodoacetate-auxin interaction.



Graph 51.

Effect of 2.4.D. in iodoacetate - malate interaction.



EFFECT OF IODOACETATE ON STEM TISSUE

Effect of iodoacetate on stem tissue

Christiansen and Thimann (1950) find the following using pea-stem sections 15 mm. long.

Indolyl acetic acid stimulates the initial respiration rate / unit dry weight 15-25% over that of sections grown in water alone.

Iodoacetate used at a concentration of 6×10^{-4}M reversed this stimulation by auxin (1 mg. / litre) and caused 50% inhibition of growth.

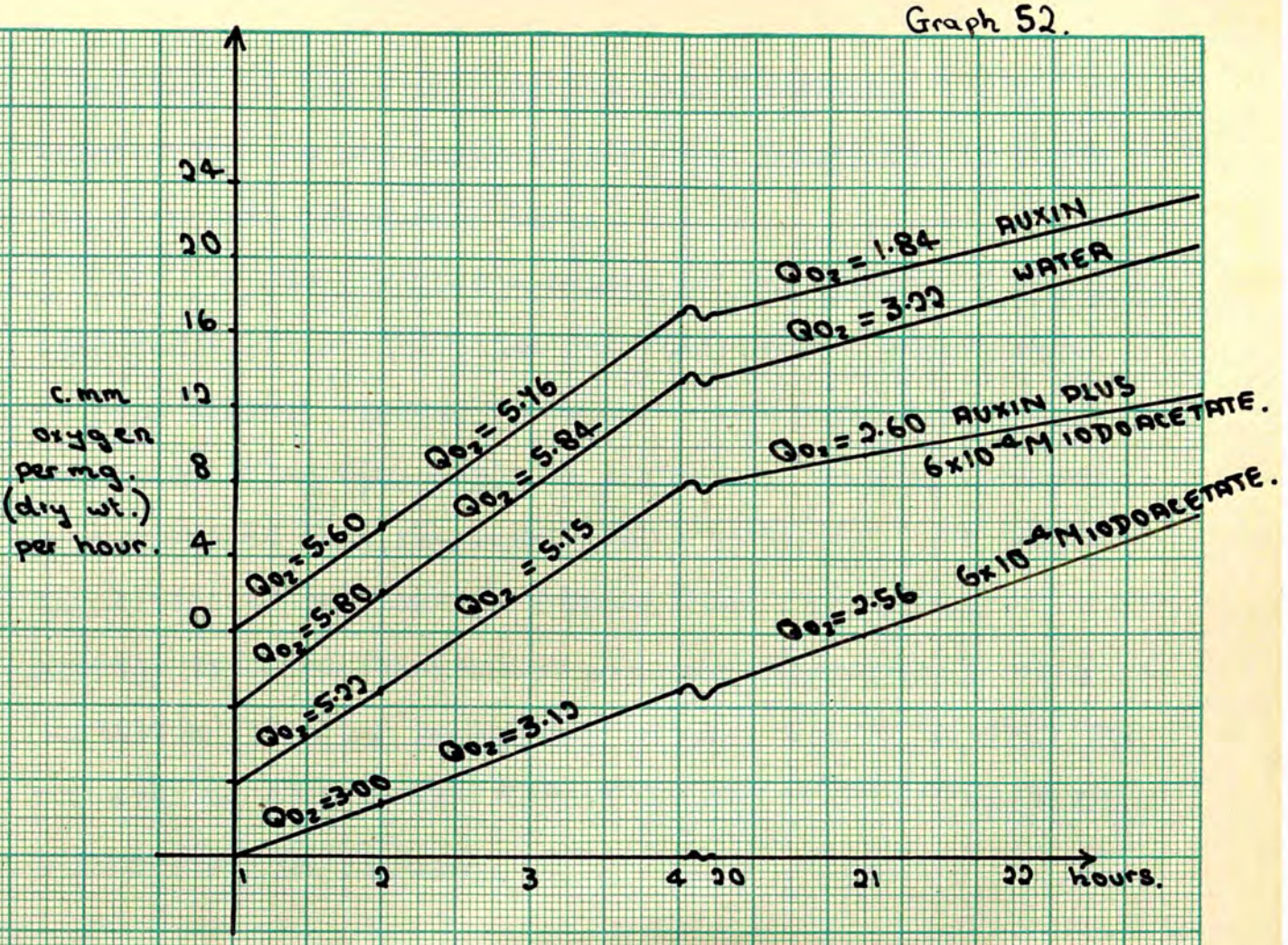
No results were included for iodoacetate in the absence of added auxin.

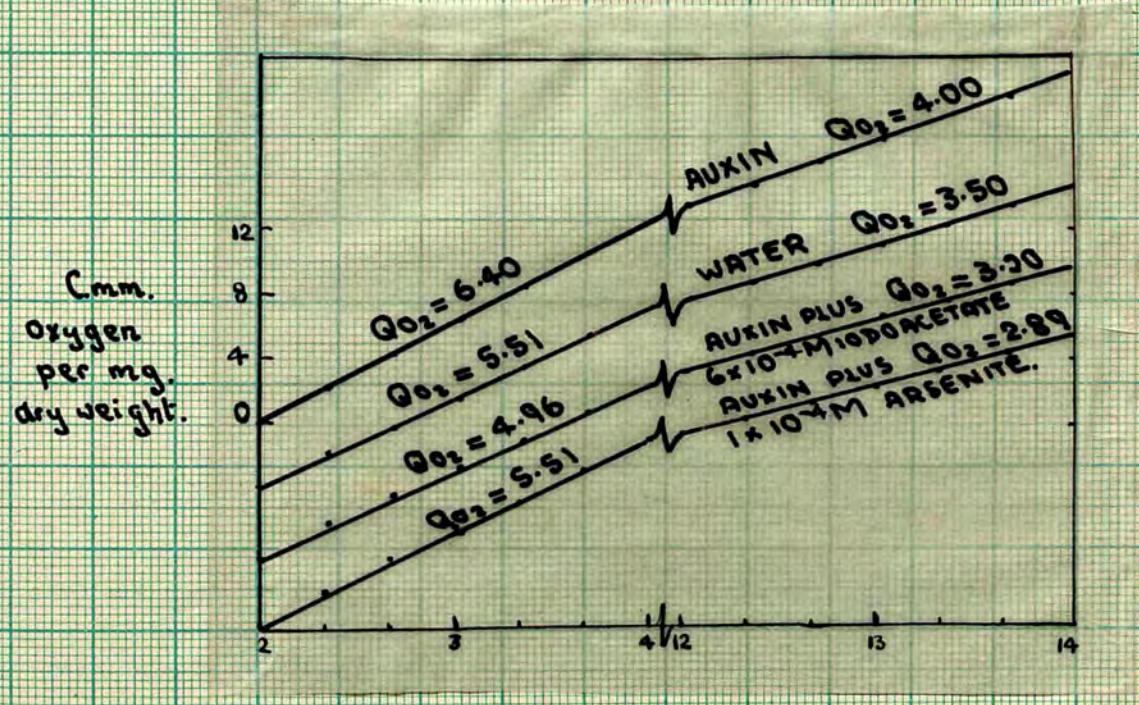
We repeated the experiments of Christiansen and Thimann exactly using 15 mm. sections taken from the third internode of etiolated seedlings. Since there was not sufficient information in their paper to work out their results on a basis of unit section, as we had done previously, we obtained dry weight values for each sample and worked out our results on a unit dry weight basis in the same way as these authors had done previously. We include here copies of their original graph and the graph we obtained using our tissue (a different variety of pea).

It can be seen that auxin has no significant effect on the Q_{O_2} over the first four hours. The same concentration as was used by Christiansen and Thimann was used here.

A concentration of 6×10^{-4}M iodoacetate is

Graph 52.





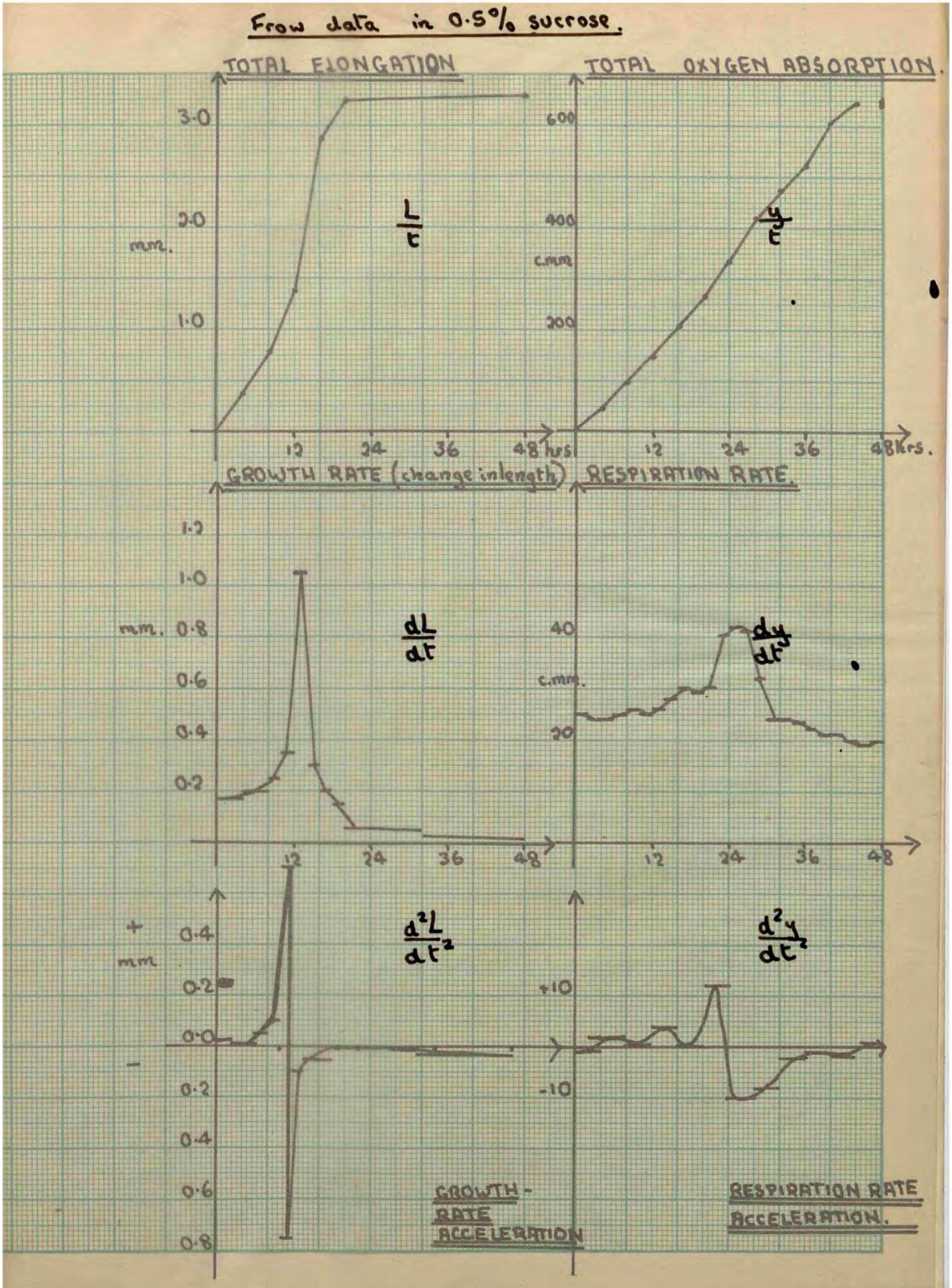
Tracing from Christiansen G.S. and Thimann K.V. (1950) (see references).

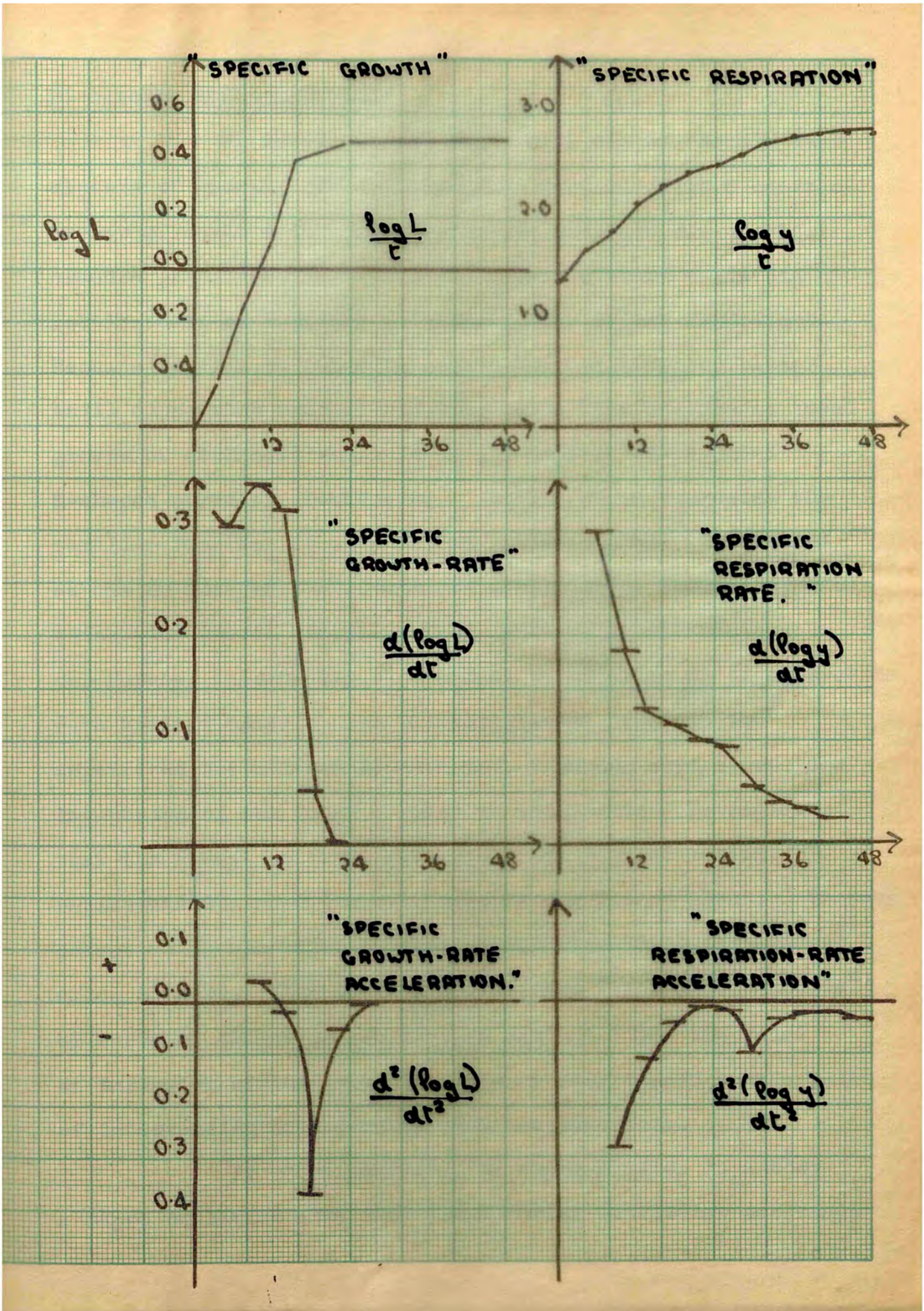
inhibitory to the same extent as was previously found; BUT iodoacetate has not reversed the stimulation due to auxin since we have found none here.

We included a sample in the absence of auxin to show iodoacetate inhibition and find that in stem tissue, as well as root tissue, there is a considerably greater inhibition than when auxin is present. It must therefore be emphasised that the reason for a relatively low iodoacetate inhibition of respiration coupled with a high inhibition of growth, is not a fundamental differential action of this inhibitor which can be applied universally, it is only the property of this inhibitor in the presence of sufficiently high auxin concentration.

DISCUSSION AND CONCLUSIONS

Frow data in 0.5% sucrose.




"SPECIFIC GROWTH"
"SPECIFIC RESPIRATION"
log L
0·6
0·4
0·2
0·0
0·2
0·4
3·0
2·0
1·0
12
24
36
48
$\frac{\log L}{t}$
$\frac{\log y}{t}$
0·3
0·2
0·1
"SPECIFIC GROWTH-RATE"
"SPECIFIC RESPIRATION RATE."
$\frac{d(\log L)}{dt}$
$\frac{d(\log y)}{dt}$
+
-
0·1
0·0
0·1
0·2
0·3
0·4
"SPECIFIC GROWTH-RATE ACCELERATION."
"SPECIFIC RESPIRATION-RATE ACCELERATION"
$\frac{d^2(\log L)}{dt^2}$
$\frac{d^2(\log y)}{dt^2}$

Discussion and conclusions

We have been concerned with one of the simplest aspects of growth, for we have taken sections with a constant number of cells (that number remained constant due to the absence of mitotic activity) and moreover these cells did not increase appreciably in diameter during the experiment. Because of this by plotting length against time (or better still elongation against time) we get a true picture of total growth of each cell which is in the form of the typical S-shaped curve of growth/time. Such a curve expresses the familiar function of exponential increase until such a time as one or more of the components of growth be exhausted, after which time growth ceases. This curve is a valuable one but further information is obtained by plotting $\frac{dL}{dt}$ (i.e. growth-rate against time). The valuable "point of inflexion" appears as the maximum growth-rate. In our curves too, the lag-period of the first 6 hours is prominent. We believe that the almost constant rate over this initial period represents the normal growth rate in the intact plant. Sucrose causes an acceleration of that growth rate which is manifest after 6 hours. The curve $\frac{d^2L}{dt^2}$ (i.e. the "acceleration curve" or curve of change of growth-rate against time) the point of inflexion of the previous curve is represented by the point of intersection of the curve with the time-axis.

To quote Medawar, "If there existed in a body a depot of living substance which turned out living substance

at first with increasing and latterly with decreasing energy, then the point of inflexion would mark a genuine climacteric." Now if growth were dependent on simultaneous increase im respiration we might expect respiration rate to increase upto the point of growth-rate inflexion, and this to be followed by a fall. We have shown this is not the case. The growth-rate begins to fall, while respiration rate / sample is still rising. However we have also shown that it is somewhat erroneous to consider respiration rates on a unit sample basis without regard for the change in length. Respiration rate / unit length decreases with time, showing growth is <u>not</u> dependent on any recognisable simultaneous respiratory increase, but in reality it is quite the reverse. Apparent respiratory rate increases on a sample basis are due to changes in length.

It can be mentioned that the quantity which has been called "specific growth-rate" also decreases with time in the same manner as does respiration rate / unit length. To obtain this quantity we must take first the curve of specific growth against time. Log L is plotted as ordinate. This curve records the rate of multiplication of living substance. If all living matter formed continues to promote formation of further new substance at a constant rate the curve Log L against time will be a straight line (as it is over the"exponential phase" of growth). The former S-shape is obliterated. Taking $\frac{d(\log L)}{dt}$ against time there is no longer a growth rate maximum. The curve $\frac{d^2(\log.L)}{dt}$ shows

continuous deceleration throughout the whole of the growth process. Although we may believe Richard's law that in a constant environment (internally and externally) growth proceeds with a uniform specific velocity, Minot's law is more appropriate under the actual conditions to which living tissue is subjected. He states that specific acceleration of growth is always negative. There is therefore a progressive dissipation of "growth energy". In theory every particle of protoplasm formed during growth should be able to reproduce itself at the same rate as it has been formed, just as each bacterium is formed by a binary fission and is capable of itself forming two organisms (Hinshelwood, 1946). However even with bacteria the case is not as simple as that. Other phenomena such as staling, ageing and depletion of substrate come into play. Specific growth-rate, which is an expression of the rate of growth of individual particles of protoplasm as they are formed, always decreases with time. This specific growth-rate declines more and more slowly with time; it is very difficult to point to the actual spot after which it can be said that growth has ceased entirely. This is well illustrated by the curve of $\frac{d^2 \log . L}{dt^2}$ against time. The respiration rate / unit length also decreases with time but much less rapidly than the growth rate. There is not sufficient evidence for any conclusive statement of connection between the two processes to be made.

We have shown that auxin has no immediate effect

on the respiration of sections when used over ranges which are stimulatory to growth, and also any growth stimulating or growth inhibiting concentrations have only those effects on respiration which are attributable to change in length with time. Stimulation comparable to that found by Berger et al (1946), Bonner (1949) or Christiansen and Thimann (1950) for aerial tissue was not obtained. Again there is no simultaneous increase in respiration with an increase in growth.

It does not seem that either sodium arsenate or sodium iodoacetate act in the root as specific inhibitors of auxin action in growth and respiration, a role which has been assigned to them by workers on Avena coleoptile metabolism (Bonner, 1950; Commoner and Thimann, 1941). Arsenate may stimulate respiration at the same time as growth is inhibited; and at higher inhibitory concentrations growth and respiration are inhibited to the same extent. A similar state of inhibition is true for iodoacetate in the absence of auxin. Auxin reduces the "effective" concentration of this inhibitor - a fact to which no attention has been given by previous workers. The reversibility of iodoacetate action in the presence of malate confirms the identification of the inhibited part of the system with the Szent-Gyorgyi acid cycle but we have no evidence here that such a cycle has exclusive control over growth.

Using an inhibitor of an entirely different type, dinitrophenol, we still fail to find any differential effect on auxin action.

By diligent search it might be possible to find an inhibitor which in root tissue completely inhibited growth but had a low rate of inhibition of respiration. Would it then be justifiable to assume that the inhibition was of "the part of respiration controlling growth?" Firstly it seems unlikely that roots and coleoptiles should have different "correlation mechanisms" for two such fundamental processes. Secondly we must make sure that the inhibitor does not act as an antagonist to any added substance (e.g. auxin) in a way that effects may be confused. Thirdly action should be considered over the whole concentration range, although the 50% growth-inhibiting mark may be considered as typical. Fourthly proper attention must be paid to penetration of all added substances and to time drifts in all measurements made. Even if proper attention was paid to all these points, it would still be necessary to furnish evidence that the action of the inhibitor is really specific. Iodoacetate, as a dehydrogenase inhibitor, may act at a large number of points in the respiratory cycle; any one of which may be taken as the "growth-controlling process". The so-called identification of this process has also fallen down in view of the variable types of substances, as well as C_4 acids now known to reverse its action.

It seems that a large part of the reasoning connected with this problem has been based on insufficient evidence in conjunction with somewhat fallacious arguments.

This thesis sets out to show how far from complete studies of this nature have been. We have been unable to produce a theory to rival that of previous authors. Such a theory seems at this stage totally unjustified.

SUMMARY

SUMMARY

1. The zone of maximum elongation was located in uniform pea roots by excising different lengths of tip, and the use of two lengths of sections, 3.39 and 1.98 mm. long.

2. Sectioning had no stimulatory effect on subsequent respiration of the root tissue.

3. A technique was elaborated by which sections were taken 2.0 mm. from the tip, and cultured in samples of 25 short or 12 long sections in shaken petri-dishes at 26°C in the dark. After desired time intervals the samples were removed, placed in Warburg flasks for determination of their oxygen absorption, and their lengths measured at the end of this determination. A new sample was used for each determination. A solution of 0.5% sucrose gave maximal elongation and was generally selected as the "control" medium.

4. A typical S-shaped curve of elongation against time was obtained, but growth continued for a longer period if sucrose was present.

5. If no sucrose was present the respiration / sample fell off slowly with time over the experimental 48 hour period. However if sucrose is present the respiration increases to a maximum which is followed by a fall after 24 hours.

6. The growth / rate/ unit time increases to a maximum at 12 hours in sucrose. The growth rate maximum and respiration rate maximum do not co-incide.

7. The respiration rate / unit length decreases with time.

8. Indolyl acetic acid increases the elongation of sections if the concentration is low but high concentrations inhibit elongation.

9. This auxin has no initial stimulatory effect on respiration, and change in respiration rates above or below normal at further time periods can entirely be accounted for by change in length of the sections.

10. The effects of the inhibitors, sodium arsenate, dinitrophenol and iodoacetic acid were determined at pH 7.0 including simultaneous respiratory and length measurements.

11. One concentration of sodium arsenate, 10^{-5}M, was stimulatory to respiration rate (after the initial period of penetration had elapsed) but at the same time inhibited the elongation of the sections 65%).

12. Higher concentrations were inhibitory to both growth and respiration.

13. No stimulation occurred unless sucrose was present, and sucrose increased the inhibitory effect of arsenate on respiration and growth.

14. The stimulation by arsenate of respiration rate is removed if auxin is present.

15. The stimulation of respiration by 10^{-5}M arsenate is completely reversed by a concentration of 10^{-3}M potassium dihydrogen phosphate.

16. Dinitrophenol has a similar effect on growth and respiration in that it stimulates the respiration at a low

concentration and inhibits it at higher concentrations. These concentrations are all inhibitory to elongation.

17. Dinitrophenol action cannot be reversed by phosphate, if the phosphate is added at the same time or subsequent to the dinitrophenol. If however phosphate is added previously the system is "protected" against dinitrophenol action. This was thought to be due to the relative rates of penetration of dinitrophenol and phosphate ions.

18. Auxin increases the stimulatory action of dinitrophenol on respiration.

19. Iodoacetate inhibits growth and respiration to the same extent in all concentrations studied. If auxin is present in low concentrations, iodoacetate may not be inhibitory but stimulatory to both processes. In high concentrations iodoacetate is again inhibitory. A theory of mutual reduction of "effective" concentrations is discussed.

20. Malate increases auxin action in growth and respiration if sucrose is present.

21. Malate "protects" against iodoacetate inhibition in the absence of auxin.

22. If iodoacetate, malate and inhibitory auxin are all present, respiration and elongation are reduced to a very low value.

23. 2.4. dichlorophenoxyacetic acid seems to have similar effects to indolyl acetic acid in instances tried.

24. A repeat of the work of Christiansen and Thimann (1950) yielded several important differences.

25. Some inaccuracies in current biochemical lines of reasoning are pointed out.

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"STUDIES IN THE GROWTH AND RESPIRATION OF ROOT TISSUE, with particular reference to auxin action."

ABSTRACT

The zone of maximum elongation was located in uniform pea roots by use of two different lengths of section taken at varying distances from the tip.

Sectioning had no stimulatory effect on the respiration of root tissue.

The elongation of sections followed a typical S-shaped curve of growth / time. If growth-rate was plotted, there was a maximum at 12 hours. This effect was only well defined if sucrose was present.

The respiration rate / sample increased upto a maximum at 24 hours in sucrose, after which it fell. The respiration rate and growth rate maxima did not co-incide. In the absence of sucrose there was no respiration rate maximum

The curve of respiration rate / unit length shows no maximum. Auxin stimulates the growth of root sections if present in very low concentrations. Higher concentrations are inhibitory.

There is no initial effect of auxin on respiration.

Effects of auxin after a lapse of time are wholly attributable to effects on the length.

Concentrations of 10^{-5} M sodium arsenate and dinitrophenol were found to be stimulatory to the respiration at the same time as growth was inhibited.

Higher concentrations of arsenate and dinitrophenol were inhibitory to both growth and respiration.

Phosphate , added at the same time as inhibitor , will prevent the normal effect of arsenate , which is slow-penetrating ; but the effect of the quickly penetrating dinitrophenol is not reversed.

Iodoacetate inhibits growth and respiration to the same extent in the absence of au xin . Auxin prevents inhibition , if used at a suitable concentration.

Malate "protects" against the action of iodoacetate in the absence of auxin.

However , if malate , iodoacetate and auxin are all present , metabolic activities are reduced to a very low level.