THE EFFECT OF SOME SUBSTITUTED PHENOXYACETIC ACIDS ON THE RESPIRATION OF THREE SPECIES OF FERN

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CHAPTER I

INTRODUCTION.

The problem of control of weeds in agricultural land has long confronted man, and toxic chemicals have been employed for half a century in eradication or suppression of weeds. The last two decades have seen a tremendous expansion in the use and variety of applications, principally because of the development of translocated organic compounds of a growth regulatory type which very often are highly selective.

Some indication of the interest in chemical weed control, for example with the substituted phenoxyacetic acids, can be seen in the fact that in the U.S.A. alone over twenty million pounds of 2.4-D is manufactured annually. The volume of research has likewise been tremendous. Norman et al (1), reviewing the field in 1950 covered some three hundred and fifty new papers for the previous year on phytotoxicity.

The mechanism of action of growth regulatory type herbicides is an important problem, particularly in so far as it may throw light on the physiology of plant growth and development, and their control by plant hormones.

The general approach is still largely empirical, involving a high degree of speculation in the selection and synthesis of new compounds followed by trial and error tests in the laboratory, glasshouse and field. Detailed physiological studies have been limited and it is only by such study that the principles of toxic action can be laid down.

This study is an attempt to examine one small facet, the effect of plant regulators on respiration, of a complex and rapidly expanding field of endeavour.

(i) Purpose of the Study

The purpose of the study is to compare the actions of an homologous series of substituted phenoxyacetic acids on the endogenous respiration of three species of Pteridophyta. Experiments are designed to show the presence of differences or similarities between the respiratory levels of the three species of Pteridophyta, together with any significant changes induced by the application of the plant regulators.

The three substituted phenoxyacetic acids used were -

(a) Para-4-chlorophenoxyacetic acid. (4-CPA)
(b) 2,4 - dichlorophenoxyacetic acid. (2,4 - D)
(c) 2,4,5 - trichlorophenoxyacetic acid. (2,4,5 - T)

Of the three species of fern examined Pteridium aquilinum has an underground rhizome, Paesia scaberula and Microsorium diversifolium have an above ground rhizome. A brief description of their appearance and habit as described by Dobbie (2) are as follows.

(a) Pteridium aquilinum var esculentum. (Forst. f.)

Commonly called "Bracken Fern" or by the Maoris "Rahurahu". In the Genus Pteridium the rhizome possesses hairs but no scales. The rhizome has an underground habit of growth. After germination the primary axis bears about seven to nine leaves, then it forks and each shank burrows down into the soil. The shanks will fork again, unequally so that in a short time a single plant can cover a wide area while its rhizomes, being underground, are well protected. When the older parts rot beyond the area of branching two individuals are left. It thus multiplies principally by vegetative propagation. The rhizome is thick, creeping below the ground producing numerous scattered fronds. Stipes are variable in length, stout, rigid, erect, smooth and shining. Fronds are usually from two to six feet long, sometimes ten to twelve feet, stiff and harsh, green to reddish green, lighter below. Sori usually extending round

entire margins of fertile segments. Outer indusium formed by reflexed leaf margin, inner often very poorly developed.

It is abundant everywhere in New Zealand except in dense forests. Found from sea level to four thousand feet.

(b) Paesia scaberula. (A. Rich).

Commonly called "Scented fern", "Lace fern" or "Hard fern". It belongs to a genus of terrestrial ferns with long creeping above ground rhizomes and finely cut fronds, usually with a zig-zag rachis. Sori long with true inner indusia, the outer being formed by the reflexed frond margin. The rhizome is wide creeping, rigid wiry and clothed with chestnut brown scales. Stipes four to twelve inches long, sinuous, rigid, erect yellow-brown, rough to the touch, more or less bristly. Fronds nine to eighteen inches high, rarely more, by four to nine inches broad, pale yellow-green, somewhat harsh to the touch. Midrib sinuous, rough. Both stipes and fronds are extremely hairy and glandular on both sides. The product of the glands gives the frond its characteristic stickiness. The sori are usually very copious when present. It grows on sunny banks to the exclusion of other plants. Found from sea level to two thousand five hundred feet.

(c) Microsorium diversifolium. (Willd) Synonomous with Polypodium diversifolium (Willd).

Sometimes called "Hound's Tongue". A broad leaved bright green species climbing over rocks and trees with a thick creeping above ground rhizome; very irregular in shape and size; the large round sori of a bright orange and most conspicuous. The rhizome is long, stout, creeping, often sea green in colour covered with black specks. Stipes jointed to the rhizomes two to eight inches long, stout, erect, smooth and glossy. Fronds numerous, scattered along the rhizome bright green, stiff and firm in texture, polished and shining, varying greatly in shape, sometimes

three to nine inches long by one and a half to two inches broad, quite entire; sometimes six to eighteen inches long by three to nine inches broad deeply cut. Veins netted and conspicuous in young fronds. Sori numerous, large, round, orange-red, forming a single row on each side of the midrib between margins and midrib or sometimes nearer the former. Found from sea level to three thousand feet everywhere, in the shade or sun.

(ii) The Importance of the Problem

Pteridium aquilinum one of the most successful and widespread species of the family Pteridophyta, is a major weed in unploughable hill country regions of Northern England and Scotland, South Eastern Australia and throughout New Zealand, more particularly in the hill regions of the North Island. Up to the present time no successful method of eradicating it has been discovered for unploughable country. Cultivation of the soil appears to be the only effective method of providing control, by breaking up and exposing the network of underground rhizomes which enable the plant to recover from the very severest mutilation of foliage.

Chemical treatment has so far been unsuccessful. Contact weed-killers such as sulphuric acid and sodium chlorate are effective only in such high concentrations that their use over a large area is uneconomic. Braid (3) (4) and Bates (5) using sodium chlorate found that two to three tons per acre could effectively kill Bracken in localised areas but there was no evidence to suggest any translocation of the herbicide through the plant and deep seated rhizomes were apt to escape. Conway and Stephens (6) using 3-p-chlorophenyl-1, 1 dimethylurea (CMU), Egler (7) and Holly et al (8) using 2 methyl 4 chlorophenoxyacetic acid (MCPA), 2,4-D and 2,4,5-T acids together with mixtures of MCPA and 2,4-D, and 2,4D and 2,4,5-T at a wide range of concentrations and at a number of seasons of the year found, that although the fronds could in some cases be destroyed, the rhizomes in every case remained unaffected. Rhizomes and fronds developing later in the season or in the year following treatment were normal in appearance and did not differ in number or height from those on untreated areas.

A preliminary spraying trial was carried out in the upper Tiritea valley, near Palmerston North, in May 1954 (Autumn) on Pteridium aquilinum and Paesia scaberula using commercial formulations (Butoxyethanol-esters) of 4-CPA, 2,4-D and 2,4,5-T, in concentrations of two pounds, one pound and half a pound acid equivalents per acre. Circular plots four feet in diameter were sprayed and in the case of Paesia scaberula a ditch six inches deep was opened around the plots to separate treated and untreated rhizomes.

After four weeks Paesia scaberula showed moderate epinastic effects in young frond growth after treatments of 4-CPA, 2,4-D and 2,4,5-T at two pounds. AE/acre and 2,4-D and 2,4,5-T at one pound AE/acre. However the plants recovered and by July there was nothing to indicate any effect of the treatments.

Pteridium aquilinum showed an even smaller response. 2,4,5-T at concentrations of two pounds, one pound and one half a pound gave mild epinastic effects to the rachis and in some cases thickening of the stipe of the frond. Beyond this, treatment was of no significance and the plants rapidly recovered.

From these results a conclusion might be made that there is a high degree of resistance to attack by plant regulators in the growing regions of the rhizome of Pteridium aquilinum and that attack through the fronds is not sufficiently effective to ensure complete control of the plant.

Examination of the physiological factors involved in the successful application of plant regulators as herbicides reveals that the regulator must first enter the plant, be translocated to the site of action and then take place in a reaction or reactions which will ultimately bring about the death of the plant. In the particular cases of the three species of Pteridophyta ultimately examined, the fronds are quite heavily cutinised, more particularly Pteridium aquilinum. Norman et al (1) have

shown that stomata appear to be important though not exclusive means of entry for oils particularly those of low surface tension; further that aqueous solutions do not ordinarily penetrate through open stomata. Diffusion through the cuticle is probably the usual means of entry.

The amount of regulator that enters is a function of the time and area of contact; both factors are related to the degree of wetting of the surface and the contact angles of discrete droplets (Fogg (9)). Paesia scaberula has a very rough frond surface and thus might be expected to provide a poor surface for entry of both aqueous solutions and oil formulations.

Non-polar molecules penetrate the cuticle (Lipoidal in nature) more readily than polar molecules. Weak acids in general are absorbed in the form of undissociated molecules rather than as ions, (9).

Several important physiological factors therefore control the entry of a plant regulator.

Once having successfully penetrated the plant, a herbicide may either act by rapidly killing the superficial cells thus preventing further spread and action unless simple diffusion takes place (very slow), or by acting more slowly, thus becoming more widely distributed. Substances entering via the root are transported by the transpiration stream. Transport from the leaf is with the products of photosynthesis (sugars). (Mitchell and Brown (10), Weaver and De Rose (11)).

If we assume that plant regulators can penetrate the cuticle of Bracken fronds and thus enter the translocatory stream it could be further assumed that they would then be carried to points of sugar storage or utilisation such as the rhizome or primary meristem of the rhizome. Does any further reaction take place at these sugar $\rightleftharpoons$ starch metabolic centres?

In the case where Bracken foliage has been destroyed without apparently affecting the rhizome, the non-effectiveness may be due to the plant

regulator never reaching the rhizome because the pathway of translocation is destroyed by direct herbicidal action of high concentrations of plant regulator within cells.

The other alternative is that if the plant regulator is applied in a low enough concentration to penetrate the cuticle, enter the translocatory stream without damaging cells and then penetrate to a site of action in the rhizome, it may be in such low concentrations in the rhizome as to be ineffectual.

By eliminating the two steps, (a) of entry and (b) translocation of plant regulators, any direct effects on the rhizome can be studied. It was with this aim in mind that the present study was carried out.

There are many interrelated processes which can be involved in a phytotoxic response to an applied plant regulator. Metabolism may be stimulated to the point where rapid growth and cell division deplete the plant of food reserves and photosynthesis is unable to supply enough energy for the plant to survive. Resistance of the plant to micro-organisms may be lowered and its death hastened by pathogens. On the other hand death may occur through direct inhibition of vital processes such as photosynthesis, respiration, water and salt accumulation.

Cell division, and changes in water uptake and growth generate a concomitant change in respiration. It was for this reason that a measure of respiratory rate was used to indicate any effects of the plant regulators on the rhizomes of the three species of fern studied.

(iii) Definition of Terms Used

The following three terms are used in agreement with a recent (12) conference on nomenclature of plant growth substances and denote:-

A. Plant regulators.

"Organic compounds other than nutrients which in small amounts promote, inhibit or otherwise modify any physiological process in plants." 4,CPA, 2,4-D and 2,4,5-T are therefore included in this definition.

B. Auxin.

"A generic term for compounds characterised by their capacity to induce elongation in shoot cells. They resemble Indole-3-acetic acid in physiological action. Auxins may and do affect other processes besides elongation, but elongation is considered critical. Auxins are generally acids with an unsaturated cyclic nucleus or their derivatives."

C. Anti-auxins.

"Are compounds which inhibit competitively the action of auxins."

Respiration. In this text, respiration refers to aerobic respiration as measured by changes in the level of oxygen uptake.

Phytotoxic concentrations of Plant regulators.

All concentrations which inhibit respiration (i.e. decrease oxygen uptake) are considered to be phytotoxic in the sense that normal death of tissue is accelerated.

Physiological concentrations of Plant regulators.

Denote all other concentrations used whether they have a stimulatory effect or none at all on tissue respiration.

Rhizome.

A stem, generally of root like appearance, having a diageotropic position, either above or below the ground (13).

Meristems. The definitions are those adopted by Eames and MacDaniels (14).

Promeristem.

The region of new growth in a plant body where the foundation of new organs or parts of organs is initiated. The remainder of the meristem represents the early stages of the tissues formed by the promeristem. No term exists for this partly developed region in which segregation of tissues is beginning but cell division continues freely.

Apical Meristem.

Initiation of growth is by one or more cells (a solitary cell in Pteridophytes) situated at the tip of the organ which maintain their individuality and position and are called apical initials or cells. Promeristem consists in part therefore of apical meristem.

Primary Meristems.

Those that build up the fundamental primary part of the plant and consist in part of promeristem. (There is no secondary development in ferns.)

CHAPTER II

A REVIEW OF SOME PREVIOUS RELATED STUDIES.

In 1880, Charles Darwin (15) published "The Power of Movement in Plants" in which he postulated a mechanism for the response of plants to the force of gravity and unilateral light. His observation that plants possess a transmissable "influence" which moves from stimulated shoot tips to zones of reaction elsewhere in the plant set in motion a series of studies, which have now developed into a large field of experimental Botany entirely devoted to the study of the plant growth regulators.

In 1911 Boysen-Jensen (16) demonstrated beyond doubt that a purely chemical mechanism underlay this transfer of stimulus, when he showed that the "influence" could pass through non-living tissue such as gelatine. It was not until 1926 (17) that this chemical substance was first isolated in the now classical experiments of F.W. Went in which the substance was found to diffuse into agar blocks if they were placed on cut surfaces of oat coleoptiles.

The first attempt to isolate and carry out chemical analysis of the structure of the "growth hormone" was made by Dolk and Thimann in 1932 (18). They described the active principle as a weak acid of approximately the same strength as acetic acid. At about the same period Kögl and Haagen-Smit (19) discovered that human urine was a particularly active source of the growth factor. Purification and elucidation of the chemical structure of crystals obtained from urine showed a substance auxentriolic acid to be present, (Auxin A.) In further work with maize germ oil and barley malt they found Auxin A together with another substance which they described as auxenolonic acid (Auxin B.). A third substance was isolated by the same workers from the urine of an

eighteen year old youth which showed particularly strong growth regulating properties (20). They called it Heteroauxin and showed its chemical structure to be β-indolyl-acetic acid.

The auxins A and B, have never since been re-isolated, though much of the literature of the nineteen-thirties is dominated by vague references to them. β-indolyl-acetic acid has however been conclusively shown to be an active principle in almost all plant life, from a vast number of papers published in the last fifteen years.

It is to the isolation of β-indolyl-acetic acid (β-IAA) in a pure form from corn meal and corn germ by Haagen-Smit et al, (21)(22) that we owe the vast expansion of the field of plant regulator studies and their practical application in Agriculture.

Following the isolation of β-IAA as an active growth regulator a number of synthetic compounds were examined for similar chemical constitution and physiological properties.

In 1935 Zimmerman et al (23) discovered α-(Indole-3)-propionic acid and γ-(Indole-3)-butyric acid. In the same year Haagen-Smit and Went (24) discovered β-indolylpyruvic acid and Zimmerman et al (23) α and β napthylacetic acids, phenylacetic acid and anthracene acetic acid.

In 1938, napthoxyacetic acid was added to the list by Irvine (25). The most significant discovery of all was the phenoxyacetic acid series by Zimmerman and Hitchcock in 1942 (26).

It was very quickly noted that most synthetic auxins if applied in sufficiently high concentrations act as selective herbicides. Since the discovery of this fact many thousands of compounds in the same chemical family have been synthesised and tested for herbicidal activity.

Methods of biological screening in the laboratory have generally been confined to measurement of the direct action of the herbicide, usually involving the measurement of growth inhibition of seedling organs

in sensitive species. Many thousands of compounds were tested on this basis in England (Slade et al (27) 1945) and America (Thompson et al (28) 1946). From this and much subsequent work it has been shown that the substituted phenoxyacetic acids are the most effective compounds.

Many secondary properties of the molecules such as persistence in soil, ease of absorption by and translocation through plants etc. may be missed in simple laboratory screening tests for example the following types. Primary root growth in Zea mais (Swanson 1946 (29)) and Cress (Lepidium sativum) Audus (30). Seedling growth of Kidney bean (Phaseolus vulgaris) Thompson et al 1946 (28). Rate of killing of samples of duck-weed (Lemna minor) by Blackman 1953 (31). The final evaluation of test materials must therefore come from extensive field experiments using a range of species under a number of treatment conditions, and the data from planned and replicated experiments subjected to statistical analysis, as for example the methods advocated by Audus (32), Blackman (33) and Sampford (34).

For compounds which are freely translocated, methods of growth analysis are to be preferred (33).

Following the discovery of the importance of the substituted phenoxy-acetic acids as herbicides many attempts have been made to elucidate the mechanism of phytotoxic as well as physiological responses to these acids. The first major work was that of Zimmerman and Hitchcock (26) who demonstrated with a number of test plants that in addition to a direct herbicidal action at high concentrations, low concentrations of particularly the chlorophenoxyacetic compounds could induce cell elongation and epinastic effects, cell proliferation, adventitious roots on cuttings, inhibition of buds, parthenocarpy, and persistence of floral parts. They also found that esters, salts and amides of the acids are also active.

In 1941, Thimann (35) concluded that the action of auxin on growth

and protoplasmic streaming was via the four-carbon acid respiratory system. His so called "master-reaction" was thought to be a single process controlled by auxin and able to generate all the changes such as cell elongation, proliferation, water uptake etc. Explanation of the diversity of effects was sought in a series of secondary phenomena arising as a result of the "master reaction", or the modifying effect of various cell conditions on the master reaction itself.

The majority of more recent work on the mechanism of action of the substituted phenoxyacetic acids has been founded on a study of the relation of structure to physiological activity exemplified by individual studies of particular facets of auxin action such as respiratory response, cell elongation and water uptake. A number of controversial theories have been presented, none of which are ~~as~~ yet conclusive and which are mainly based on interpretation of other workers experiments. As long as the mode of action of endogenous plant regulators is imperfectly understood, any theories of exogenous response cannot be more than speculation. The theories can be divided into two sections.

(a) Those where the plant regulator participates as a coenzyme in some enzymatic metabolic reaction or so called "master reaction" which is common to various kinds of plant cells but leads to different end results according to the environmental conditions and the physiological and morphological structure of the cells concerned. This theory includes the work of Wort (36), Commoner et al (35), West et al (37), Crafts (38), Galston et al (39)(46), Goldacre (41), Hofman et al (42), Rhodes and Ashworth (43) and Leaper and Bishop (44).

(b) The primary action of the growth regulator is to affect some more physico - chemical property of the cell such as degree of association or dissociation of protoplasmic proteins. For example exposure or masking of active sites of enzymes can increase or decrease their activity.

Changes in structural viscosity of protoplasm could be expected to influence such processes as cell expansion, permeability and protoplasmic streaming. Veldstra (45) was the first to advocate this type of action followed by Muir and Hansch (46), Smith and Wain (47), and Norman and Weintraub (48).

The order of activity in promoting growth of Avena coleoptiles in the chlorine substituted phenoxyacetic acids (46) is:-

Phenoxyacetic acid	0.03
Ortho-chlorophenoxyacetic acid	0.06
Meta - " " " "	2.0
Para - " " " "	5.0
2,4-Di-chlorophenoxyacetic acid	25.0
2,6 " " " " "	Inactive
3,5 " " " " "	Inactive
2,4,5-Tri-chlorophenoxyacetic acid	25.0
2,4,6- " " " " "	Inactive.

Any comprehensive theory explaining the mode of action must include an explanation of why some chlorine substituted phenoxyacetic acids are active and others not, as well as why other plant regulators, not very similar in chemical structure are also active, presumably at the same sites. Muir and Hansch (46) have enumerated nine important requirements in addition to an unsaturated ring structure and carboxyl groups (which all plant regulators possess) that must be present in an explanation of a theory of action of plant regulators.

1. The effect of optical isomerism in side chains.
2. Effect of geometrical isomerism of the side chain.
3. Ortho effect in phenoxyacetic acids.
4. Ortho effect in benzoic acids.
5. Inhibiting effect of lactones and maleic hydrazide.
6. Inhibiting effect of cysteine.
7. Inhibiting effects of reagents specific for sulfhydryl groups.
8. Inactivity of phenoxyacetic acids without two open para - positions.
9. Effect of electron releasing and electron attracting ring substituents.

To date there has been no theory sufficiently versatile to accommodate all the above considerations.

The position at present therefore is that the exact mechanism by which plant regulators exert their influence is not clear, although the evidence supporting a nucleophilic reaction of masking and unmasking of sites of enzyme activity would seem to outweigh that of other theories.

In the last seven or eight years interest has been focussed on the relation between growth and respiratory activity and the effect that plant regulators have on both these processes. Smith (49)(50) and Avery (51) have reviewed the field up to 1951. In stem and coleoptile tissue work it is evident that plant regulators such as 2,4-D stimulate both growth and respiration in low concentrations, and inhibit in high at concentrations specific for the different tissues. Commoner and Thimann (35) and Kelly and Avery (52) have demonstrated inhibition of coleoptile or stem elongation and oxygen uptake in the range 10-100 ppm. with 2,4-D and IAA. French and Beevers (53) working with corn coleoptiles demonstrated a relation between growth and respiration with four plant regulators including 2,4-D. In stem tissue it would seem therefore, that there is some definite relation between respiration and growth as affected by 2,4-D for example.

With roots no such relation has been conclusively shown. Nance (54) has shown up to 20% stimulation of oxygen uptake in wheat roots with 5 p.p.m. of 2,4-D, but no accompanying growth measurements were taken, although nitrate uptake was inhibited by 60% at this concentration. Smith et al (55) working with rhizomes and roots of Bindweed showed an average of 70% increase in oxygen uptake as a result of spraying intact plants with 2,4-D at 1000 mgs/litre. No growth measurements were taken however and the conclusion was that the increase in respiration was due to an increased activity of phloem causing increased food translocation

from the rhizomes and roots to the above ground parts of the plants.

Mitchell et al (56) observed an inhibition of oxygen uptake in root and stem slices by 0.002 M. 2,4-D (350 p.p.m.), the percentage inhibition being greater in roots than stems. Audus (57) demonstrated growth inhibitions in roots of Pisum sativum and Lepidium sativum at concentrations of 2,4-D between 0.1 to 10 p.p.m. No respiratory measurements were taken however.

Evidence for a relation between growth and respiration in roots from the above quoted work is most fragmentary and in all cases a measurement of either oxygen uptake or growth alone has been taken.

In 1953 Audus and Garrard (58) working with roots of Pisum sativum concluded that β-IAA in both growth-stimulatory and growth-inhibitory concentrations has no direct effect on the respiratory enzyme system of growing root cells. Small respiratory responses were best explained as resulting from differential changes in section size and correlated changes in the enzyme complements of the growing cell.

They suggest that much of the stimulation of section respiration attributed to a direct effect of auxin on the respiratory systems of various materials (e.g. Avena coleoptile - pea stems etc.) may be due to indirect effects arising from respiratory--enzyme synthesis in the normal course of augmented growth. Further they have levelled valid criticism at much of the previous work published giving little indication of statistical significance of the small stimulations of respiration observed. Their experience with stem and root sections of pea indicate that coefficients of variation of initial respiration (100 x Standard Deviation / $\bar{X}$) of exactly comparable examples is 10% i.e. the order of least significance (5% level) to be expected between treated and control means of a tenfold replicated experiment. Most work has had

fewer replications indicating the necessity for checking of data of the order 0-20% stimulation and inhibition.

There is much scope therefore for simultaneous determinations of growth and respiration in relation to application of auxin or plant regulators.

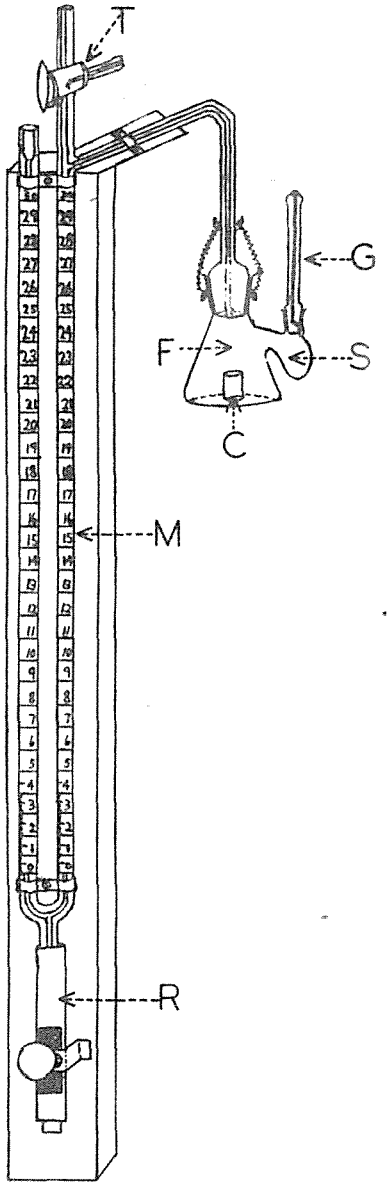


Fig. 1. The Warburg constant volume manometer.

F = flask
S = sidearm
G = sidearm stopper with gas vent
C = center well (for alkali)
M = manometer proper
R = fluid reservoir which, by adjustment of the screw clamp, serves to alter the level of the fluid in the manometer
T = three-way stopcock

CHAPTER III

THE METHOD OF PROCEDURE

Part 1.

The Measurement of Respiration.

In all experiments, respiration was measured by determining the oxygen uptake in micro litres per milligram dry weight of the material used, in a Warburg constant volume respirometer using the "Direct Warburg Method". The essential principle involved is, that if at constant temperature one holds the volume of a gas constant any changes in the amount of gas can be measured by changes in pressure. The apparatus (59) consists of a detachable flask containing a small well, attached to a manometer containing a liquid of known density. The flask is immersed in a water bath at a constant temperature and between readings the system is shaken to facilitate rapid gas exchange between the fluid and gaseous phase. (See figure one).

The manometer has one side open to the atmosphere. A given point on the closed side of the manometer is chosen and before recording any pressure changes the liquid in the closed arm of the manometer is adjusted to this point. Carbon dioxide is removed from the gaseous phase of the closed flask by a solution of potassium hydroxide contained in the centre well. Any pressure changes are therefore due to changes in the pressure of oxygen when allowance has been made for atmospheric fluctuations measured by a thermobarometer control flask.

The following standard techniques were adopted.

A. Washing of glassware:-

Extreme care was necessary with washing, because of the use of plant

regulators in the majority of experiments. After use, Warburg flasks and stoppers were first washed in tap water, placed in white spirit to remove grease, washed again with tap water and then immersed in a concentrated solution of chromic acid for at least eight hours. After a very thorough washing in tap water the flasks were rinsed with glass distilled water and dried in an oven. The importance of thorough removal of chromic acid has been emphasised by Science (Anon. (60)).

B. Temperature of water bath:-

Temperature was maintained in all the experiments at 30 $\pm$ 0.05°C by a Sunvic electronic relay and the vigorous action of an electric stirrer.

C. Absorption of Carbon Dioxide:-

In all experiments the centre well of the flasks contained a piece of filter paper one inch by three quarters of an inch, folded accordion-wise with the projecting end cut in two or three places so that when 0.3 mls of 20% potassium hydroxide were added, the pieces sprang open exposing a large area of potassium hydroxide to the gaseous phase. The paper also prevented "creeping" of potassium hydroxide during tipping.

D. Rate of shaking:-

Shaking was maintained in all experiments at approximately 110-112 strokes per minute on the basis of a standard procedure adopted by Umbreit Burris and Stauffer in "Manometric Techniques" (59).

E. Equilibration:-

After assembling the apparatus and immersing in the water bath, stopcocks were left open for a period of thirty minutes to allow all the glassware to come to a steady temperature. Treatments were not applied until one hundred and fifty minutes after closing the stopcocks, in order that the total oxygen uptake would be fairly large compared with errors in reading. In all experiments a sufficiently large quantity

of tissue was used to make errors of measurement negligible compared with total uptake between readings.

F. Flask constants:-

In all experiments control flasks contained a total of 2.5 mls of liquid consisting of the following portions.

1. 0.3 mls of 20% potassium hydroxide together with a filter paper roll contained in the centre well.
2. 2.2 mls of either standardised buffer or glass distilled water according to the particular experiment being performed, contained in the main body of the flask.

Treatment flasks contained in all experiments -

1. 0.3 mls of 20% potassium hydroxide plus filter paper in the centre well.
2. 1.7 mls of buffer or glass distilled water in the main body of the flask.
3. 0.5 mls of plant regulator in the sidearm of the flask. The total liquid as with control vessels 2.5 mls.

G. Standardised buffer solution:-

In all experiments where phenoxyacetic acid treatments were applied a Phosphate-Citrate buffer (McIlvaine (61)) was used to suspend the tissue slices. In all cases pH of the buffer was 5.0 and the phosphate molarity 0.05. Andus (62), Simon and Beevers (63)(64) and Simon and Blackman (65) have emphasised the necessity for testing weak acids and bases at pH levels at which there is very little dissociation. The toxicity of a weak acid decreases in the presence of increasing concentrations of the anion. It would be desirable to control pH levels at two or more units below the pKa of the weak acids being used. However in the case of 4CPA, 24D and 245T the pKa's are respectively 3.11, 2.81 and 2.57 (66) so that a buffered solution on the principles outlined above would be impracticable. pH values for expressed cell sap,

measured with a glass electrode were as follows.

1. Pteridium aquilinum - 5.80 ± 0.05
2. Paesia scaberula - 5.85 ± 0.05
3. Microsorium diversifolium - 5.35 ± 0.05

A buffer solution of pH 5.0 was therefore used on the basis that it was close to the cell sap pH of the materials used, bearing in mind the necessity for a low pH. The pH values for expressed cell sap possibly may not be a true indication of the internal pH of the cell due to liberation of enzymes, mixture of cell walls with cell contents and general disintegration during the cell sap expression.

Fresh solutions of buffer were made periodically to avoid any bacterial contamination and checked with a glass electrode.

H. Manometer fluid:-

"Brodies" solution was used of the following composition.

23 grammes of NaCl, 5 grammes of Sodium choleate (Merck) in 500 cc. H_2O. Coloured with Evan blue (200 mg's/litre). Density is 1.030. Po value = 10010.

I. Test solutions of plant regulators:-

The aqueous solutions of 4-CPA, 2,4-D and 2,4,5-T were all made up on an equi-molar basis.

	Mol. Wt. (By exp't)(67)	Calc. M.W.	Sol's at 20 ± 1°C.(68)
4-CPA	-	186.51	681 or 674 mgs/l/H_2O.
2,4-D	221	220.96	546 or 540 " "
2,4,5-T	256	255.41	194 or 189 " "

The reason for using an equi-molar basis rather than an equal percentage weight solution follows work of Fults and Payne (69) who emphasised that equal concentrations on a parts per million basis of an homologous series of chemicals may differ widely on a molar basis. If it is desired to evaluate the relative properties of the substituted

TABLE I.

The Concentrations of the Aqueous Solutions of Plant Regulators used in Experiments

	Stock	Stock x 10^{-1}	Stock x 10^{-2}	Stock x 10^{-3}	Stock x 10^{-4}		
4-CPA	3.812×10^{-3}	3.812×10^{-4}	3.812×10^{-5}	3.812×10^{-6}	3.812×10^{-7}	Molarity	Before Tipping
	711	71.1	7.11	0.711	0.0711	p.p.m.	
2,4-D	842.5	84.25	8.425	0.842	0.084	p.p.m.	
2,4,5-T	Sodium Salt					Molarity	
	973.5	97.35	9.735	0.9735	0.09735	p.p.m.	
	9.148×10^{-4}	9.148×10^{-5}	9.148×10^{-6}	9.148×10^{-7}	9.148×10^{-8}	Molarity	After Tipping
4-CPA	170.64	17.064	1.706	0.1706	0.0170	p.p.m.	
2,4-D	202.20	20.22	2.022	0.202	0.0202	p.p.m.	
2,4,5-T	233.64	23.36	2.336	0.2336	0.02336	p.p.m.	

phenoxyacetic acid series molecules an equi-molar basis is essential.

To that end then, test solutions were made by direct weighing and making up to volume. All other concentrations were made by dilution in such a manner that each succeeding dilution was one tenth as concentrated as the preceding one. The following standard solutions were adopted. "Stock" solutions of 4-CPA, 2,4-D and 2,4,5-T acids were 0.003812 molar.

i.e.	4-CPA	-	0.7110 grms/litre	≡	711 mgms/litre
	2,4-D	-	0.8425 " "	≡	842.5 " "
	2,4,5-T	-	0.9735 " "	≡	973.5 " "

With 4-CPA and 2,4-D warming the stock solution was sufficient to dissolve all the acid. With 2,4,5-T the stock concentration was made up to the Sodium salt by addition of a small amount of sodium hydroxide and then brought to pH 7 by neutralisation with H_2SO_4. The sodium salt of 2,4,5-T was used only for the stock and one tenth stock concentrations, a further solution of the acid (0.09735 grms/litre ≡ 0.00003812 molar) was used for more dilute concentrations. (See Table one.)

The following table shows the relative concentrations and equivalent parts per million of the solutions placed in the side arm of the flasks, followed by the resultant concentrations after tipping and intermingling with the contents of the main body of the flask.

J. Tissue sections:-

Tissue slices of the fern rhizomes examined were cut to a uniform thickness. Briggs and Robertson (70) demonstrated in disks of carrot tissue that diffusion of carbon dioxide and oxygen are independent of disk thickness between 0.05 and 0.2 cm. Their work indicates that a similar effect can be expected in other types of tissue. Pteridium aquilinum and Microsorium diversifolium sections were therefore cut to a thickness of 0.5 mm. on a hand microtome. Because of the small rhizome and the

time factor involved in cutting sections, Paesia scaberula was cut into 0.3 cm. sections. The mean oxygen uptake in microlitres per mgm. dry weight of 0.5 mm. slices (3 replications) compared with oxygen uptake of 0.3 cm. disks (3 replications) taken from a similar position on the primary meristem of the rhizome of Paesia scaberula showed no significant difference.

Students "t" test between means

Mean (3 Rep's) θ_2 Uptake (270 mins)		d.f.	"t" - calc.	"t" - 5% level sign	Result
0.5 mm. Sects	0.3 cm. Sects.				
22.74	18.94	4	0.2586	2.776	—

It was assumed therefore that no significant discrepancy would occur by the use of 0.3 cm. sections in this particular case.

K. Washing time for sections:-

Immediately after cutting, all tissue sections were washed in a steady stream of tap water for thirty minutes before being placed in the Warburg flasks. The purpose of this short period of washing was to remove wound hormones, liberated enzymes, cell sap and other cell debris produced during the tissue sectioning. Considerable controversy has existed over the necessity for washing tissue slices. Some workers have not washed tissue at all (71)(72)(73)(74). Others have washed from periods of quick rinses up to 650 hours. The general pattern of respiratory response with increased washing time has been indicated by Schade et al (75) and Stiles and Dent (76). They show that increased washing time leads to an increase in respiration. Barrow et al (71) have emphasised that long periods of washing probably damage the cell membrane and cause the loss of water soluble co-enzymes. It would appear therefore that long periods of washing are undesirable where a

measure of the true endogenous respiration only is looked for.

L. Measurement of Oxygen uptake.

In all experiments conducted, respiration was measured by the oxygen uptake in micro-litres per milligramme dry weight of the tissue used. A constant was determined for each flask using the formula

$$K = \text{flask constant} = \frac{Vg \frac{273}{T} + V_f \alpha}{Po}$$

after Umbreit (59) where

- Vg = volume of gas in the flask down to the zero point. (Determined by "Schale's quick method" (77).
- T = temperature of bath in absolute degrees.
- V_f = total volume of fluid in vessel.
- α = Solubility of gas involved in liquid in vessel.
- Po = Density of mercury/density of manometer fluid.

The difference in gas pressure measured by the changes in level of the manometer fluid, multiplied by the constant K is equal to the micro litres of gas given out or absorbed. Oxygen uptake was measured in micro litres per milligram dry weight. $Q.O_2$ (N) gives the truest estimation, being a direct measure of the protein or living tissue that actively respires. Estimation on a fresh weight basis is unsatisfactory because the residue of water from washing is difficult to remove. Link and Goddard (78) studying oxygen uptake in crown gall of tomato came to the conclusion that total N. does not appear to be a more adequate basis than fresh weight for calculating rates of oxygen uptake. Newcomb (79) and Shirk (80) in comparisons of fresh weight and dry weight bases of oxygen uptake show that oxygen comsumption on a fresh weight basis is considerably lower than on a dry weight basis. (As would be expected).

The time taken to do Kjeldahl micro-estimations of nitrogen was deemed too great, in view of the already lengthy periods of the experiments and the lack of laboratory assistance. All manometers were corrected for fluctuations in atmospheric pressure with a thermobarometer.

Part 2.

Selection of Material

In order to reduce biological variability in the tissues used, the following technique of selection was adopted for each experiment. (Twelve flasks were used in each experiment.)

1. Pteridium aquilinum var. esculentum.

Twelve to fifteen mature, undeformed and disease free rhizome tips were taken from the ground and washed in tap water to remove soil debris. Actively growing rhizomes could be distinguished by the relatively long creamy coloured promeristems, free from hairs as opposed to apices of rhizomes produced by breakage or decay of the main body of the rhizome, or "frond buds" as described by Stevens (81). Approximately two millimetres of the tip was removed and discarded from each meristem, and twenty sections each 0.5 mm. in thickness were removed with a hand microtome. Approximately 250 sections were then washed in running tap water for thirty minutes and from this population twelve disks per flask were selected at random. Limitations on further reduction in variability were:-

(i) By increasing the number of disks per flask the constant V_f becomes altered due to the apparent increase in liquid volume. Further, a state is reached where the buffer volume in inadequate for the quantity of tissue present.

(ii) The time factor set a limit on gathering and sectioning more than twelve to fifteen rhizomes, when a further period of eleven to twelve hours was necessary for the main part of the experiment.

2. Paesia scaberula.

One hundred and fifty to one hundred and sixty rhizome tips were selected from the field, washed to remove debris, and carefully wiped to remove fine hairs from the basal region of the primary meristem.

The first one to two mm. of the tip were discarded and three sections, each of three mm. length removed with a hand microtome. An explanation of why 0.3 mm. disks were used, is given in Chapter IV, Part 1, Section (i) of the preliminary experiments. The diameter of most rhizomes was approximately two mm. Fifteen sections, selected at random from a population of four hundred to four hundred and fifty disks were allocated to each flask. Disks were washed for 30 minutes in running tap water.

3. Microsorium diversifolium.

Fifteen rhizome tips were selected and gently scraped to remove protective scales. The first two to three mm. of the promeristem were rejected. Twenty sections each of 0.5 mm. were removed with a hand microtome from each rhizome. Twenty sections were allocated to each flask from the bulk total of three hundred. It was found necessary to use more tissue than with the other two fern species because of the lower oxygen uptake. Disks were washed for 30 minutes under running tap water.

The removal of the extreme tip in each of the three species followed the results of preliminary experiments showing the considerably higher respiratory rate of the promeristem (1-2 mm.) compared with the remaining 10-15 mm. of still actively dividing but older primary meristem.

GRAPH 1
20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
OXYGEN UPTAKE IN MICRO LITRES PER MILLIGRAM DRY WEIGHT
0 1 2 3 4 5 6 7 8 9 10 11 12
TIME IN THIRTY MINUTE PERIODS.

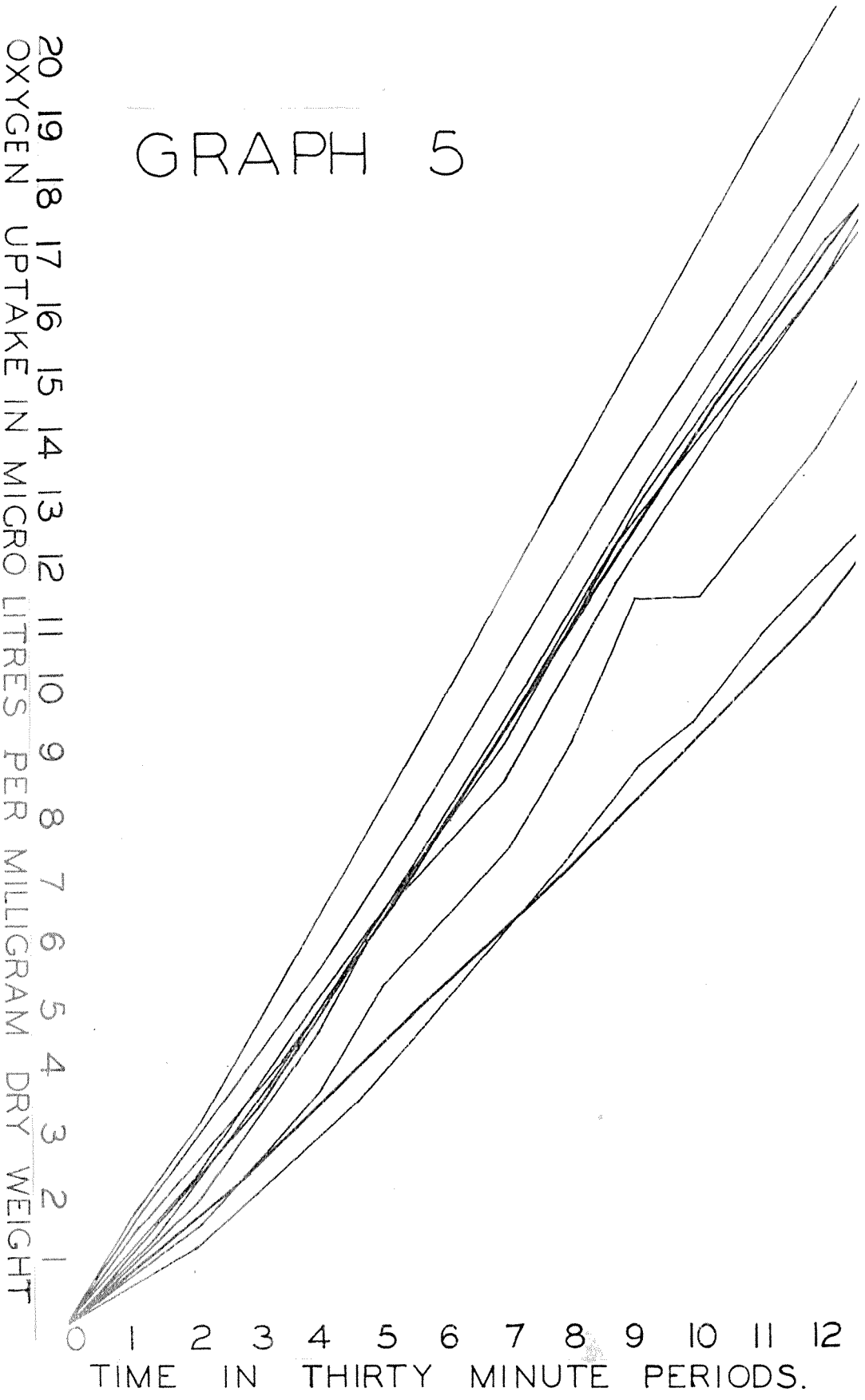
GRAPH 2

20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
OXYGEN UPTAKE IN MICRO LITRES PER MILLIGRAM DRY WEIGHT
0 1 2 3 4 5 6 7 8 9 10 11 12
TIME IN THIRTY MINUTE PERIODS.

GRAPH 3
20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
OXYGEN UPTAKE IN MICRO LITRES PER MILLIGRAM DRY WEIGHT
0 1 2 3 4 5 6 7 8 9 10 11 12
TIME IN THIRTY MINUTE PERIODS.

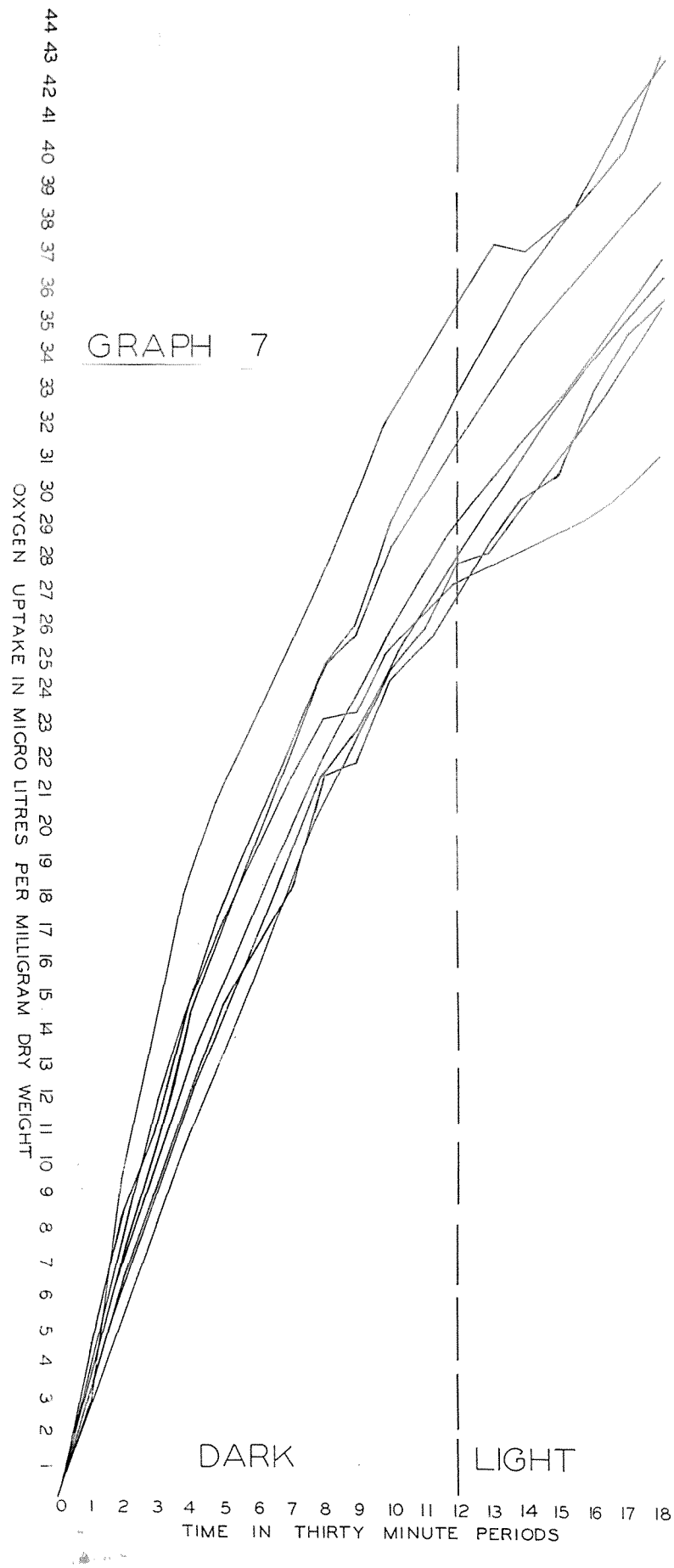
GRAPH 4
II
I
III
21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
OXYGEN UPTAKE IN MICRO LITRES PER MILLIGRAM DRY WEIGHT
0 1 2 3 4 5 6 7 8 9 10 11 12
TIME IN THIRTY MINUTE PERIODS.

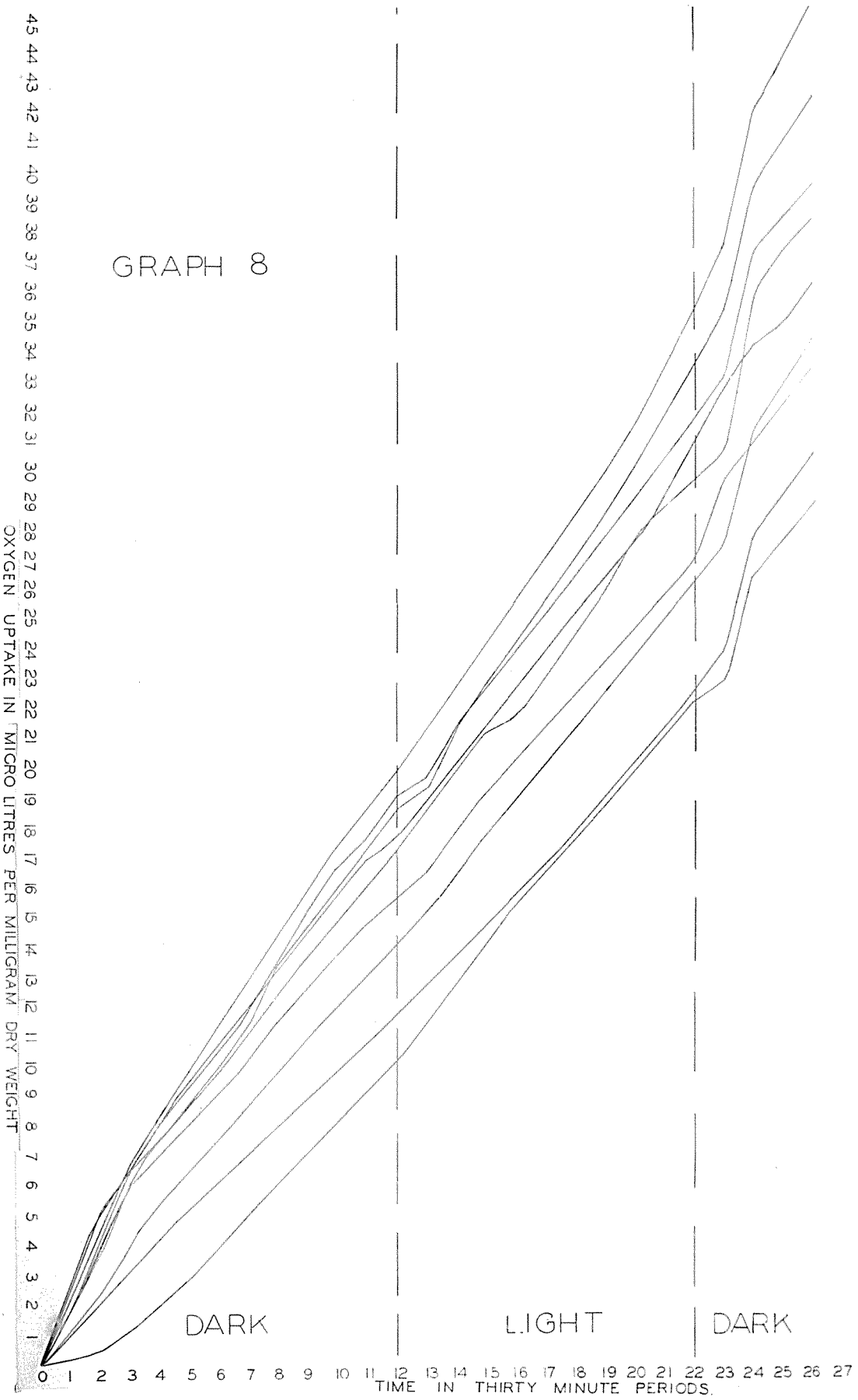
GRAPH 5


OXYGEN UPTAKE IN MICRO LITRES PER MILLIGRAM DRY WEIGHT
20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
0 1 2 3 4 5 6 7 8 9 10 11 12
TIME IN THIRTY MINUTE PERIODS.

GRAPH 6

30 29 28 27 26 25 24 23 22 21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
OXYGEN UPTAKE IN MICRO LITRES PER MILLIGRAM DRY WEIGHT
0 1 2 3 4 5 6 7 8 9 10 11 12
TIME IN THIRTY MINUTE PERIODS.


GRAPH 7
OXYGEN UPTAKE IN MICRO LITRES PER MILLIGRAM DRY WEIGHT
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44
DARK
LIGHT
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
TIME IN THIRTY MINUTE PERIODS


GRAPH 8
OXYGEN UPTAKE IN MICRO LITRES PER MILLIGRAM DRY WEIGHT
45 44 43 42 41 40 39 38 37 36 35 34 33 32 31 30 29 28 27 26 25 24 23 22 21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
DARK
LIGHT
DARK
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
TIME IN THIRTY MINUTE PERIODS.

CHAPTER IV

PART 1. PRELIMINARY EXPERIMENTS.

(i) Examination of Variability in the Test Material

Three experiments were carried out in the Warburg Respirometer on successive days using discs of Microsorium diversifolium suspended in distilled water. The object was to observe the degree of variability in O_2 uptake within each experiment, also between days, using four rhizomes each day from each of which thirty discs were taken. Ten sections, each 0.5 mm. in thickness were used in each flask and thirteen flasks plus one thermobarometer were used in each experiment. The results are summarised in graphical form. (See Graphs 1,2,3 and 4.)

The conclusion was that over a short period of six hours the oxygen uptake in each flask showed striking linearity although the variation within each experiment was quite high and between days also high. The coefficient of variation based on the mean and average deviation for example at 360 minutes was 16.70 (Exp. I), 9.46 (Exp. II) and 10.26 (Exp. III).

A further experiment (IV) was carried out on Bracken fern to observe the general order of variability in distilled water. (See Graph 5.) At 360 minutes the coefficient of variability based on the mean and average deviation was 16.06 (thirteen replications). In this experiment twelve 0.5 mm. sections per flask were selected from 200 discs obtained by cutting 20 discs from each of ten rhizomes. Oxygen uptake is again quite linear.

Hard fern was examined for variability (Expt.V) taking 10-12, 0.5 mm. sections per rhizome from twenty rhizomes. Fifteen sections were

placed in each flask in distilled water. At 360 minutes the coefficient of variability based on the mean and average deviation was 12.68 for twelve replications. As with Bracken and Microsorium the oxygen uptake is quite linear. (See Graph 6.)

It was apparent from these experiments that a method of randomised selection from as large a population as was practically possible, together with the necessary biometrical evaluation was essential if differences due to application of plant regulators to tissue were to be separated from those due simply to biological variation.

Because of the smallness of the rhizome of Paesia scaberula and the consequent lengthy period taken to cut 0.5 mm. sections, a further preliminary experiment was conducted to observe any differences in respiration between different portions of the rhizome, also whether 0.3 cm. discs differed significantly in respiration from 0.5 mm. discs taken from the same region of the rhizome meristem. There was no significant difference between 0.3 cm. discs and 0.5 mm. discs from the same region. Discs from the apical 3 mm. have a higher respiratory level than the remaining 6 mm. as might be expected, because of the more actively dividing cells in the apical portion of a rhizome. (See experimental procedure).

In the remainder of the experiments conducted on Paesia scaberula 0.3 cm. disks were used.

The apical 1 - 2 millimetres containing the most actively dividing cells of the primary meristem together with the single "imitial" cell of promeristem was discarded with each of the fern species used for the remainder of the experiments in order that variability would be reduced.

(ii) Distilled Water as a Suspensory Medium for Tissue Slices.

Because all of the preliminary experiments were conducted in a distilled water medium, tests were initially carried out to determine whether any rupture of cells occurred through uptake of water, with a

consequent increase in turgor pressure to the point where cell membranes are ruptured, liberating the cell contents.

Tests were carried out on tissue of the rhizome meristem in Pteridium aquilinum, Paesia scaberula and Microsorium diversifolium.

Tissue was sectioned to 0.5 mm. discs and then rapidly weighed. Known amounts were placed for thirty minutes,

(a) In 1% Sucrose.
(b) In distilled water.

At the end of this period the sections were dried quickly on filter paper and again rapidly weighed.

It was assumed that a 1% sucrose solution would have a very similar osmotic pressure to the cells, thus preventing any loss of cell contents.

The results are shown in table form.

	Sugar Solution			Distilled Water		
Species	Tissue Weight in air.	Dried Wt. After immers in sugar	% change	Tissue wt. in air.	Dried Wt. After immers in H_2O	% Change
Microsorium Diversifolium	1.2342	1.4494	17.44 Incr.	1.2546	1.4322	14.16 Incr.
Paesia Scaberula	.8650	.9606	11.05 "	.7340	.8528	16.18 "
Pteridium Aquilinum	1.5726	1.5802	0.48 "	1.0603	.9624	9.12 Loss

In every case, except Pteridium aquilinum suspended in water, there was an increase in weight. The majority of this increase can be attributed to the inefficiency of removing all the moisture from the surface of the tissue slices with filter paper. Some of the increase in weight may be due to an accumulation of water either by osmosis or an active cellular process, or small quantities of sucrose.

Doubt as to whether the 9.12% loss of weight in water of Pteridium aquilinum was a true one. (In view of the very slight increase in weight

of the sugar treated tissue) was cleared by oven drying both the sugar and water treated tissue and expressing the resultant dry matter as a percentage of the original air dried material.

(a) D.M. percentage of original air dried weight (Sugar treated) = 8.15%
(b) " " " " " " " (H_2O treated) = 6.63%

These very similar percentages indicated that there had been no real loss of weight, rather that the drying process with filter paper was liable to error.

The conclusion was made therefore that with the three species examined there was no danger of loss of cell contents if tissue was suspended in distilled water for relatively short periods of time.

(iii) The Effect of Light on Respiration.

As both Paesia scaberula and Microsorium diversifolium contain considerable amounts of chlorophyll, two experiments were conducted with Microsorium to determine the magnitude of the differences in respiratory level when experiments are run in light and darkness. (Graphs 7 and 8). In preliminary experiment VI (Graph 7) twelve flasks each containing 10, 0.5 mm. sections of Microsorium suspended in distilled water were allowed to take up oxygen in darkness for 360 minutes. At the end of this period the cloth cover was removed and the respirometer exposed to ordinary laboratory sunlight together with the light from two 75 watt tungsten filament lamps for a further three hours.

The experiment was repeated on the following day (Exp.VII) when sunlight was more intense, running for six hours in darkness, five hours in light and two more hours in darkness. (Graph 8.)

It was concluded from these two experiments that light has a slight but insignificant effect on respiration, hence to ensure uniformity of conditions the remainder of the experiments were conducted in darkness obtained by covering the respirometer with a black cloth draped down

over all sides. Flasks when being tipped are exposed for periods of up to twenty seconds (maximum) to the light of the laboratory. It was considered that such periods were insignificant especially in view of the fact that at tipping periods the laboratory was always darkened by lowering window blinds and reducing artificial light.

(iv) Volatility Effects.

In several preliminary experiments it was noted that flasks containing plant regulators of relatively high concentration in the sidearm, appeared to show some degree of stimulation of oxygen uptake before tipping. It was considered possible that some vapour or droplets were escaping into the main body of the flask. In order to test this theory two experiments were conducted.

I. Measuring oxygen uptake for a longer period before tipping in order to detect any differences between control and treatment vessels.

II. Flasks containing glass distilled water alone, and plant regulator in the side arm were placed in the water bath (30°C) and oscillated for 150 minutes. The contents of the main body of the flask were then tested with chromatropic acid (89.90) a reagent which gives a red to purple colour with substances which can by hydrolysis, oxidation, or reduction be reduced to form formaldehyde (e.g. 2,4-D acid). As little as 0.05 gamma of 2,4-D per ml. can be detected.

Both experiments were very carefully conducted and all equipment meticulously washed. Experiments I and II gave negative results from which it was concluded that the standard of washing flasks, hands, pipettes etc. had not been sufficiently high, and in subsequent experiments with the standard procedure outlined in Chapter III this conclusion was verified, as no further difficulty was encountered.

PART 2. DESIGN OF EXPERIMENTS.

(i) Available Test Materials and Methods.

Blackman (33) in discussing the assessment of relative toxicity of plant regulators has emphasised the necessity of measuring responses to a number of dosages if accurate comparisons of relative toxicity are to be obtained. Using a wide range of concentrations and the technique of probit analysis he has shown that responses are greatly dependent on the species examined. In fact, order of toxicity may be reversed from one species to another.

Simon (65) in a paper discussing phytocidal effects of plant regulators says -

"Determinations of relative toxicity are liable to serious error,

(a) If restricted to a single species of test plant.
(b) Carried out at any single stage of development.
(c) Only a single concentration of each compound is employed."

Similar principles hold in the assessment of relative effectivity of plant regulators whether in respiration, growth or developmental studies, or auxin responses in plants as emphasised by the following workers, Thompson et al (28), Zimmerman and Hitchcock (82)(Growth), Zimmerman and Hitchcock (26)(Development), Leaper and Bishop (44)(Growth), Smith (50) (Respiration), and Kelly and Avery (52) (Respiration).

In order to assess the action of the three plant regulators 4-CPA, 2,4-D and 2,4,5-T on Pteridium aquilinum it was considered necessary to observe their simultaneous effects on several other fern species. It was hoped that any general pattern of response by the Pteridophyta would thus be disclosed. The two ferns Paesia scaberula and Microsorium diversifolium were selected for inclusion in the tests for the above reasons and in addition because of their well developed rhizome systems,

the focal point of the respiratory study.

Five concentrations of growth regulator were used, ranging from the highest soluble concentrations for an aqueous solution (9.148×10^{-4} M) in successive dilutions by 10^{-1}M down to 9.148×10^{-8}M. The range therefore included concentrations known to have phytotoxic as well as physiological effects on some monocotyledons and dicotyledons (Smith(50)).

(ii) Statistical Method.

A Comparison of Analytical Methods of Assessing Treatment Differences.

In all of the fifteen major experiments three treatments were replicated three times, and in addition, to provide a basis for comparisons, three control vessels were used. All vessels were corrected with a thermobarometer flask.

One of the major difficulties encountered in the use of the Warburg respirometer is the limitation on the number of flasks (usually twelve or fourteen) that can be used in any one experiment. Physiological differences in tissue responses on successive days are very often encountered, making it difficult to compare results between experiments. It is desirable therefore to have as many treatments as possible within any one experiment or alternatively to have a method of statistical evaluation that will provide a comparison between experiments.

Audus (32) in a rather critical paper has pointed out the dangers of bad experiments and bad planning. To use his own words,

> "Proper design means ability to measure between and within experiments. If the data is biological it must have a statistical method of verification using principles as laid down by Fisher (1947)."

Four methods of analysing oxygen uptake were therefore studied in relation to their efficiency of measuring detectable differences between

treatments and between experiments.

A. Direct measurement of oxygen uptake expressed either on an hourly basis or the cumulative total over a relatively short period such as six hours. Such measurements are usually expressed as micro-litres per milligramme dry weight, fresh weight or nitrogenous material. Comparison of treatment effects is made on the basis of a common comparison with the average value of the three control flasks. Changes are therefore expressed as a percentage of the control uptake. An analysis of variance can be carried out on the data to measure the significance of mean differences at the end of a period of six hours of treatment. Simultaneously standard errors of the means can be determined.

Such a method has the disadvantage that any errors in control values are reflected throughout the treatments.

B. French and Beevers (53) developed a method to help eliminate the problem of obtaining a wide range of concentrations, or using a number of different chemicals, within any one experiment, given a limited number of vessels to work with. The basis of the method is that the effect of each treatment is expressed as a percentage of its own control rate. Variation in initial rates before tipping is allowed for by calculating a control value for each flask during treatment assuming that the percentage differences between "to be treated" and control flasks observed before tipping would have persisted throughout the subsequent period had no addition been made. An example of their method follows -

Method of adjusting respiration rates for variation between samples.
(Data from an experiment with IAA.)

Treatment	I Initial O_2 uptake $1\frac{1}{4}$ hours	II Total Resp. $5\frac{3}{4}$ hours	III O_2 uptake $4\frac{1}{2}$ hours after tip (II - I)	IV Correction factor (III/I)	V Calculated controls (IV x I)	VI % of Controls (III/V x100)
H_2O	43	212	169	3.93	169	100
10^{-6}M. IAA.	39	216	177	"	153	116
10^{-5}M.	45	250	205	"	177	116
3×10^{-5}M	41	244	203	"	161	126
10^{-4}M.	46	278	232	"	181	128

(R.C. French and Harry Beevers - Am. J.Bot. 40 (1953) 661.)

One advantage of such a method is that if oxygen uptake is not linear the control flasks will still reflect this situation and percentage changes will still be valid.

In the cases of Pteridium aquilinum and Paesia scaberula tissues suspended in buffer (0.05 M. Phosphate-Citrate at pH 5.0), oxygen uptake from control flasks shows a high degree of linearity. Microsorium diversifolium however shows a definite increase in hourly uptake over a period of at least six hours. French and Beevers method could therefore be applied to such data.

No attempt was made by French and Beevers to apply any form of analysis of variance to the method and in most of their experiments only one replication of each treatment was used. To do an analysis of variance using Beevers method the assumption is made that the ratio of rates of uptake before and after treatment is constant for all flasks and this is estimated before analysis from the control flasks only.

The disadvantage is that the method uses the variance of only the three controls so that any serious error in them will upset the significance of the treatment effects.

The method of French and Beevers was tested on data obtained from a preliminary experiment on Pteridium, using 4-CPA, 2,4-D and 2,4,5-T acids at stock concentrations and the standard procedure outlined in Chapter III. Constants for the three control flasks were calculated using 150 minutes as the tipping time, and examined for variability. It was found that the constants were very similar. The average of the total deviations from the average constants, determined at thirty minute periods (three replications), expressed as a percentage of the constant was as low as 1.165%. The tabulated figures are presented in Table II.

TABLE II.

Time in Minutes	Flask One			Flask Two			Flask Three			Deviation as a % of Average Constant.
	Oxygen Uptake	Constant	Deviation from Average	Oxygen Uptake	Constant	Deviation from Average	Oxygen Uptake	Constant	Deviation from Average.	
150	17.16	1.000	.000	18.28	1.000	.000	19.72	1.000	.000	.000
180	20.46	.192	.007	22.19	.213	.014	23.56	.194	.005	1.306
210	22.77	.327	.017	24.86	.359	.015	26.60	.348	.004	1.040
240	26.73	.557	.024	29.45	.611	.030	31.10	.577	.004	.998
270	29.50	.791	.018	32.81	.794	.021	34.22	.735	.038	.996
300	32.93	.919	.036	36.85	1.015	.060	38.13	.933	.022	1.235
330	36.63	1.134	.036	40.82	1.233	.063	42.26	1.143	.027	1.077
360	39.60	1.308	.032	44.11	1.413	.073	45.45	1.304	.039	1.074
390	43.23	1.519	.001	47.74	1.611	.091	47.92	1.430	.090	1.197
420	45.34	1.642	.005	50.48	1.761	.114	50.09	1.540	.107	1.372
450	48.18	1.807	.002	53.53	1.928	.119	53.14	1.694	.115	1.310
480	51.10	1.977	.003	56.85	2.109	.129	56.33	1.856	.124	1.292
510	53.59	2.122	.005	59.52	2.256	.129	59.23	2.003	.124	1.212
540	56.76	2.307	.003	62.88	2.439	.125	62.49	2.168	.136	1.145
570	59.00	2.438	.017	64.59	2.533	.112	64.88	2.290	.131	1.073
600	61.51	2.584	.030	67.13	2.672	.118	67.20	2.407	.147	1.155

C. A third method of analysis studied was an analysis of covariance (33) (Snedicor Chapter 12) of uptakes at the end of a given period of treatment (510 minutes), using uptakes before treatment (150 minutes) as concomitant data. In effect the method compares the slope of the line formed by plotting oxygen uptake against time before, and after treatment. The advantage of this method over French and Beever's is that allowance is made for the variance of all the treatments + controls rather than just controls. Further no assumption is made about the relationship between rates of uptake before treatment and after treatment, except that there is some linear relation which is determined during the course of the analysis.

D. A further method using an analysis of covariance was studied. In both methods (2) and (3), intermediate data recorded at sixty minute intervals between initial closing of stopcocks, tipping time and final readings are not used to give their full contribution to the error terms of the analysis. By assuming that oxygen uptake is linear in form (over a relatively short period) the intermediate data can be computed as rates of uptake (measured by linear regression coefficients), and provided readings are recorded at equal intervals of time these regression coefficients are easily worked out. Thus all errors of measurement are incorporated in the analysis of covariance. By this method the final treatment means can be adjusted to values which allow for all errors incorporated in the experiment through variation.

The assumption that oxygen uptake is linear was tested in Pteridium aquilinum and Microsorium diversifolium for a period of ten hours, two hours longer than the majority of experiments were run for.

The method of testing the departure from linearity in the time response curves is outlined symbolically below.

Test of Curvature in Time Response Curves

Let y's be the responses (oxygen uptake) corresponding to x_1's, the coded times (0 - 20 in example). Also let x_2's denote squares of x_1's (i.e. for $x_1 = 4$, $x_2 = 16$, and so on.)

Compute

(1) n = no. of observations (20 in example)

(2) Σx_1

(3) Σx_1^2

(4) $SSx_1 = \Sigma x_1^2 - (\Sigma x_1)^2/n = q$ (say)

(5) Σy^2 and Σy

(6) $SSy = \Sigma y^2 - (\Sigma y)^2/n$

(7) $\Sigma x_1 y$

(8) $SPx_1y = \Sigma x_1 y = (\Sigma x_1)(\Sigma y)/n = s$ (say)

(9) $b = \frac{SPx_1y}{SSx_1}$

(10) Linear regression eqn. $(y-\bar{y}) = b(x - \bar{x})$

(11) Part of SSy accounted for by linear regression

$$= \frac{(SPx_1y)^2}{SSx_1} = A \text{ (say)}$$

(12) Variance from regression line

$$= \frac{SSy - A}{(n-2)} = E \text{ (say)}$$

(13) SE of b

$$= \sqrt{\frac{E}{SSx_1}}$$

(14) Test of significance of b

Source of variation	S.S.	d.f.	M.S.	F
Linear regression	A	1	A	A/E
Residual	SSy - A	(n-2)	E	
Total	SSy	(n-1)		

(15) Σx_2 $(= \Sigma x_1^2)$

(16) Σx_2^2

(17) $SSx_2 = \Sigma x_2^2 - (\Sigma x_2)^2/n = p$ (say)

(18) $\Sigma x_1 x_2$

(19) $SPx_1x_2 = \Sigma x_1 x_2 - (\Sigma x_1)(\Sigma x_2)/n = r$ (say)

(20) $x_2 y$

(21) $SPx_2y = \Sigma x_2 y - (\Sigma x_2)(\Sigma y)/n = t$ (say)

(22) Find b_1 and b_2 from equations

$$q\,b_1 + r\,b_2 = s$$
$$r\,b_1 + p\,b_2 = t$$

(23) Quadratic regression equation

$$(y - \bar{y}) = b_1(x_1 - \bar{x}_1) + b_2(x_2 - \bar{x}_2)$$

(x_1 can now be written as x and x_2 as x^2)

(24) Part of SSy accounted for by quadratic regression

$$= b_1 s + b_2 t = B \text{ (say)}$$

(25) SE of b_1 =

$$\sqrt{G\left(\frac{p}{pq - r^2}\right)}$$

(26) SE of b_2

$$\sqrt{G\left(\frac{q}{pq - r^2}\right)}$$

(27) Test of departure from linearity in regression.

Source of variation	S.S.	d.f.	M.S.	F
Linear regression	A	1	A	
Quadratic regression	B	2		
Increment due to b_2	B-A	1	B-A	$\frac{B-A}{G}$
Residual	(SSy-B) = F	(n-3)	$\frac{F}{n-3} = G$	
Total	SSy	n-1		

The test of departure from linearity in the regressions for Pteridium aquilinum (Exp. VIII) showed a high degree of significance at the 99% level of probability indicating that oxygen uptake was quite definitely linear. The conclusions from the test are presented in tabular form.

Pteridium aquilinum. (Curvature in time response curve).

(i) Linear regression.

Source of variation	S.S.	d.f.	M.S.	F
Linear regression	6409.4809	1	6409.4809	7068 **
Residual	16.3228	18	.9068	
Total	6425.8037	19		

(ii) Quadratic regression.

Source of variation	S.S.	d.f.	M.S.	F
Linear regression	6409.4809	1	6409.4809	**
Quadratic "	6423.5035	2		
Inc. due to Quad. regression	14.0226	1	14.0226	**
Residual	2.3002	17	.1353	
Total	6425.8037	19		

** Significant p is less than .01

In the case of Microsorium diversifolium the departure from linearity test was non significant at the 99% level of probability indicating that oxygen uptake plotted against time was not linear but curved. (Experiment IX)

Microsorium diversifolium.

(i) Linear regression

Source of Variation	S.S.	d.f.	M.S.	F
Linear regression	3699.8340	1	3699.8340	11.75 **
Residual	5352.2735	17	314.8396	
Total	9052.1075	18		

(ii) Quadratic regression

Source of Variation	S.S.	d.f.	M.S.	F
Linear regression	3699.8340	1	3699.8340	**
Quadratic "	3783.8312	2		
Inc. due to b_2	83.9972	1	83.9972	-
Residual	5268.2763	16	329.267	
Total	9052.1075	18		

** Significant

Each of these tests was carried out on a single flask chosen at random. By inspection of other graphed uptakes it was apparent that Microsorium diversifolium showed an even greater curvilinearity than that of the result actually tested. Paesia scabemla showed a high degree of linearity on inspection of graphed uptakes.

It was apparent therefore that an analysis of covariance using regression coefficients (Method 4) to adjust the final treatment means could only be used on Pteridium aquilinum and Paesia scaberula.

A comparison of the relative efficiencies of methods (1) (2) and

(3) was obtained from a preliminary Experiment No. X by expressing the residual mean squares of (2) and (3) as a percentage of the residual mean square of (1) (The uncorrected analysis).

Method (2) was found to be 208% more efficient than (1)

Method (3) was found to be 422% more efficient than (1)

In the particular experiment examined, none of the treatments showed significance under the three methods of analysis. Method (2) appeared to be the closest to reaching significance. Using rates of uptake (Method (4)) a significant "F" was obtained at the 5% level.

The summarised results are presented below.

Analyses of Oxygen Uptake Curves Experiment X.

Pteridium aquilinum treated with 4-CPA, 2,4-D and 2,4,5,-T. Three replications of each treatment and three controls.

1. Ordinary analysis of Uptakes at 510 minutes.

Source of Variation	d.f.	Mean Square	F	
Treatments	3	0.7158	0.11	-
Residual	8	6.2762		

\- Non Significant

Means and Standard Errors are:-

Control	53.5	±	1.5
4-CPA	52.9	±	"
2,4-D	53.7	±	"
2,4,5-T	54.0	±	"

2. Analysis using French and Beevers correction.

Source of Variation	d.f.	Mean Square	F	
Treatments	2	13.2492	4.38	-
Residual	6	3.0225		

\- Non Significant

Means and Standard Errors are:-

Control	53.5	±	1.0
4-CPA	52.1	"	"
2,4-D	56.1	"	"
2,4,5-T	53.1	"	"

3. Analysis of Covariance of Uptakes at 510 minutes.

Using uptakes at 150 minutes (before application of treatment) as concomitant data.

Source of Variation	d.f.	Adjusted Mean Squares	F
Treatments	3	3.6416	2.45 -
Residual	7	1.4855	

- Non Significant

Means and Standard Errors are:-

Control	53.41	±	0.70
4-CPA	52.30	"	"
2,4-D	55.17	"	"
2,4,5-T	52.23	"	"

4. Analysis of covariance on rates of uptake.

(Using linear regression coefficients of intermediate data to adjust final means.)

Source of Variation	d.f.	Adjusted Mean Squares	F
Treatments	3	.10689	4.37 * (5% level)
Residual	7	.02448	

* significant

The adjusted final means and their standard errors are:-

Control	6.98	±	.09
4-CPA	6.78	"	"
2,4-D	7.27	"	"
2,4,5-T	6.92	"	"

The relative efficiencies of each of the methods can best be illustrated by listing for each method the Standard error of the mean expressed as a percentage of the control mean.

1. 2.80 per cent
2. 1.87 " "
3. 1.31 " "
4. 1.29 " "

Methods 3 and 4 have a definite advantage over 1 and 2, method 4 being preferred to method 3.

To verify that the results of the test of relative efficiencies was not due purely to chance, a further set of similar analyses was computed for data known to be non-linear (Microsorium diversifolium - Exp. XI). As was to be expected, the first three methods showed a similar increase in efficiency going from methods one to three as in the analysis previously described when standard errors of the mean were expressed as percentages of the control mean.

i.e. 1. 10.37% , 2. 3.48% , 3. 2.17 %

Analyses of oxygen Uptake Curves. (Exp. XI.)

Microsorium diversifolium treated with 4-CPA, 2,4-D and 2,4,5-T. Three replications of each treatment and three controls.

1. Ordinary analysis of variance of final uptakes.

Source of Variance	d.f.	Mean Square	F
Treatments	3	60.7845	1.15 -
Residual	8	52.9484	

- Non significant

Means and Standard Errors are:-

Control	40.5	±	4.2
4-CPA	47.6	"	"
2,4-D	42.6	"	"
2,4,5-T	36.8	"	"

2. Analysis using French and Beevers correction.

Source of Variation	d.f.	Mean Square	F
Treatments	2	116.8096	19.62 **
Residual	6	5.9535	

** Significant

Means and Standard Errors:-

Control	40.48	±	1.41
4-CPA	35.83	"	"
2,4-D	41.61	"	"
2,4,5-T	31.40	"	"

3. Analysis of Covariance of uptakes at 510 minutes.

Using uptakes at 150 minutes (before application of treatment) as concomitant data.

Source of Variation	d.f.	Adjusted mean squares	F
Treatments	3	48.8946	17.66 **
Residual	7	2.7681	

* Significant

Means and Standard Errors:-

Control	44.22	±	0.96
4-CPA	41.68	"	"
2,4-D	45.52	"	"
2,4,5-T	36.11	"	"

Method 4 using linear regression coefficients to adjust final means could not be used because of the non-linearity of oxygen uptake of Microsorium tissue suspended in buffer solution.

After consideration of the above analyses the following methods were adopted. In experiments 1 - 5 and 10 - 15 of the major series of experiments with Pteridium aquilinum and Paesia scaberula, the

significance of the differences between treatment and control means was assessed by an analysis of covariance, using linear regression coefficients (based on rates of oxygen uptake) to correct the final treatment means. (Method four.)

Major experiments 5 - 10 with Microsorium diversifolium were analysed by an analysis of covariance. (Method three.)

CHAPTER V.

THE EFFECT OF THE PLANT REGULATORS ON RESPIRATION IN THE THREE PTERIDOPHYTE SPECIES

Part 1.

Pteridium Aquilinum.

(i) In the sixteen major experiments to be described the following pattern was adopted. Tissue sections were allocated to each Warburg flask as described in the section on experimental procedure and test solutions placed in the sidearms. The apparatus was assembled and allowed to equilibrate for thirty minutes and then the stopcocks were closed. The first reading of oxygen uptake was taken at the end of thirty minutes, thereafter at sixty minute intervals to the end of the experiment. Test solutions were tipped into the main body of the flask at the end of 150 minutes after first noting the oxygen uptake. Readings were continued at hourly intervals for another six hours (510 minutes total), at which time the stopcocks were opened.

Tissue was removed and washed thoroughly in tap water to remove any traces of potassium hydroxide, and then dried in an oven at 105°C overnight. Oxygen uptakes in micro-litres per milligram dry weight were determined and corrected by the vessel constants.

In experiments one to eleven inclusive an analysis of covariance was carried out on each experiment using initial rates of uptake based on the linear regression coefficients determined for each flask before tipping, and final rates of uptake based on the linear regression coefficients after tipping, as the concomitant data.

These linear regression coefficients (Snedecor (83)) were determined for each flask in the following manner.

If Y_1, Y_2, Y_3 ..... Y_9 are the oxygen uptakes at equal intervals of time (60 minutes) then b_i (linear regression coefficient for the uptakes before tipping) is equal to

$$\frac{Y_3 - Y_1}{2}$$ (when Y_3 is the final uptake immediately before tipping).

b_f. (Linear regression coefficient for the uptakes after tipping) is equal to -

$$\frac{3Y_3 - 2Y_4 - Y_5 + Y_7 + 2Y_8 + 3Y_9}{28}$$

Y_3 is the final oxygen uptake before tipping and Y_9 is the final treatment oxygen uptake.

b_i therefore represents the average rate of uptake before treatment.

b_f likewise represents the average rate of uptake after treatment.

The analysis of covariance (83, Chapter 12) was then carried out in the normal manner using b_i's and b_f's as the concomitant data.

Tests of significance and adjusted final treatment means together with standard errors of the means are set out in tabular form for the experiments conducted on (A) Bracken Fern (B) Hard Fern. Plant regulator concentrations are those obtained after tipping.

A. Experiment 1. Pteridium aquilinum treated with 9.148×10^{-4}M aqueous solutions of 4-CPA, 2,4,-D and 2,4,5-T.

Var.	d.f.	X	xy	y	y'	d.f'	M.S.	F
B	3	.8055	- 2.4882	10.7605				
E	7	7.4613	+ 2.0112	3.6976	3.1555	6	.5259	7.146 *
B+E	10	8.2668	- .4770	14.4581	14.4306	9		
D					11.2751	3	3.7583	

* Significant

Adjusted final treatment means.

Control	7.370	±	.725
4-CPA	5.143	"	"
2,4-D	4.839	"	"
2,4,5-T	5.137	"	"

Significance in experiment one is obtained only at the 95% level of probability.

Throughout all experiments * denotes a significant result, (-) a non significant result.

Experiment 2. Pteridium aquilinum treated with 9.148×10^{-5} M aqueous solutions of 4-CPA, 2,4-D and 2,4,5-T.

Var.	d.f.	x	xy	y	y'	d.f.'	M.S.	F.
B	3	.2290	- .0647	.0705				
E	8	1.0781	.8954	.9501	.2065	7	.0295	3.233 -
B+E	11	1.3071	.8307	1.0206	.4927	10		
D					.2862	3	.0954	

Adjusted final treatment means.

Control	6.979	±	.09
4-CPA	6.785	"	"
2,4-D	7.265	"	"
2,4,5-T	6.924	"	"

Experiment 3. Pteridium aquilinum treated with 9.148×10^{-6} M aqueous solutions of 4-CPA, 2,4-D and 2,4,5-T.

Var.	d.f.	x	xy	y	y'	d.f.'	M.S.	F
B	1	.0043	.0179	.0749				
E	4	.4219	.4518	.5375	.0537	3	.0179	2.29 -
B+E	5	.4262	.4697	.6124	.0948	4		
D					.0411	1	.0411	

Adjusted final treatment means.

Control	6.472	±	.134
4-CPA	6.637	±	.134

Experiment three was repeated because of several accidents which did not permit the use of 2,4-D and 2,4,5-T treatments. Experiment four is therefore a repetition of experiment three.

Experiment 4.

Var.	d.f.	x	xy	y	y'	d.f'	M.S.	F
B	3	.8052	.1460	1.2960				
E	6	1.3130	.0996	.4732	.4657	5	.0931	4.56 -
B+E	9	2.1182	.2456	1.7692	1.7407	8		
D					1.2750	3	.4250	

Adjusted final treatment means.

Control	5.333	±	.176
4-CPA	5.634	"	"
2,4-D	5.786	"	"
2,4,5-T	4.912	"	"

Experiment 5. Pteridium aquilinum treated with 9.148×10^{-7}M aqueous solutions of 4-CPA, 2,4,-D and 2,4,5-T.

Var.	d.f.	x	xy	y	y'	d.f.'	M.S.	F
B	3	.4502	.5839	1.1734				
E	7	3.3983	3.4361	3.8197	.3454	6	.0575	2.72 -
B+E	10	3.8485	4.0200	4.9931	.8148	9		
D					.4694	3	.1564	

Adjusted final treatment means.

Control	6.971	±	.138
4-CPA	7.546	"	"
2,4-D	7.131	"	"
2,4,5-T	7.321	"	"

Experiment 6. Pteridium aquilinum treated with 9.148×10^{-8} M aqueous solutions of 4-CPA, 2,4-D and 2,4,5-T.

Var.	d.f.	x	xy	y	y'	d.f'	M.S.	F
B	3	.1235	.0838	.2375				
E	8	2.0706	2.4314	3.2108	.3557	7	.0508	1.372 -
B+E	11	2.1941	2.5152	3.4483	.5650	10		
D					.2093	3	.0697	

Adjusted final treatment means.

Control	7.745	±	.130
4-CPA	7.823	"	"
2,4-D	7.754	"	"
2,4,5-T	7.471	"	"

A summary is presented of the results from experiments one to six inclusive, together with the detectable differences between final adjusted means (Snedecor - Student's "t" test - page 57 (83)), obtained for those experiments producing a significant result in the analysis of covariance. "F" values are significant at the 99% level of probability, unless otherwise mentioned, in all analyses of covariance. The 95% level of probability is used in Students "t" test for differences between means of treatments and control values.

TABLE III. A SUMMARY OF THE EFFECTS OF THE THREE PLANT REGULATORS ON PTERIDIUM AQUILINUM.

The Response of Pteridium Aquilinum to 4-CPA, 2,4-D and 2,4,5-T

Exp. No.	Conc. (Molar)	"F"	Sign. F. (99%)	Final Mean Control	Final Mean 4-CPA	Final Mean 2,4-D	Final Mean 2,4,5-T	Standard Error of mean	Signif. of Diff. (5%)	Calculated detect. difference	Observed detect. difference
1.	9.148×10^{-4}	7.146	*95%	7.370				± .725			
					5.143			" .725	*	1.633	- 2.227
						4.839		" .725	*	1.485	- 2.531
							5.137	" .725	*	1.519	- 2.233
2.	9.148×10^{-5}	3.233	=	6.979				" .09			
					6.785			" .09	-		
						7.265		" .09	-		
							6.924	" .09	-		
3.	9.148×10^{-6}	2.29	=	6.472				" .134	-		
					6.637			" .134	-		
4.	9.148×10^{-6}	4.56	=	5.333				" .176			
					5.634			" .176	-		
						5.786		" .176	-		
							4.912	" .176	-		
5.	9.148×10^{-7}	2.72	=	6.971				" .138			
					7.546			" .138	-		
						7.131		" .138	-		
							7.321	" .138	-		
6.	9.148×10^{-8}	1.372	=	7.745				" .130			
					7.823			" .130	-		
						7.754		" .130	-		
							7.471	" .130	-		

* Significant = Non Significant

Part 1.

(ii) Paesia Scaberula - Experimental Results.

B. Experiment 7. Paesia scaberula treated with 9.148×10^{-4}M aqueous solutions of 4-CPA, 2,4-D and 2,4,5-T.

Var.	d.f.	x	xy	y	y'	d.f.'	M.S.	F
B	3	.1158	- .9278	12.6538				
E	8	1.9266	+ .4527	.4742	.3678	7	.0525	80.253**
B+E	11	2.0424	- .4751	13.1280	13.0175	10		
D					12.6497	3	4.2165	

Adjusted final treatment means

Control	3.461	±	0.132
4-CPA	3.269	"	"
2,4-D	3.032	"	"
2,4,5-T	.870	"	"

Experiment 8. Paesia scaberula treated with 9.148×10^{-5}M aqueous solutions of 4-CPA, 2,4-D and 2,4,5-T.

Var.	d.f.	x	xy	y	y'	d.f.'	M.S.	F
B	3	.1104	- .1212	2.4976				
E	8	.2904	.2523	.5470	.3280	7	.0468	19.042**
B+E	11	.4008	.1311	3.0466	3.0018	10		
D					2.6738	3	.8912	

Adjusted final treatment means

Control	5.41	±	0.125
4-CPA	5.55	"	"
2,4-D	5.24	"	"
2,4,5-T	4.33	"	"

Experiment 9. Paesia scaberula treated with 9.148×10^{-6} M aqueous solutions of 4-CPA, 2,4-D and 2,4,5-T.

Var.	d.f.	x	xy	y	y'	d.f.'	M.S.	F
B	3	.1374	.0402	.0326				
E	8	.1729	.0894	.1266	.0804	7	.0115	.713 -
B+E	11	.3103	.1296	.1592	.1051	10		
D					.0247	3	.0082	

Adjusted final treatment means.

Control	5.055	±	0.061
4-CPA	4.950	"	"
2,4-D	4.933	"	"
2,4,5-T	5.028	"	"

Experiment 10. Paesia scaberula treated with 9.148×10^{-7} M aqueous solutions of 4-CPA, 2,4-D and 2,4,5-T.

Var.	d.f.	x	xy	y	y'	d.f.'	M.S.	F
B	3	.0544	.0362	.0709				
E	8	.0933	.1498	.4927	.2522	7	.0360	.714 -
B+E	11	.1477	.1860	.5636	.3294	10		
D					.0772	3	.0257	

Adjusted final treatment means.

Control	4.788	±	0.109
4-CPA	4.502	"	0.109
2,4-D	4.599	"	"
2,4,5-T	4.637	"	"

Experiment 11. Paesia scaberula treated with 9.148×10^{-8}M aqueous solutions of 4-CPA, 2,4-D and 2,4,5-T.

Var.	d.f.	x	xy	y	y'	d.f.'	M.S.	F
B	3	.1094	.1366	.2354				
E	8	.4083	.4146	.4231	.0019	7	.0003	291.3**
B+E	11	.5177	.4512	.6585	.2653	10		
D					.2624	3	.0874	

Adjusted final treatment means.

Control	5.052	±	0.01
4-CPA	4.858	"	"
2,4-D	4.909	"	"
2,4,5-T	4.644	"	"

Although experiment eleven gave an apparently significant result, (Inhibition) which appeared very unlikely at the very low concentrations used, the mean value of the control was considered to be erroneous, purely by chance (1 chance in 99). Hence the experiment was repeated (11A) showing that there was no significant effect due to treatments.

Experiment 11A. Paesia scaberula treated with 9.148×10^{-8}M aqueous solutions of 4-CPA, 2,4-D and 2,4,5-T.

Var.	d.f.	x	xy	y	y'	d.f.'	M.S.	F
B	3	.0558	.0332	.2151				
E	8	.2546	.2124	.3184	.1413	7	.0202	3.262 -
B+E	11	.3104	.2456	.5335	.3392	10		
D					.1979	3	.0659	

Adjusted final treatment means.

Control	3.324	±	0.08
4-CPA	3.567	"	"
2,4-D	3.667	"	"
2,4,5-T	3.623	"	"

The results of treatment of Paesia scaberula are summarised in Table IV.

TABLE IV

SUMMARY OF THE EFFECTS OF THE THREE PLANT REGULATORS ON PAESIA SCABERULA

The Response of Paesia Scaberula to 4-CPA, 2,4-D and 2,4,5-T											
Exp. No.	Conc. (Molar)	F	Sign. F. (99%)	Final Mean Control	Final Mean 4-CPA	Final Mean 2,4-D	Final Mean 2,4,5-T	Standard Error of mean	Signif. of Diff. (5%)	Calculated detect. difference	Observed detect. difference
7.	9.148×10^{-4}	80.25	**	3.461				± .132			
					3.269			" .132	-	.448	- .192
						3.032		" .132	-	.444	- .429
							.870	" .132	*	.454	- 2.591
8.	9.148×10^{-5}	19.04	**	5.41				" .125			
					5.55			" .125	-		
						5.24		" .125	-		
							4.33	" .125	*	.471	- 1.08
9.	9.148×10^{-6}	.713	=	5.055				" .061			
					4.950			" .061	-		
						4.933		" .061	-		
							5.028	" .061	-		
10.	9.148×10^{-7}	.714	=	4.788				" .109			
					4.502			" .109	-		
						4.599		" .109	-		
							4.637	" .109	-		
11.	9.148×10^{-8}	291.3	**	5.052				" .01			
					4.858			" .01	*	.0345	- .194
						4.909		" .01	*	.0334	- .143
							4.644	" .01	*	.0334	- .408
11A.	9.148×10^{-8}	3.262	=	3.324				" .08			
					3.567			" .08	-		
						3.667		" .08	-		
							3.623	" .08	-		

Part 1.

(iii) Microsorium diversifolium - Experimental

Experiments twelve to sixteen inclusive were analysed by an analysis of covariance (Snedecor (83)) using uptakes at 150 minutes (immediately before tipping) and 510 minutes (end of treatment period) as concomitant data. The tests of significance together with the means and standard errors are tabulated for each experiment. A summary of the five experiments including the detectable differences between treatment means for the significant experiments is presented below.

Experiment 12. Microsorium diversifolium treated with 9.148×10^{-4}M aqueous solutions of 4-CPA, 2,4-D and 2,4,5-T.

Var.	d.f.	x	xy	y	y'	d.f.'	M.S.	F
B	3	.5400	-18.5545	829.0544				
E	6	3.6106	+10.6588	37.9734	6.5254	5	1.3051	215.943 ***
B+E	9	4.1506	- 7.8957	867.0278	852.0078	8		
D					845.4824	3	281.8274	

Adjusted final means.

Control	40.730	±	.660
4-CPA	39.454	"	"
2,4-D	33.724	"	"
2,4,5-T	17.190	"	"

Experiment 13. Microsorium diversifolium treated with 9.148×10^{-5}M aqueous solutions of 4-CPA, 2,4-D and 2,4,5-T.

Var.	d.f.	x	xy	y	y'	d.f.'	M.S.	F
B	3	1.7232	3.3352	128.7346				
E	8	5.6301	22.9206	107.2814	13.9698	7	1.9957	21.428 ***
B+E	11	7.3533	26.2558	236.0160	142.2667	10		
D					128.2969	3	42.7656	

Adjusted final means.

Control	39.126	±	.816
4-CPA	36.678	"	"
2,4-D	40.164	"	"
2,4,5-T	31.634	"	"

Experiment 14. Microsorium diversifolium treated with 9.148×10^{-6}M aqueous solutions of 4-CPA, 2,4-D and 2,4,5-T.

Var.	d.f.	x	xy	y	y'	d.f.'	M.S.	F
B	3	9.5890	46.5469	235.0597				
E	8	6.6237	15.4602	51.3260	15.2408	7	2.1772	5.21*
B+E	11	16.2127	62.0071	286.3857	49.2962	10		
D					34.0554	3	11.3518	

(Significant at 5% level)

Adjusted final means.

Control	48.404	±	.852
4-CPA	44.381	"	"
2,4-D	40.748	"	"
2,4,5-T	42.353	"	"

Experiment 15. Microsorium diversifolium treated with 9.148×10^{-7}M aqueous solutions of 4-CPA, 2,4-D and 2,4,5-T.

Var.	d.f.	x	xy	y	y'	d.f.'	M.S.	F
B	3	.4631	3.3205	38.8513				
E	8	1.4893	4.6373	32.5048	18.0654	7	2.5808	2.693=
B+E	11	1.9524	7.9578	71.3561	38.9208	10		
D					20.8554	3	6.9518	

Adjusted final means

Control	35.154	±	.928
4-CPA	37.214	"	"
2,4-D	34.336	"	"
2,4,5-T	33.517	"	"

Experiment 16. Microsorium diversifolium treated with 9.148×10^{-8} M aqueous solutions of 4-CPA, 2,4-D and 2,4,5-T.

Var.	d.f.	x	xy	y	y'	d.f.'	M.S.	F
B	3	1.6281	8.2094	56.7816				
E	8	2.6733	6.3358	33.9813	18.9653	7	2.7093	2.782=
B+E	11	4.3014	14.5452	90.7629	41.5783	10		
D					22.6130	3	7.5376	

Adjusted final means.

Control	44.828	±	.950
4-CPA	43.180	"	.950
2,4-D	45.783	"	"
2,4,5-T	41.880	"	"

TABLE V

SUMMARY OF THE EFFECTS OF THE THREE PLANT REGULATORS ON MICROSORIUM DIVERSIFOLIUM.

The Response of Microsorium diversifolium to 4-CPA, 2,4-D and 2,4,5-T.

Exp. No.	Conc. (Molar)	F	Sign. F. (99%)	Final Mean Control	Final Mean 4-CPA	Final Mean 2,4-D	Final Mean 2,4,5-T	Standard Error of mean	Signif. of Diff. (5%)	Calcul. detect. difference	Observed detect. difference
12.	9.148×10^{-4}	215.24	***	40.730				± .660			
					39.454			" .660	—		
						33.724		" .660	*	3.002	- 7.006
							17.190	" .660	*	2.797	-23.540
13.	9.148×10^{-5}	21.428	***	39.126				" .816			
					36.678			" .816	—		
						40.164		" .816	—		
							31.634	" .816	*	2.733	- 7.492
14.	9.148×10^{-6}	5.214	*	48.404				" .852			
					44.381			" .852	—		
						40.748		" .852	—		
							42.353	" .852	—		
15.	9.148×10^{-7}	2.693	=	35.154				" .928			
					37.214			" .928	—		
						34.336		" .928	—		
							33.517	" .928	—		
16.	9.148×10^{-8}	2.782	=	44.828				" .950			
					43.180			" .950	—		
						45.783		" .950	—		
							41.880	" .950	—		

(Exp. 14) Significant at 5% level.

Part 2. Discussion. Pteridium Aquilinum

(i) The only significant response with Pteridium is seen in experiment one where all three acids at a concentration of 9.148×10^{-4}M have an inhibitory effect on respiration. (Table III). Significance is reached only at the 95% level, meaning that the odds of getting these results other than by chance are 95 times in 100.

The order of variability is quite high for this particular experiment (Standard error expressed as a percentage of the control mean = 9.84). In the subsequent five experiments variability is reduced to around the two per cent level (as expressed by standard error as a per cent of control mean).

Any treatment effects obscured in experiment one by the high variability would presumably still have been indicated in experiment two but there is no indication of any inhibition, in fact 2,4-D shows a tendency (non-significant) towards stimulation of respiration.

In experiment four both 4-CPA and 2,4,-D show a tendency (non-significant) to stimulation, and experiment five indicates that all three acids are tending to stimulate rather than inhibit. In experiment six all three acids are obviously in such low concentrations as to have no effect at all on respiration. (Table III).

The conclusion can therefore be made that on rhizome apical meristem tissue in Pteridium aquilinum the three plant regulators studied have no significant effect on respiration at concentrations below 9.148×10^{-4}M. (170.64 p.p.m. of 4-CPA, 202.20 p.p.m. of 2,4-D and 233.64 p.p.m. of 2,4,5-T.)

A comparison can be made of these responses with respiratory studies undertaken on other types of tissue (Mono and di-cotyledons). Kelly and Avery (52) working with "Avena" coleoptile tissue have demonstrated

increases of 20% or more in oxygen uptake for 2,4-D concentrations ranging from less than 1 to 100 mg's./litre. With young pea stem tissue it was found that the concentration of 2,4-D required to give a 20% stimulation, was of the order of one over a thousand that needed for "Avena".

Brown (84) sprayed Bean and Morning-glory plants with 2,4-D (1000 mg/litre) and demonstrated up to 80% greater CO_2 output than in unsprayed plants. Smith et al (55) showed that rhizomes and roots of bindweed showed an average of 70% increase in oxygen uptake as a result of spraying intact plants with 2,4-D at 1000 mg/litre. Similarly Nickell (85) observed with "Rumex" virus tissue a 20% increase in respiration over control values at concentrations of 0.1 mg/litre.

Smith (49) demonstrated an 80% inhibition of respiration in bean stem slices treated with 100 p.p.m. of 2,4-D in an aerated aqueous medium. At concentrations between one and ten p.p.m. results ranged from slight inhibition to marked acceleration of respiration. Mitchell et al (56) in an extensive study of root and stem slices of a number of species (carrot, bean, corn, tobacco, tomato, tomato crown gall, and sunflower tissue culture) treated with 350 p.p.m. of 2,4-D and 410 p.p.m. IAA demonstrated inhibitions of respiration ranging from 50% to 88% control values.

On the basis of this previous work, one might expect that both a definite stimulation and inhibition of respiration would be observed in tissue of Pteridium aquilinum over the range of concentrations tested, at least with 2,4-D, if not also with the other two plant regulators. However, the results indicate that this is not so, but that concentrations above 200 p.p.m. of 2,4-D, 170.64 p.p.m. of 4-CPA and 233.64 p.p.m. of 2,4,5-T inhibit respiration by approximately 30%. Concentrations below this have no apparent effect on respiration.

Explanation of the lack of marked effects on respiration may lie in the following hypotheses.

I. "Pteridium aquilinum does not possess similar respiratory enzymes to the mono and dicotyledenous tissue mentioned above so that the key reactions which are accelerated or retarded by plant regulators cannot take place." Inhibition of respiration above 200 p.p.m. could be explained simply by a high concentration effect of a chemically toxic substance.

The fact that epinastic effects and gross malformations of fronds have been observed in spraying trials would indicate that the fronds at least, possess enzymes common to mono and dicotyledenous stems. (Accelerated growth is invariably associated with an increase in respiratory activity (51)).

The hypothesis can therefore be restricted to the statement "that the rhizome of Pteridium aquilinum does not appear to have an exactly similar respiratory enzyme system to that of mono and dicotyledenous plants."

II. Mitchell (56) has shown that bean and corn roots are inhibited by 2,4-D only at high concentrations (10^{-2} - 10^{-3}M) and that at concentrations as low as 10^{-6}M respiration is only slightly stimulated. At similar concentrations on the same material Indoleacetic Acid (IAA)(10^{-2} - 10^{-3}M) shows a stimulatory effect but at lower concentrations (10^{-3} - 10^{-6}M) no effect at all. Audus and Shipton (86) studying auxin antagonism in cress (Lepidium sativum) and pea (Pisum sativum) root growth, using IAA, 2,4-D, phenoxyacetic acid (POXA) and 2,4-dichloranisole (DCA) came to the conclusion that IAA is not the natural root hormone holding growth below a maximum. They postulated the presence of a simple inhibitor which normally maintains extension growth below its maximum level.

On this basis they classify active plant regulators (acting on roots!) as -

(a) Those that augment growth in low concentrations and inhibit progressively in high e.g. IAA.

(b) Those that only inhibit growth in all concentrations - for example most of the "synthetic auxins" including 2,4-D and phenoxyacetic acid.

Quoting Audus and Shipton:

"In high concentrations of both types of compound, root growth inhibition sets in, and the actual site of this inhibiting action in the growth system may be quite different from that at which the naturally occurring inhibitor works. It is this high concentration inhibition by externally applied auxins that runs parallel with auxin action in the extension growth of coleoptiles and shoots, highly active auxins being very effective inhibitors e.g. IAA and 2,4-D, - weak auxins having small inhibitory powers, e.g. phenoxyacetic acid."

If growth and respiration are linked, as most experimental evidence indicates, the hypothesis of Andus and Shipton will apply to the inhibition of respiration in the rhizome of Pteridium aquilinum if the rhizome is considered to have the same response to plant regulators as a normal mono or dicotyledenous root.

The resultant hypothesis is therefore "that the rhizome of Pteridium aquilinum, although anatomically classified as an underground stem, behaves in its response to plant regulators as a normal root."

Part 2.

(ii) General Discussion

The results of experiments seven to sixteen (Tables IV and V) indicate a similar pattern of response to the plant regulators as that seen earlier with Pteridium aquilinum.

Considering firstly Paesia scaberula (Table IV) it is apparent that 2,4,5-T is the only regulator showing any significant effect (9.148×10^{-4} and 9.148×10^{-5}M). The inhibition of respiration induced by 2,4,5-T is more pronounced at lower concentrations with Paesia than it is with Pteridium. At concentrations below 9.148×10^{-5}M there appears to be no further effect on respiration by all three plant regulators.

Microsorium diversifolium (Table V) has a similar response, with 2,4,5-T again being most effective at concentrations of 9.148×10^{-4} and 9.148×10^{-5}, also 2,4-D showing an inhibition only at 9.148×10^{-4}M.

In all the experiments seven to sixteen there is no significant stimulation of respiration. Neither is there any non-significant tendency towards a stimulation at concentrations below 9.148×10^{-5}M.

In arriving at an explanation of this pattern of response it is not reasonable to apply an hypothesis of "a root-like response" to plant regulators as with Pteridium aquilinum because the rhizomes of both Paesia and Microsorium have an above ground habit and both contain considerable amounts of chlorophyll.

All three rhizomes do however show some common traits which help to throw some light on the problem; they are all plagiotropic, normally growing at an angle of 90° to the force of gravity. With Paesia and Microsorium both of which contain chlorophyll and are often exposed to full sunlight, a simple theory of auxin control of growth as the Cholodny-Went hypothesis (where the rate of growth in plant

parts exhibiting a geotropic response is due to an accumulation of an essential metabolite which has been displaced to the lower side of the organ under the influence of gravity) is not sufficient to explain their mode of growth, always horizontal. e.g. What makes a rhizome grow horizontally before the auxin becomes displaced downwards under the influence of gravity?

Bennet-Clark and Ball (13) in a study of the plagiotropic behaviour of rhizomes of Aegopodium podograria found that by inverting rhizomes through 180° so that the lower surface becomes the upper, the rhizomes go through a rapid series of upward and downward bending motions until finally they adopt a horizontal position similar to the original one. They say "the fact that recovery from the stimulus brings about a condition apparently exactly similar to that existing before the stimulus but with a different anatomical region as the physical lower side seems to make necessary at least a two hormone or two particle hypothesis."

They postulate either

(a) a growth hormone together with an anti-hormone neutralising it on the lower side of the rhizome.

or (b) a second hormone capable of evoking growth acceleration but located on the opposite side of the rhizome acting as a neutralising agent.

Their work was carried a step further by a chromatographic assay of growth substances carried out by Bennet-Clark and Kefford (87) and Kefford (88). They demonstrated on chromatograms the presence of two areas of growth promotion and one of growth inhibition. One of the areas of growth promotion has been demonstrated as solely due to IAA. They have tentatively called the other two growth regulators "accelerator α" and "inhibitor β". All three growth regulators were isolated

in etiolated broad bean and pea shoots and roots, etiolated sunflower shoots, maize roots and potato etiolated shoots and tubers. The most significant result arising from this work was the demonstration in etiolated broad bean shoots, of IAA as the predominating growth substance in the stem and the substance β predominating in the first lateral bud. The latter is suggested as an explanation of apical dominance and the predominance of β in potato tuber skin is suggested as an explanation of dormancy in tubers.

The hypothesis of Audus and Shipton (86), earlier mentioned in connection with Pteridium, that root growth is maintained below a maximum level by an inhibitor would seem most likely on the basis of the evidence just presented. Similarly the "diageotropic response" contemplated by Bennet-Clark and Ball (13) in rhizomes would seem to be substantiated by the definite proof of the presence of an inhibitor, since further substantiated by Ball (14).

On the basis of the probable presence of a plant growth inhibitor in all three species of rhizome examined in this study, the following possible mechanism of response to applied plant regulators is presented.

It is postulated that the three rhizomes examined contain a natural growth inhibitor (possibly the β isolated by Kefford (13)) which acts as a balancing co-factor with IAA in controlling the plagiotropic pattern of growth. This inhibitor has an anti-auxin activity (12) which inhibits competitively the action of auxins applied in low concentrations. The three plant regulators used in this study, which are know to have strong auxin activity, particularly 2,4-D and 2,4,5-T, inhibit growth and respiration at concentrations above that necessary to counteract the competitive anti-auxin effect of the postulated inhibitor. The site of inhibition may not be the same with the natural inhibitor and the applied regulator.

Low concentrations of applied plant regulator are ineffective due to the presence of competive anti-auxin activity of the postulated inhibitor.

CONCLUSION.

The general conclusion arising from the study is that Pteridium aquilinum has a high resistance to applied plant regulators because of the nature of its rhizome. Firstly that the rhizome is situated at a considerable distance from the usual site of application (fronds) such that concentrations of plant regulator penetrating to the rhizome tissue are necessarily low. Secondly that the rhizome, in company with the rhizomes of Paesia scaberula and Microsorium diversifolium would appear to have a natural plant regulatory system that opposes by an antiauxin action the low concentrations of applied plant regulator that arrive at the site of action.

It is suggested that further study to reveal which concentrations of applied plant regulator will actually bring about death (whether physiological or phytotoxic) could be along the lines of a correlated growth and respiration study. Such a study in addition to helping solve the problem of how an applied plant regulator exerts its influence would also help to elucidate the mechanism of diageotropic response of rhizomes, and therefore indirectly the natural plant regulator composition of rhizomes and any similarities they may have to roots.

It is further suggested that where possible a biometrical evaluation of such studies would prove to be of great assistance in clarifying numerical results.

SUMMARY.

1. The pattern of response of respiration in meristematic tissue of rhizomes of three species of Fern; Pteridium aquilinum, Microsorium diversifolium and Paesia scaberula, to the application of three plant regulators, 4-CPA, 2,4-D and 2,4,5-T has been studied using a Warburg respirometer technique.

2. All three species exhibited some significant degree of inhibition of respiration at concentrations of 9.148×10^{-5} Molar and higher.

3. 4-CPA inhibited respiration at 9.148×10^{-4}M with Pteridium aquilinum only.

4. 2,4-D caused a significant inhibition of respiration at 9.148×10^{-4}M with Pteridium aquilinum and Microsorium diversifolium.

5. 2,4,5-T caused inhibition of respiration at 9.148×10^{-5}M with Paesia scaberula and Microsorium diversifolium, in addition to a significant inhibition with all three species at 9.148×10^{-4}M.

6. No significant stimulations of respiration were recorded for the range of concentrations used, (9.148×10^{-4}M down to 9.148×10^{-8}M).

7. The theoretical significance of these findings is discussed.

BIBLIOGRAPHY

(1) A.G. NORMAN, C.E. MINARIK & R.L. WEINTRAUB. - Herbicides. Ann. Rev. Plant Physiol (1950) 1, 141-168.

(2) H.B. DOBBIE. - New Zealand Ferns (4th Edit.) (1951) 175-177.

(3) K.W. BRAID. - Bracken Control, Artificial and Natural. J. Brit. Grassl. Soc. (1947) 2, 181-9.

(4) K.W. BRAID.- An Investigation into the claims of the Smear Method for the application of Sodium Chlorate on Bracken. Agr. Res. Council Report. 324/52 (1950).

(5) G.H. BATES. - A method for the application of Sodium Chlorate to Bracken. British Rubber Publicity Assoc. Bull. 14 (1950) 1-5.

(6) E. CONWAY & R. STEPHENS. - How the Bracken Plant reacts to Treatment. N.A.A.S. Quart. Rev. (1954) 25 (Autumn) 1-15.

(7) F.E. EGLER. - Herbicide Effects in Connecticut Vegetation. Bot. Gaz. (1951) 112. 76-85.

(8) K. HOLLY, E.K. WOODFORD & G.E. BLACKMAN. - The Control of Some Perennial Weeds in Permanent Grassland by Selective Herbicides. Agriculture (1952) 59, 19-23.

(9) G.E. FOGG. - Quantitative studies on the Wetting of Leaves by Water. Proc. Roy. Soc. (London) (B) (1947) 134, 503-22.

(10) J.W. MITCHELL & J.W. BROWN. - Movement of 2,4-Dichlorophenoxyacetic acid Stimulus and its Relation to the Translocation of Organic Food Materials in Plants. Bot. Gaz. (1946) 107, 393-407.

(11) R.J. WEAVER & H.R. De ROSE. - Absorption and Translocation of 2,4-Dichlorophenoxyacetic Acid. Bot. Gaz. (1946) 107, 509-521.

(12) Plant Growth Regulators Conference. - Nomenclature. Pl. Physiol (1954) 29 (3) 307.

(13) T.A. BENNET-CLARK & N.G. BALL. - The Diageotropic Behaviour of Rhizomes. J. Exp. Bot. (1951) 2, 170-203.

(14) N.G. BALL. - The Effects of Certain Growth - regulating Substances on the Rhizomes of Aegepodium podograria.
J. Exp. Bot. (1953) 4, 349-62.

(15) CHARLES DARWIN. - The Power of Movement in Plants. London (1880).

(16) P. BOYSEN-JENSEN. - The Transmission of Phototropic stimulus in Avena (La transmission de l'irritation phototropique dans l'Avena.)
Bull. Acad. Roy. Danmark (1911) 1, 3-24.

(17) F.W. WENT. - On growth-accelerating substances in the Coleoptile of Avena sativa.
Proc. Kon Akad. Wetensch. Amsterdam (1926) 30, 10-19.

(18) H.E. DOLK & K.V. THIMANN. - Studies on the Growth Hormone of Plants.
Proc. Nat. Acad. Sc. (Wash.) (1932) 18, 30-46.

(19) F. KÖGL & A.J. HAAGEN-SMIT. - Über die chemie des Wuchstoffs. (On the Chemistry of Growth Substances)
Proc. Kon. Akad. Wetensch Amsterdam (1931) 34, 1411-1416.

(20) F. KÖGL, A.J. HAAGEN-SMIT & H. ERXLEBEN. - Über ein neues Auxin (Heteroauxin) aus Harn. (1934) XI Mitteilung. Z. Physiol Chem. 228, 90-103.

(21) A.J. HAAGEN-SMIT, W.D. LEACH & W.R. BERGREN. - The Estimation, Isolation and Identification of Auxins in Plant Materials.
Am. J. Bot. (1942) 29, 500-506.

(22) A.J. HAAGEN-SMIT, W.B. DANDLIKER, S.H. WITTWER & A.E. MURNEEK. - Isolation of 3-Indoleacetic acid from Immature Corn Kernels.
Am. J. Bot. (1946) 33, 118-120.

(23) P.W. ZIMMERMAN, A.E. HITCHCOCK & F. WILCOXON. - Several Esters as Plant Hormones.
Contr. Boyce. Thompson Instit. (1936) 8, 105-112.

(24) A.J. HAAGEN-SMITH & F.W. WENT. - A Physiological Analysis of the Growth Substance.
Proc. Kon. Akad. Wetensch (Amsterdam) (1935) 38, 852-857

(25) V.C. IRVINE. - (Abstract of Thesis)
Univ. Colo. Studies (1938) 26, 69-70.

(26) P.W. ZIMMERMAN & A.E. HITCHCOCK. - Substituted Phenoxyacetics and Benzoic Acids. Relation of Structure to Physiological Activity.
Contr. Boyce Thompson Instit. (1942) 12, 321.

(27) R.E. SLADE, W.G. TEMPLEMAN & W.A. SEXTON. - Plant Growth Substances as Selective Weedkillers.
Nature (London) (1945) 155, 497-8.

(28) H.E. THOMPSON, C.P. SWANSON & A.G. NORMAN. - New Growth Regulating Compounds. I. Summary of Growth Inhibiting Activities of Some Organic Compounds as Determined by Three Tests. Bot. Gaz. (1946) 107, 476-507.

(29) C.P. SWANSON. - A simple bio-assay method for the Determination of Low Concentrations of 2,4-D. acid in Aqueous Solutions. Bot. Gaz. (1946) 107, 507-509.

(3) L.J. AUDUS. - Biological Detoxication of 2,4-D. acid in soils: Isolation of an Effective organism. Nature (London) (1951) 166, 356.

(31) G.E. BLACKMAN & E.C. ROBERTSON-CUNNINGHAME. - The Influence of pH on the Phytotoxicity of 2,4-D acid to Lemna minor. New Phytol. (1953) 52, 71-75.

(32) L.J. AUDUS. - A Critical Approach to Auxin Antagonists and Synergists. New Phytol (1954) 53, 461.

(33) G.E. BLACKMAN. - Studies in the Principles of Phytotoxicity. I. The Assessment of Relative Toxicity. J. Exp. Bot. (1952) 3, 1-27.

(34) M.R. SAMPFORD. - Studies in the Principles of Phyto-toxicity. II. Experimental designs and Techniques of Statistical Analysis for the Assessment of Toxicity. J. Exp. Bot. (1952) 3, 28-46.

(35) B. COMMONER & K.V. THIMANN. - The Relation between Growth and Respiration in the Avena Coleoptile. J. Gen. Physiol. (1940) 24, 279.

(36) D.J. WORT. - The Effects of 2,4-D on the Activity of Plant Enzymes. Dept. Bot. Univ. Brit. Columbia Vancouver B.C. (Thesis) 1951.

(37) F.R. WEST & J.H.M. HENDERSON. - The effect of 2,4-D acid and other Substances on Seedling roots of Blue Lupin Science (1950) 111, 579-81.

(38) A.S. CRAFTS. - Selectivity of Herbicides. Pl. Physiol. (1946) 21,345-61.

(39) A.W. GALSTON, J. BONNER & R. BAKER. - Flavoprotein and Peroxidase as constituents of Indoleacetic acid oxidase of peas. Am. J. Bot. (1950) 37, 677-8.

(40) A.W. GALSTON & M.E. HAND. - Studies on the Physiology of Light Action. I. Auxin and the Light Inhibition of Growth. Am. J. Bot. (1949) 36, 85-94.

(41) P.L. GOLDACRE. - The Mechanism of Action of 2,4-D. Aust. J. Sc. Res. (1949) 2 (B) 154-56.

(42) E. HOFMAN & B.V. SCHMELING. - Effect of 2,4-D Acid on Metabolism and Enzyme Content of Plants.
Naturwissenschaften (1953) 40, 23-4

(43) A. RHODES & R. de B. ASHWORTH. - Mode of Action of Growth Regulators in Plants.
Nature (London) (1952) 169, 76-77.

(44) J.M.F. LEAPER & J.R. BISHOP. - Relation of Halogen Position to Physiological Properties in the Mono, - Di - and Trichlorophenoxyacetic acids.
Bot. Gaz. (1951) 112, 250-58.

(45) H. VELDSTRA. - Researches on Plant Growth Substances V. Relation Between Chemical Structure & Physiological Activity II. Contemplations on Place and Mechanism of the Action of the Growth Substances.
Enzymologia (1944) 11 (2) 137-163.

(46) R.M. MUIR & C. HANSCH. - On the Mechanism of Action of Growth Regulators.
Pl. Physiol (1953) 28, 218-32.

(47) R.L. WAIN & M.S. SMITH. - The Plant Growth Regulating Activity of dextro and laevo α- (2-napthoxy) propionic acid.
Proc. Royal Soc. London (B) (1951) 139, 118-127.

(48) A.G. NORMAN & R.L. WEINTRAUB. - Structure activity relations in Plant Growth Regulators.
1st Symposium - Chem. Biolog. Correl. (1950) 46.
Nat'l. Res. Council. Nat. Acad. Sc. (U.S.)

(49) F.G. SMITH. - The effect of 2,4-Dichlorophenoxyacetic acid on the Respiratory Metabolism of Bean Stem-Tissue.
Pl. Physiol. (1948) 23, 70-83.

(50) F.G. SMITH. - Respiratory changes in Relation to Toxicity. Plant Growth Substances. Univ. Wisconsin Press. (1951) 111-119.

(51) G.S. AVERY (Jun). - Stimulation of Respiration in Relation to Growth.
Plant Growth Substances - Symposium Wiscon. Press (1951) 105-109.

(52) SALLY KELLY & G.S. AVERY. - The Effect of 2,4-Dichlorophenoxyacetic acid and other Physiologically Active Substances on Respiration.
Am. J. Bot. (1949) 36, 421.

(53) R.C. FRENCH & HARRY BEEVERS. - Respiratory and Growth Responses Induced by Growth Regulators and Allied Compounds.
Am. J. Bot. (1953) 40, 660-66.

(54) J.F. NANCE. - Inhibition of Salt Accumulation in Excised Wheat Roots by 2,4-D acid.
Science (1949) 109, 174-176.

(55) F.G. SMITH, C.L. HAMNER & R.F. CARLSON. - Changes in Food Reserves and Respiratory Capacity of Bindweed Tissues accompanying Herbicidal Activity of 2,4-D acid.
Pl. Physiol (1947) 22, 58-65.

(56) J.E. MITCHELL, R.H. BURRIS & A.J. RIKER. - Inhibition of Respiration in Plant Tissues by Callus Stimulating Substances and Chemicals.
Am. J. Bot. (1949) 36, 368-78.

(57) L.J. AUDUS. - Studies on the Phytostatic action of 2,4-D acid and Coumarin. - The Reversibility of Root-Growth Inhibitions.
New Phytol (1948) 47, 196-219.

(58) L.J. AUDUS & H. GARRARD. - Studies on the Growth and Respiration of Roots I. The effects of Stimulatory and Inhibitory Concentrations of beta-IAA on root sections of Pisum sativum.
J. Exp. Bot. (1953) 4, 330-48.

(59) W.W. UMBREIT, R.H. BURRIS & J.F. STAUFFER. - Manometric techniques and Related methods for the Study of Tissue Metabolism. 1.

(60) Use of Sulphuric acid - dichromate mixture in cleaning glassware.
(Anon) Science (1946) 104 (2705) 426-27.

(61) A.J. VOGEL. - A textbook of Quantitative Inorganic Analysis.
(1947) 809.

(62) L.J. AUDUS. - Studies on the pH - Relationships of Root Growth and its Inhibition by 2,4-D acid and Coumarin.
New Phytol (1949) 48, 97-114.

(63) E.W. SIMON & H. BEEVERS. - The Effect of pH on the Biological Activities of Weak Acids and Bases. I. The most usual Relationship between pH and activity.
New Phytol (1952) 51, 163-190.

(64) E.W. SIMON & H. BEEVERS. - Quantitative Relationship between pH and Activity of Weak Bases and Acids in Biological Experiments.
Science (1951) 114, 124.

(65) E.W. SIMON & G.E. BLACKMAN. - Significance of Hydrogen ion concentration in study of Toxicity.
S.E.B. Symposia III. Selective Toxicity and Anti-Biotics.

(66) J. Van OVERBEEK, RENÉ BLONDEAU & VESTA HORNE. - Difference in activity between 2,4-D acid and other Auxins, and its significance in Herbicidal action.
Pl. Physiol (1951) 26, 687-96.

(67) R. POKORNY. - Some Chlorophenoxyacetic acids.
Am. Chem. Soc. Journal (1941) 63, 1768.

(68) M.E. SYNERHOLM & P.W. ZIMMERMAN. - The Preparation of Some Substituted Phenoxy Alkyl Carboxylic Acids and Their Properties as Growth Substances.
Contr. Boyce, Thompson Instit. (1945) 14, 91-103

(69) J.L. FULTS & M.G. PAYNE. - A Biometrical Evaluation of the Growth Regulating and Herbicidal Properties of Some Organic Compounds.
J. Am. Soc. Agron. (1947) 39, 667-81.

(70) G.E. BRIGGS & R.N. ROBERTSON. - Diffusion and Absorption in Disks of Plant Tissue.
New Phytol (1948) 47, 265-83.

(71) E.S.G. BARRON, G.K.K. LINK, R.M. KLEIN & B.E. MICHEL. - The Metabolism of Potato Slices.
Arch. Biochem. (1950) 28, 377-98.

(72) SALLY KELLY. - The Relationship between Respiration of Water Uptake in the Oat Coleoptile.
Am. J. Bot. (1947) 34, 521-26.

(73) B.E. MICHEL. - Effects of Indoleacetic Acid upon Growth and Respiration of Kidney Bean.
Bot. Gaz. (1951) 112, 418-36.

(74) G.S. CHRISTIANSEN & K.V. THIMANN. - The Metabolism of Stem Tissue during Growth and its Inhibition.
Arch. Biochem. (1950) 26, 248-59.

(75) A.L. SCHADE, L. BERGMANN & A. BYER. - Respiration of White Potato. I. Preliminary investigation of the Endogenous Respiration of Potato Slices and Catecholoxidase.
Arch. Biochem (1948) 18, 85-96.

(76) W. STILES & K.W. DENT. - Researches on Plant Respiration. VI. The Respiration in Air and in Nitrogen of thin slices of Storage Tissue.
Ann. Bot. (N.S.) (1947) 11, 1-34.

(77) OTTO SCHALES. - Quick method for Calibration of Warburg Manometers with Mercury.
Arch. Biochem. (1944) 3, 475-6.

(78) G.K.K. LINK & D.R. GODDARD. - Studies on the Metabolism of Plant Neoplasms I.
Bot. Gaz. (1951) 113, 185-90.

(79) E.H. NEWCOMB. - Tobacco Callus Tissue Respiration.
Am. J. Bot. (1950) 37, 264-271.

(80) H.G. SHIRK. - Freezable water content and the oxygen Respiration in wheat and ryegrain at Different Stages of Ripening.
Am. J. Bot. (1942) 29, 105-9.

(81) RUTH STEVENS. - Bracken Control.
Brit. Weed Control Conf. (1953) 249.

(82) P.W. ZIMMERMAN & A.E. HITCHCOCK. - Comparative Effectiveness of Acids, Esters and Salts as Growth Substances and Methods of Evaluating them.
Contr. Boyce. Thompson Instit. (1937) 8, 337-350.

(83) G.W. SNEDECOR. - Statistical Methods. (1938) 98-101, 104-159.

(84) J.W. BROWN. - Effect of 2,4-D acid on the Water Relations, the Accumulation and Distribution of Solid Matter, and the Respiration of Bean Plants.
Bot. Gaz. (1946) 107, 332-343.

(85) L.G. NICKELL. - The Effect of Certain Plant Hormones and Colchicine on the Growth and Respiration of Virus Tumour Tissue from Rumex acetosa.
Am. J. Bot. (1950) 37, 829-55.

(86) L.J. AUDUS & M.E. SHIPTON. - 2,4-Dichloranisole. Auxin interactions in Root Growth.
Physiologia Planta. (1952) 5, (3) 430.

(87) T.A. BENNET-CLARK & N.P. KEFFORD. - Chromatography of the Growth Substances in Plant Extracts.
Nature (London) (1953) 171, 645-7.

(88) N.P. KEFFORD. - The Growth Substances Separated from Plant Extracts by Chromatography.
J. Exp. Bot. (1955) 6, 129-51.

(89) DUANE Le TOURNEAU & NORMAN KROG. - The use of Chromatropic Acid for the Quantitative Determination of 2,4-D acid.
Pl. Physiol. (1952) 27, 822-7.

(90) V.H. FREED. - Qualitative Reaction for 2,4-D Acid.
Science (1948) 107, 98.

APPENDIX A.

Tabulated below are oxygen uptakes in micro-litres per milligram dry weight corrected by each flask constant, for each flask in the seventeen major experiments.

Exp. No.	Flask Numbers 1	2	3	4	5	6	7	8	9	10	11	12	Time in Minutes
1.	2.29	2.01	2.25	-	2.53	2.78	1.82	2.99	2.26	2.04	3.27	2.64	30
	7.90	7.41	7.48	-	7.92	8.50	6.00	9.63	7.18	6.46	9.71	7.97	90
	13.15	12.65	12.98	-	13.70	14.38	10.41	18.17	13.75	12.44	17.57	14.41	150
	19.43	18.63	19.69	-	17.75	18.63	14.10	22.40	17.48	15.25	21.18	18.48	210
	26.58	25.58	26.10	-	21.84	22.83	18.42	26.70	21.74	18.56	24.23	21.06	270
	41.51	39.61	41.08	-	32.39	33.28	28.11	37.10	33.18	28.28	33.56	34.76	390
	48.70	46.40	48.45	-	37.78	39.11	33.94	43.34	40.03	34.68	39.48	42.68	450
	55.37	53.18	55.38	-	43.16	44.46	39.99	48.62	46.48	39.78	44.97	50.87	510
2.	1.79	2.12	1.91	2.15	1.91	1.97	2.25	1.63	1.81	1.71	1.98	2.08	30
	6.64	7.28	6.98	7.49	6.66	6.73	7.48	5.94	6.77	6.42	6.86	7.35	90
	11.31	12.68	12.27	13.42	11.71	11.61	11.05	11.00	12.59	11.95	12.43	12.58	150
	16.80	18.80	17.97	19.35	17.11	16.87	18.15	18.25	18.31	18.08	18.18	18.73	210
	23.26	25.72	24.91	26.60	23.58	23.23	24.75	24.66	25.28	24.75	24.08	25.48	270
	37.94	40.48	39.60	41.99	37.82	36.41	37.34	39.57	41.64	40.04	39.46	40.42	390
	44.93	47.68	46.30	49.19	43.93	43.14	44.22	47.10	48.94	46.99	46.64	46.94	450
	51.80	55.12	53.62	57.03	51.30	50.24	50.71	53.83	56.66	54.34	54.06	53.60	510

APPENDIX A (continued)

Exp. No.	Flask Numbers 1	2	3	4	5	6	7	8	9	10	11	12	Time in Minutes
3.	1.91	1.99	2.47	2.18	2.29	2.2.	-	-	-	-	-	-	30
	7.01	6.68	7.93	7.43	7.61	7.04	-	-	-	-	-	-	90
	12.36	12.24	14.30	13.42	13.28	12.82	-	-	-	-	-	-	150
	17.98	17.87	20.56	19.23	19.36	17.96	-	-	-	-	-	-	210
	24.30	23.50	27.37	26.07	26.06	24.59	-	-	-	-	-	-	270
	37.40	36.04	41.60	39.97	40.32	37.73	-	-	-	-	-	-	390
	43.59	42.21	48.49	46.63	47.31	43.99	-	-	-	-	-	-	450
	50.16	47.80	55.82	53.65	53.69	50.93	-	-	-	-	-	-	510
4.	1.49	1.66	-	1.67	2.09	-	1.96	1.45	1.45	1.54	1.52	1.55	30
	4.60	5.42	-	5.55	7.00	-	5.31	5.22	5.38	5.58	5.15	5.75	90
	8.14	9.71	-	9.70	12.12	-	8.48	9.62	9.58	9.89	9.04	9.58	150
	11.59	13.56	-	13.24	18.98	-	12.50	12.41	13.41	14.91	12.72	13.27	210
	15.82	18.20	-	17.68	25.70	-	16.87	16.47	18.49	19.27	17.41	17.31	270
	26.20	29.66	-	29.99	33.74	-	27.54	28.36	28.80	29.29	28.35	31.68	390
	32.67	36.66	-	37.78	41.15	-	33.93	34.68	35.06	34.88	34.66	37.86	450
	38.17	42.08	-	42.87	46.93	-	40.09	40.94	40.66	39.44	39.66	42.44	510

APPENDIX A (continued)

Exp. No.	FLASK NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	Time in Minutes
5.	2.68	2.54	2.26	2.62	3.05	-	2.80	2.94	3.14	2.86	2.73	2.82	30
	8.46	8.18	7.07	8.15	9.15	-	8.68	9.04	10.31	10.07	9.03	7.39	90
	14.68	14.10	12.09	13.89	15.17	-	14.14	14.62	16.79	16.34	14.49	11.91	150
	21.04	20.12	17.71	20.58	22.02	-	21.22	22.13	25.12	25.28	22.08	18.19	210
	28.05	26.84	23.31	27.65	29.12	-	27.49	29.14	32.70	32.96	28.77	23.59	270
	42.02	40.86	35.08	42.35	44.49	-	41.54	44.07	48.30	49.70	43.69	36.18	390
	49.60	48.82	41.60	50.22	53.45	-	48.99	52.09	56.17	58.76	51.37	42.84	450
	56.40	56.72	47.99	57.57	61.93	-	55.95	59.47	63.57	66.91	58.51	48.65	510
6.	2.56	2.80	2.81	2.90	2.88	2.31	1.62	2.78	2.94	2.80	2.97	2.77	30
	8.17	9.02	9.10	9.38	8.91	7.62	6.91	8.80	9.18	8.81	9.19	8.76	90
	14.02	15.77	15.75	16.26	15.42	13.16	12.13	15.15	15.88	15.05	15.64	14.93	150
	20.56	23.58	23.51	24.00	22.75	19.29	18.09	22.23	23.39	21.99	22.82	21.57	210
	27.92	31.83	32.06	32.52	30.85	26.70	24.93	30.01	31.98	29.52	30.83	29.14	270
	42.70	48.19	48.74	49.51	46.73	41.77	38.59	46.19	49.57	44.96	47.44	44.31	390
	49.83	56.15	57.05	57.88	54.24	49.06	44.99	53.76	57.51	52.14	55.08	51.16	450
	56.30	64.11	65.83	66.16	61.30	56.60	51.06	61.22	65.16	58.85	62.59	57.81	510

APPENDIX A (continued)

Exp. No.	FLASK NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	Time in Minutes
7.	1.93	2.06	2.15	2.43	2.16	2.16	2.24	1.91	2.01	1.88	2.65	1.93	30
	6.12	6.55	6.64	7.19	6.60	6.53	6.77	6.03	6.22	5.61	8.22	5.96	90
	10.11	10.71	10.80	11.74	10.76	10.82	11.31	10.29	10.49	9.56	13.79	10.12	150
	13.71	14.64	14.65	17.66	14.49	14.53	14.86	13.75	14.02	11.98	17.16	12.39	210
	17.49	18.62	18.73	22.32	18.29	18.39	18.72	17.50	17.90	13.38	19.31	14.61	270
	24.16	25.59	25.60	29.35	24.15	24.32	24.30	23.40	24.13	14.14	20.54	15.66	390
	26.89	28.40	28.39	32.05	26.42	26.63	26.28	25.74	26.38	14.23	20.69	15.20	450
	30.20	31.73	31.92	35.07	29.08	29.35	28.63	28.40	29.96	14.54	21.25	15.62	510
8.	2.18	2.33	2.42	2.56	2.52	2.41	2.38	2.25	2.36	2.47	2.54	2.85	30
	6.20	6.67	6.79	7.29	6.79	6.71	6.70	6.51	6.81	7.15	7.01	6.90	90
	10.26	11.13	11.25	12.07	11.47	11.10	11.17	11.07	11.25	11.94	11.65	11.38	150
	14.28	15.64	15.80	16.41	15.85	15.45	15.39	15.38	15.54	15.87	15.47	15.21	210
	18.54	20.35	20.43	21.44	20.61	20.14	20.00	19.94	20.28	19.36	18.61	18.61	270
	28.24	31.57	31.51	33.17	31.98	31.00	30.53	30.03	30.89	27.22	26.07	26.48	390
	33.66	38.32	37.86	39.73	38.69	37.23	36.44	35.42	36.71	32.65	30.77	31.88	450
	38.64	44.67	44.26	46.53	45.83	43.71	42.20	40.57	42.04	40.29	38.39	39.49	510

APPENDIX A (continued)

Exp. No.	FLASK NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	Time in Minutes
9.	2.19	2.32	2.36	2.22	2.27	2.33	2.41	2.27	2.23	2.21	2.22	2.19	30
	6.61	7.10	7.05	6.73	6.83	7.24	7.38	7.04	6.96	6.71	6.84	6.65	90
	10.73	11.52	11.35	10.88	10.84	11.64	11.73	11.38	11.11	10.75	10.86	10.53	150
	14.82	15.98	15.64	14.98	14.90	16.09	15.95	15.50	15.07	14.72	14.84	14.41	210
	19.09	20.56	20.08	19.16	19.08	20.77	20.52	19.02	19.44	19.18	19.26	18.72	270
	29.05	31.19	30.42	28.80	28.56	30.99	30.52	30.10	29.37	28.89	29.05	28.37	390
	34.62	37.01	36.30	34.07	34.03	36.58	35.69	35.51	34.70	34.31	34.34	33.95	450
	40.60	43.26	42.88	40.02	40.44	42.73	41.68	41.96	40.72	40.65	40.59	40.33	510
10.	2.07	2.18	2.12	2.12	2.27	2.15	2.07	2.11	2.26	2.25	2.16	2.22	30
	6.25	6.55	6.23	6.30	6.78	6.34	6.51	6.39	6.65	6.65	6.36	6.51	90
	10.32	10.77	10.18	10.40	11.08	10.49	10.74	10.66	10.97	11.05	10.65	10.76	150
	14.24	14.87	14.03	14.19	15.08	14.41	14.44	14.62	14.91	15.04	14.49	14.39	210
	18.46	19.21	18.10	18.44	19.47	18.60	18.80	19.05	19.44	19.48	19.65	18.85	270
	27.22	28.53	26.75	27.01	29.20	27.22	27.59	28.24	28.52	28.75	28.54	27.80	390
	32.49	34.20	31.92	32.17	34.52	33.52	33.74	33.91	34.23	34.45	33.74	33.90	450
	37.40	40.14	37.19	37.20	40.25	38.71	38.38	38.95	39.08	39.43	38.50	38.85	510

APPENDIX A (continued)

Exp. No.	1	2	3	4	5	6	7	8	9	10	11	12	Time in Minutes
11.	2.35	2.26	2.67	2.58	2.25	1.79	2.39	1.30	2.21	2.11	2.25	2.42	30
	6.75	6.54	7.24	7.24	6.37	5.43	6.80	5.70	6.39	6.37	6.55	6.94	90
	10.62	10.44	11.38	11.22	10.09	8.85	10.62	9.62	10.10	10.10	10.38	10.88	150
	15.28	15.00	16.34	16.14	14.17	13.30	15.22	14.45	14.57	14.68	14.84	15.59	210
	19.42	19.11	20.72	20.59	17.96	17.17	19.33	18.71	18.80	18.76	18.93	19.73	270
	29.87	29.29	31.33	31.15	27.15	26.52	29.46	28.67	28.52	28.15	29.18	30.00	390
	35.07	34.51	36.87	36.43	31.60	31.10	34.60	33.65	33.47	32.73	34.08	34.94	450
	40.84	40.35	43.10	42.27	36.74	36.36	40.28	39.06	38.86	37.35	39.48	40.41	510
11.a.	1.71	1.87	1.80	1.92	2.03	1.70	1.84	1.48	2.00	1.79	1.79	1.71	30
	5.45	5.63	5.67	5.65	6.17	5.43	5.71	4.91	5.87	5.56	5.72	5.43	90
	8.88	9.25	9.48	9.27	10.18	9.10	9.21	8.23	9.65	9.09	9.38	8.97	150
	12.49	12.63	13.09	12.38	13.81	12.52	12.45	11.37	13.13	12.24	12.96	12.46	210
	15.74	15.85	16.43	15.89	17.75	16.31	16.14	14.69	16.91	15.64	16.49	15.88	270
	22.17	22.46	23.04	22.78	24.95	23.46	22.87	21.44	24.26	22.26	24.00	22.73	390
	25.48	25.69	26.31	26.29	28.89	27.32	26.75	24.94	27.96	26.10	28.12	26.27	450
	29.15	28.82	29.85	30.08	33.28	31.87	31.13	27.84	32.83	29.37	33.05	30.47	510

APPENDIX A (continued)

Exp. No.	1	2	3	4	5	6	7	8	9	10	11	12	Time in Minutes
12.	6.32	6.70	-	7.02	6.90	5.70	-	7.43	6.41	8.06	6.09	6.94	150
	38.63	39.01	-	40.26	41.20	34.98	-	35.17	33.24	20.99	16.26	16.74	510
13.	6.51	6.60	7.47	7.28	7.78	8.10	6.24	5.71	8.30	6.89	6.35	7.74	150
	36.96	37.40	40.32	36.50	42.51	38.95	36.56	34.42	45.47	31.04	28.79	34.00	510
14.	10.12	9.94	10.45	9.09	9.02	8.82	6.30	7.81	9.19	8.91	7.10	8.98	150
	49.83	51.26	54.20	46.46	44.23	43.60	38.30	38.83	44.80	42.66	38.55	42.48	510
15.	5.49	6.26	6.52	6.95	6.40	5.80	6.00	5.99	5.54	6.23	6.44	5.96	150
	31.81	36.46	36.80	39.84	35.80	38.35	32.34	34.85	33.12	33.51	35.50	32.27	510
16.	7.18	7.52	8.76	6.45	7.25	7.21	7.46	7.24	6.98	7.00	6.20	7.42	150
	44.41	45.93	48.39	43.15	42.73	41.86	46.25	43.84	47.28	41.18	37.92	44.05	510

APPENDIX B.

Tabulated below are oxygen uptakes in micro-litres per milligram dry weight corrected by each flask constant, for flasks in the preliminary experiments.

Experiment I.

Time in Minutes	FLASK NUMBERS 1	2	3	4	5	6	7	8	9	10
30	.109	.33	.25	.60	1.85	.79	.63	1.44	.66	.11
60	.657	1.10	.75	1.08	2.71	1.14	.72	1.72	1.04	.66
90	2.08	2.97	2.00	3.13	4.81	3.07	2.17	4.17	2.75	3.52
120	2.95	4.29	2.83	4.45	6.29	4.10	3.16	5.61	3.61	4.18
150	3.72	5.39	3.67	5.54	7.28	4.78	3.62	6.33	4.56	4.41
180	4.70	6.71	4.59	6.86	8.64	5.92	4.70	8.20	5.79	5.51
210	5.69	7.92	5.42	8.31	10.49	7.18	5.70	9.70	6.93	7.49
240	6.78	9.13	6.17	9.27	11.48	8.20	6.60	11.37	7.79	7.93
270	7.77	10.56	6.93	10.24	12.22	8.89	6.96	12.09	8.36	7.27
300	9.08	12.10	8.26	12.41	14.32	10.14	8.59	14.25	9.78	9.70
330	9.85	13.09	9.01	13.73	15.43	11.28	9.41	15.26	10.54	10.80
360	10.95	14.63	10.02	15.06	16.54	12.19	10.40	16.70	11.78	11.68

APPENDIX B (continued)

Experiment II

Time in Minutes	FLASK NUMBERS 1	2	3	4	5	6	7	8	9	10
30	1.43	1.86	.63	.92	1.17	.44	.37	1.37	.96	.86
60	2.96	3.94	2.23	2.34	2.44	2.19	1.54	3.18	2.74	2.37
90	4.29	6.02	3.83	3.77	3.72	3.95	2.82	4.87	4.42	3.78
120	5.63	7.99	5.32	4.99	5.00	5.56	4.23	6.25	6.09	5.07
150	7.25	9.63	6.81	6.73	6.81	7. 32	5.64	7.95	7.88	6.58
180	9.07	11.82	8.41	8.56	8.30	9.22	6.95	9.64	9.67	7.99
210	10.21	13.46	9.79	9.89	9.58	10.98	8.08	10.81	10.75	9.07
240	11.46	15.00	11.18	11.11	10.75	12.30	9.21	12.08	12.42	10.36
270	12.89	16.75	12.67	12.24	12.14	13.77	10.34	13.56	13.86	11.55
300	14.22	18.50	14.05	13.46	13.52	15.52	11.56	14.94	15.17	12.85
330	15.56	20.14	15.54	14.79	15.33	17.14	12.87	16.43	16.49	14.04
360	16.99	22.22	17.04	15.91	16.40	18.75	13.91	17.70	17.92	15.22

Experiment IV

Time in Minutes	FLASK NUMBERS 1	2	3	4	5	6	7	8	9	10
30	.88	1.10	1.48	.84	1.74	.75	.66	1.02	1.68	1.35
60	1.90	2.21	2.41	1.61	3.13	1.56	1.18	1.97	2.70	2.34
90	3.33	3.51	3.62	2.61	4.78	2.51	2.07	3.47	4.27	3.78
120	4.62	5.26	5.11	3.46	6.52	3.74	2.96	5.13	5.73	5.22
150	6.72	6.56	6.41	4.38	8.26	5.50	3.92	6.55	7.08	6.39
180	7.34	8.12	8.18	5.46	10.17	6.32	5.25	8.21	9.00	7.92
210	8.77	9.36	9.48	6.23	11.83	7.54	6.21	9.63	10.23	9.27
240	10.60	11.05	11.16	7.16	13.48	9.18	7.47	11.53	12.15	11.16
270	12.24	12.67	12.64	8.31	15.39	11.62	8.80	13.03	13.83	12.78
300	13.60	14.04	14.04	9.31	17.13	11.49	9.54	14.61	15.18	14.31
330	15.02	15.47	15.25	10.31	18.70	12.71	10.87	16.11	16.76	15.66
360	16.38	16.77	16.46	11.31	20.53	13.87	11.91	17.69	18.33	17.10

APPENDIX B (continued)

Experiment III.

Time in Minutes	FLASK NUMBERS 1	2	3	4	5	6	7	8	9	10
30	.82	.48	1.1	.79	.85	1.07	1.08	.98	1.09	1.49
60	1.76	1.14	2.2	1.92	1.27	2.14	2.53	1.83	2.18	2.78
90	2.85	2.00	3.76	3.16	2.44	3.26	3.97	2.67	3.64	4.70
120	3.26	2.57	4.46	3.84	2.87	3.77	4.57	3.24	4.13	5.56
150	3.94	3.34	5.44	4.74	4.04	4.79	5.90	3.94	5.22	6.63
180	4.89	3.91	6.55	5.53	4.47	5.40	6.74	4.79	6.07	7.06
210	5.71	4.67	7.53	6.44	5.43	6.01	7.71	5.49	7.04	7.41
240	6.25	5.06	8.23	7.11	5.75	7.14	8.67	5.92	7.65	7.91
270	7.20	5.82	9.34	8.13	6.60	7.44	9.39	7.05	8.74	8.98
300	8.29	6.87	10.32	8.92	7.34	8.26	10.36	7.75	9.59	9.63
330	8.56	7.16	10.60	9.37	7.66	8.97	10.96	8.03	10.08	9.63
360	9.79	8.40	12.13	10.62	8.83	9.99	12.41	9.72	11.42	10.91

Experiment V.

Time in Minutes	FLASK NUMBERS 1	2	3	4	5	6	7	8	9	10
30	1.56	1.58	2.48	1.87	2.04	2.43	2.47	1.22	1.12	1.82
60	3.92	3.96	4.96	4.02	4.59	4.86	4.72	2.20	2.02	3.91
90	6.01	6.87	7.68	6.71	7.39	8.00	7.42	3.67	3.83	6.00
120	8.62	9.52	10.41	9.12	10.45	10.15	9.22	5.14	6.31	8.61
150	10.19	11.37	12.40	11.00	12.75	11.86	11.25	6.12	8.34	10.44
180	12.81	13.75	14.63	12.88	15.55	14.72	13.50	8.08	10.84	12.52
210	14.90	16.13	16.61	14.76	18.10	16.58	15.30	10.04	13.30	14.35
240	16.21	17.72	18.35	15.84	19.89	17.87	20.25	11.27	15.10	15.92
270	21.18	23.01	24.05	20.67	25.24	23.59	20.92	15.92	20.97	21.14
300	21.96	23.08	24.55	20.94	25.75	24.73	23.17	16.66	21.87	21.40
330	24.05	25.92	26.04	22.82	27.79	26.88	24.75	18.37	24.12	22.70
360	25.88	27.77	27.77	24.16	29.58	28.88	26.77	20.09	26.15	24.53

Experiment VI

Time in Minutes	FLASK NUMBERS 1	2	3	4	5	6	7	8
30	3.26	3.86	2.95	3.36	4.60	3.26	4.02	2.85
60	7.25	8.41	9.93	6.51	8.60	6.73	7.54	5.98
90	9.85	11.37	13.13	9.45	11.00	8.77	10.56	7.12
120	13.35	15.47	18.52	11.97	15.00	12.44	14.58	11.11
150	15.20	17.74	21.21	14.91	17.20	14.48	16.85	13.11
180	17.35	20.02	23.35	16.59	19.40	16.72	19.61	15.67
210	19.89	22.52	25.50	17.85	21.40	19.17	22.13	17.38
240	22.23	24.79	27.65	21.63	23.20	21.82	24.89	21.94
270	23.63	25.70	28.99	21.84	23.40	22.64	25.90	21.37
300	25.97	28.43	32.48	24.57	25.40	25.09	29.17	24.79
330	27.37	29.57	33.83	25.20	26.00	26.52	30.43	25.38
360	29.25	31.62	35.44	26.88	27.40	28.35	32.94	27.95
390	30.18	32.52	37.32	28.77	27.40	29.58	34.45	28.21
420	31.59	34.58	37.05	30.03	28.20	31.62	36.46	29.94
450	32.52	35.49	37.85	30.45	28.60	32.84	37.47	30.78
480	33.93	36.62	38.93	32.97	29.40	33.86	38.98	32.20
510	35.10	37.76	40.27	34.65	29.80	34.88	41.49	33.91
540	36.73	39.13	42.96	35.49	31.00	36.51	42.50	35.34

Experiment VII

Time in Minutes	FLASK NUMBERS 1	2	3	4	5	6	7	8	9
30	1.80	1.81	1.88	2.24	1.00	2.32	2.67	.12	.87
60	3.87	3.96	4.28	4.65	2.57	3.81	5.19	.41	2.28
90	5.95	6.43	6.51	6.72	4.29	6.30	6.53	1.11	3.27
120	7.06	7.59	8.06	8.45	5.43	7.63	7.72	1.93	4.36
150	8.03	8.58	9.26	9.83	6.57	8.46	9.65	3.04	5.34
180	9.14	10.06	10.63	11.55	7.57	9.96	10.84	3.87	6.21
210	10.11	11.38	11.66	12.76	8.43	10.62	10.98	4.29	6.32
240	11.77	13.53	14.06	14.66	9.86	12.61	13.06	6.64	7.95
270	12.60	14.52	15.09	15.69	11.01	13.44	14.55	7.20	8.72
300	13.71	15.84	16.80	17.42	11.86	15.10	16.33	8.03	9.81
330	14.81	17.32	17.49	18.45	12.87	16.10	16.92	9.14	10.79
360	15.65	18.64	19.20	19.83	14.15	17.09	17.52	10.24	11.55
390	16.48	19.30	19.72	20.87	15.01	18.75	19.00	11.08	12.42
420	18.28	21.45	21.60	22.59	16.44	19.42	20.34	12.60	13.51
450	19.25	22.93	22.80	24.15	18.01	21.41	21.68	14.54	14.82
480	20.22	24.25	23.83	25.35	18.59	21.58	22.72	15.51	15.58
510	21.46	25.74	25.21	27.08	20.15	23.07	24.35	16.62	16.78
540	22.43	27.22	26.41	28.63	21.45	24.56	25.32	17.58	17.98
570	23.82	28.75	27.95	30.18	22.59	26.06	26.43	18.83	19.29
600	24.65	30.36	28.81	31.74	23.59	28.05	27.62	19.80	20.27
630	25.89	32.01	30.18	33.63	25.02	28.88	29.40	20.91	21.47
660	27.00	33.66	31.89	35.53	29.45	33.03	31.18	22.43	22.67
690	29.97	35.47	33.27	37.60	27.59	30.71	32.98	22.85	24.08
720	31.02	39.69	37.55	42.26	31.60	36.18	19.30	26.59	28.01
750	32.40	41.25	38.58	43.81	32.60	37.51	19.89	27.42	28.99
780	33.51	42.73	39.78	45.88	34.46	38.51	21.38	28.94	30.73

Oxygen uptake figures used for experiments IX, X and XI of the preliminary experiments are those for respectively 12, 2 and 13 of the major series of experiments.